eman ta zabal zazu

Universidad del País Vasco

Euskal Herriko Unibertsitatea

Doctoral Thesis

Characterization of an animal model of cognitive impairment associated with schizophrenia. Effects of α_2-adrenoceptor compounds.

Blanca Pérez del Palomar Asín

2019

"All our dreams can come true if we have the courage to pursue them"

Walt Disney

AGRADECIMIENTOS

Jamás podría imaginar que llegado este momento de escribir los agradecimientos, me iba a quedar mirando la pantalla sin saber reaccionar, como me quedé aquel fatídico día en el que puse la primera palabra de lo que ahora es mi tesis...Y eso que éste siempre había sido mi apartado más esperado y deseado; no sólo porque implica que esta etapa ha llegado a su fin, sino porque invita a poner en la balanza estos últimos años y reflexionar sobre qué es lo que ha pasado durante este tiempo. Y ahora que ha llegado el momento, me ha pillado con la guardia baja.

Tengo que confesar que cuando meses atrás pensaba en este ansiado momento, no me podía imagina lo difícil que me resultaría afrontarlo; son muchos los sentimientos, emociones y pensamientos que se amontonan en mi cabeza en cada segundo. Siendo sincera conmigo misma, he de decir que estos cuatro años han conformado una de las mejores y peores etapas de mi vida. No me arrepiento ni por un instante del camino que tomé. Pero sí he de reconocer que ciertos momentos, especialmente aquellos que resultaron más duros, me hicieron replantearme qué había hecho con mi vida y porqué me había metido en este lío cuando podría haber elegido caminos mucho más sencillos. Y son precisamente esos momentos de crisis existenciales, los que una vez son superados, te muestran todo lo que te han hecho aprender y madurar. No sólo a nivel profesional, sino especialmente a nivel personal, que es aún más importante. Sin duda alguna, estos últimos años han sido una lección de vida para mí. Una carrera de fondo con muchas "asignaturas" de diversa índole que había que ir superando. Algunas no duraban mucho y se superaban con la práctica; no obstante otras han durado hasta el día de hoy, y algunas durarán para siempre. Estos cuatro años de "carrera" me han enseñado a conocerme más en profundidad a mí misma y a reconocer cuáles son mis puntos fuertes y mis debilidades, y a intentar, al menos, potenciar unos y corregir los otros. Como a muchos les ha pasado antes que a mí, he chocado contra la pared el triple de veces de las que he salido exitosa, pero de eso consta todo proceso y de ello se aprende, que es lo importante.
Cualquier persona que me conozca un poco sabe que me resulta muy difícil resumir, y expresar en pocas páginas todo lo que me gustaría decir a todas esas personas que tengo en mente, es casi misión imposible para mí… Así que aquí comienza mi último reto durante esta tesis, reto que curiosamente, sea uno de los más difíciles. *Pero vamos allá…*

Javier, lo primero, muchísimas gracias por ser mi director de tesis y por darme la opción de poder formar parte del grupo y aspirar a conseguir mi sueño, el de tener una carrera investigadora. Además de eso, quiero agradecerte tu apoyo especialmente durante estos últimos meses en los que, a pesar de tropezar numerosas veces durante el camino, has tenido la paciencia de ayudarme a seguir hasta la meta. Gracias por todo lo que me has enseñado durante este proceso, y también por tu exigencia; la cual, aunque a veces resultaba dura, sin duda me ha ayudado a prepararme para afrontar un futuro como investigadora.

Ane, la madre del "Pharmateam", gracias por estar siempre ahí y por tener a mano un buen consejo para el día a día. Pocas personas conozco con el don de tu inmensa paciencia (*y por tu gusto por una ducha con agua ardiendo también en pleno verano, ¡di que si!* ;)
Koldo, importante pilar que sustenta el "Pharmateam", y uno de los mejores expertos en temas del buen comer y del buen beber (*como buen autóctono*), y siempre con la colección de "anécdotas congresiles" dispuestas a relucir para hacernos pasar un buen rato. Gracias por los consejos y por todos esos buenos ratos (¡*y por ser ejemplo de puntualidad en el grupo*!).
Rebe, la eterna sabiduría personificada. Siempre sacándonos de los apuros, con respuestas a todas las preguntas, y disponible al 100% para ayudar a quién sea con lo que sea. Gracias por tu

disposición y buenos consejos. Y cómo no, en el pack viene Raúl, uno más del "Pharmateam", que allá por donde pasa siembra alegría y es capaz de provocarte una sonrisa en los días más grises. Gracias por darnos una razón para reír cada día, y por supuesto, por ser tan buen anfitrión durante aquel fin de semana en León, inolvidable.
Leyre, vivo ejemplo de que si uno lucha por lo que quiere, lo puede lograr. Espero poder aprender de tu determinación y aplicarla en mi día a día.
Guada, no hemos coincidido mucho, pero tu gran sentido del humor y buenos consejos son de sobra conocidos en el lab.
Igortxu, gran persona y compañero donde los haya. Siempre con alguna anécdota personal graciosa para echar unas risas y afrontar el día con otra actitud. Eskerrik asko por ofrecerme tu ayuda cuando la he necesitado, por tus cariñosos abrazos y por los todos los buenos consejos que me has ido dando durante estos años, no dudes que me han aportado mucho.
Carol, la chica de la eterna sonrisa. Gracias por tu disposición y ayuda, por tu alegría, por los consejos, y por darme la oportunidad de disfrutar de la celebración de uno de los momentos más importantes en tu vida; tu boda.
Amaia, eskerrik asko urte hauetan zehar egindako guztiagatik. Eskerrik asko beti laguntzeko denbora bilatzeagatik, baina batez ere, nigan izan duzun konfiantzarengatik; zuk beti nire gaitasunetan sinetsi duzu, nik sinesten ez nuenean ere. Eskerrik asko hain irakasle ona izateagatik, zure gertutasunagatik eta zure maitasun amaigabegatik.
Evita, la "labmanager" del "Pharmateam", ¡qué te voy a decir que no sepas! Quién nos iba a decir que nuestra relación iba a ser como es a día de hoy, ¿verdad? Precisamente por haber superado diversas fases durante estos años puedo decir que hemos logrado forjar una fuerte, intensa y sincera amistad. Han sido innumerables las anécdotas que hemos compartido juntas: cambios de ratones, viajes con las "labgirls", vídeos pasando la aspiradora y cantando en pijama (*INOLVIDABLE*), noches de hoteles de lujo en Madrid donde nos sobraba más de media cama a cada una, fiestas en tu terraza, risas eternas haciendo skypes con Julie, condiciones de hipoxia durante las largas jornadas del TS, SanFermines, Celedón...Incluso has sido mi salvadora en situaciones de emergencia, como cuando me quedé atascada en la nieve o encerrada en la uni... En definitiva, gracias por todos esos momentos, por tus consejos, y por tu ayuda (*y "asistencia*") siempre que lo he necesitado. Espero que aún queden muchos más por compartir y disfrutar (*y para empezar, ¿qué tal esa apuesta que finalmente he acabado ganando?? Ya estoy saboreando el Lambrusco!)*
Leire Corada, el gran soporte real del "Pharmateam". ¡Qué habríamos hecho sin ti en tantas ocasiones! Siempre con una sonrisa y una solución para cualquiera de tus problemas, con una visión positiva y siempre mirando hacia adelante. Gracias por preocuparte siempre y por estar ahí para animarme y apoyarme. Y gracias también por todos esos momentos de cotilleos en el despacho, capaces de hacerte olvidar hasta los días más penosos.

Toca el turno de las "labgirls", y comenzaré por las que ya "volaron" del lab.
Aintzane, la rubia más actualizada en todos los temas de interés ;) Hemos coincidido más fuera que dentro del labo, pero en todas esas ocasiones has tenido sabias y buenas palabras para mí y consejos que me han aportado muchísimo. Nunca olvidaré tu despedida en Oxford, tu foto con la camiseta de la Real y tu gran papel como "gremlin", *awesome*!
Patri, mi gran consejera y amiga. A pesar de no haber compartido mucho dentro del labo, desde fuera siempre has estado ahí para escucharme, aconsejarme y ayudarme cuando te lo he pedido. Tu experiencia, serenidad y tu gran personalidad son envidiables, y a pesar de la distancia, te he sentido muy cerca de mí cuando más te he necesitado. Gracias por todo, creo que sobran las palabras pues tú bien sabes todo lo que me has aportado.
Itzi, un ejemplo a seguir dentro y fuera del laboratorio. Estuvimos poco tiempo juntas, aunque más que suficiente para darme cuenta de que eres la perfección en persona, pocas personas son

tan eficaces y resolutivas como tú en el trabajo. Además de eso, generosa, buen compañera y solidaria con los demás, gracias por toda tu ayuda durante estos años.
Irene, instructora y compañera de técnica. Tú me abriste paso en mis inicios encargándote de mí, guiándome, enseñándome y transmitiéndome toda tu experiencia. Gracias por tu inmensa paciencia, y por estar ahí cuando te necesité.

Y ahora vamos a por las que son más contemporáneas a mi época y con las que he pasado gran parte de mi vida en el lab.
Inés, mi más real contemporánea, a cuyo lado comencé oficialmente mi carrera investigadora. Hemos vivido muchas experiencias juntas, sobre todo durante aquel "curioso" año de máster, repleto de vivencias y recuerdos que no nos dejaron indiferentes. Gracias por estar siempre dispuesta a ayudar y a aconsejar cuando se te necesita (*y por compartir conmigo nuestro "no gusto" por el queso :P*). Mucho ánimo en esta última etapa, para cuando te des cuenta, estarás escribiendo este apartado.
Iria, mi *pulpeira* favorita. Desde que llegaste congeniamos y fuimos compartiendo juntas innumerables vivencias de todo tipo. Siempre has tenido la capacidad de detectar cuando algo no me iba bien, y de alguna manera, te encargabas de hacérmelo olvidar y contagiarme con tu actitud siempre optimista. Gracias por todas esas cervezas *(¿y mojitos?)* con confesiones y por todas las geniales experiencias vividas durante estos años. Esta etapa no habría sido lo mismo sin ti.
Itzi, la "txikitina" del grupo y que ya no lo es tanto ;) con esa energía y optimismo que te caracteriza siempre. Todavía recuerdo tus inicios; fuiste de las primeras a las que pude considerar "alumna" y tu disposición y ganas lo hicieron todo mucho más fácil. Sigue así, y llegarás muy lejos.
Marta, mi fuente de apoyo e inspiración del lab. Desde que llegaste demostraste tu madurez, entereza y gran personalidad, y estoy segura de que esa disposición y entrega en tu trabajo te llevarán muy lejos. Gracias por todos tus abrazos, tus consejos, tus chistes y tu paciencia, y gracias también por aquellos maravillosos días en San Diego que jamás olvidaré. Te aseguro que me has aportado mucho más de lo que tú puedes imaginar. Y Carlos, gracias a ti también, que estando en situación similar a la mía, me has servido de inspiración a través de lo que Marta me contaba. ¡Es maravillosa esa alegría que contagiáis!
Natalia, ¡mi pupila! Irradias felicidad allá por donde pasas. Tus ganas de aprender, tu disposición y tu constancia te han llevado a conseguir lo que has logrado hoy, y te servirán para alcanzar todo lo que te propongas en la vida. Gracias por dejarte enseñar, por tu humildad y por tu cariño, has sido indispensable para mí durante estos meses.
Amaia, nuestro terremoto personificado. El carácter y la energía que te caracterizan te ayudarán a sobrellevar toda situación que se te presente. Dispuesta donde las haya, te deseo que sigas adelante y no tires la toalla por muchos obstáculos que te encuentres, ya que siempre hay una salida. Sigue así y llegarás a donde te propongas. Miles de gracias por tu apoyo durante estos últimos meses, por sacarme del búnker para compartir un café y desconectar de la tesis y por animarme día a día a seguir adelante.
Teresa, la última en llegar, pero pisando fuerte. Estoy segura de que todo te irá estupendamente con la constancia y dedicación que estás demostrando. Mucho ánimo y siempre para adelante.
Maider, estuviste poco en el lab pero desde luego dejaste huella. Gracias por tu apoyo, tus ganas de aprender, tu iniciativa y tu buen humor. Siempre dispuesta a ayudar y aprender de todo el mundo. Una pena que no eligieras este camino, el "Pharmateam" perdió con tu marcha un muy buen fichaje.

Y para dar por cerrada la sección "Pharmateam" quería terminar contigo Jorge, que por supuesto, no me he olvidado de ti, más bien todo lo contrario. Creo que no encuentro manera para expresar ni por escrito ni con palabras todo lo que has significado para mí. Desde esas primeras preguntas en clase de Farma sentada en la primera fila (*creo que jamás olvidaremos el útero grávido, ¿verdad?*), pasando por aquel examen que me permitiste repetir para subir nota, y llegando al momento en el que me brindaste la oportunidad de poder pasar a formar parte del "Pharmateam". Y a partir de ahí, en la etapa tesis,… ¿Qué puedo decir? No me puedo sentir más afortunada por haber tenido a alguien como tú como mentor, director y tutor, pero sobre todo, como compañero y amigo, de los de verdad. A nivel profesional, me has enseñado prácticamente todo lo que sé, has confiado en mí por encima de todo incluso cuando ni siquiera yo misma lo hacía, y nunca me has abandonado. Pero es que a nivel personal, me has ayudado a crecer como persona y a afrontar todas las adversidades para poder seguir adelante. Gracias por no juzgarme, por confiar en mí, por saber escuchar y por entenderme. Gracias por todos esos ratos, por las conversaciones telefónicas eternas, por todas esas reflexiones profundas en Oxford acompañadas de buena cerveza y *live music*, por tu sinceridad y franqueza, por tu transparencia, por tu positividad, por tus principios. Gracias, porque este trabajo también es tuyo. Sabes que sin ti, no habría llegado hasta donde estoy ahora. Espero que no olvides esto, y que a partir de ahora, de la manera que sea, sigamos trabajando juntos, compartiendo ilusiones y proyectos que lograremos hacer florecer con esfuerzo conjunto, como hasta ahora. Gracias de corazón.

Ahora me traslado por un momento al otro lado del océano...
My dear USA lab! I cannot find the words to explain how grateful I feel for having had the chance of meeting you all and to work with you in the lab during those wonderful months last year. You made me feel part of the amazing family you have there, I was one more since the first day. All my dear colleagues (Gray, Manish, Marie, Kyle, Bhooma, Loc, Makenzie, Sasha, Jenny, Joel, Sarah, Kia, Rasheed, Sree, Alex, Michael…*sorry if I miss someone, it was such a big crowd!*), thank you all for your patience and willingness to help me, to always support me, and for trusting me. I cannot say thank you enough to all of you... And what should I say for Jordan and Ream? You were just like lovely parents during that time, always thinking about my well-being, doing everything you could to help me in any way, transmitting me your enormous generosity, kindness, and love. I really could not ask for more...Honestly, I have to admit those months were some of the best times of my life. And Jordan, special thanks to you for your efforts helping me with this thesis in a very complicated and busy moment for you, both professionally and personally. You have no idea how important your help and support have been during this time. And of course, thank you both for offering me a chance to continue my scientific career with your amazing lab, there is no way I could thank you enough for that! See you all very soon! <3
Y sin abandonar USA, no puedo dejar de agradecer a mis *American-Spanish* people todo el apoyo y ayuda que me habéis brindado durante aquellos meses, pero ahora también, a través de la distancia, apoyándome en todo momento durante esta dura etapa. Muchos no llegaréis a leer esto, pero no me puedo quedar sin expresaros mi eterna gratitud.
Rocío, mi cántabra favorita; gracias infinitas por tu inmensa ayuda y tu apoyo incondicional donde los haya para superar todos los obstáculos. Un ejemplo de entereza, determinación y positividad.
Rebeca, vecina, confidente y gran amiga. Fueron unos meses intensos en STL en los que compartimos mucho, pero durante mi etapa de escritura de tesis tu apoyo ha sido clave, tus consejos y tu experiencia siempre me han ayudado a verlo todo más positivo. Mil gracias, no sabes las ganas que tengo de volver a compartir contigo todo lo que te he ido apuntando en mi lista de "*things to do in STL when I am back*", asi que, *get ready*!
Sonia, durante esta etapa el poder compartir contigo mis ralladuras, dramas, subidones y emociones han sido el mejor combustible para seguir adelante. Y como te dije, tu visita esos días para mí fue un punto de inflexión; sin él no habría podido llegar (*al menos en buen estado*) a

donde he llegado hoy. Mil gracias por todo, por supuesto que no habrá distancia que separe lo que estas tesis han unido.
Yolanda, imposible no pensar en ti cuando miro esta tesis; eres una artistaza de pies a cabeza y no me puede hacer más ilusión que formes parte de este trabajo de manera tan especial. Muchas gracias, no sólo por haber contribuido a hacer de este trabajo algo precioso y a que me acuerde de ti cada vez que la mire, si no porque tu energía, alegría y forma de ver la vida me han empujado mucho para seguir adelante durante estos duros meses. Madrid contigo fue un chute de energía. No sabes cómo desearía que volvieras a STL en un futuro cercano. Ánimo campeona.
Y mi querida Noe, mi gasteiztarra favorita, a ti qué te puedo decir que no sepas o que no te haya dicho ya... Tú has sido sin duda alguna un pilar clave en mi vida desde que pisé por primera vez STL y luego tras abandonarlo. Desde la distancia, con nuestros skypes, audios, llamadas y mensajes, has logrado levantarme una y otra vez incluso cuando más me había hundido. Desde que te conocí, hasta el día de hoy, no has podido ser más generosa y buena conmigo, y no hay forma para agradecerte todo eso. Nos une algo especial; no sólo la carrera profesional o la ciudad de origen, es algo mucho más fuerte. Prepárate que ese viaje a NY nos espera, ¡y muchísimo más!

Volviendo a los orígenes…
Alazne e Ylenia, gracias por no olvidaros nunca de mí y por siempre sacar un ratito para un café. Gracias por escucharme todos mis rollos, me habéis aportado muchísima energía y positividad.
Katia, la distancia nos unió aún más que la cercanía, y desde luego no hay palabras para darte las gracias por tu cariño, preocupación y apoyo. Gracias por sacarme de la rutina y por siempre tener una visión positiva de la vida.
Jorge, siempre con alguna broma preparada para sacarme una sonrisa, siempre poniéndome en un pedestal (*sin merecerlo*) y haciéndome sentir que era capaz de todo. Te aseguro que si algún día consigo obtener ese premio Nobel que tan seguro estás que lograré, serás el chófer oficial de mi limusina como tanto anhelas :P
Estitxu, ¿hay palabras? Si creía que no podía haber forma de intensificar nuestra amistad, la distancia me quitó la razón. Cómo expresar mi gratitud por tu apoyo diario, tus mensajes de ánimo comenzando con "*Hola, reina mora!*", tu disposición para escuchar y aconsejar, fuera cual fuera el motivo de crisis. Gracias por ayudarme a sobrellevar estos duros meses y por ponerme como prioridad. Por todo lo que hemos compartido, por las confesiones, por las escapadas, por tu confianza... En definitiva, por estar ahí como solo tú sabes, mil gracias.
Leire, y por supuesto Iva, mis "supernaiset" favoritas. Expertas en hacer que la distancia no sea un obstáculo. No os he podido echar más de menos en estos meses, pero desde luego no hay palabras para expresaros lo importante que ha sido vuestro apoyo incondicional. Esas escapadas anuales de fin de semana por lugares de Europa (*tradición que rompí pero pretendo recuperar*) eran chutes de vida, de energía y positividad. No sé si conozco personas más enriquecedoras. Gracias por manteneros a mi lado a pesar de las dificultades, os quiero muchísimo.
Beatriz, mi *lehengusina* favorita. Gracias por ser mi confidente, por darme tus consejos de vida, por confiar en mí y por no juzgarme nunca…Gracias por ser mi compañera de viajes, y cómo no, de múltiples juergas. Gracias por ayudarme a olvidar *la cárcel* durante unos días acompañándome a Dublín (i*nvitarme a Guinness también colaboró a ello :P),* y por estar siempre dispuesta a compartir vivencias conmigo. Nos une la sangre sí, pero también algo mucho más fuerte, que es un inmenso cariño y una gran y leal amistad.
Ainhoa, mi tía favorita. Nuestra pasión por los "*Takies*" ha sobrepasado muchas fronteras, y este año de manera aún más especial en aquel viaje a Manchester. Mil gracias, me dio un chute de energía cuando más lo necesitaba. Gracias por estar siempre dispuesta a escucharme, aconsejarme y ayudarme, sea cual sea el problema. Siempre serás la hermana mayor que nunca tuve. *Never Forget…*

Marian, gracias por todo lo que tú ya sabes. Y no sólo por mi parte, sabes que mis aitas y yo te debemos mucho. Tu saber estar, tu disposición y tu cariño nos han ayudado a salir adelante a todos y a mí la primera. Te queremos muchísimo. Iñaki, aunque nos faltas desde hace ya tiempo, te seguimos manteniendo en el corazón. Gracias por tus "abrazos de oso" cuando más los he necesitado. Siempre estarás entre nosotros.
Abuela Mª Carmen y abuelo Vicente, no habéis logrado ver terminado este trabajo, pero sé que sin vuestra ayuda y confianza en mí, no lo habría conseguido. Abuela Celsa y abuelo Jose Mari, por fin os puedo decir que esto… ¡se ha acabado! Gracias por vuestra preocupación y por preguntarme siempre "*y qué tal van los estudios*"…

Y para finalizar, mis pilares vitales, mis aitas. Eternamente agradecida se queda inmensamente corto. Sin vosotros no sólo no estaría aquí, sino que me habría perdido por el camino ya mucho tiempo atrás. Y lo primero que quiero es pediros disculpas por todos esos momentos en los que no he sido yo, en los que me he convertido en alguien desconocido y durante los cuales ha sido tan difícil llevarme. Siento mucho el haberos hecho sufrir con mis problemas, inquietaros con mis eternas dudas, y por mi humor irascible y agresivo durante tanto tiempo… Nunca me arrepentiré lo suficiente por haberos hecho pasar esos malos ratos... *Aita*, gracias por tu ayuda constante e incondicional siempre, por sacarme de todos los apuros sin pedir nada a cambio, por siempre buscar lo mejor de lo mejor de lo mejor para mí. Todos estos años de esfuerzo habrán merecido la pena cuando vea esa expresión de orgullo en tu cara, esa que pones y no dices nada, y en la que tímidamente se asoma una pequeña lagrimilla que intentas disimular. *Ama*, podría escribir otras mil tesis solo dándote las gracias, pero creo que sabes lo que eres y significas para mí. Sin ti, ya no sólo acabar esta tesis, sino llegar a dónde he llegado hoy, totalmente imposible. Perdóname mil veces por todo lo que te he hecho pasar, y gracias infinitas por no abandonarme nunca y estar a mi lado de manera incondicional y absoluta. Vendrán tiempos mejores pronto para las dos, lo sabes, y lucharé por encima de todo para intentar devolveros aunque solo sea una pequeña parte de todo lo que habéis sacrificado por mí. Os quiero, *aitatxos*.

Por último, gracias a todas aquellas personas que han formado parte de mi vida durante estos años y que no he mencionado de manera específica pero que vosotros sabéis que habéis estado ahí. Gracias a todos y a cada uno de vosotros, se ha ido forjando la persona que escribe estas líneas ahora mismo.

GRACIAS

Y bien, ahora sí, creo que ha llegado el momento de poner el punto final real a esta tesis. Y esa sensación que me invade ahora mismo, no se puede expresar con palabras…

α;→

This Doctoral Thesis has been performed thanks to the financial support of a Predoctoral Training Contract (FPU) from the Spanish Ministry of Education, Culture and Sports during the years 2015-2019.

Additionally, the present work has also been funded by the Spanish Ministry of Economy and Competitiveness (SAF2013-48586-R, SAF2017-88126-R), the Basque Government (IT-616-13, IT1211-19), the Instituto de Salud Carlos III, and the Centro de Investigación Biomédica en Red en Salud Mental (CIBERSAM).

♒

A mi ama,

a quien debo absolutamente todo…

❀∞❀

ABBREVIATION INDEX

αAR	α-adrenoceptor
AAV	Adeno-associated viral vector
AC	Adenylyl cyclase
aCSF	Artificial cerebrospinal fluid
AD	Alzheimer's disease
ADHD	Attention deficit hyperactivity disorder
Akt	Protein Kinase B
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
A/P	Anterior-posterior
ArchT	Archaerhodopsin
AUC	Area under the curve
βAR	β-Adrenoceptor
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
°C	Degrees Celsius of temperature
Ca^{2+}	Calcium
cAMP	Cyclic adenosine monophosphate
CAV-2	Canine adenovirus type 2
cGMP	Cyclic guanosine monophosphate
CIAS	Cognitive impairment associated with schizophrenia
CM	Condensed milk
cm	Centimeters
CNS	Central nervous system

COMT	Catechol-*O*-methyl-transferase
CSF	Cerebrospinal fluid
5-CSRTT	Five-choice serial reaction time task
DA	Dopamine
DAT	Dopamine (DA) transporter
DBH	Dopamine-β-hydroxylase
DIO	Doble-floxed inverse orientation
DISC-1	Disrupted in schizophrenia-1
DNA	Deoxyribonucleic acid
(±)-DOI	2,5-Dimethoxy-4-iodoamphetamine
DOPAC	3,4-Dihydroxyphenyl-acetic acid
DR	Dopamine (DA) receptor
DSP-4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine
dsRNA	Double-stranded RNA
DTT	Dithiothreitol
D/V	Dorso-ventral
Ed	Extraction fraction
EDTA	Ethylenediamine tetraacetic acid
Ef1α	Eukaryotic translation elongation factor 1 alpha
Emax	Maximal effect
ErbB	ErbB-type tyrosine kinase receptor
FGA	First-generation antipsychotic
FST	Forced swimming test
GABA	γ-Aminobutyric acid
GD	Gestational day
GPCR	G protein-coupled receptor

GTPγS	Guanosine 5'-O-[gamma-thio]triphosphate
h	hour
HCl	HydroChloric Acid
HCN	Hyperpolarization/cyclic nucleotide-gated channel
HDAC	Histone deacetylase inhibitor
HPLC	High-performance liquid chromatography
5-HT	5-Hydroxytryptamine (serotonin)
5-HTR	Serotonin (5-HT) receptor
Hz	Hertz
IL	Interleukin
i.p.	Intraperitoneal
ITI	Inter-trial interval
i.v.	Intravenous
K^+	Potassium
KCNQ	Potassium voltage-gated channel
K_d	Dissociation constant
kDa	Kilodalton
kg	Kilogram
KO	Knockout
KOH	Potassium hydroxide
LC	Locus Coeruleus
L-DOPA	3,4-Dihydroxyphenylalanine
LH	Limited hold
LI	Latent Inhibition
LPS	Lipopolysaccharide
LSD	Lysergic acid diethylamide

M	Molar
MAO	Monoamine oxidase
MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
mg	Miligram
mGluR	Metabotropic glutamate receptor
MIA	Maternal Immune Activation
min	Minute
MK-801	(5S,10R)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate) or Dizocilpine
MK-912	(2*S*,12*bS*)-1',3'-dimethylspiro[1,3,4,6,7,12*b*-hexahydro-[1]benzofuro[2,3-a]quinolizine-2,4'-1,3-diazinane]-2'-one;hydrochloride
µl	Microliter
ml	Mililiter
M/L	Medial-lateral
µM	Micromolar
mM	Milimolar
MRI	Magnetic resonance imaging
N	Normal
Na^+	Sodium
NA	Noradrenaline
nA	Nanoampere
NaOH	Sodium hydroxide
NET	Noradrenaline (NA) transporter
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
nM	Nanomolar
NMDA	N-methyl-D-Aspartate

NO	Nitric oxide
NORT	Novel object recognition test
NR1	Subunit 1 of NMDA receptor
NRG-1	Neuroregulin-1
OCT	Organic cation transporter
OSA	1-octanesulfonic acid
PANSS	Positive and Negative Syndrome Scale
PBS	Phosphate-buffered saline
PCP	Phencyclidine
PFA	Paraformaldehyde
PET	Positron emission tomography
PFC	Prefrontal Cortex
PGK	Phosphoglycerate kinase
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PMAT	Plasma membrane monoamine transporter
PND	Postnatal day
Poly (I:C)	Polyinosinic:polycytidylic acid
POP	Propyl oligopeptidase
PPI	Prepulse Inhibition
PSA	Prostate specific antigen
PSAM	Pharmaco-selective actuator module
PV	Parvoalbumin
RCF	Relative centrifugal force
RT	Retention time

s	Seconds
SD	Stimulus duration
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
SERT	Serotonin (5-HT) transporter
SGA	Second generation antipsychotic
SPECT	Single-photon emission computerized tomography
TEMED	N, N, N, N'-tetramethylethylenediamine
TH	Tyrosine hydroxylase
THC	Δ^9-Tetrahydrocannabinol
TLR	Toll-Like receptor
TNF-α	Tumor necrosis factor α
UHPLC	Ultra high-performance liquid chromatography
V	Volt
VMAT	Vesicular monoamine transporter
VTA	Ventral tegmental area
WM	Working memory
[X]	Concentration of X
$[X]_{in}$ or C_{in}	Concentration of X accessed through the probe
$[X]_{out}$ or C_{out}	Concentration of X present in the dyalisate

INDEX

4. RESULTS 167

1. INTRODUCTION

1.1. SCHIZOPHRENIA

1.1.1. General considerations

Schizophrenia is a chronic, heterogeneous, multifaceted and debilitating disorder triggered by a series of interacting genetic, epigenetic, developmental and environmental factors that collectively interfere with normal brain development and maturation (Millan et al., 2016). This incapacitating syndrome affects around 1 % of the population and it is estimated to be the seventh most costly illness because of the high frequency of hospitalizations, need for psychosocial services and loss of productivity (Davis et al., 1997; Awad & Voruganti, 2008). Schizophrenia remains a leading cause of years lived with disability (World Health Organization, 2014), and active psychosis has been ranked among the most disabling disorders by severity in the general population (Kroken et al., 2014).

The onset of the illness, often referred as the prodromal phase (before the manifestation of the first psychotic episode) consist of a decline in cognitive and social functioning, and usually begins in the early adolescent years, preceding the onset of psychotic symptoms by > 10 years (Kahn & Keefe, 2013). The outcome of schizophrenia can range from complete recovery to the chronic need for care. On average, the life expectancy of people affected by this disorder is reduced by 20 years, compared with the general population (Laursen et al., 2014). Generally, patients with schizophrenia experience serious impairments in multiple domains of everyday life; including the ability to maintain social relationships, sustain employment and live independently (Harvey, 2014).

Many illness categories are defined by unitary molecular pathophysiology; however, this is not the case with schizophrenia, which diagnosis is defined by the behavioral presentation and illness course (Ibrahim & Tamminga, 2011). For diagnosing schizophrenia and closely related disorders, the guidelines most used by psychiatrists are the Tenth Revision of the International Classification of Disease (ICD-10) and the Fifth Edition of the Statistical Manual of Mental Disorder (DSM-5). Even if during decades schizophrenia has been conceptualized as a single illness with multiple manifestations, new models of research suggest that it is a combination of individual component symptom complexes, in which these components are composed of similar symptoms that have common pharmacology, course, pathophysiology, and treatment (Hyman & Fenton, 2003). The typical component symptom complexes reported are psychosis (e.g., hallucinations, delusions, and thought disorder), cognitive dysfunction (e.g., reductions in attention, memory, and executive function), negative symptoms (e.g., anhedonia, asociality, and alogia), along with depressed mood (**Figure 1.1**). If divided into categories we could differentiate in more detail:

- Positive symptoms: behaviors and thoughts that are not normally present ("loss with reality"): delusions, hallucinations and disorganized speech and behavior.
- Negative symptoms: social withdrawal, affective flattening, anhedonia (inability to feel pleasure) and diminished initiative and energy.
- Cognitive symptoms: a broad set of cognitive dysfunctions: difficulties in concentration and attention, learning, memory and executive functions.

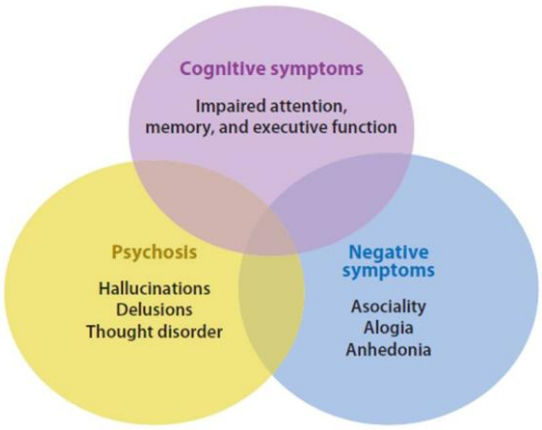

Figure 1.1: Component symptom complexes in schizophrenia. Schizophrenia has been viewed as a single illness with mixed manifestations. A new model proposes that schizophrenia is best conceptualized as a clinical syndrome, a complex of individual component symptom complexes with a distinct course, pathophysiology, pharmacology, and treatment response. Adapted from *Ibrahim & Tamminga, 2011*.

1.1.2. Risk factors

There are various modifiable and non-modifiable risk factors influencing the development of schizophrenia that put certain groups at particular risk of suffering from the disease:

1.1.2.1. Genetic risk factors

The genetics of schizophrenia are complex with: first, a few and rare variants of large effect *vs.* innumerable variants of very small and collective impact; second, a role for rare copy number variants encompassing multiple genes and stretches of DNA, and third, both inherited and *de novo* anomalies (Millan et al., 2016). Using genome-wide association studies, a substantial number of genes have been postulated to be candidates underlying genetic risk of schizophrenia: neuregulin-1 (NRG-1), catechol-*O*-methyl-transferase (COMT), brain-derived neurotrophic factor (BDNF), disrupted in schizophrenia-1 (DISC-1), N-methyl-D-aspartate (NMDA) receptor subunit (NR1) and regulator of G protein signaling 4, between others (Lee et al., 2012) In addition to that, there are other genetic-related factors that could also play an important role, as:

- Sex: schizophrenia is reported to be slightly more frequent in men than women (risk ratio 1.4/1) and it is usually more severe in men (Castle & Murray, 1991). Besides, men tend to develop this disorder earlier than women (Castle et al., 1998; Aleman et al., 2003).
- Paternal age: some studies have reported that men who are older when fathering a child have a greater chance of having a child who develops schizophrenia than younger men. However, it is unclear whether this risk is due to biological or psychological factors (Malaspina et al., 2001).

1.1.2.2. Maternal infections

There is accumulating evidence expressing that maternal infection during pregnancy is one of the relevant environmental risk factors of neurodevelopmental brain dysfunctions in the offspring (Meyer et al., 2009). It has been found in several retrospective studies a higher risk of schizophrenia and related psychotic disorders in offspring of mothers with viral or bacterial infections during early-to-middle stages of pregnancy (Brown & Susser, 2002; Brown, 2006; Patterson, 2007; Boksa, 2008). What is more, for at least some of the infectious agents implicated in the prenatal infectious etiology of schizophrenia, the establishment of prospective epidemiological approaches have provided clear serologic evidence, some of them appearing to be relatively strong in magnitude (Brown et al., 2004). In fact, after the rubella pandemic in 1964, the incidence of two neurodevelopmental disorders, autism and schizophrenia, rose from less than 1 % in the general population to about 13 and 20 % in the exposed population, respectively (Estes & McAllister, 2016a). Subsequent studies pointing out historic outbreaks of flu, mumps, measles chickenpox, and polio revealed an association with several mood disorders, schizophrenia and autism (Reisinger et al., 2015). However, not all epidemiological studies have replicated these associations (Selten et al., 2010). Still, numerous prospective studies following birth cohorts (Knuesel et al., 2014; Estes & McAllister, 2015) are consistent with an association between viral infection and psychiatric disorders in offspring, and they add other classes of pathogens to the list: particularly, bacterial infections (pneumonia, sinusitis, and tonsillitis) and the parasite *Toxoplasma gondii* (Patterson, 2009; Knuesel et al., 2014).

The common point to all the implicated pathogens is the maternal immune response; the infection acts as a disease primer, producing the release of many proinflammatory cytokines and activation of T_H17 cells in the mother's bloodstream (increased IL-6 and IL-17, among others). This fact, combined with a particular genetic background, autoimmune status and a possible second hit during childhood and/or adolescence (stress, drug abuse) will determine the consequences of the maternal immune activation (MIA) to increase the likelihood of developing psychiatric disorders as adults (Meyer, 2014; Estes & McAllister, 2015; 2016a; Choi et al., 2016) (**Figure 1.2**). It seems clear that disturbances directed at the maternal host during pregnancy can lead to direct physiological changes in the fetal environment and negatively affect the normal course of early brain development in the offspring (Rees & Harding, 2004; Rees & Inder, 2005). This can have long-lasting consequences for the development of postnatal brain dysfunctions, in which the primary cerebral insult or pathological process occurs during

early brain development and long before the illness is clinically manifest (Weinberger, 1987; Murray et al., 1991; Rapoport et al., 2012; Ross et al., 2006; DiCicco-Bloom et al., 2006).

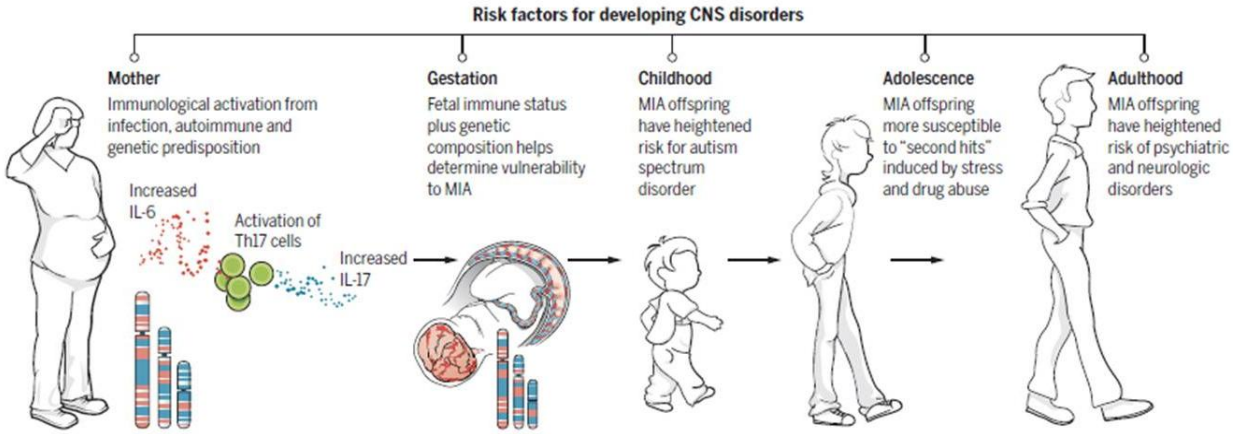

Figure 1.2: Schematic representation for how MIA can lead to a psychiatric disorder in offspring. Adapted from *Estes & McAllister, 2016b.*

1.1.2.3. Other environmental risk factors

Several psychosocial and biological factors have been described to play a role in the emergence of schizophrenia:

- Urban environment: in disadvantaged areas of inner cities schizophrenia is most common, finding first noted in Chicago (Stilo & Murray, 2010). Also, it has been shown an increased incidence of schizophrenia in people raised in urban areas compared with those raised in rural areas (Pedersen & Mortensen, 2001).
- Migration status: it has been demonstrated an increased incidence of schizophrenia among many migrant groups compared with individuals who do not have a personal/family history of migration. Nevertheless, recent literature has focused on African-Caribbean migrants to European countries, who show much higher rates of this disorder than white or Asian migrants to Europe, or migrants to other continents (Cantor-Graae & Selten, 2005). Interestingly, studies of the relatives of African-Caribbean patients with schizophrenia who live in England showed that the risk of developing the disorder is much lower in the siblings living in the Caribbean than in those residing in England (Hutchinson et al., 1996). These findings suggest the influence of an environmental factor in the European host country but not in the country of origin (Boydell et al., 2001).
- Drug abuse: It is already known that persistent abuse of amphetamine, methamphetamine, cocaine, as well as cathinone-derived "legal highs" can produce resembling states to that of paranoid schizophrenia (Murray et al., 2013). Furthermore, cross-sectional and prospective epidemiological studies (Gage et al., 2016; Mustonen et al., 2018), as well as biological evidence (Murray et al., 2017),

support a causal link between cannabis use and psychotic disorder. Meta-analysis shows a dose-response association with the highest odds of psychotic disorder in those with the heaviest cannabis use (Marconi et al., 2016). In a multicentre case-control study with patients from eleven sites across Europe and one from Brazil, they found striking variations in the incidence of psychotic disorder depending on the frequency of daily cannabis use and the use of high-potency cannabis (Di Forti et al., 2019). In fact, they reported that daily cannabis use was associated with three-times increased odds for psychotic disorder compared with never users, increasing to nearly five-times increased odds for daily use of high potency types of cannabis. This results correlated with places where high-potency cannabis was widely available, such as Amsterdam, London, and Paris. This data is consistent with previous evidence suggesting that the use of a high concentration of Δ^9-Tetrahydrocannabinol (THC) has a more harmful effect on mental health than the use of weaker forms (Di Forti et al., 2015; Murray et al., 2016; Freeman et al., 2018)

- Social adversity: childhood adversities including physical abuse, sexual abuse, maltreatment, and bullying are associated with increased risk of later schizophrenia (Stilo & Murray, 2010). Whether these environmental factors are independent risk factors is debated (Kahn et al., 2015).

- Prenatal and perinatal events: those individuals who experience an excess of complications in fetal life and/or at birth have an increased risk of developing schizophrenia. Cannon and colleagues (Cannon et al., 2002) published a meta-analysis demonstrating associations between complications of pregnancy and abnormal fetal growth with this disorder. Besides, people who were born in late winter and spring are slightly (7-10 %) over-represented among schizophrenia patients. This increase could be justified due to a higher probability of exposure to maternal respiratory infections or malnutrition in the mother, including deficits in folic acid or vitamin D during the winter months; although none of this explanations have been firmly established (Kahn et al., 2015). Concretely, it is important to highlight malnutrition in the mother as an important prenatal risk factor. Most studies on this issue are based on the Dutch famine of 1944–1945 and the Chinese famine of 1959–1961 (Susser & St Clair, 2013). The first study regarding the Dutch famine and risk of schizophrenia found out that those who were prenatally exposed to famine in the first trimester had more than the two-fold likelihood of suffering schizophrenia, specifically among women (Susser & Lin, 1992). The second Dutch study, retrieving hospital register data, suggested a significant association of prenatal exposure to famine with roughly two-fold odds of schizophrenia in both men and women (Susser et al., 1996). Subsequent studies on the Dutch famine and schizophrenia observed similar findings (Hoek et al., 1996; 1998; Hulshoff Pol et al., 2000). The Chinese studies regarding famine exposure and schizophrenia emerged recently and found similar associations with the Dutch studies. Actually, two studies using hospital records of schizophrenia patients replicated the Dutch findings and both reported two-fold increased odds of schizophrenia in

adulthood among those who were exposed in utero to the Chinese famine (St Clair et al., 2005; Xu et al., 2009).

1.2. MONOAMINE SIGNALING SYSTEMS

During the last decades, the relationship between the processes of synaptic transmission and the pathophysiology of schizophrenia has gained strength, and several neurotransmitters and their specific signaling systems have been proposed as molecules and pathways involved in the pathophysiology of this disorder.

1.2.1. The noradrenergic system

The noradrenergic system consists of central and peripheral pathways that play an important role in many processes of daily life: attention, sleep and wakefulness, learning, memory, emotion, reproduction and physiological responses to stress, to name a few (Huether, 1996; Sved et al., 2001; Sara, 2009). It is also involved in affective and anxiety-related disorders, and much of this knowledge was acquired by data obtained from studies that used psychotropic drugs, including tricyclic antidepressants and selective inhibitors of noradrenaline (NA) reuptake (Charney, 2003).

1.2.1.1. Synthesis, release, reuptake, and catabolism

NA belongs to the group of amines called catecholamines because of the presence of a catechol ring and an amine side chain; in which dopamine (DA) is also included, and from which NA synthesis is carried out.

NA synthesis begins in the cellular cytoplasm with the hydroxylation of the amino acid L-tyrosine by the tyrosine hydroxylase (TH) resulting in 3,4-dihydroxyphenylalanine (L-DOPA), and its subsequent decarboxylation, by the L-amino acid aromatic decarboxylase, that results in DA. The TH has low enzymatic activity and catalyzes the first step of the synthesis, thus is considered the limiting enzyme of this process. Then DA is transported to the interior of the synaptic vesicles where the enzyme dopamine-β-hydroxylase (DBH) is located, catalyzing the reaction that transforms DA into NA (Chaudhry et al., 2008); therefore, it is considered the limiting enzyme of this process, along with the TH. DBH is a mixed-function oxidase; a tetrameric glycoprotein that is found either in the membrane of the vesicles or soluble in its interior (Lamouroux et al., 1987) and which requires molecular oxygen and uses ascorbic acid as a cofactor (Craine et al., 1973). DBH is released from the nerve terminals together with NA, at the same time and proportionally to the neurotransmitter, by a process of exocytosis (**Figure 1.3**).

Once released into the synaptic space, NA acts on its specific membrane receptors. Its function can be finished mainly by two different mechanisms: diffusion processes that allow its action on extrasynaptic receptors (Aoki et al., 1998) and/or reuptake processes via an extraneuronal transporter, such as the NA transporter (NET). This transporter allows the passage of NA from the extracellular fluid to the interior of the terminal passing through the plasma membrane (Chaudhry et al., 2008). It is a protein constituted by twelve transmembrane segments (Amara & Kuhar, 1993) and is located in noradrenergic terminals. Its mRNA is expressed predominantly

in the locus coeruleus (LC) and other noradrenergic regions, including the area A4, A5 and A7, the lateral tegmentum and the nucleus of the solitary tract. Once the protein is translated, it is transported to the terminal areas that project from the LC and other noradrenergic nuclei; thus, its expression pattern corresponds to noradrenergic innervation sites (Tejani-Butt, 1992). Studies with radioligands have shown NET labeling in areas such as the LC, the thalamus, the hypothalamus, and the neocortex, among others; nevertheless, the caudate-putamen nucleus is practically devoid of this transporter. In addition, although it contains few noradrenergic fibers, it seems that NET could be expressed in noradrenergic terminals that greatly innervate the raphe nuclei too (Nestler et al., 2001). Apart from the reuptake by the NET, it should be noted that a small portion of the synaptic NA can leak into the circulation or be taken up by another category of transporters (Uptake 2 in **Figure 1.3**) and metabolized to form normetanephrine (Tellioglu & Robertson, 2001). These other transporters include organic cation transporter (OCTs) and plasma membrane monoamine transporter (PMATs) (Bacq et al., 2012). OCT2, OCT3, and PMAT, in particular, are expressed in various brain areas, including the cortex and a direct role in *in vivo* clearance of 5-hydroxytryptamine (5-HT) and NA were demonstrated in the mouse brain for OCT2 (Bacq et al., 2012; Blakely & Bauman, 2000). Because 70–90% of the synaptic NA is taken up by the NET, blockade of NET is likely to produce a shift towards the normetanephrine pathway and away from the 3,4-dihydroxyphenyl glycol pathway (**Figure 1.3**) (Zhou, 2004).

After its reuptake, NA can be metabolically inactivated or introduced back into the vesicles. Its metabolism is carried out by a deamination reaction by the monoamine oxidase (MAO). This enzyme is normally localized intraneuronally, in the outer membrane of the mitochondria. It should be noted that the MAO presents two isoforms, A and B. These two MAO isoforms are distinguished on the basis of their substrate preferences and sensitivity to inhibition. In the human central nervous system (CNS), the MAO_A is principally responsible for the deamination of 5-HT and NA, while the MAO_B is responsible for the catabolism of DA and β-phenylethylamine (Westlund et al., 1985; Fowler et al., 2015). It is also recognized that regarding the MAO, there are significant differences between the rodent and human brains with respect to the regional and cellular distribution of the two isoforms, as well as regarding the substrate specificity and sensitivity (Youdim, 1988; Fowler et al., 2015). In contrast to the rat brain, MAO_B is the major form in the human and guinea pig CNS (Youdim, 1988; Engel et al., 2004). In fact, the highest MAO_A concentrations are in the catecholaminergic neurons of the LC, and of MAO_B in the serotonergic and histaminergic neurons of the raphe and posterior hypothalamus (Foley et al., 2000; Fowler et al., 2015). Also, there are especially high concentrations of both forms in the human basal ganglia (Azzaro et al., 1985; Fowler et al., 2015). The topographic location of the MAO subtypes, thus, does not correspond with that of their presumed natural substrates. The MAO_B may indirectly regulate extraneuronal transmitter levels, particularly those of DA, by regulating the levels of a release-promoting substance, such as β-phenylethylamine (Foley et al., 2000). MAO_A, on the other hand, acts to maintain low intraneuronal concentrations of DA, NA, and 5HT.

This deamination reaction by the MAO enzyme results in 3,4-hydroxyphenyl glyceraldehyde. This compound is either transformed into 3,4-dihydroxyphenyl glycol, or it is oxidized by the

enzyme aldehyde dehydrogenase into dihydroxymandelic acid. Regarding cerebral NA, the reduction of aldehyde appears to be the major metabolic pathway (Zhou, 2004). The second enzyme that participates in the metabolic process of NA is the COMT, which exits in two isoforms, the membrane-bound and the soluble form (Rivett et al., 1983; Schendzielorz et al., 2013). It has been reported to be both pre- and postsynaptically in the brain neurons and glial cells (Mannisto & Kaakkola, 1999; Schendzielorz et al., 2013) but this matter still remains controversial. Its metabolic activity produces 3-methoxy-4-hydroxyphenyl glycol, the main metabolite of NA.

In addition, it is also well known that the reuptake NA can also be stored inside the synaptic vesicles, involving an active transport mediated by the vesicular monoamine transporter 2 (VMAT2) that regulates NA passage inside the vesicle (Chaudhry et al., 2008). Because the VMAT2 transporter has more affinity for NA than the MAO enzyme, more than 70 % of the reuptake NA is stored in synaptic vesicles, thus being available for release again (Eisenhofer, 2001). Apart from that, it should be noted the existence of a third type of transporter called extraneuronal transporter, responsible for a minor clearance pathway of catecholamines (NA, DA) in non-neuronal cells. Finally, something to bear in mind is that the reuptake processes are not able to reuptake the totality of released neurotransmitter. Hence, a diffusion process can occur allowing the neurotransmitter to travel far from the nerve terminal and possibly allowing the stimulation of extrasynaptic receptors (Tellioglu & Robertson, 2001).

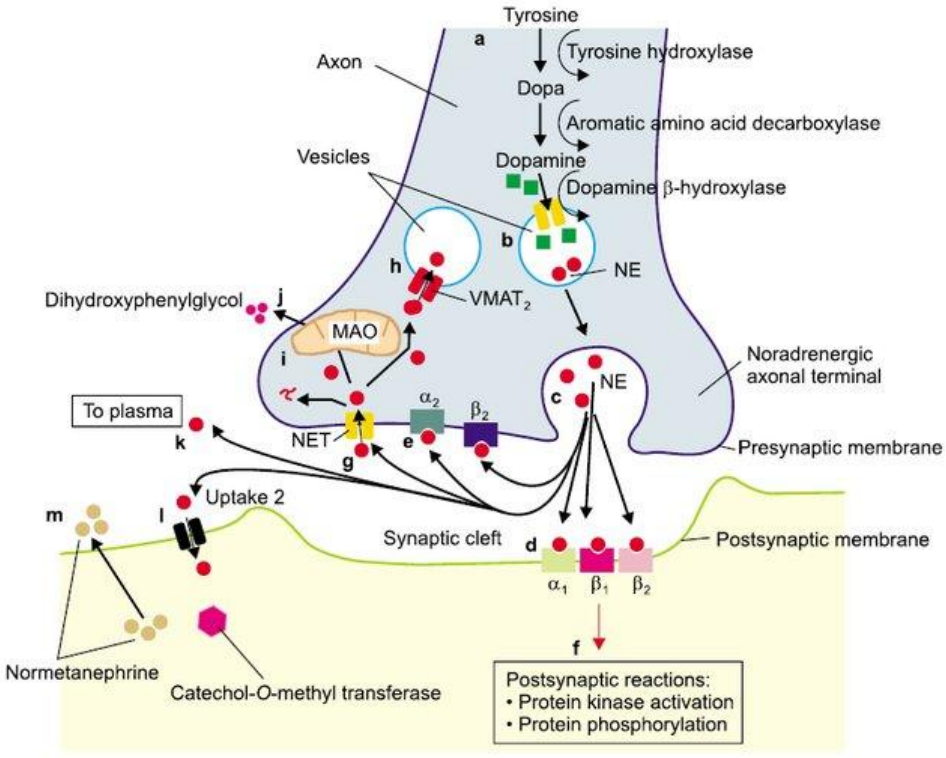

Figure 1.3: Representative scheme of the synthesis, release, reuptake, catabolism, and action of noradrenaline (NE). MAO: Monoamine oxidase; VAMT2: vesicular monoamine transporter 2; NET: noradrenaline transporter. Modified from *Zhou, 2004.*

1.2.1.2. Adrenoceptors

NA effects are mediated by the adrenoceptors (ARs), that also mediate the effects of adrenaline and are expressed in numerous types of peripheral tissues and the CNS.

These receptors are classified into two main families based on their structural, pharmacological, and signaling properties: α (α_1 and α_2) family and β (β_1, β_2, and β_3) family. Both types of receptors belong to the superfamily of G-protein-coupled receptors with seven transmembrane domains. Thanks to the improvements in molecular cloning technology, nine ARs have been identified: three α_1ARs (α_{1A}, α_{1B}, α_{1D}AR), three α_2ARs (α_{2A}, α_{2B} α_{2C}AR) and three β-adrenoceptors (βARs) (β_1, β_2, β_3AR) (Bylund et al., 1994).

1.2.1.2.1. α_1-Adrenoceptors

Inside the α_1AR family, three subtypes can be differentiated: α_{1A}, α_{1B}, and α_{1D}, with different pharmacological properties and amino acid sequences (Zhong & Minneman, 1999). All of them are coupled to $G_{q/11}$ proteins; thus activate the enzyme phospholipase C (PLC) and, consequently, generate inositol 1,4,5-triphosphate and diacylglycerol, leading to activation of protein kinase C (PKC) and the increase of intracellular Ca^{+2} (Birnbaum et al., 2004).

In the CNS, this family of receptors is located pre- and post-synaptically in the PFC, in superficial and deep layers. Also, approximately 20 % of these receptors are distributed in γ-aminobutyric acid (GABA)ergic neurons (Xing et al., 2016). The α_1AR subtype is considered to be principally a postsynaptic receptor, but similar to other postsynaptic receptors, it can modulate the release of neurotransmitters, for instance, in non-noradrenergic neurons, acting as heteroreceptors (Tanoue et al., 2003). Several studies have implicated functional roles for α_1ARs in the CNS. For example, α_1ARs are involved in locomotion, cognitive functions and in the control of motor activity (Stone et al., 2001). Among some other functions, they are responsible for mediating the contraction of the cardiovascular smooth muscle and therefore, they can modulate blood pressure. Precisely, α_{1B}ARs seem to have an important role in vulnerability to addiction (Drouin et al., 2002). In addition, α_1AR agonists have effects on passive avoidance learning in aged mice (Stone et al., 1999) and spontaneous movement (Tanoue et al., 2003). The presence of high densities in brain nuclei such as the thalamus, the cerebral cortex, the hypothalamus, and the hippocampus has been described (Palacios et al., 1987).

1.2.1.2.2. α_2-Adrenoceptors

This family of receptors includes the α_{2A}, α_{2B}, and α_{2C}ARs, widely distributed in both CNS and in the periphery (McCune et al., 1993; Bylund et al., 1994; Scheinin et al., 1994; Nicholas et al., 1996), although their density and expression differ greatly in the distinct brain areas and peripheral tissues. These α_2AR subtypes have similar tissue distribution patterns, but they diverge in the physiological and pharmacological profiles (MacDonald et al., 1997; Gilsbach & Hein, 2012). They are normally coupled to the inhibitory $G_{i/o}$ proteins and decrease adenylyl cyclase (AC) activity, thus leading to the inactivation of the protein kinase A (PKA); but it has

also been described that depending on the endogenous agonist concentration, they can also couple to the G_s protein and consequently increase AC activity (Jones et al., 1991; Eason et al., 1992; MacDonald et al., 1997; Jasper et al., 1998).

While all three receptors are present in the CNS, the α_{2B}AR is mainly expressed in the thalamus and does not seem to contribute to CNS auto- and heteroreceptor function (Brum et al., 2006). The α_{2A}ARs and α_{2C}ARs, on the other hand, are the primary α_2ARs modulating neurotransmission in the CNS (Scheibner et al., 2001a,b; Brum et al., 2006), and both the α_{2A} and α_{2C}ARs are recognized to play a very distinct and specific role in memory, cognition, and mood disorders, each one in a very different manner (supplementary information in *section 1.7*).

1.2.1.2.3. β-Adrenoceptors

The β-ARs include three subtypes: β_1, β_2, and β_3AR (Bylund et al., 1994) and are coupled to G_s proteins promoting the activity of AC, increasing the concentration of intracellular cyclic adenosine monophosphate (cAMP) and, consequently, the activity of PKA. They are the ones that have the lowest affinity for NA (Xing et al., 2016).

In general, in the human brain, they have been located in the cerebral cortex, hippocampus, caudate nucleus-putamen, and nucleus accumbens. To a greater extent they have been observed in the thalamus, the hypothalamus and the cerebellum (Minneman et al., 1979; Pazos, 1988). The βARs are densest in the intermediate layers of the PFC, with the adult cortex highly enriched in β_1ARs (Rainbow et al., 1984; Summers et al., 1995). Studies in primate PFC have also localized β_2ARs on both glutamatergic pyramidal neurons and GABAergic interneurons (Aoki et al.,1998). In contrast, β_3AR has a very low presence at the cerebral level (Pupo & Minneman, 2001). Regarding functional effects of these receptors in the brain, they are poorly defined; but they may have an important role in arousal, mood, and memory; and therefore, be involved in aging-associated changes in these functions (Scarpace & Abrass, 1988).

1.2.1.3. Noradrenergic pathways in the CNS

The LC noradrenergic nucleus was the first neuromodulatory system to be delineated anatomically and specified neurochemically (Dahlström & Fuxe, 1964), and it is the most intensely investigated network. The small LC nucleus is situated deep, adjacent to the fourth ventricle, and innervates and supplies NA throughout the CNS. It can modulate the activity of a high number of brain areas including the brainstem, the cerebellum, the diencephalon and the paleo- and neocortex (Jones et al., 1977), standing out as a critical component of the neural architecture (**Figure 1.4**). Through extensive axonal branching, a single cell can have terminals in diverse remote brain regions, including the forebrain, the brainstem and the cerebellum. The entire cerebral cortex, including the frontal cortex and all sensory regions, receive input from the LC. It also sends projections to thalamic nuclei and limbic structures, including the amygdala, the hippocampus and the septum (Sara, 2009). These noradrenergic projections from the LC nucleus to almost all brain regions are considered the sole source of NA in the

forebrain (reviewed by Foote & Morrison, 1987). This unusual anatomical organization arose much of the early interest and speculation about the role of this system in cognitive processes (early reviews by Amaral & Sinnamon, 1977; van Dongen, 1981).

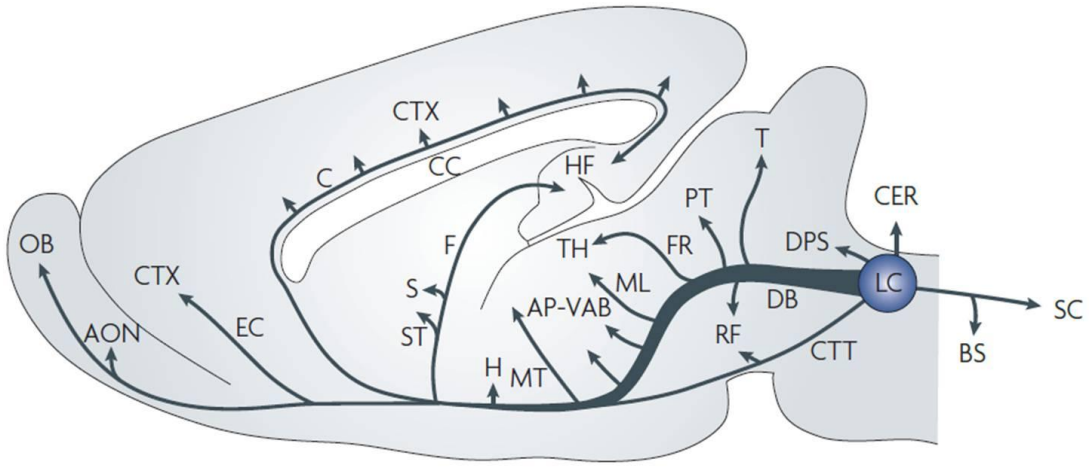

Figure 1.4: Sagittal schematic view of noradrenergic efferent projections in the rat brain. Abbreviations: LC: Locus coeruleus, S: septum, EC: entorhinal cortex, AON: anterior olfactory nucleus, AP-VAB: ansa peduncularis–ventral amygdaloid bundle system, BS: brainstem nuclei, CC: corpus callosum, CER: cerebellum, CTT: central tegmental tract, CTX: cortex, DB: dorsal bundle, DPS: dorsal periventricular system, F: fornix, FR: fasiculus retroflexus, H: hypothalamus, HF: hippocampal formation, ML: medial lemniscus, MT: mamillothalamic tract, OB: olfactory bulb, PRC: perirhinal cortex, PT: pretectal area, RF: reticular formation, SC: spinal cord, ST: stria terminalis, T: tectum, TH: thalamus. Modified from *Sara, 2009.*

NA has been widely reported to be implicated in cellular excitability, synaptic plasticity and long-term potentiation (a form of synaptic plasticity that results in a long-lasting increase in the strength of the synaptic transmission) (Harley, 1987; 2007). Other studies have demonstrated its role in the activation and tuning sensory signals in the thalamus and the cortex (Berridge & Waterhouse, 2003). Also, pharmacological studies have provided evidence that NA, interacting with other neuromodulators and hormones, modulates memory formation, mainly through actions in the amygdala and the hippocampus (see Cahill & McGaugh, 1996 for a review).

Some other approaches have revealed a noradrenergic influence in frontal cortical regions that are engaged in attention and working memory (WM) functions (Arnsten & Li, 2005; Robbins & Roberts, 2007). On top of that, in behaving primates and rodents, electrophysiological studies have shown a clear relationship between activity in LC neurons and cognitive behaviors (Arnsten et al., 1988; Aston-Jones & Cohen, 2005; Bouret & Sara, 2005; Arnsten, 2013).

Early theories of the function of the LC-noradrenergic system focused on vigilance and sleep-wake cycles (Hobson et al., 1975; Aston-Jones & Bloom, 1981). Indeed, noradrenergic neurons can fire following tonic and phasic activity patterns. During quiet wakefulness, LC neurons fire at a regular slow rate (~1 Hz), whereas they show bursts of firing in response to arousing stimuli and fire at a diminished rate during drowsiness and slow-wave sleep (SwS) (Aston-Jones

& Cohen, 2005). Although the evidence regarding the role of the LC neurons in vigilance, the LC has been suggested to play a role in many other important functions too, including attention, sensory processing, synaptic plasticity, network resetting, memory formation, memory retrieval, decision making and performance facilitation (Sara, 2009).

A clear relationship between LC neural activity and behavior has been revealed thanks to electrophysiological studies in rodents and behaving primates. Recordings from LC neurons in rats performing a differential conditioning task showed that LC neurons respond to reward and punishment and later to stimuli in any modality that are associated with reinforcement (Sara & Segal, 1991; Bouret & Sara, 2005). Besides, LC neurons respond most vigorously to any changes in stimulus-reinforcement contingencies (e.g. extinction or reversal). Importantly, adjustment of LC responses to changes in stimulus-reinforcement contingencies precedes changes in behavioral adaptation capacity in both rats and monkeys (Sara & Segal, 1991; Bouret & Sara, 2005). Taken together, these observations led several investigators to suggest that the LC-NA system facilitates attentional and cognitive shifts and behavioral adaptation to changes in environmental imperatives (Sara & Segal, 1991; Yu & Dayan, 2005; Bouret & Sara, 2005).

1.2.2. The dopaminergic system

DA is the most basic of the catecholamine neurotransmitters in the CNS. Since its identification as an independent neurotransmitter in the brain (Carlsson et al., 1958; Carlsson, 1959), a large number of molecular and behavioral studies have been carried out to understand the functional roles of DA. However, there is a general agreement to suggest that the role of DA is not to mediate direct synaptic driving of neurotransmission in the brain, but instead to modulate excitatory and inhibitory neurotransmission (Kupfermann, 1979).

1.2.2.1. Synthesis, release, reuptake, and catabolism

Since DA and NA share the synthesis process, see *section 1.2.1.1.* However, a few details in relation to its synthesis, release, reuptake, and catabolism are described below.

Once DA has been synthesized, is released to the synaptic space through a process of exocytosis and binds to its specific dopaminergic receptors, its action ends principally by a reuptake process back to the nerve terminal. The reuptake of DA inside the cell is mediated by its specific transporter, the DA transporter (DAT), dependent on Na^{+} and Ca^{+2} influx (Uhl & Johnson, 1994; Blakely & Bauman, 2000). Structurally, it is very similar to the NET, in charge of the reuptake of NA. DAT is mainly located at the level of the caudate nucleus and putamen, as well as the nucleus accumbens in the human and rodent brain (Nirenberg et al., 1996). It is situated specifically in dopaminergic nerve terminals and moderate levels have been observed in the pale globe, amygdala, and hypothalamus. However, in areas such as the PFC and the mesencephalon, the density of this protein is scarce (Sesack et al., 1998). As it happens with NA, DA can also be stored inside the synaptic vesicles through an active transport system through the VMAT2 for a new posterior release, or it can undergo a metabolic process (Oberhauser & Fernandez, 1996) by the MAO enzyme, in humans mainly the MAO_B isoform

(Westlund et al., 1985; Youdim, 1988; Foley et al., 2000). The metabolite resulting from MAO action is 3,4-dihydroxyphenyl-acetic acid (DOPAC), which once released into the synaptic cleft, it is metabolized by the enzyme COMT, giving rise to the principal metabolite of DA, homovanillic acid. COMT is considered to be involved in the catabolism of DA at the glial level or postsynaptic neurons (Karhunen et al., 1995) because no activity of this enzyme is detected in dopaminergic terminals.

1.2.2.2. DA receptors

DA exerts its action by binding to specific membrane receptors. So far, five different subtypes of DA receptors (DRs) have been isolated, divided into two families according to their biochemical and pharmacological characteristics: the D_1-like family receptors, including subtypes D_1R and D_5Rs; and the D_2-like family receptors, including subtypes D_2R, D_3R and D_4Rs (Civelli et al., 1993; Sokoloff & Schwartz, 1995).

DA receptors are G protein-coupled receptors (GPCRs). The D_1-like family stimulates G_S, which is positively coupled to AC, leading to elevated cAMP levels and activation of PKA. In contrast, D_2-like family activates G_i proteins, which directly inhibits the formation of cAMP by inhibiting AC. In addition, the D_2R family has also been shown to signal through an independent pathway involving the formation of a complex composed of the protein kinase B (Akt), protein phosphatase-2A, and β-arrestin 2 (Beaulieu & Gainetdinov, 2011).

Furthermore, DRs can also signal independently of cAMP/PKA to modulate intracellular Ca^{2+} levels and regulate ligand- and voltage-gated ion channels. This is particularly true for $G\alpha_{i/0}$-coupled receptors, such as members of the D_2-like family, which target several effector proteins through the liberation of the $G_{\beta\gamma}$ subunit of heterotrimeric G proteins upon receptor activation. Membrane-bound $G_{\beta\gamma}$ subunits can diffuse along the plasma membrane to directly activate ion channels or second messengers. The best example is the gating of G protein-activated inward-rectifier potassium (K^+) channels in D_2R-expressing midbrain DA neurons (Beckstead et al., 2004).

D_1R and D_2Rs are the two most abundant receptor subtypes expressed in the brain, with D_1Rs displaying the most widespread distribution and highest expression levels. D_1R and D_2Rs are most prominently found in the dorsal striatum, ventral striatum (nucleus accumbens) and olfactory tubercle, which collectively constitute the principal recipient structures of midbrain DA axons. Significant levels of D_1R and D_2R mRNA are also found in other forebrain structures, including cortex. The expression of D_3R, D_4R and D_5Rs in the brain is considerably more restricted and weaker than of D_1R and D_2Rs. D_1-like and D_2-like receptors are expressed in both striatal projection neurons and interneurons, as well as in subpopulations of pyramidal neurons, interneurons and glial cells in cortex. In these brain regions and others, D_1- and D_2-like receptors are localized presynaptically in nerve terminals and axonal varicosities, as well as postsynaptically in dendritic shafts and spines (Bentivoglio & Morelli, 2005). Thus, no simple and general division of labor exists between D_1R and D_2R families with respect to receptor distribution in projection *vs*. locally-projecting neurons, or pre- *vs*. postsynaptic membrane specializations.

In the cerebral cortex, the cellular distribution of DA receptors is not as well delineated (Tritsch & Sabatini, 2012). The distribution and density of mesocortical DA fibers and cortical DA receptors varies between species, as well as between and within cortical areas in a given species (Bentivoglio & Morelli, 2005), limiting the ability to extract general DA signaling principles. In the PFC, the principal cortical recipient of DA afferents, D_1R and D_2Rs are the two most abundant subtypes expressed. Actually, a large number of histological studies have confirmed that D_1Rs are the most widespread and strongly expressed DA receptors in PFC. D_1Rs and D_2Rs are distributed in both pyramidal neurons and interneurons throughout layers 2 to 6, but most prominently in deep cortical layers (Bentivoglio & Morelli, 2005; Santana et al., 2009), where DA innervation is densest. Generally, D_1-Rs display a more widespread distribution and higher expression level than D_2Rs (Santana et al., 2009; Tritsch & Sabatini, 2012). The cellular distribution of D_5Rs in pyramidal neurons overlaps with that of D_1Rs (Bergson et al., 1995), and D_3R and D_4Rs mostly distribute to GABAergic interneurons (Mrzljak et al., 1996; Khan et al., 1998). Therefore, unlike striatum, DA receptors in PFC may only be expressed in a fraction of projection neurons, indicating that a considerable number of pyramidal cells may not be subject to direct modulation by DA (Tritsch & Sabatini, 2012). Moreover, DA receptor expression in PFC pyramidal neurons does not delineate a functionally-homogeneous group of cells, as only a small proportion of corticostriatal (6–11%), corticothalamic (~25%) and corticocortical neurons (4–10%) expressed D_1Rs or D_2Rs (Gaspar et al., 1995).

Despite the fact that the D_2-like receptor affinity for DA has been shown to be 10- to 100- fold greater than that of D_1-like receptors, in the striatum at least both high and low affinity states of D_1Rs and D_2Rs (Beaulieu & Gainetdinov, 2011), and in their high-affinity states, both exhibit similar nanomolar affinities for DA (Tritsch & Sabatini, 2012).

1.2.2.3. Dopaminergic pathways in the CNS

DA neurons exhibit two types of spike firing patterns: tonic and burst spike firing. Tonic spike is driven by an endogenous pacemaker conductance and is the baseline spontaneous activity state (Grace & Bunney, 1984), not depending on an excitatory driving force. In contrast, transient, burst spike firing, is known to be triggered by external stimuli, especially those associated with the presentation of unexpected reward or sensory signals that predict rewards (Schultz et al., 1993). Furthermore, DA neurons exhibit transient suppression of tonic spike firing with aversive stimuli or omission of expected rewards (Tobler et al., 2003; Ungless et al., 2004).

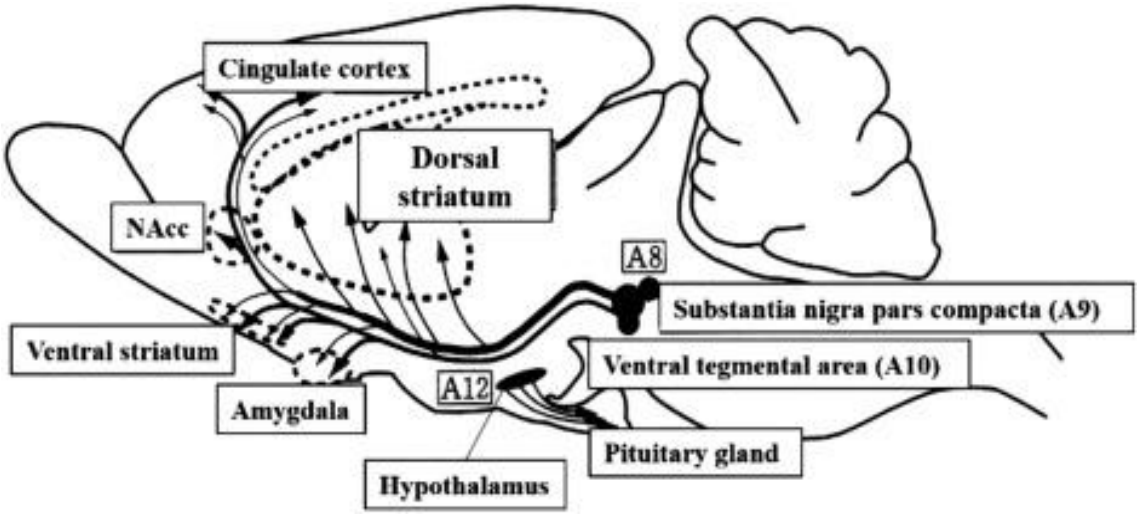

Figure 1.5: Schematic representation of the dopaminergic pathways in the rat brain. Abbreviations: NAcc: nucleus accumbens. From *Zheng & Hasegawa, 2017.*

Even if DA neurons are relatively small in number (about one million), DA is the prevalent catecholamine neurotransmitter in the CNS (Meisenzahl et al., 2007). DA neurons project into the forebrain along three major pathways described below.

The nigrostriatal system is composed of DA neurons located in the substantia nigra pars compacta that project into the dorsal striatum (Anden et al., 1964). This system is believed to have a principal involvement in motor control since degeneration of DA neurons in the substantia nigra pars compacta is the primary pathology of Parkinson's disease (Lloyd & Hornykiewicz, 1970; Hornykiewicz, 1971). Particularly important about the functional significance of nigrostriatal DA projections is its involvement in motor learning and habit formation (Graybiel, 1998; Jog et al., 1999). However, animal and human studies have also revealed that this projection is involved in nonmotor cognitive functions also (Graybiel, 1997; Schultz, 2002; Carbon & Marie, 2003) (**Figure 1.5**).

The mesolimbic DA system is composed of DA neurons located in the ventral tegmental area (VTA) that project to the ventral striatum including the nucleus accumbens, olfactory tubercle, and limbic structures such as the basolateral amygdala and hippocampus (Fallon & Moore, 1978; Fallon et al., 1978; Scatton et al., 1980; Voorn et al., 1986; Brinley-Reed & McDonald, 1999). DA release in the amygdala and hippocampus are thought to be involved in emotional learning (Rosenkranz & Grace, 2002; Bissiere et al., 2003) and long-term memory (Li et al., 2003; Lisman & Grace, 2005), respectively. Limbic and cortical inputs converge in the ventral striatum, and information encoded on these brain structures is integrated there to organize goal-directed behavior (Mogenson et al., 1980; Groenewegen et al., 1996). Thus, the mesolimbic DA projections to the ventral striatum modulate this information integration, thereby influencing motor behavior. In particular, these DA projections into the ventral striatum are considered to be crucial for motivation and reward-seeking (Everitt & Robbins, 2005) (**Figure 1.5**).

Finally, the mesocortical DA system is composed of DA neurons also located in the VTA that project to the prefrontal cortex (PFC), anterior cingulate, and entorhinal cortex in rodents (Fallon et al., 1978) as well as additional neocortical and even cerebellar areas in primates and humans (De Keyser et al., 1989; Melchitzky & Lewis, 2000; Moore et al., 2003). The PFC is considered to be the highest center of cognition (Goldman-Rakic, 1995; Robbins, 2000; Funahashi, 2001) and DA release in the PFC is essential for its function. As such, a number of cognitive functions including short-term memory (Funahashi et al., 1993; Goldman-Rakic, 1995), attention (Gorenstein et al., 1989; Knight et al., 1995; Muir et al., 1996b), future planning (Baker et al., 1996; Owen et al., 1990), and set-shifting (Milner, 1963; Owen et al., 1993) have been proposed to be mediated by this dopaminergic system the PFC. Precisely, interruption of the mesocortical DA innervation of the PFC is known to produce impairments in these functions (Seamans et al., 1998; Ragozzino, 2002; Floresco et al., 2006) (**Figure 1.5**).

The VTA is a structure composed of DA neurons and GABAergic neurons. Some of the GABA neurons are interneurons and others are projection neurons that innervate both the PFC and the ventral striatum. Physiological and anatomical studies have revealed that the mesolimbic and mesocortical DA pathways are organized into two independent closed loops (Carr & Sesack, 2000; Floresco et al., 2001; Sesack & Carr, 2002; Floresco et al., 2003; Lisman & Grace, 2005).

On the one hand, regarding the mesocortical DA system, PFC pyramidal neurons (in layers V and VI) are reported to project selectively onto VTA dopamine neurons that project back to the PFC (**Figure 1.6)** (Sesack & Carr, 2002). In addition, PFC afferents are also shown to target GABA interneurons that, in turn, regulate the activity of DA neurons that project to the ventral striatum, as well as GABA neurons that project directly to the ventral striatum. Consequently, this anatomically closed-loop induces higher DA release in the PFC during periods of PFC activation, while at the same time it suppresses DA release in the ventral striatum.

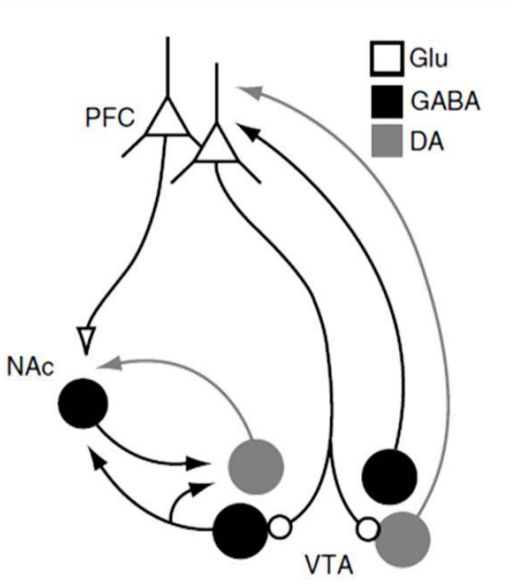

Figure 1.6: Schematic representation of the relationship between the PFC, ventral striatum, and VTA in the mesocortical system. The reciprocal interaction between the PFC and VTA would allow PFC activation to selectively induce DA release in the PFC and suppress DA release in the ventral striatum simultaneously. Abbreviations: DA: dopamine; GABA: γ-Aminobutyric acid; Glu: glutamate; NAc: nucleus accumbens; PFC: prefrontal cortex; VTA: ventral tegmental area. Adapted from *Goto & Grace, 2007.*

On the other hand, the mesolimbic DA system also forms another closed loop circuit with the hippocampus and ventral striatum. Thus, the HPC send excitatory projections into the ventral striatum (Kelley & Domesick, 1982; Groenewegen et al., 1987), which in turn regulates other basal ganglia nuclei including the ventral pallidum and pedunculopontine tegmentum (Floresco et al., 2003). Through this loop, the efferents from the HPC control the DA release in the ventral striatum (Floresco et al., 2001).

Apart from this, it is important to highlight the interaction of the noradrenergic and dopaminergic networks in the functions that are affected by both systems. In fact, taking into consideration the reciprocal connections between the two systems and the systems' mutual connections to the PFC, it is clear that they are highly interdependent on each other (**Figure 1.7**). Moreover, there are remarkable similarities between the activity patterns of dopaminergic and noradrenergic neurons, suggesting that DA and NA are released simultaneously (Briand et al., 2007; Masana et al., 2012a). Indeed, extracellular DA in the cerebral cortex might originate not only from dopaminergic but also noradrenergic terminals, where it could act as a precursor of NA and as a cotransmitter (Devoto et al., 2001; 2005). This idea is also supported by the fact that extracellular DA is modified by drugs acting on noradrenergic but not, on dopaminergic transmission (Devoto et al., 2005). Also, α_2AR agonists and antagonists have been shown to produce coincident effects in extracellular DA and NA in PFC (Devoto et al., 2001; Devoto & Flore, 2006). Moreover, is likely that in the PFC, a consistent fraction of DA is recaptured by the NET into noradrenergic terminals; as it has been postulated that both neurotransmitters compete for the same transporters (Devoto et al.,

2001; Moron et al., 2002; Devoto et al., 2005; Devoto & Flore, 2006; Masana et al., 2012a) and that the NET has more affinity for DA than the DAT itself (Moron et al., 2002; Devoto & Flore, 2006).

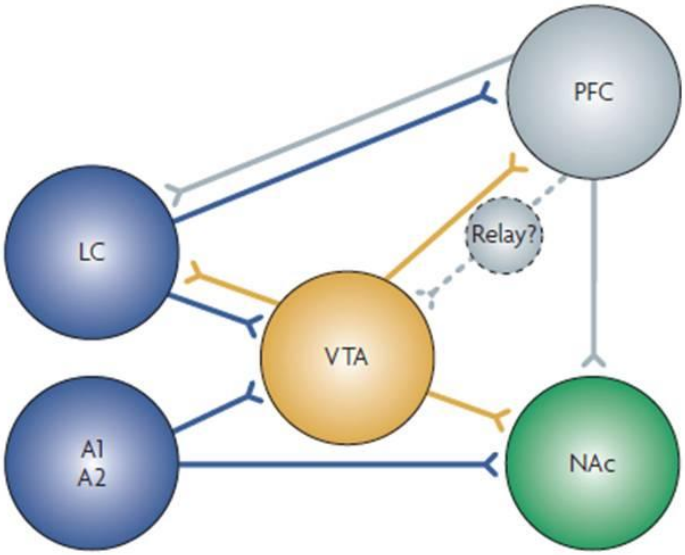

Figure 1.7: Anatomical connections and interactions between the noradrenergic and dopaminergic systems. LC activation elicits burst firing in the VTA, resulting in DA release in the nucleus accumbens. LC activation also affects neurons in the PFC that project indirectly to the VTA (relay). The release of glutamate in the VTA results in increased excitability and more DA release in the nucleus accumbens. The VTA projects to the LC (Ornstein et al., 1987), as does the PFC (Sara & Hervé-Minvielle, 1995). A1 and A2 are brainstem noradrenergic cell groups. Termini showed in blue release NA; termini shown in grey release glutamate; termini shown in yellow release DA. Abbreviations: LC: locus coeruleus; VTA: ventral tegmental area; PFC: the prefrontal cortex. From *Sara, 2009.*

1.2.3. The serotonergic system

During the 50s, 5-HT was observed to have an important role in peripheral tissues in relation to vasoconstriction and contraction of the gastrointestinal smooth muscle (Rapport et al., 1948; Erspamer & Asero, 1952). Later, its presence is also observed in the mammalian brain (Twarog & Page, 1953), understanding that 5-HT is present throughout the body. About the 90% of 5-HT is found in enterochromaffin cells of the intestinal mucosa, between the 8-10% is present at the blood level, the pineal gland, the spleen, the liver, the lungs, and the skin, and only 1-2 % are located in the CNS (Lozeva-Thomas, 2004).

In the CNS, 5-HT participates in various processes, including circadian rhythms, food intake, sleep cycles, aggressive behavior, locomotion, thermoregulation, nociception, sexual activity, memory, and learning, among others (Lucki, 1998; Meneses, 1999; Buhot et al., 2000). Some studies also suggest that it plays a role during development and in the adult brain regarding the formation of the synapse (Matsukawa et al., 1997). More importantly, in humans, it has been described that 5-HT participates in emotional processes and mood states, playing a

crucial role in different psychiatric conditions, including anxiety, depression, schizophrenia, and anorexia nervosa (Terry et al., 2008).

1.2.3.1. Synthesis, release, reuptake, and catabolism

5-HT is a biogenic amine composed of an indole ring and an ethylamine side chain. Most of its synthesis, around 90% of 5-HT, takes place in the enterochromaffin cells of the gastrointestinal tract; then it is released into the blood and stored in the platelets by an active transport by a platelet serotonin transporter (SERT) localized in the platelet membrane (Brenner et al., 2007), from where it is transported to all the vascularized tissues. In addition, 5-HT is also synthesized in the serotonergic neurons of the CNS. The synthesis starts from its precursor, the amino acid, which is obtained from the diet. L-tryptophan is then oxidized by the action of the enzyme tryptophan hydroxylase giving rise to 5-hydroxytryptophan, and it is considered the limiting step of the synthesis. Next, 5-hydroxytryptophan is decarboxylated by the enzyme L-amino acid decarboxylase, resulting in 5-HT. Finally, it is stored in vesicles by the action of the VMAT2 (Chaudhry et al., 2008), often as a co-transmitter along with several peptide hormones such as somatostatin, P substance or vasoactive intestinal polypeptide (VIP).

In response to depolarization of the cell and Ca^{+2} entry in the terminal, 5-HT is released into the synaptic space by a process of exocytosis. Once released, part of this 5-HT acts on its specific membrane receptors, part diffuses and another part is reuptake by the nerve ending.

The reuptake process puts an end to the serotonergic action and it carried out by the SERT which is dependent on Na^{+} and Cl^{-}. The majority of SERTs are located on the outer cell membrane, either perisynaptically or along the axon (Zhou et al., 1998). It is expressed in numerous brain regions with different density, such as raphe nuclei, hypothalamus, thalamus, amygdala, caudate-putamen nucleus, hippocampus, insular cortex, PFC and cortex of the cerebellum (Cortes et al., 1988; Laruelle et al., 1988; Kish et al., 2005). In addition, SERT expression is tightly related to the processes of synthesis, storage, and sensitivity of 5-HT receptors; since changes in 5-HT concentrations and receptors' functions have been observed in mice lacking this transporter (Bengel et al., 1998; Li, B. M. et al., 1999).

Back inside the cell, 5-HT can be stored inside the synaptic vesicles through VMAT2 to be recycled or it can undergo an intracellular metabolism process. In the latter, 5-HT is metabolized principally by oxidative deamination catalyzed by the MAO_A enzyme, followed by oxidation mediated by aldehyde dehydrogenase; resulting in the 5-hydroxyindoleacetic acid, its main metabolite. 5-hydroxyindoleacetic then diffuses into the extracellular space and the cerebrospinal fluid (CSF) and is finally eliminated through urine (**Figure 1.8**).

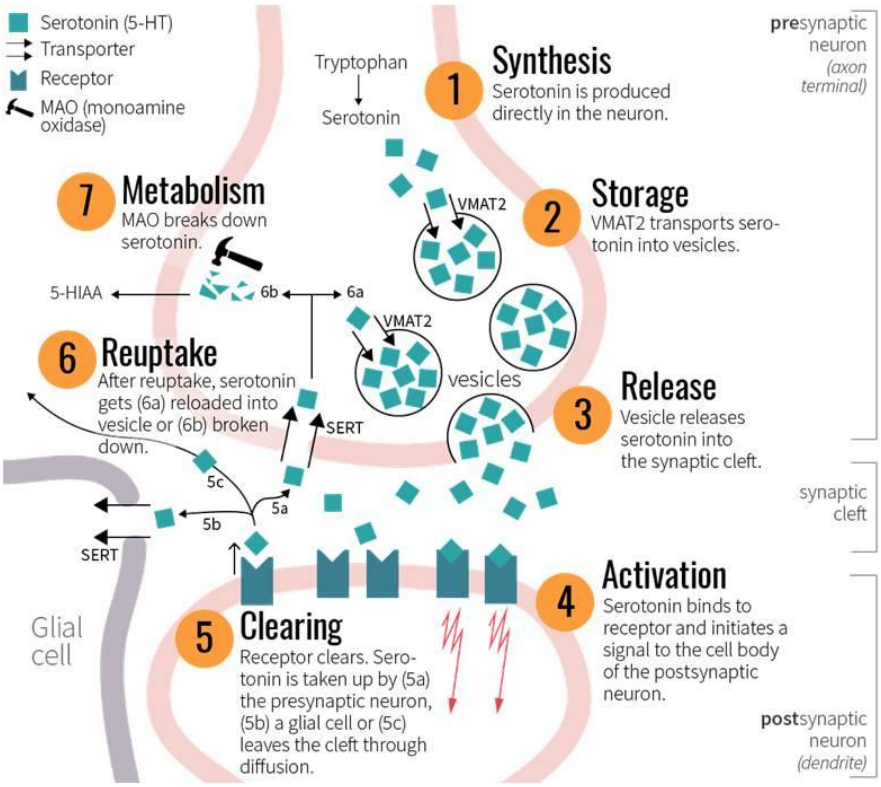

Figure 1.8: Representative scheme of the processes of synthesis, release, reuptake and catabolism of 5-HT. Abbreviations: VMAT2: vesicular monoamine transporter 2; SERT: serotonin transporter; 5-HIAA: 5-hydroxyindoleacetic acid; MAO: monoamino oxidase. Adapted from *https://sapiensoup.com/serotonin, 2017*

1.2.3.2. 5-HT receptors

The serotonergic system is a complex system where 5-HT can act through a multitude of 5-HT receptors (5-HTR). Thanks to the advance of techniques such as molecular cloning and application of signal transduction and pharmacological specificity studies, the identification of the different families of 5-HTRs has been possible: from 5-HT_1R to 5-HT_7R, consisting of fourteen receptor subtypes, that are structurally and pharmacologically different in mammals (Hoyer et al., 1994). All the 5-HTRs belong to the superfamily of GPCRs with the exception of the 5-HT_3R, that is included in the family of ligand-activated ion channels (Boess & Martin, 1994). Regarding its location, 5-HTRs are found in peripheral and CNS, as well as in intestinal and cardiovascular tissues and at blood level.

The 5-HT_1R family is composed of five receptor subtypes: 5-HT_{1A}R, 5-HT_{1B}R, 5-HT_{1D}R, 5-HT_{1E}R, and 5-HT_{1F}Rs. There is no 5-HT_{1C} designation as this receptor is classified as 5-HT_{2C} due to the similarities with this subfamily (Hoyer et al., 1994). This receptor family is coupled to $G_{i/o}$ proteins inhibiting the formation of cAMP. A key mechanism of control of 5-HT release is the auto-inhibition through the 5-HT_{1A} autoreceptors located presynaptically (Innis & Aghajanian, 1987; Celada et al., 2013) inhibiting neurotransmitter release for the serotonergic neurons in the raphe nuclei. 5-HT_{1A}Rs are also expressed in limbic and cortical forebrain areas and in the spinal cord. Its density is particularly high in the lateral septum, hippocampal CA1 and dentate gyrus, frontal and cingulate cortices. The hypothalamus, amygdala and interpeduncular

nucleus also contain high densities of this receptor. 5-HT_{1A}R is expressed postsynaptically to 5-HT axon terminals in the forebrain (Pompeiano et al., 1992; Kia et al., 1996) and the regional distribution is similar in rodent and human brains (Pazos & Palacios, 1985; Pompeiano et al., 1992; Kia et al., 1996; Sargent et al., 2000; Martinez et al., 2001). In rodent PFC, 5-HT_{1A}Rs are present in approximately 50% of pyramidal neurons and 25% of GABAergic interneurons (Santana et al., 2004), and in human PFC this proportion is even higher, up to 80% (de Almeida & Mengod, 2008). This suggests a prominent role of 5-HT_{1A}R in cortical function (Celada et al., 2013). 5-HT_{1A}Rs expressed in PFC pyramidal neurons that project to the brainstem might be important in the modulation of PFC-dorsal raphe pathways, which are reported to be sensitive to postsynaptic 5-HT_{1A}R activation (Celada et al., 2001) and have been implicated in the PFC-dependent behavioral responses (Warden et al., 2012). On the other hand, 5-HT_{1B}Rs are expressed in the basal ganglia, the striatum and the PFC; acting too as autoreceptors and also as heteroreceptors controlling the release of other neurotransmitters (Pauwels, 1997).

The family of 5-HT_2Rs include the 5-HT_{2A}R, 5-HT_{2B}R, and 5-HT_{2C}Rs. Regarding molecular structure, pharmacology and signal transduction pathways, they are similar to each other, as the amino acid sequences of these three receptor subtypes have a high degree of homology. They are coupled to $G_{q/11}$ proteins, activating the PLC as a second messenger and favoring the synthesis of inositol phosphate and glycerol, as well as stimulating the mobilization of intracellular Ca^{+2} (Boess & Martin, 1994). As for the 5-HT_{2A}Rs it is present in cortical regions, caudate nucleus, nucleus accumbens, olfactory tubercle and hippocampus (Pazos & Palacios, 1985); but also in GABAergic interneurons (Morilak et al., 1994) and in glutamatergic pyramidal neurons of the cortex (Wright et al., 1995). In general, the activation of 5-HT_2Rs gives rise to a neuronal excitation in a great variety of cerebral regions. Particularly, 5-HT_{2A}R stimulation causes the activation of a biochemical cascade that alters the expression of a certain number of genes (e.g. the gene encoding the BDNF (Vaidya et al., 1997). Regarding the 5-HT_{2B}R location in the brain, immunoreactivity is observed in areas such as the cerebellum, lateral septum, dorsal hypothalamus and amygdala in rat brain (Duxon et al., 1997). It has been suggested that 5-HT mediates mitogenic effects on neural development through this receptor (Choi et al., 1997). Indeed, the administration of 5-HT_{2B} agonists at the level of the amygdala presented anxiolytic effects in different trials that study social interaction (Duxon et al., 1997). 5-HT_{2C}R distribution is mainly limited to the CNS and the choroid plexus. In the CNS, it is present principally in the cortex and in limbic structures such as the nucleus accumbens, the hippocampus, and the amygdala, as well as in the basal ganglia, especially the caudate nucleus and the substantia nigra (Palacios et al., 1991). Different studies conclude that activation of the 5-HT_{2C}R exerts an inhibitory tonic influence on dopaminergic and adrenergic transmission, but not serotonergic transmission (Millan et al., 1998; Jorgensen et al., 1999). In fact, mice genetically lacking this receptor suffer from cognitive impairment, increased food intake and obesity, spontaneous seizures, increased responses to stress and compulsive behavior (Chou-Green et al., 2003).

5-HT_3Rs are the only ones in the serotonergic family of receptors that are not coupled to G proteins, belonging to the group of ionic receptors activated by ligand. When activated, they give rise to a rapid depolarization with a subsequent opening of non-selective channels (Na^+,

$Ca^{+2,}$ and K^{+}). They are present in several brain regions, including the hippocampus, the area postrema, or the dorsal motor nucleus of the solitary tract (Laporte et al., 1992). At the cellular level, postsynaptic receptors mediate rapid excitatory synaptic neurotransmission in rat cortical interneurons, in the amygdala, and in the visual cortex (Sugita et al., 1992; Roerig et al., 1997; Ferezou et al., 2002). Until recently, their most known function was the regulation of intestinal secretion and motility due to the receptors present in the gastrointestinal tract (De Ponti & Tonini, 2001).

5-HT_4Rs are coupled to Gs proteins that activate in a positive way the activity of the AC enzyme and promote the formation of cAMP (Hoyer et al., 2002). Large numbers of these receptors are found in the limbic system and areas such as the olfactory tubercle, the frontal cortex, the ventral pale, the septal region, the hippocampus, and the amygdala; supposedly involved in memory and learning functions (Marchetti-Gauthier et al., 1997). On the other hand, these receptors also contribute to controlling the dopaminergic secretion (Bonhomme et al., 1995), as it has been observed in the mammalian brain.

Included in the 5-HT_5R family there are the designated 5-HT_{5A}R and 5-HT_{5B}R. The 5-HT_{5A}R has been identified in the mouse, rat, and human. The 5-HT_{5B}R also is expressed in the mouse and rat, but not in the human (Nelson, 2004). Both receptors have a limited distribution in the CNS, being the 5-HT_{5A}R the one that shows a relatively broader distribution (Grailhe et al., 1999). The 5-HT_5R family has not been extensively characterized pharmacologically. Apparently, the 5-HT_{5A}R is coupled to the $G_{i/o}$ proteins, inhibiting the formation of cAMP. Both receptors show their highest affinity for the lysergic acid diethylamide (LSD), which appears to act as a partial agonist at the 5-HT_{5A}R. Nothing is known about the role of the 5-HT_{5B}R in vivo (Nelson, 2004). This family of receptors remains as one of the least studied and understood of the serotonergic family of receptors (Wisden et al., 1993; Rees et al., 1994; Nelson, 2004).

5-HT_6Rs are coupled to G_s proteins activating the AC and inducing the formation of cAMP (Millan et al., 2008). Their expression is limited to the CNS exclusively. The mRNA coding for the rat and human receptor is located in the striatum, the amygdala, the cortex, and the olfactory tubercle, but so far it has not been found in peripheral organs (Hoyer et al., 2002). It is still unknown which its function is in the brain; however, it has been observed that blocking these receptors increases the concentration of other neurotransmitters such as acetylcholine, excitatory amino acids, NA and DA (Mitchell & Neumaier, 2005). Based on these, it is suggested that this receptor subtype could be involved in cognitive memory and learning processes.

Finally, 5-HT_7Rs are coupled to G_s proteins, they favor the formation of cAMP (Bard et al., 1993). In the CNS, they are located in the membranes of cortical neurons and more discreetly, they are present in limbic regions, in the thalamic nucleus, and in regions of the cerebral cortex. A very low expression has been reported in the substantia nigra, the hypothalamus and the dorsal raphe nucleus (To et al., 1995; Gustafson et al., 1996). Some of the known functions in the CNS include endocrine and thermal regulation, and involvement in mood, cognition, and sleep.

1.2.3.3. Serotonergic pathways in the CNS

The serotonergic system in the CNS is composed of an assembly of neurons that are divided into nine-cell groups named B1-B9 (Dahlstrom & Fuxe, 1964), constituting what is known as raphe nuclei, located along the midline of the mesencephalon and brain stem (**Figure 1.9**). It is important to consider that not all neurons of these raphe nuclei are serotonergic, their proportion varies among the different nuclei (Kohler & Steinbusch, 1982; Molliver, 1987; Tork, 1990).

The dorsal raphe nucleus is composed of the B7 and B6 neuron groups. Group B8 corresponds to the medial raphe nucleus. Group B9 originates in the ventrolateral tegmentum of the brainstem bridge and forms a lateral extension of the medial raphe nucleus. Finally, the caudal group, composed of B1-B4, contain a small number of serotonergic cells that give rise to axons that project to the brainstem and the spinal cord (reviewed by Hensler, 2006). These serotonergic neuron projections constitute one of the most complex and extensive anatomical and functional systems of the mammalian brain. In general, these raphe nuclei receive information from other cellular groups located in the substantia nigra and the VTA, the superior vestibular nucleus, the LC and the nucleus of the solitary tract. Other afferents include neurons of the hypothalamus, cortex and limbic structures such as the amygdala (reviewed by (Jacobs & Azmitia, 1992).

Among all the serotonergic nuclei, the two most important ones are the dorsal raphe nucleus and the medial raphe nucleus. Specifically, the dorsal raphe nucleus innervates the frontoparietal cortex, the amygdala, the lateral septum, the cortex of the nucleus accumbens, the ventral hippocampus and several hypothalamic nuclei. It is responsible for sending axons to the dopaminergic neurons of the substantia nigra; which, in turn, innervate the amygdala, the caudate-putamen and subthalamic areas that are responsible for involuntary motility. This nucleus receives axons from cortical areas that stimulate and inhibit serotonergic activity through the release of glutamate and GABA, respectively (Celada et al., 2001). Likewise, it receives dopaminergic axons from the subcortical nucleus capable of exciting serotonergic neurons. On the other hand, the medial raphe nucleus innervates the dorsal hippocampus, the medial septum, the central area of the nucleus accumbens and several hypothalamic nuclei. In addition, it sends axons to the dopaminergic neurons of the VTA that innervate, in turn, the frontal cortex. Both nuclei, dorsal and medial raphe nuclei, exchange serotonergic axons in proportion to their population of serotonergic neurons (reviewed by (Lechin et al., 2006)).

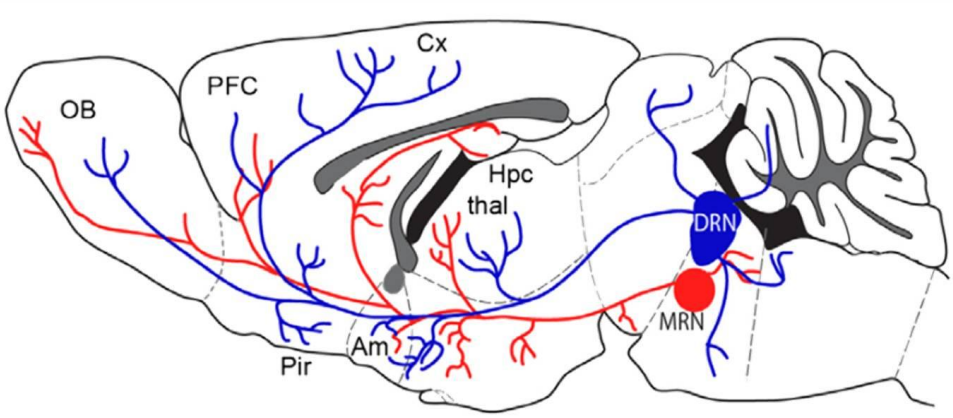

Figure 1.9: Schematic representation of the serotonergic projections. Abbreviations: DRN, dorsal raphe nucleus; MRN, median raphe nucleus; Hpc, hippocampus; thal, thalamus; Cx, cortex; PFC, prefrontal cortex, OB, olfactory bulb; Am, amygala; Pir, piriform cortex. Adapted from *Gaudry, 2018.*

1.3. NEUROBIOLOGY OF SCHIZOPHRENIA

As it has been seen through the different sections of this work, there are many neurotransmission systems and signaling pathways that play an important role in schizophrenia. If these abnormalities are a consequence of the progress of the disease or if, on the contrary, they are responsible for its development and progression, it is a subject that is still not entirely clear.

1.3.1. Clinical features and morphological alterations

Recent advances in magnetic resonance imaging (MRI) and diffusion tensor imaging have enabled the exploration of the function and structure of the interconnected neural networks that are implicated in schizophrenia (Ross et al., 2006).

In schizophrenia, the most consistent structural abnormalities found include lateral and third ventricular enlargement, medial temporal lobe volume reductions and superior temporal gyrus volume reductions, particularly on the left. There is also moderate evidence for frontal lobe volume reduction, particularly of prefrontal and orbitofrontal regions, and parietal lobe abnormalities (Dickey et al., 2003; Antonova et al., 2004; Honea et al., 2005). Apart from that, abnormalities in basal ganglia, corpus callosum, thalamus, and cerebellum are also evident. Some studies, but not all, have suggested that structural changes may be progressive (Rapoport et al., 2005).

It has been suggested from structural neuroimaging studies, that abnormal processes in schizophrenia occur at different stages of neurodevelopment. There is evidence that an early (pre- or perinatal) neurodevelopmental lesion can make the brain more vulnerable to anomalous late neurodevelopmental processes, particularly postpubertal. These anomalous processes may interact with other environmental factors (e.g., stress, substance use) associated with the onset of psychosis, which together can lead to sequelae of neurodegenerative nature (Pantelis et al., 2005; Rapoport et al., 2005). Actually, via MRI, abnormal brain structure may be detectable prior to the onset of psychotic symptoms (Lymer et al., 2006).

Also, studies using MRI for evaluating executive function and memory, have reported abnormalities on the dorsolateral PFC, medial temporal lobe, hippocampus, parahippocampal gyrus, anterior cingulate, medial frontal and posterior parietal cortex, striatum, thalamus and cerebellum (Dickey et al., 2003; Turner et al., 2006). DTI, a technique based on the direction of water diffusion and that is still under development, has suggested the possibility of white matter disorganization in brain regions, such as prefrontal and temporal white matter (Kanaan et al., 2005; Kubicki et al., 2005).

Another line of evidence suggests a reduction of cortical neuropil volume without a comparable neuronal loss (Selemon & Goldman-Rakic, 1999). Besides, quantitative and qualitative deficits in neuronal processes and synaptic connectivity in schizophrenia have been reported from some ultrastructural, immunohistochemical and other quantitative

neuropathological studies (**Figure 1.10**). Finally, genetic expression arrays studies report that genes related to GABA neurotransmission, synaptic transmission and metabolism may be implicated in the disease (Katsel et al., 2005). In fact, abnormal expression of genes related to the myelination has been identified; suggesting the possibility of glial and white matter abnormalities that could be fundamental to the development of the disorder (Ross et al., 2006).

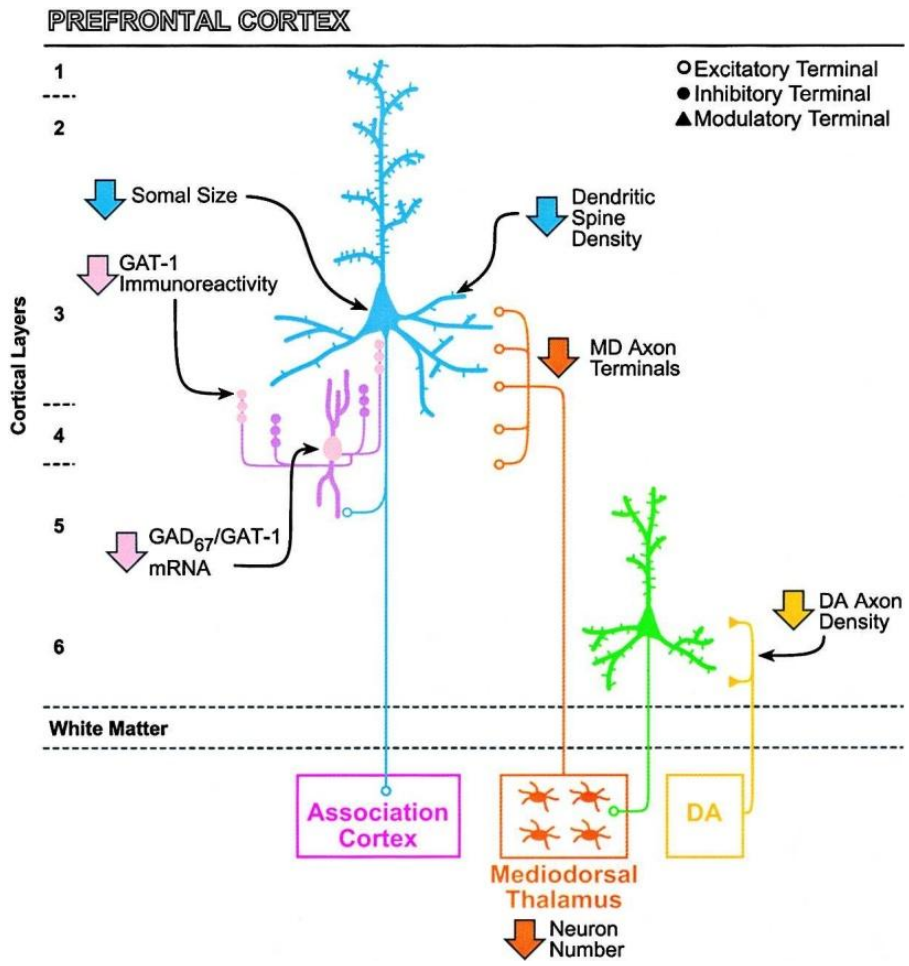

Figure 1.10: Schematic diagram summarizing disturbances in the connectivity between the mediodorsal thalamic nucleus and the dorsal PFC in schizophrenia. From *Lewis & Lieberman, 2000.*

1.3.2. The noradrenergic system in schizophrenia

In the last decades, the dominant and most popular hypothesis has been centered on the possibility of a disturbed brain DA metabolism in this disorder. During the last years, other neurotransmitters have also been considered (5-HT, glutamate, GABA, among others). Even if as early as in 1971 NA was proposed to be involved in schizophrenia (Stein & Wise, 1972), this catecholamine has only received marginal attention within the theoretical constructs of this disorder.

Practically all clinically effective and routinely used neuroleptic drugs act on more than a single type of neurotransmitter receptor, be it DA, 5-HT, NA, acetylcholine or histamine, to name a few. In regard to NA, the majority of antipsychotics act as antagonists of α_2ARs (Minzenberg & Yoon, 2011).

As previously commented, NA has been proposed to participate in a wide variety of brain functions: sleep-wakefulness cycles, arousal, attention, novelty-oriented behavior, anxiety, fear, aggressiveness, stress, memory consolidation, learning, various aspects of psychomotor behavior, and many neuroendocrine and autonomic functions, among others. The basis of all these NA-dependent functions, the so-called “higher brain functions”, is probably provided by the widespread distribution and branching of the noradrenergic system in the brain (Yamamoto & Hornykiewicz, 2004). Precisely because of this important noradrenergic innervation of the human neocortex and limbic system, this catecholamine should be considered crucial in schizophrenia.

Indeed, the review made by Yamamoto and Hornykiewicz (Yamamoto & Hornykiewicz, 2004) provides numerous evidence of the possible alterations of the noradrenergic system and its potential role in schizophrenia. For instance, in brain postmortem studies, it was reported a significantly above-normal NA concentration in schizophrenia patients (Farley et al., 1978). Also, studies of CSF of schizophrenic patients revealed elevated concentrations of NA in the lumbar region (Gomes et al., 1980; Lake et al., 1980; Sternberg et al., 1981). Specifically, those patients with elevated mean CSF NA concentrations correlated positively with higher scores in the subscale of positive symptoms, supporting the idea that high CSF NA may be associated with paranoid schizophrenia (Kemali et al., 1990). Regarding blood plasma studies, above-normal NA concentrations have been repeatedly found in treated and non-treated patients, correlating positively with the positive and paranoid symptomatology (Dajas et al., 1983; Bondy et al., 1984). In the case of 3-methoxy-4-hydroxyphenylglycol plasma concentrations, the main metabolite of NA, increased concentrations were reported in patients with a paranoid diagnosis and high score in the positive symptom scale (Kaneko et al., 1992), suggesting the association between increased plasma NA and 3-methoxy-4-hydroxyphenylglycol concentrations with the paranoid and positive symptomatology of the disorder.

Taking these evidence into consideration, the noradrenergic theory of schizophrenia could postulate that the psychopathology of the positive and negative symptoms may be interpreted from hypervigilant and hypovigilant states of consciousness, respectively. Positive symptoms

could be associated with hypervigilant states of consciousness; and conversely, negative symptoms could be linked to hypovigilant states. In an extremely alert state, as stress sensitivity is increased, even slight stimuli often provoke disproportionate fear and aggressiveness, which may develop into persecutory ideas or delusions. In contrast, an extremely hypovigilant state often results in blunted affect and cognitive impairment (Yamamoto & Hornykiewicz, 2004).

Concerning the pathogenesis of the brain atrophy, it is well confirmed the association of ventricular enlargement with a diffuse reduction of blood flow and metabolic rate in the PFC and the gray matter (Berman et al., 1987; Hazlett et al., 1993). Besides, there is enough evidence that the cerebral blood flow and metabolic rate are controlled by the central NA system Besides, there is enough evidence that the cerebral blood flow and metabolic rate are controlled by the central NA system (e.g., Bolme et al., 1975; Wolkin et al., 1994). Therefore, it is possible that long-term NA dysfunction influences cerebral blood flow and metabolic rate chronically, which may result in brain atrophy, another characteristic feature of this disorder.

1.3.3. The dopaminergic system in schizophrenia

Since the first description of amphetamine induction of schizophrenia-like symptoms in healthy subjects (Connell, 1958) and the observation that D_2R antagonists are effective in the treatment of this disorder, the *dopaminergic hypothesis* aiming at DA as the main neurotransmitter involved in the disease has been the most known an accepted hypothesis in the etiology of schizophrenia (Carlsson, 1974; Seeman, 1987).

These classical studies suggested that schizophrenia symptoms may be caused by an excess in DA release; however, more recent studies claim that this may be an oversimplification. In fact, results show that there is an increase of DA release in the striatum only during specific types of system activation, and this increase is correlated with the positive psychotic symptoms of this disorder (Laruelle et al., 1999). In contrast, it has been proposed that the functional deficits observed in the PFC of schizophrenia patients could be due to a deficit in DA activity, which may underlie the negative or cognitive dysfunction of schizophrenia (Abi-Dargham et al., 2002; Davis et al., 1991).

Recently, the disturbed DA system in schizophrenia has been described as an opposing relationship between PFC and striatal DA release. Primate (Kolachana et al., 1995; Saunders et al., 1998), rodent (Jackson et al., 2001), and human studies (Jackson et al., 2001), have shown that the weakened PFC activity in schizophrenia may be correlated with an exaggerated DA release in the striatum. This idea could be explained taking into consideration the anatomical organization of PFC-VTA and hippocampus-VTA interactions (**Figure 1.12**). PFC projections onto GABA interneurons in the VTA can suppress spike firing in the DA neurons that project into the striatum. Therefore, with PFC deficits, the PFC drive of VTA DA neurons is diminished, leading to abnormally augmented DA release in the striatum. Studies from (Goto & Grace, 2005) showed that increasing DA release in the ventral striatum facilitates limbic inputs and attenuates PFC inputs; whereas decreasing DA release, shifts the balance in favor of the PFC

inputs. Thus, DA maintains the balance between limbic and PFC drive of ventral striatum neurons.

A key mechanism for this inverse relationship between PFC activity and striatal DA release may be drawn from the known reciprocal interactions between the hippocampus and PFC (**Figure 1.11A)**. The hippocampus sends direct projections into the PFC (Jay et al., 1989; Fuster, 1997), while the PFC sends indirect projections into the hippocampus through the temporal cortex (Groenewegen & Uylings, 2000; Kyd & Bilkey, 2003). Since it has been suggested that the PFC exerts an inhibitory influence over limbic structures (Fuster, 1997; Grace & Rosenkranz, 2002) when there is a higher degree of PFC activity in relation to the hippocampus, DA release in the ventral striatum could be suppressed. whereas higher activity within the hippocampus would lead to augmented DA release in the ventral striatum.

Accordingly, in schizophrenia, when PFC activity is abnormally attenuated, there would be a reduction in mesocortical DA release, as well as a decreased PFC-mediated inhibition of HPC activity. As a consequence, there would be an increase in HPC drive of striatal neuron activity and mesolimbic DA release. The increase in striatal dopaminergic D_1R and D_2Rs stimulation would then cause a further attenuation of PFC input and facilitation of HPC input to the striatum (**Figure 1.11B**) (Meyer-Lindenberg et al., 2002; 2005; Goto & Grace, 2005).

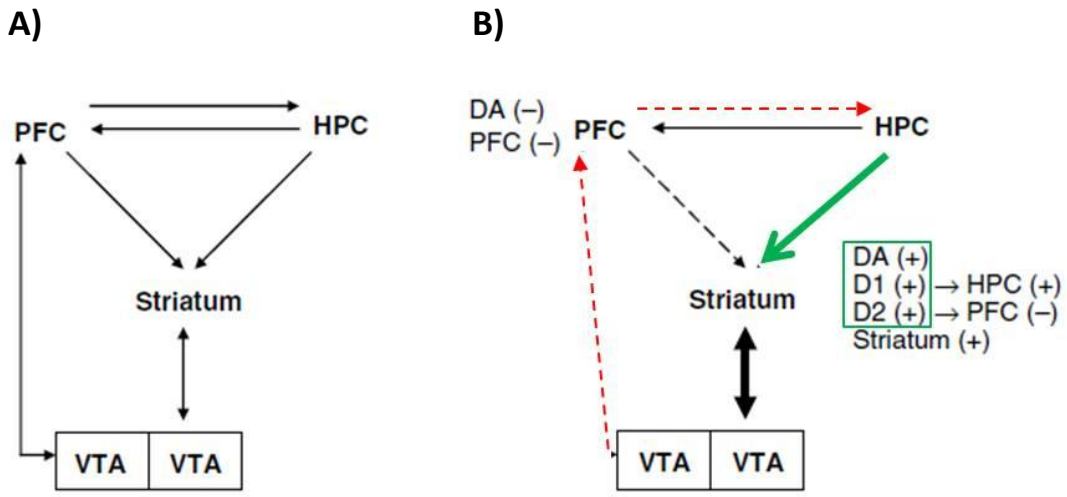

Figure 1.11: Schematic representations of the mesocortical and mesolimbic DA system and its regulation by interactions among PFC-HPC-striatum. **A)** The reciprocal interaction between the PFC and the HPC maintains the balance of mesocortical and mesolimbic DA release. **B)** When PFC function is attenuated, as may occur in SCH, there would be an increase in HPC drive of striatal neuron activity and mesolimbic DA release, enhancing D_1R and D_2R stimulation. This would cause a further PFC input attenuation and facilitation of HPC input to the striatum. Abbreviations: HPC, hippocampus. (+) and (–), denotes increase and decrease, respectively. Modified from *Goto & Grace, 2007*.

After the discovery that schizophrenia symptoms respond to treatment by D_2R antagonists (Carlsson, 1974), the therapeutic approach to treating it by antipsychotic drugs has targeted brain DA systems. Nevertheless, this simple approach has not produced consistent results. In fact, postmortem tissue and imaging studies reporting increased D_2R density in schizophrenia

have been controversial, with many studies reporting no difference (Farde et al., 1987; Nordstrom et al., 1995). In contrast, given that one of the core deficits in schizophrenia are cognitive dysfunctions associated with PFC activity (Weinberger et al., 1994), some studies have focused on alterations of D_1Rs in the PFC of schizophrenia patients. Indeed, even if the results are still incomplete, increases (Okubo et al., 1997) or decreases (Abi-Dargham et al., 2002), of D_1Rs in the PFC of schizophrenia patients have been reported. Given that D_1R stimulation facilitates calcium influx via NMDA channels (Greengard et al., 1999; Tseng & O'Donnell, 2004), alterations in D_1Rs are also consistent with the hypo-NMDA function theory of schizophrenia pathophysiology (Jentsch et al., 1999; Goff & Coyle, 2001; Coyle et al., 2003). This theory is based on observations that NMDA antagonists can lead to schizophrenia-like symptoms in normal patients.

It is known that DA stimulation of D_1Rs in the PFC exhibits an inverted U-shaped relationship, with optimal DA release required for mediating effective cognitive functions (Lidow, 2003; Robbins, 2005). Therefore, increases or decreases in D_1Rs would shift the relationship between optimal DA release and over- or understimulation of D_1Rs, with the consequent impact on PFC function (Callicott et al., 2003; Manoach, 2003).

On the other hand, even if elevated presynaptic striatal DA function is a robust feature of schizophrenia, the relationship between this DA abnormality and the response to DA-blocking antipsychotics is still unclear, as it is the exact mechanism by which D_2R antagonism achieves therapeutic efficacy in schizophrenia. Several lines of evidence have been followed to study this issue. Positron emission tomography (PET) studies have reported that DA synthesis capacity is elevated in schizophrenia, with effect sizes ranging from 0.63 to 1.89 (Howes et al., 2007). Furthermore, elevated DA synthesis capacity precedes the onset of psychosis (Howes et al., 2009; 2011) and other molecular imaging studies indicate that both DA release and baseline DA concentrations are elevated in these patients (Laruelle et al., 1996; Abi-Dargham et al., 2000). In one study (Demjaha et al., 2012) using PET it was demonstrated that DA synthesis capacity was lower in schizophrenia patients with treatment-resistant illness than in those who showed a good response to antipsychotic treatment. This could suggest that treatments that involve the blockade of D_2Rs may be more effective in patients who have an elevation of DA synthesis capacity, but less useful in patients with relatively normal DA synthesis capacity, considering them to be treatment-resistant or non-responders.

Furthermore, the relationship between increased striatal DA and increased baseline D_2Rs occupancy in schizophrenia has been also reported (Reith et al., 1994; Hietala et al., 1995; Dao-Castellana et al., 1997; Hietala et al., 1999; Meyer-Lindenberg et al., 2002; McGowan et al., 2004). Single-photon emission computerized tomography (SPECT) studies with schizophrenia patients and control subjects showed that amphetamine-induced DA release and baseline D_2R occupancy by DA are very tightly correlated in drug-naïve schizophrenia patients but not in control subjects, being higher in patients compared with controls (Abi-Dargham et al., 2009). Indeed, this correlation between amphetamine-induced DA release and the level of baseline intrasynaptic DA in schizophrenic patients indicates that the increase in striatal DA activity varies among patients. As these findings correspond to patients never

exposed to antipsychotic drugs previously, it confirms that the DA pathology in the disorder is related to the disease process, not a result of previous antipsychotic exposure. Another study (Abi-Dargham et al., 2000) showed that the increase in D_2R occupancy was predictive of good treatment response of positive symptoms to antipsychotic drugs and that low D_2 baseline occupancy was a predictor for dysphoric effects of treatment with antipsychotics (Voruganti et al., 2001).

1.3.4. The serotonergic system in schizophrenia

It has long been recognized that hallucinogenic drugs, such as LSD, psilocybin and mescaline, recruit specific serotonin 5-HT_{2A}R-mediated signaling pathways to exert their psychotic-like effects (Vollenweider et al., 1998; Gonzalez-Maeso et al., 2007; Geyer & Vollenweider, 2008). Indeed, they are agonists/partial agonists at the serotonin 5-HT_{2A}Rs and produce symptoms in healthy volunteers that resemble and share similarities with the core symptoms of schizophrenia (Gouzoulis-Mayfrank et al., 2005; Carhart-Harris et al., 2012).

This kind of observations gave rise to what is known as the serotonergic hypothesis of schizophrenia, postulating that a stress-induced serotonergic overdrive from the dorsal raphe nucleus can disrupt cortical neuronal function. This, along with the hyperactivity of 5-HT in the cerebral cortex, has been proposed as one of the presumable causes of schizophrenia (Eggers, 2013). Indeed, there is a good correlation between serotonin 5-HT_{2A}R binding affinities and hallucinogenic potencies of psychoactive drugs such as LSD and N, N-dimethyltryptamine in humans (Glennon et al., 1984). This, in addition to the higher affinity of atypical antipsychotic agents (such as clozapine, risperidone, and olanzapine) for 5-HT_{2A}Rs as compared to D_2Rs, has led to the serotonin hypothesis of schizophrenia with 5-HT_{2A}Rs being recognized as key players (Geyer & Vollenweider, 2008; Gonzalez-Maeso & Sealfon, 2009; Moreno & Gonzalez-Maeso, 2013).

However, regarding the 5-HT_{2A}R and its role in schizophrenia, there are also some inconsistencies. Studies on human *postmortem* brains from schizophrenia patients show some discrepancies in the variation of 5-HT and 5-HT_{2A}R levels. Therefore, so far it is not clear enough whether an increase or a decrease in those levels occurs in schizophrenia (Crow et al., 1979; Bleich et al., 1988; Ohuoha et al., 1993; Muguruza et al., 2014).

Nevertheless, it seems necessary to highlight the importance of the serotonergic system for the information provided by studies performed in animal models. Psychedelic drugs such as ketamine or phencyclidine (PCP) increase 5-HT release in the rat frontal cortex (Quarta & Large, 2011). Similarly, cocaine, amphetamine, and amphetamine-related compounds, with the exception of 2,5-Dimethoxy-4-iodoamphetamine ((±)-DOI), augment 5-HT output in rat frontal cortex (Matsumoto et al., 2014). Moreover, the 5-HT release was decreased after local administration in rat frontal cortex of (±)-DOI (1 mg/kg) (Martin-Ruiz et al., 2001).

Regarding antipsychotics, there is evidence that they can also modulate 5-HT output in animal models. In fact, the atypical antipsychotic risperidone induces 5-HT release in rat frontal cortex (Kuroki et al., 1999; Huang et al., 2006a). It has been reported also that the increased 5-HT

efflux in the medial PFC provoked by PCP or ketamine can be reversed by clozapine, olanzapine and prazosin but not the classical antipsychotic haloperidol (Amargos-Bosch et al., 2006). Besides, co-administration of PCP and ketamine attenuate the high 5-HT release evoked by the recreational drugs like amphetamine and cocaine. These findings could suggest that an increased serotonergic transmission in the medial PFC is a functional consequence of NMDA receptor hypofunction and that this effect is blocked by atypical antipsychotic drugs (Amargos-Bosch et al., 2006). However, inconsistencies have also been reported regarding the effect of the different drugs acting through the serotonergic system. For instance, Kuroki and collaborators (Kuroki et al., 1999) observed that clozapine increases 5-HT output in the frontal cortex, while olanzapine, sulpiride, haloperidol, and M100907 have no effect on extracellular 5-HT concentrations in that region.

1.4. COGNITION

Cognitive impairment is considered a core feature of schizophrenia (Kahn & Keefe, 2013) which is manifested even before the clinical diagnosis, during the prodromal phase of the disorder (Cornblatt et al., 1998; Erlenmeyer-Kimling et al., 2000). Actually, it was Kraepelin who originally described the disorder as "dementia praecox" (Angst, 2002); characterized by a generally slow cognitive decline, starting almost a decade before the onset of the psychosis during adolescence. As reduced cognitive function correlates positively with improved prognosis and functional outcome (Bowie & Harvey, 2005; Bowie & Harvey, 2006; Keefe, 2008), treatments to enhance cognitive function are an important therapeutic strategy. Unfortunately, truly effective pro-cognitive treatment has yet not been developed (Geyer et al., 2012).

Cognitive disorders involve dysfunction of the most highly evolved cortical regions with particular vulnerabilities in the PFC. The PFC subserves the highest order cognitive functions and guides thought, actions and emotions using representational knowledge (Anderson et al., 1999). PFC circuits hold information "in mind" to provide the foundation for abstract thought and mental manipulation (Robbins, 1996; Wallis et al., 2001). It allows us to organize and plan for long-term ambitions as well as short term goals, to provide directed meaning and a purpose in life (Goldman-Rakic, 1994). It protects goal-directed behavior from distractions and compulsions (Thompson-Schill et al., 2002), thus being essential for self-control. It is also the key for high order decision making (Lee et al., 2007) and meta-cognition. For all this, it has probably become a converging biological system underlying cognitive dysfunction in schizophrenia (Pratt et al., 2008).

1.4.1. Structure and functions of PFC

The PFC includes the frontal cortex without motor cortex. It is a key region involved in executive functions such as WM, attention, decision making and also in emotional processing; including affection, emotion, and social behavior. These functions, very important in daily living, are the ones that normally result affected in people suffering from schizophrenia (Bowie & Harvey, 2006).

1.4.1.1. Definition, anatomical considerations, and connectivity

The PFC was originally defined as the frontal cortex containing granular layer IV (Siddiqui et al., 2008). Based on the cytoarchitecture, Brodmann defined the area "praefrontalis" as area 11 within the large ''regio frontalis'', which included his areas 8, 9, 10, 11, 12, 13, 44, 45, 46 and 47 (Rose & Woolsey, 1948; Uylings et al., 2003). After further analysis of connections and functions, the human/primate PFC consists of a much larger area containing both granular and agranular areas. Efforts have been made to define the rodent PFC, but anatomically equivalent regions in rodents are agranular (**Figure 1.12, Left panel**). Therefore, after many studies trying to compare between primates and other species of animals, a sensible criterion for the PFC that can be extended into other species beyond human/primate has been the distribution of

thalamic fibers. Thus, the PFC is defined as the part of frontal cortex connected with the mediodorsal (MD) nuclei of the thalamus (Rose & Woolsey, 1948; Uylings et al., 2003; Wise, 2008). Apart from that, PFC is also connected to diverse regions that, at the same time, can be subdivided into regions with different connections and functions.

1.4.1.2. Functional subdivisions

The PFC accomplishes cognitive feats in a topographically organized manner. Dorsal and lateral PFC circuits regulate attention, thought, and action (Goldman-Rakic, 1987) and the more ventral and medial PFC circuits regulate emotion and physiological state (Price et al., 1996). In humans, the neocortex contains six layers of neurons (**Figure 1.12, Right panel**) which are intricately interconnected for form neural networks (Arnsten & Jin, 2012). To identify equivalent regions between primates and rodents, morphological comparisons have been made (Ongur & Price, 2000; Vogt & Paxinos, 2014) and they have shown differences in their PFCs: for instance, in rodents there are not granular areas, and their PFC is not spread over to merge into both lateral and medial surfaces of the hemispheres, conversely to what happens in the primate PFC.

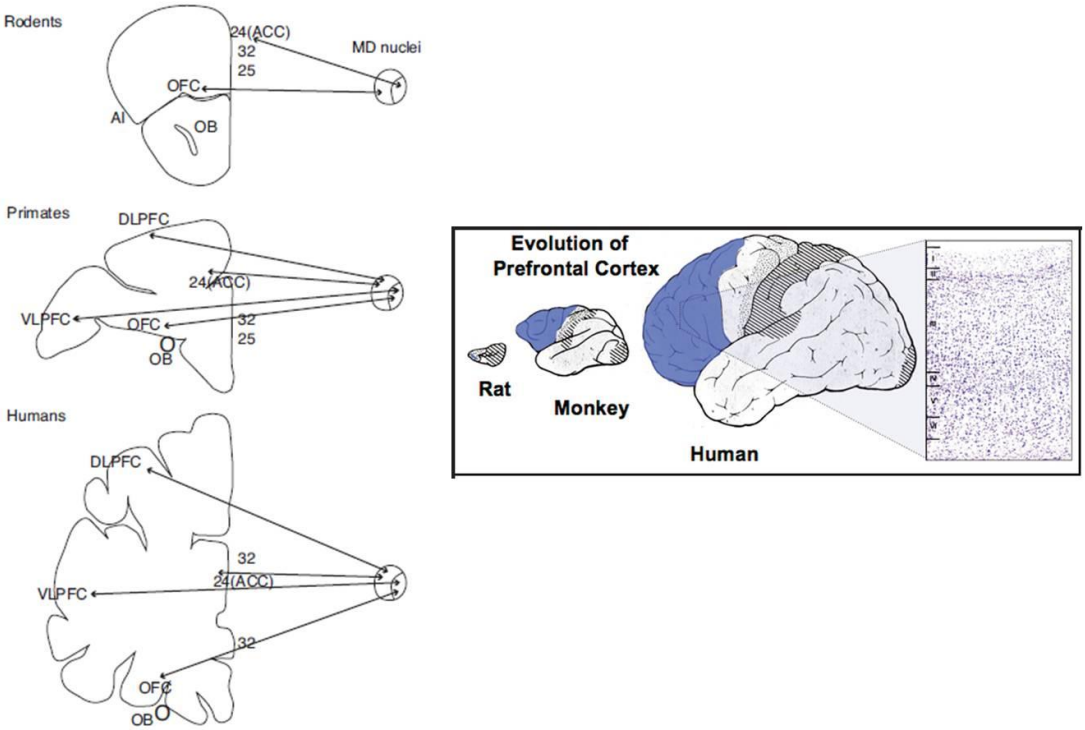

Figure 1.12: **Left panel**: Comparison of the PFC between primates and rodents. The PFC is defined as the frontal cortex that is connected with the MD nuclei of the thalamus. **Right panel**: The PFC expands tremendously in brain evolution, comprising a very small proportion of the brain in rodents such as the rat and increasing dramatically in primates, with special prominence in the human brain. The PFC is highlighted in blue. An inset of the human dorsolateral PFC is shown at the right; this Nissl-stained section shows the six layers of dorsolateral PFC. Abbreviations: ACC: anterior cingulate cortex; OFC: orbitofrontal cortex; OB: olfactory bulb; VLPFC: ventrolateral PFC. Adapted from *Arnsten and Jin, 2012; Sakurai et al., 2015*.

It should be considered that superficially similar behavioral phenotypes may not be equivalent between primates and rodents (Brown & Bowman, 2002; Wise, 2008) and that there are several fundamental differences (Preuss, 1995; Rilling & Insel, 1999; Wise, 2008). Despite all of this, functionally equivalent regions in the PFC between primates and rodents have been mapped (**Table 1.1**) by numerous lesion studies and others (Heidbreder & Groenewegen, 2003; Arnsten, 2013).

Below there is a summary of the PFC functional subdivision in a task-related manner (See **Table 1.1** for more information about which area mediates each function in primates, rodents, and humans):

- Working memory: this function involves the maintenance and integration of relevant information to guide decision making and behavior (Brown & Bowman, 2002; Chudasama & Robbins, 2006).

- Reversal learning: requires animals to reverse learned associations between actions and subsequent rewards or punishments, and involves behavioral flexibility (Chudasama & Robbins, 2006; Young et al., 2011). Chudasama (Chudasama, 2011) reviewed about other forms of decision making task including the reinforcer devaluation, in which animals adjust to changes in reward value and make choices between immediate small rewards and later larger rewards.

- Attention: this is a crucial behavior. Animals allocate resources for efficient information processing and focus on more important and relevant environmental events. There are several types of attention: selective, divided, and sustained attention, each associated with different PFC regions (for review, see Chudasama, 2011). This function is usually measured by tasks such as the five-choice serial reaction time task (5-CSRTT) using a touch-screen panel for both primates and rodents (Maddux & Holland, 2011).

- Set shifting: This function requires the recognition of changes in the dimension of cues, inhibitory control, and decision making. There are two kinds of set shiftings: one is an extradimensional shift in which relevant cue is changed from one dimension to another (for example, from shape to color); and the other is an intra-dimensional in which relevant cue is changed (for example, from a triangle to a square) but within the same dimension.

- Social behavior: includes a wide range of behaviors: maternal nurturing, juvenile playing among siblings, mating, and aggression. Basic social interactions between adult animals of the same sex require social motivation, recognition of social cues, social memory, processing of reward, fear, emotions, and decision making (Millan & Bales, 2013).

Table 1.1: Comparison of subdivisions and functions of PFC between primates and rodents. Adapted from *Sakurai et al., 2015.*

Human/primates	BA47, 11, 12, 13, 14, 10 Orbitofrontal	BA14, 10, 8, 9 Medial[a]	BA32, 33, 24, 25, 26 Cingulate[b]	BA10, 8, 9, 46 Dorsolateral	BA44, 45, 47 Ventrolateral
Functions	• Decision making • Reward/punishment • Flexibility • Reversal learning • Solving conflict • Emotional motivation • Social behavior	• Fear conditioning	• Solving conflicts • Reward based learning • Set shifting • Attention • Error detection • Decision making (cost-benefit analysis) • Mood • Emotional regulation • Social behavior • Fear conditioning	• Working memory • Suppression • Flexibility • Switching • Set shifting • Planning • Guessing • Attention • Response selection • Reasoning • Decision making (accuracy, impulsivity) • Motor planning • Motivation	• Working memory • Motor language (Broca's area)
Rodents[c]	Orbitofrontal	Medial PFC (IL, PL)	Anterior cingulate	Medial PFC (IL, PL) and anterior cingulate	
Functions	• Reward association • Flexibility • Reversal learning • Social behavior	• Fear conditioning	• Solving conflicts • Set shifting • Attention • Decision making (cost-benefit analysis) • Emotional regulation • Social behavior • Fear conditioning	• Working memory • Inhibitory control • Flexibility • Set shifting • Attention • Incentive learning • Response selection • Decision making, (accuracy, PL; impulsivity, IL)	

[a] Continuous structures that spread over the dorsolateral PFC and OFC.
[b] BA 23, posterior cingulate cortex, is not part of the PFC and is unique to primates (Vogt & Paxinos, 2014).
[c] Important to note that areas listed for rodents are functionally similar to some human/primate areas, but not necessarily all areas, nor they necessarily are responsible for the same functions (Sakurai et al., 2015). There may also be heterogeneity in the area along the anterior-posterior axis in rodent brains (Wise, 2008).

1.4.2. Cognition and monoamine neurotransmission systems

1.4.2.1. The noradrenergic system on cognitive functions

Pharmacology and/or neurotoxic lesions in rodents, monkeys, and humans for the manipulation of the noradrenergic system has provided substantial evidence for the modulatory influence of NA on cognitive functions that depend on the frontal cortex (Sara, 2009). Mair and cols. (Mair et al., 2005) worked with rats that had lesions to the ascending noradrenergic projections; and they showed marked cognitive deficits, especially when the tasks were high demanding (reviews of earlier works Robbins, 1984; 1997). In non-human primates, optimal concentrations of NA in the PFC are necessary for WM and selective attention. In fact, administration of local clonidine (an α_2AR agonist) induced low NA function and impaired performance, by inhibition of NA release at presynaptic sites. However, the same drug in higher concentration but at post-synaptic sites produced the opposite effect, facilitating performance, especially in aged monkeys (Arnsten & Goldman-Rakic, 1985; Ramos & Arnsten, 2007).

It is important to note that, unlike other cortical regions, the frontal cortex receives substantial dopaminergic input, which has been implicated in the same cognitive processes. As it was

commented before, noradrenergic and dopaminergic neurons respond to similar environmental stimuli, so NA and DA should be released simultaneously in the frontal cortex, where they can act together to modulate network activity. The considerable evidence that DA, NA, and other neuromodulators act in concert to mediate cognition in the PFC has been extensively reviewed (Briand et al., 2007).

In addition, PET studies in humans have suggested that clonidine acts on attentional processes by modulating the connectivity between brain regions that are part of the functional network implicated in attention, as it is the LC (Coull et al., 1999). Thus, NA affects cognitive processes (especially attention and WM) by facilitating the functional integration of the brain regions implicated in these processes, rather than by exerting the effect locally in a discrete brain region (Eschenko & Sara, 2008; Sara, 2009).

In relation to another principal cognitive process such as memory, the noradrenergic system has also been postulated to play a fundamental role in its modulation (McGaugh & Roozendaal, 2009; Sara, 2009). The memory process itself could be divided into three consecutive stages. First, the "learning" process; where a stimulus is presented and attention is selectively dedicated to that stimulus. Second, the "acquisition" process, when that information is consolidated and leads to memory stabilization. Finally, the "retrieval", defined as a result of the integration of incoming information with the memory network that is triggered by that information and by the context in which that experience occurred in the past. In humans, this retrieval is expressed through the verbal report (**Figure 1.13**) (Sara, 2009)

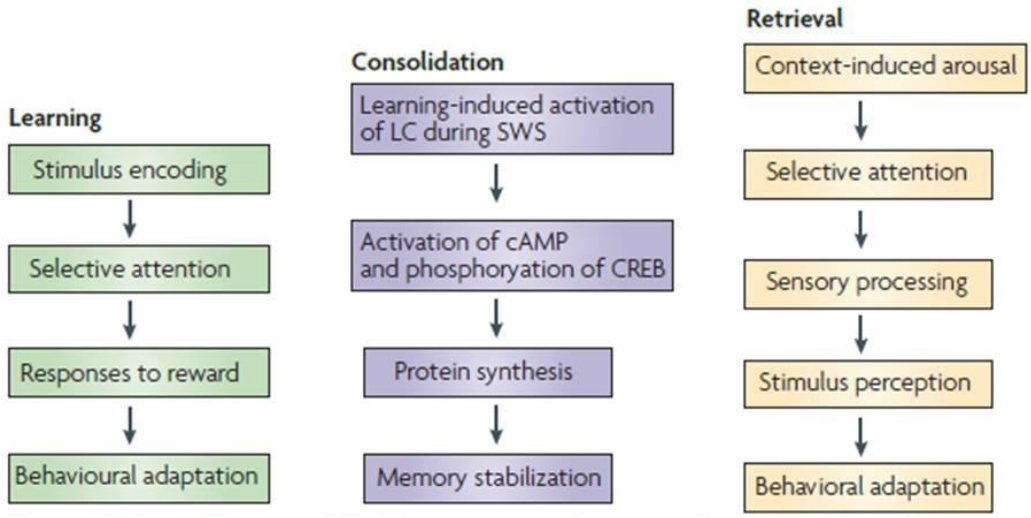

Figure 1.13: Influence of the noradrenergic system on stages of memory. Abbreviations: CREB: cAMP-responsive element binding protein; SWS: slow-wave sleep. Adapted from *Sara, 2009.*

NA interacts with other transmitters, neuromodulators and stress hormones in the amygdala or the hippocampus to promote long-term memory formation (McGaugh & Roozendaal, 2009). The evidence of this influence of NA on memory processes comes mainly from pharmacological studies in which noradrenergic transmission is manipulated after memory

acquisition (consolidation period) and the animal is tested later when the effect of the drug has presumably dissipated (Sara & Devauges, 1989; Sara et al., 1999; Eschenko & Sara, 2008).

Some studies have shown that β-ARs may play a role in a late stage of memory formation: e.g. after learning, rats injected intracerebrally with a β-AR antagonist showed amnesia when they were tested 48 h later; but it the injection was made immediately after learning, there was no effect. This data suggest that there is a time window after learning experience during which the noradrenergic system is activated to reinforce long-term memory processing (Sara et al., 1999). It is proposed that this NA-dependent memory consolidation could happen during slow-wave sleep; as neurons of the LC, which are usually inactive during sleep, show a transient increase in activity during this phase after periods of an intensive learning experience (Eschenko & Sara, 2008). Furthermore, there is also evidence suggesting that activation of the cAMP-PKA cascade is necessary for the effects of the noradrenergic system, as interruption of any step leading to PKA activation prevented long-term memory formation (Huang et al., 2006b).

Studies of mutant mice temporarily lacking DBH, an essential enzyme for the synthesis of NA, support the idea that the noradrenergic system has a specific role in the retrieval phase of memory. These animals were able to learn a contextual fear-conditioning task but showed a deficit in retention 48 hours after training. However, this retrieval deficit was counterbalanced when restoring NA function by injecting a NA precursor between training and retention (Murchison et al., 2004). Furthermore, injection of propranolol in rats before the contextual fear-conditioning test impaired retrieval 24 h after training, but not 1 h or 1 week after training. These results suggest that the noradrenergic system is necessary for the retrieval of recent memories but not remote memories (Murchison et al., 2004).

Regarding humans, the explicit role of the LC-noradrenergic system in memory retrieval comes from a functional MRI study (Sterpenich et al., 2006). In this study, human subjects were presented with neutral faces in emotional or neutral contexts (they measured their emotional response by pupil size during the encoding phase and their brain was imaged during the retrieval phase). Activation of the LC region was observed during retrieval only if there had been an emotional response during the encoding phase. Moreover, when the correctly remembered faces had been encoded in an emotional context, this same LC region was more tightly connected to the amygdala. No other brain region that was activated by the retrieval effort showed this increased functional connectivity with either the LC or the amygdala. In conclusion, the authors suggested that this coordinated action of the LC and the amygdala activates forebrain fronto-hippocampal networks that are essential for memory retrieval (Sterpenich et al., 2006), confirming the previously proposed role for LC neurons in mediating the effects of contextual reminders on forgetting and retrieval (Sara, 1985; 2009).

1.4.2.2. The dopaminergic system on cognitive functions

Similar to NA, DA also plays a crucial role in learning and in memory processes, as it is involved in the induction of synaptic plasticity in the brain regions that receive DA neuron projections

(Schultz, 2002). Depending on the specific regions involved, DA can mediate different types of learning and memory processes (Schultz et al., 2003).

DA projections into the dorsal striatum are critical for motor learning and habit formation (e.g., playing a musical instrument or riding a bicycle) (Graybiel, 1998). It seems that DA innervations of limbic structures play a different role in the mediation of learning processes. For instance, DA innervation of the hippocampus appears to be involved in the formation of long-term memory (Lisman & Grace, 2005), while DA projections into the amygdala mediate emotional memory, such as aversive conditioning (Nader & LeDoux, 1999; Rosenkranz & Grace, 2002; Rodrigues et al., 2004).

The function in the learning processes of DA innervation of the ventral striatum is not that clear, but it is likely that mesolimbic projections to the ventral striatum are involved in the processes that happen when acquiring aspects of goal-directed motor control (Everitt & Robbins, 2005). Studies carried out by Goto and cols. (Goto & Grace, 2005) suggest that D_1-dependent hippocampus-ventral striatum information processing mediates learning of a response strategy; when in fact, D_2-dependent PFC-ventral striatum information processing is crucial for flexible switching of this response strategy in guiding goal-directed behavior.

The cognitive functions associated with the PFC, such as short-term storage of memory, flexible switching of response strategy, attention and future planning, may not involve synaptic plasticity in this region. However, PFC is known to exhibit synaptic plasticity in its network (Laroche et al., 1990; Herry & Garcia, 2002), and mesocortical DA release is essential for induction of such synaptic plasticity. So, this idea suggests that any cognitive function requiring proper DA release in the PFC could involve DA-dependent synaptic plasticity (Otani et al., 1998; Gurden et al., 1999).

Regarding WM, it has been reported that DA effects within the PFC have a bimodal function. That is, there is an optimal level of DA stimulation required for proper PFC functioning, with either over- or understimulation of D_1Rs leading to dysfunctional states (Granon et al., 2000) an example of the classic "inverted U"-shaped relationship. According to primate studies, D_1Rs play a pivotal role in short-term memory functions (especially for spatial WM) (Goldman-Rakic, 1995). Whether D_2Rs also exhibit a U-shaped functional relationship in the PFC is not known. Nevertheless, evidence suggests that short-term memory can be affected by the administration of D_2 agonists or antagonists into human subjects (Kimberg et al., 1997; Mehta et al., 2001). Although this supports a D_2R involvement in short-term memory in humans, the location of this D_2 action is not known.

1.4.2.3. The serotonergic system on cognitive functions

5-HT acts as a tissue hormone, neurotransmitter, and neuromodulator in both central and peripheral systems (Fidalgo et al., 2013). It has been tied to cognitive decline and multiple other behavioral and psychological symptoms of dementia. The latter has been studied more in-depth due to their relationship with Alzheimer's disease (AD) (Dillon et al., 2013; Lanctot et al., 2001). As commented before, the projections from the raphe nuclei are widespread across

the CNS, and the system has been highly implicated in influencing cognition and memory in health and across multiple pathological disease states (Cowen & Sherwood, 2013; Jenkins et al., 2016).

It is considered that the serotonergic system affects cognition through its activity in two main brain areas: the hippocampus and PFC. 5-HT in the hippocampus is involved in spatial navigation, decision making, and social relationships (Clark et al., 2004; Rubin et al., 2014); while, in the PFC, 5-HT plays a major role in WM, attention, decision making, and reversal learning in both human and animals (Robbins, 2000; Clark et al., 2004).

With advancing age, declines in certain 5-HTRs in cognition-related brain areas are well-documented. In fact, during aging, the activity and density of 5-HT_{1A}R, which is known to affect declarative and non-declarative memory, decrease by approximately 10% every ten years. At a similar rate, in the hippocampus and frontal cortex of healthy individuals, age-dependent decreases in 5-HT_{2A}R are also observed (Meltzer et al., 1998), correlating clinically with cognitive decline (Hasselbalch et al., 2008). Although the overall expression of the 5-HT_4R is unlikely to change with aging (Madsen et al., 2011), a 5-HT_4R agonist reverses scopolamine-induced cognitive defects pre-clinically (Lelong et al., 2003), likely through regulation of synaptic plasticity (Vidal et al., 2011). Overall, brain area- and 5-HTR-specific alterations that occur with aging may lower cognitive function and influence the rate of cognitive decline associated with these neurodegenerative and psychiatric disorders (Chaudhry et al., 2008).

Apart from that, the altered expression of 5-HTRs directly contributes to cognitive decline. As previously commented, the 5-HT_{1A}R declines progressively with aging in the hippocampus and DR of patients with mild cognitive impairment and AD (Kepe et al., 2006), therefore increasing this cognitive dysfunction. The 5-HT_{1B}R is similarly reduced in the frontal and temporal cortices and is also associated with the level of cognitive dysfunction (Garcia-Alloza et al., 2004). For the 5-HT_{2A}R, there is a correlation between the severity of cognitive impairment and the receptor's decreased expression in the temporal lobe (Versijpt et al., 2003; Lai et al., 2005). Even if the expression of the 5-HT_4R does not seem to change with age, there is a significant reduction in the post-mortem hippocampus and cortices of AD patients (Reynolds et al., 1995; Lai et al., 2003); also, preclinical evidence demonstrates improved cognition with partial agonism of the 5-HT_4R in an AD mouse model (Cochet et al., 2013). Furthermore, 5-HT_7R agonism is suggested to improve cognitive function in rodent AD models (Perez-Garcia & Meneses, 2005).

Finally, the SERT has also been studied in relation to cognition. The 5-HTT-linked polymorphic region is a well-described and common polymorphism in the SERT gene causing a low expression of the SERT (Damsbo et al., 2019). This polymorphism is one of the most investigated polymorphisms related to 5-HT regulation and antidepressant treatment response. It has been suggested that the low expression of SERT produced by this polymorphism could increase cognitive dysfunction, especially in patients with poor cognitive abilities (Marini et al., 2011).

1.4.3. Cognition in schizophrenia

1.4.3.1. Cognitive impairments and network disturbances

Several studies have suggested that patients with schizophrenia suffer from WM impairments that are independent of psychotic symptoms (Fleming et al., 1995; Gold, 2004). WM in healthy individuals depends on the coordinated firing of subsets of pyramidal neurons in the dorsolateral PFC. This synchronized activity of cortical pyramidal cells is regulated by a subset of GABA interneurons of the dorsolateral PFC that express the calcium-binding protein parvalbumin, including chandelier cells. Studies in patients with schizophrenia have reported reductions in GAD67, an enzyme that synthesizes GABA, precisely in parvalbumin-positive GABA interneurons. These reductions in GAD67 mRNA result in 1) reduced GABA synthesis and release in a specific population of interneurons and 2) decreased signaling from parvalbumin-positive GABA cells to pyramidal neurons (Lewis et al., 2001). These data suggest that impairments in WM in schizophrenia could be mediated through a reduction in GABA-mediated inhibitory modulation; as a result of a decline in GABA-synthesizing enzymes and GABA synthesis in certain interneuron population in the cortex (Akbarian et al., 1995; Benes & Berretta, 2001; Lewis et al., 2001).

There is a large body of evidence implicating deficits in PFC function in schizophrenia (Saykin et al., 1994; Heinrichs & Zakzanis, 1998; Flashman & Green, 2004; Dickinson et al., 2004), and impaired WM is a recognized endophenotype of this disorder, being present even in undiagnosed relatives of patients (Glahn et al., 2003; Snitz et al., 2006; Cannon & Keller, 2006; Lewis & Gonzalez-Burgos, 2008; Pratt et al., 2008). In the early phase of schizophrenia, the most pronounced deficits observed are in verbal and visual memory, as well as in the speed of information processing (Rajji et al., 2009). In the chronic phase of the illness, cognitive deficits appear to become more stable, and cognitive function is generally impaired relative to that in age-matched healthy controls (Harvey, 2014). More importantly, the functional outcome in chronic patients correlates with the degree of cognitive alterations (Goldberg et al., 1993; Lewis & Gonzalez-Burgos, 2008; Semkovska et al., 2004); this fact suggests that the status of the cognitive function before the chronic phase could determine the degree of functional impairment during the chronic phase.

Regarding morphological disturbances, it has been repeatedly reported that reduced white matter is one of the structural alterations associated with schizophrenia (Kuperberg et al., 2003; Thermenos et al., 2013). In patients with schizophrenia performing a spatial WM task, PFC activity associated with encoding, maintenance, and response appears to be compromised: e.g., patients show a greater decay of blood oxygen level-dependent activity in the PFC than controls during the maintenance phase of the task, which is associated with poor task performance (Driesen et al., 2008). Besides, patients that were not under treatment also showed hypofrontality and reduced metabolism in the PFC, supporting the idea that these changes are associated with the disorder and not due to confounding factors, such as medical treatment (Weinberger et al., 1992b; Callicott et al., 2003; Molina et al., 2005; Lewis & Gonzalez-Burgos, 2008).

Network studies suggest that in schizophrenia there are reductions in the global communication efficiency, implicating reduced connectivity between more segregated parts of the brain (van den Heuvel & Fornito, 2014) in a wide variety of systems: the frontoparietal control network (Repovs et al., 2011), the cingulo-opercular network (Palaniyappan et al., 2013), the default mode network (Whitfield-Gabrieli & Ford, 2012), and the frontostriatal and frontotemporal circuits (Hoffman et al., 2011). In particular, frontal and temporal brain regions showed altered clustering (Bassett et al., 2008), longer communication pathways (van den Heuvel & Hulshoff Pol, 2010), and reduced global efficiency (Wang et al., 2012). Many functional studies have also found changes in a wide variety of systems dependent on the PFC (Liang et al., 2006; Cole et al., 2011; Zalesky et al., 2011; Fornito et al., 2013). In schizophrenia patients, a lack of inhibition of the default mode network has also been reported (Anticevic et al., 2013; van den Heuvel & Fornito, 2014), with patients not being able to switch appropriately from the default mode network to other networks that are necessary for executing certain cognitive tasks such as WM and attention (Sakurai et al., 2015). Probably, these network changes reported in schizophrenia are not merely the result of a relative global reduction in connectivity strength. They likely involve topological changes in the brain networks (Collin et al., 2013) and reduced hierarchical structure; both indicative of a disruption in the organization of connectivity among cortical regions (Bassett et al., 2008). Thus, these data suggest that in schizophrenia, both formation and switching of networks necessary for certain cognitive functions are affected (Sakurai et al., 2015).

The severity of positive, negative, and cognitive impairments in schizophrenia patients has been linked to connectivity and topological aberrations (van den Heuvel & Fornito, 2014). Differences in altered connectivity are associated with differences in symptoms (Meyer-Lindenberg et al., 2006), and at the same time, these are related to certain behavioral alterations. For instance, the severity of positive symptoms is correlated with increased functional connectivity of the medial PFC in schizophrenia (Whitfield-Gabrieli et al., 2009), but is also correlated with reduced functional connectivity between dorsal regions of the caudate and dorsolateral PFC (Fornito et al., 2013). Therefore, variations in the type and degree of the symptomatology could reflect different affected neuronal circuits.

It is known the prominent role of gamma band activity in cognitive function, and in schizophrenia gamma band amplitude has been reported to be reduced in a wide range of cognitive and perceptual paradigms: e.g. WM, executive control and perceptual processing (Ford et al., 2008; Haenschel et al., 2009; Minzenberg et al., 2010). Both the amplitude (reflecting local activity) and the phase synchrony (reflecting long-range connections) of gamma oscillations are important factors that are reduced in schizophrenia (Spencer et al., 2003; Uhlhaas et al., 2006), which would lead to a functional disconnection syndrome (Stephan et al., 2009). As these gamma oscillations require the maturation of parvalbumin-positive interneurons mediated by NMDA receptors, it has been proposed that precisely that NMDA receptor-mediated maturation of these particular interneurons could be a major pathway in schizophrenia pathogenesis (Lewis et al., 2012; Jiang et al., 2013).

1.4.3.2. The developmental hypothesis of schizophrenia and cognitive impairment

Classically, pharmacological studies have predicted the importance of neurotransmitter disturbance in schizophrenia pathogenesis. Here it is principally included the hyperdopaminergic hypothesis, postulating that hyperactive D_2R signaling is responsible for psychotic aspects of schizophrenia (Kellendonk, 2009); the principal reason why antipsychotic treatment is focused on blocking the dopaminergic system. However, the limitation of this hypothesis is that negative symptoms, cognitive impairments, and cortical atrophy are poorly responsive to typical antipsychotic medications (Coyle & Tsai, 2004). Furthermore, since many psychoactive drugs including cannabinoid, amphetamine, and ketamine can induce behavioral phenotypes similar to schizophrenia, disturbance of the pathways involving these drugs may also contribute to the disorder (Moghaddam & Krystal, 2012; Steeds et al., 2015). Ketamine concretely, a dissociative anesthetic and non-competitive NMDA receptor antagonist, is capable of impairing performance during cognitive tasks that require cortex and/or hippocampal involvement in a way similar to what is observed in schizophrenia patients during cognitive task performance (Newcomer et al., 1999). These observations had led to the hypoglutamatergic hypothesis, which postulates that this disorder could result from a hypofunction of a subset of NMDA receptors (Coyle & Tsai, 2004).

Postmortem brain analyses have also identified many cell types and processes affected in schizophrenia brains including GABAergic interneurons, in particular, parvoalbumin (PV)-positive interneurons as well as oligodendrocytes and myelination processes (Akbarian et al., 1993a,b; 1995; Garey et al., 1998; Glantz & Lewis, 2000; Black et al., 2004; Lewis et al., 2012; Glausier & Lewis, 2013; Glausier et al., 2014). In addition to that, both *postmortem* analyses and imaging studies have reported microglia activation, suggesting that inflammation is also involved in the complex pathogenesis and progression of the disorder (Bergink et al., 2014; Ohgidani et al., 2015). Also, epigenetic changes caused by adaptation processes to situations created by genetic and environmental insults have also been implicated in schizophrenia (Hasan et al., 2013; Akbarian, 2014).

All the evidence mentioned above led to "the developmental hypothesis of schizophrenia", which proposes that disruption of early brain development by a combination of genetic and environmental factors (through gene-environment interactions, G x E) increases the risk of later developing this disorder (Murray et al., 1992; Weinberger, 1996; Insel & Wang, 2010). As commented previously, the development of neural networks and cognitive functions involves many biological processes which can take decades to complete (e.g., the initial formation of connections and circuitries, followed by activity-dependent refinement of these connections). Various insults during early development would make the circuitry vulnerable to stresses during the later maturation process (Sakurai et al., 2015). As the PFC has an extended maturation period (**Figure 1.14**), a sustained window of plasticity may extend the developmental time window during which these insults further affect the circuitry, making cognitive function particularly vulnerable (Uhlhaas, 2013). Affected processes include especially local circuitry formation and maturation and within them, other as sensory gating,

connectivity, myelination, neuromodulation, and activity-dependent maturation (Bassett et al., 2008; Hoffman et al., 2011; Wang et al., 2012; Niwa et al., 2013). All these changes would merge manifesting a network disruption, and finally, cognitive impairment. Further, as many networks that are necessary for a wide range of cognitive functions are connected to the PFC, manifestations of this impairment would be very diverse between cases (van den Heuvel et al., 2013). It is also important to mention that the timing of these insults during development would affect the final outcome of altered networks; as for which ones are damaged and how extensive this damage is (Fornito et al., 2013).

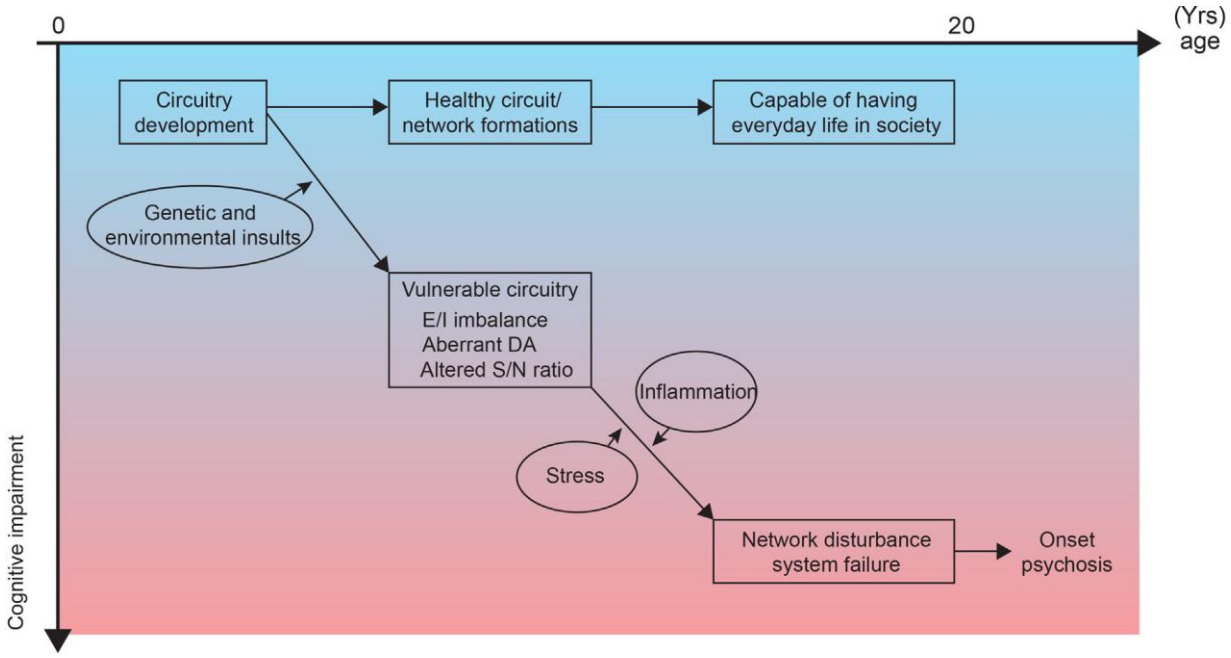

Figure 1.14: Schema of PFC neural circuitry/network development over the decades. It can proceed without any issues, but if the process is affected by genetic or environmental issues, it makes the circuitry vulnerable and it can be further impaired by synaptic stress and inflammation during adolescence. This would result in network disturbances and system failure, leading to the onset of the disorder/psychosis. Adapted from *Sakurai et al., 2015.*

1.5. TREATMENT OF COGNITIVE DEFICITS IN SCHIZOPHRENIA

Approximately 60 years have passed since the discovery of chlorpromazine, starting a new era in the management of schizophrenia; but still, antipsychotics remain the cornerstone of treatment of this disorder (Kane & Correll, 2010). The finding of the blockade of D_2Rs as the main effect of chlorpromazine antipsychotic action led to the development of "typical" or first-generation antipsychotics (FGAs); which include examples as haloperidol, fluphenazine, perphenazine, and loxapine, among others. All antipsychotics in this category act by blocking receptors of the DA pathway and they have been used in the clinic for years, as they were effective in treating positive symptoms. Nevertheless, their lack of efficacy in treating negative and cognitive symptoms and their secondary side effects have led to the search for new molecules for the treatment of schizophrenia (Tandon & Jibson, 2002).

Thus, different antipsychotics have been developed, referred to as second generation or "atypical" antipsychotics (SGAs). This group is best represented by clozapine, but we could also mention risperidone, olanzapine, aripiprazole, and quetiapine, among others. However, this classification into FGAs and SGAs has been challenged, as all of them are believed to act via reducing dopaminergic tone and both classes are heterogeneous in molecular structure, extra-dopaminergic targets and adverse effects (De Hert et al., 2011). Some of the antidopaminergic-related adverse effects of these drugs include hyperprolactinemia, dystonia, parkinsonism, akathisia, and tardive dyskinesia. Indeed, there is an extrapyramidal adverse effect, such as parkinsonism, that had been believed to be the unavoidable result of antipsychotic efficacy conferred by D_2R blockade (Kahn et al., 2015).

The essential characteristic that differentiates the subsequently developed SGAs from the FGAs is that they are believed to block 5-HTRs at lower concentrations than they block DA receptors and/or, they might block subcortical D_2Rs more than striatal D_2Rs (Kahn et al., 2015). Apart from that, these drugs are associated with less parkinsonism, akathisia and tardive dyskinesia than FGAs at therapeutic doses. Even so, no SGA is entirely free of associated parkinsonism, and all currently available antipsychotics are believed to work predominantly via D_2R blockade (Correll, 2010). Furthermore, most SGAs are associated with other different adverse effects, such as weight gain, diabetes and, therefore, increased risk of cardiovascular complications. Both FGAs and SGAs are effective in reducing the severity of positive symptoms and usually, there is a good response to this medication in the 30-40 % of the treated patients (Mailman & Murthy, 2010; Smith et al., 2010). Nevertheless, their efficacy regarding both negative and cognitive symptoms remains minimal (Miyamoto et al., 2012); in fact, they can even aggravate this symptomatology, especially with regard to cognitive function (Munafo et al., 2006; Zhang et al., 2006).

Apart from the dopaminergic system, there is strong preclinical data indicating the involvement of the glutamatergic and cholinergic systems especially regarding negative and cognitive symptoms in schizophrenia; although therapies targeting these systems have not gone beyond successful Phase II studies (Koster et al., 2014). The strategy has shifted from trying to reduce the risk of predominantly antidopaminergic-related adverse effects to

developing antipsychotics with little effect on cardiac conduction and without cardio-metabolic side effects (weight gain, dyslipidemia, glucose abnormalities, and metabolic syndrome). But above all, the non-psychotic cognition symptoms of schizophrenia are powerful determinants of poor psychosocial function (Green, 1996); they appear to be the best predictor of long-term outcome in patients with schizophrenia and considered the most disabling and persistent features of the illness (Ibrahim & Tamminga, 2011).

Therefore, the development of specific treatments for cognitive symptoms is urgent, and the search for new antipsychotic molecules should be based on achieving greater effectiveness in this field; improving tolerability and adherence and reducing adverse effects to the maximum.

1.5.1. Clinical targets for treating cognition

Since the development of the FGAs, and later the SGAs, many molecules that promised to be effective in treating symptoms in preclinical studies (especially negative and cognitive symptoms) have been ineffective in clinical trials. Therefore, the development of new effective molecules still remains a challenge. Even so, as the medical need remains imperative, research continues. Some of the neurotransmission systems considered most feasible targets include especially, monoaminergic systems, but also cholinergic, GABAergic and glutamatergic systems, among others.

1.5.1.1. Cholinergic targets

Agonism of the central cholinergic function at the nicotinic or muscarinic receptor is the target judged most favorable for improving cognition; hence, allosteric and orthosteric compounds are being tested (Ibrahim & Tamminga, 2011). The medial temporal lobe, one of the areas known to be important in schizophrenia pathology, has the highest densities of nicotinic receptors. In schizophrenia, this area shows reductions in the expression of the $\alpha_7$nicotinic receptors (Martin et al., 2004). These receptors have been shown to play an important role in cognition in both animals and humans and have potential therapeutic applications in cognitive impairment in schizophrenia. Activation of $\alpha_7$nicotinic receptors increases cholinergic neurotransmission and the release of DA and glutamate, exerting procognitive effects in rats (Picciotto et al., 1998; Pichat et al., 2007; Barik & Wonnacott, 2009; Huang et al., 2014). Therefore, many $\alpha_7$nicotinic cholinergic receptor agonists have been identified and tested for cognition treatment. For instance, encenicline is an $\alpha_7$nicotinic receptor agonist that potentiates the response to the natural agonist acetylcholine and which impact on cognition is be mediated in part by modulating multiple neurotransmitter systems including DA, acetylcholine, and glutamate in the PFC and other brain regions (Huang et al., 2014). This compound has shown procognitive effects in normal subjects (Barbier et al., 2015) and in a posterior study with schizophrenic patients treated with concomitant atypical antipsychotics it demonstrated consistent beneficial effects across multiple measures of cognition function, such as the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery and the Positive and Negative Syndrome Scale (PANSS) (Keefe et al., 2015). Also, ABT-126 is a potent and selective $\alpha_7$nicotinic receptor agonist that

has demonstrated efficacy in animal models of cognitive impairment. In a placebo-controlled study in humans, this compound has been reported to produce a dose-related trend toward a cognitive improvement in treated schizophrenic patients who were clinically stable (Haig et al., 2016), but this result was not observed in smoker patients. Indeed, in a posterior study but with smoker schizophrenic patients, ABT-126 did not demonstrate a procognitive effect in these patients, although a trend for improvement in negative symptoms was observed with the high dose (Haig et al., 2018).

Muscarinic cholinergic receptors have also been implicated in schizophrenia. *Postmortem* studies have reported reductions in M_1 and M_4 muscarinic receptor density or binding in several brain regions including the frontal cortex, striatum, and hippocampus (Hyde & Crook, 2001; Mancama et al., 2003; Friedman, 2004), and SPECT studies have shown decreases in muscarinic receptor binding in schizophrenia in the PFC (Davis et al., 1997; Friedman, 2004). As an example, xanomeline is a potent nonselective agonist of muscarinic M_1/M_4 receptors. In a phase II study with 20 patients, xanomeline administration resulted in a significant improvement of cognitive measures as verbal learning and WM. However, it was associated with gastrointestinal effects such as vomiting and nausea (Shekhar et al., 2008). Over the past years, progress has been made in developing M_1-selective agonists that are less likely to cause muscarinic side effects; e.g. molecules as N-desmethyclozapine (NMDC) or ACP-104, which are still in Phase II clinical trials (Tamminga, 1999; Weiner et al., 2004).

1.5.1.2. Dopaminergic and noradrenergic targets

In a nonhuman primate model of WM, Goldman-Rakic et al. (Goldman-Rakic et al., 2004), demonstrated the importance of D_1R signaling in WM performance, a critical domain of dysfunction in schizophrenia. Some other lines of evidence suggest that DA signaling could be deficient at the D_1R in schizophrenia patients (Okubo et al., 1997; Akil et al., 2003). Moreover, different studies with animals showed that D_1- but not D_2R antagonists can disrupt WM, although D_1R agonism enhances cognition (Goldman-Rakic et al., 2004). All this evidence supports the strategy that an increase of D_1R signaling could improve cognition, especially WM, in schizophrenia. So far, however, no molecules have been detected to be effective in this regard.

Apart from that, a different strategy regarding DA system has been the activation of neurokinin 3 receptors, coupled to G_q proteins and located in brain areas of interest to schizophrenia, such as the frontal and cingulate cortex, hippocampus, LC or substantia nigra, among others (Spooren et al., 2005; Meltzer & Prus, 2006; Griebel & Holsboer, 2012). Indeed, the administration of neurokinin 3 receptor antagonists has shown to increase DA concentrations in PFC, suggesting a strategy to overcome the cognitive deficits present in schizophrenia due to the cortical dopaminergic hypofunction (de la Flor & Dawson, 2009; Dawson et al., 2010). Supporting this, osanetant, an NK_3 receptor antagonist, showed efficacy for total and positive symptoms measured on the PANSS and comparable to haloperidol (Meltzer et al., 2004). However, these findings were not reproduced by talnetant (Meltzer & Prus, 2006; Dawson et al., 2010), nor by AD2624, other NK_3 receptor antagonists, which failed

to produce any effect over placebo in schizophrenic patients with antipsychotic treatment (Litman et al., 2014).

In addition to DA, it is also well known that NA also has a powerful influence on PFC cognitive functions, especially acting through actions at αARs (Arnsten & Goldman-Rakic, 1985; Li & Mei, 1994). However, action at these receptors would depend on the NA concentrations present in the PFC. In fact, moderate concentrations of NA enhance PFC cognitive functions acting through postsynaptic α_2ARs; but high concentrations of NA released, e.g. under stressful conditions, diminish PFC function via α_1ARs (Arnsten et al., 1999; Birnbaum et al., 1999; Mao et al., 1999). This impairment of the cognitive functions through α_1ARs comes from the activation of the intracellular PKC pathway, known to be overactive in schizophrenia and bipolar disorder (Manji & Lenox, 2000; Mirnics et al., 2001; Lidow, 2003). Therefore, the blockade of this pathway or stimulation of α_2ARs (Baldessarini et al., 1992; Friedman et al., 2001) has become targets for pharmaceutical development. Actually, $\alpha_2$agonist such guanfacine and guanabenz have resulted successful in enhancing PFC cognitive functions in humans, and serve as a proven translation from the preclinical studies in animals to clinical utility (Arnsten, 2004).

1.5.1.3. Glutamatergic and GABAergic targets

Following the hypoglutamatergic hypothesis, drugs that improve glutamatergic function, especially at the NMDA receptor, are possible candidates in the treatment for improving cognition. Enhancement of NMDA signaling can improve cognition in animals, conversely to what happens with ketamine, which adversely affects cognition in normal and schizophrenic subjects (Tamminga, 1999; Krystal et al., 1994; Lahti et al., 2001). The glutamatergic synapse is quite complex, and drugs can exert an agonist action at the NMDA receptor itself at the coagonist site (glycine), through NMDA receptor or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking, or even through downstream signaling systems associated with the NMDA receptor (Ibrahim & Tamminga, 2011). Drug candidates for cognitive enhancement acting as agonists at the NMDA receptor include glycine, D-serine, D-alanine, sarcosine, and D-cycloserine, but a meta-analysis suggested that these compounds are only slightly effective in treating cognitive deficits (Tsai & Lin, 2010). Regardless of that, glycine-reuptake blockers are thought to enhance glutamatergic transmission by increasing levels of the coagonist glycine at the NMDA receptor. Indeed, bitopertin, a selective glycine-reuptake inhibitor, has shown antipsychotic-like activity in modulating both glutamatergic and dopaminergic neurotransmission in animal models of schizophrenia (Alberati et al., 2012). In animals and human individuals, a linear, dose-dependent increase in glycine concentrations occurred in CSF after bitopertin administration (Hofmann et al., 2016). Findings from a recent phase II trial (Umbricht et al., 2014) showed the efficacy of bitopertin when added to antipsychotics in patients with predominantly negative symptoms, and with higher doses also positive symptoms appeared to improve. However, in a further placebo-controlled phase III study, bitopertin only offered a significant improvement in one out of six active treatment groups of patients with suboptimally controlled psychotic symptoms (Bugarski-Kirola et al., 2016). On the other hand and also related to the NMDA receptors, it is known that their activation increases intracellular levels of cyclic guanosine monophosphate (cGMP) and the

subsequent activation of protein kinases involved in long-term potentiation and synaptic plasticity required for memory formation and learning (Luscher & Malenka, 2012). Phosphodiesterase 9A hydrolyzes cGMP and is highly expressed in the neocortex and hippocampus (Andreeva et al., 2001), controlling the intracellular cGMP levels. Thus, it could become a potential strategy to treat patients with cognitive impairment associated with schizophrenia (CIAS) by increasing cGMP levels and therefore, glutamatergic neurotransmission (Reneerkens et al., 2009). In fact, BI409306, a potent and selective Phosphodiesterase 9A inhibitor, demonstrated good trial conduct and supportive phase I (Moschetti et al., 2016; Boland et al., 2017) and nonclinical data (Rosenbrock et al., 2015; 2015). However, it failed showing efficacy in a phase II study in patients with CIAS clinically stable and receiving antipsychotic treatment (Brown et al., 2019).

Moreover, metabotropic glutamate receptors (mGluRs) potentiate presynaptic glutamate release and therefore, postsynaptic NMDA neurotransmission. These receptors colocalize with NMDA receptors in human cortex and they are also under investigation for cognitive enhancement in schizophrenia. Actually, the activation of group II mGluRs ($mGlu_2Rs$) located presynaptically on hyperactive glutamatergic neurons where they function as heteroreceptors could attenuate schizophrenia symptoms (Schwartz et al., 2012). The $mGlu_2Rs$ are particularly expressed in PFC, hippocampus, striatum, thalamus, and amygdala (Marek, 2010), regions known to be implicated in schizophrenia. Concretely, $mGlu_2R$ overlaps with the $5\text{-}HT_{2A}R$ in the cortex (Sharma et al., 2009), and when located presynaptically, modulates glutamate release and synaptic neurotransmission (Schoepp, 2001). Using the PCP-like drug MK-801 as a psychosis model, it was demonstrated that the $mGlu_2R$-dependent antipsychotic-like behavioral effect of the $mGlu_{2/3}R$ agonist LY379268 was absent in $5\text{-}HT_{2A}R$- knockout (KO) mice, while the $5\text{-}HT_{2A}R$-dependent antipsychotic-like effect of clozapine was missing in $mGlu_2R$-KO mice; suggesting the $5\text{-}HT_{2A}$–$mGlu_2R$ complex as a promising target (Fribourg et al., 2011). Indeed, some molecules acting as positive allosteric modulators of $mGlu_2Rs$ have made it into clinical trials.

In an early clinical attempt, the $mGlu_{2/3}R$ agonist LY2140023 (an oral prodrug of LY4404039, which had demonstrated antipsychotic potential in animal studies (Schoepp & Marek, 2002)) was compared with olanzapine in a randomized, double-blind, placebo-controlled phase II study for the treatment of schizophrenic patients (Patil et al., 2007). This study was an immediate success, as patients administered twice daily with LY2140023 showed significant improvement in positive and negative symptoms compared to the placebo, although not in cognitive symptoms. However, the subsequent clinical trials were not as promising, since they failed to show any significant improvement of LY2140023 over treatment with other atypical antipsychotics with placebo in either positive or negative symptoms, neither in cognitive symptoms nor in the safety profile of the drug. The incidence of serious adverse effects was comparable between groups (Adams et al., 2013; Stauffer et al., 2013; Adams et al., 2014). One possible reason why patients in the study did not respond to this treatment could be the previous antipsychotic medication they had been receiving. In fact, posterior studies compared results obtained with early-in-disease and late-in-disease patients and patients with more prominent $5\text{-}HT_{2A}R$ antagonism antipsychotic treatment and more prominent D_2R antagonism

antipsychotic treatment. It was concluded that only early-in-disease patients or patients previously treated with prominent D_2R antagonism drugs showed significantly greater improvements when treated with the $mGlu_{2/3}R$ agonist LY2140023 (Kinon et al., 2015). Therefore, the correct identification of subgroups of patients that could benefit from this type of treatment is essential.

Previous studies have shown that chronic treatment with the atypical antipsychotic clozapine induces a down-regulation in the level of expression of $mGlu_2R$ in the mouse frontal cortex (Gonzalez-Maeso et al., 2008). This observation together with the antipsychotic properties of drugs that bind and activate the $mGlu_2R$, led to speculate that down-regulation of $mGlu_2R$ expression might restrain the therapeutic effects of atypical antipsychotic drugs (Kurita et al., 2012). Among the diversity of adjunctives that have been added to the antipsychotic treatment in order to improve their efficacy, the histone deacetylase inhibitors (HDACs) are included. These drugs seem to be efficacious when given chronically in combination with atypical antipsychotics including clozapine, olanzapine or risperidone. HDACs remove acetyl groups from lysine residues in the amino-terminal tails of core histones, which shifts the balance toward chromatin condensation, thus silencing gene expression (Borrelli et al., 2008; Arrowsmith et al., 2012). In order to elucidate why glutamatergic treatments are not always effective in patients, subsequent studies have evaluated the effect of chronic antipsychotic treatments on the epigenetic status of the $mGlu_2R$ promoter in mouse and human frontal cortex. Interestingly, it has been observed that chronic clozapine and risperidone were able to decrease histone H3 acetylation, which correlates with transcriptional activation (Borrelli et al., 2008). Also, the expression of HDAC2 protein and its binding to the $mGlu_2$ promoter was increased by chronic clozapine treatment. Furthermore, decreased density of $5\text{-}HT_{2A}R$ binding sites was also reported after chronic atypical antipsychotic treatment. This effect seems to be associated with the regulation of the transcriptional function of the HDAC2 promoter by activation of the $5\text{-}HT_{2A}R$ (Kurita et al., 2012). The over-expression of HDAC2 in the frontal cortex is sufficient to down-regulate $mGlu_2R$ expression and its physiological inhibitory effects, resulting in exacerbation of schizophrenia-like behavior.

It is known that activation of $mGlu_2R$ represses cellular, electrophysiological and behavioral responses that require the $5\text{-}HT_{2A}R$ (Moreno et al., 2009). Drugs that activate the $mGlu_2R$ have the potential for the treatment of schizophrenia, whereas $5\text{-}HT_{2A}R$ agonists result in the opposite effect, such as hallucinogenic compounds. Taking this evidence in consideration, it could be said that decreased density of $5HT_{2A}R$ binding sites by chronic treatment with atypical antipsychotic drugs results in a compensatory mechanism of repressive chromatin structure at the promoter region of the $mGlu_2$ gene. Consequently, it results in less inhibitory effects of the $mGlu_2$ receptor on $5HT_{2A}R$-regulated pathways and behaviors (Kurita et al., 2012). Hence, all this data support the hypothesis that compensatory epigenetic events at the $mGlu_2$ promoter may be responsible for the high incidence of patients who do not benefit from conventional therapy with atypical antipsychotic drugs and provide a biochemical explanation for the lack of efficacy of the glutamatergic drugs such as LY2140023 in the treatment of schizophrenia. Therefore, identification of these epigenetic mechanisms and how they influence the

antipsychotic efficacy is critical for developing new effective antipsychotic treatments that target this neurotransmission system.

Another possible strategy for the schizophrenia treatment could consist of augmenting GABA neurotransmission at the α-subunit of the GABA receptor in PFC. It seems that deficits in WM and cognitive control in schizophrenia are associated with impairments in PFC function, including gamma-band oscillations. These abnormalities are thought to reflect a deficiency in the synchronization of pyramidal cell activity that is dependent, in part, on GABA neurotransmission through GABA type A receptors containing α(2) subunits (Lewis et al., 2008).

1.5.1.4. Other targets

In addition to the development of direct and indirect agonists and modulators of acetylcholine, DA, NA, glutamate and GABA receptors, other types of potential targets for drug development have been identified. Actually, proteases such as propyl oligopeptidase (POP) have been proposed as potential targets for the treatment of cognitive disorders (Mannisto et al., 2007). POP is a serine protease that hydrolyzes peptide bonds at the carboxyl end of L-proline residues. It has been reported that POP proteolytic activity in plasma is increased in patients with schizophrenia (Maes et al., 1995; Fernández-Atucha et al., 2015). Furthermore, in a psychosis-like model by the administration of the NMDA antagonist MK-801, an increase in POP activity was reported in rats (Arif et al., 2007). Moreover, POP-KO mice show altered brain plasticity and behavior compatible with a functional role of POP in normal brain development (Hofling et al., 2016). Apart from this, other experimental data have shown POP inhibition has neuroprotective, anti-amnesic and cognition-enhancing properties in scopolamine-treated rats (Toide et al., 1995), whereas it decreases extracellular acetylcholine concentrations in the cortex and hippocampus of normal rats (Jalkanen et al., 2014). These findings stimulated the development of POP inhibitors for the treatment of cognitive disorders (Mannisto et al., 2007; Lopez et al., 2013). However, for unknown reasons, the development of these compounds was stopped in clinical trial stages. Despite this, one study evaluated the efficacy of IPR19, a POP inhibitor, for the treatment of cognitive deficits in three mouse models resembling schizophrenia-like behavioral symptoms. This compound was able to reverse the performance deficits in different behavioral tests and also ameliorate the prepulse inhibition (PPI) dysfunctions (Prades et al., 2017). Therefore, POP inhibition approach could represent another strategy to develop drugs for the treatment of cognitive deficits in schizophrenia.

1.6. ANIMAL MODELS OF SCHIZOPHRENIA

Animal models have been fundamental providing knowledge of the neurobiological mechanisms of human brain disorders. Although several animal models of schizophrenia are in use, finding a model that replicates the wide variety of symptoms observed in the disease has proven difficult. Obviously, no animal model can fully recapitulate a human disorder like schizophrenia. Indeed, animal models should be refocused around component models of symptoms, replicating specifically the known characteristic manifestations, e.g. psychosis, cognitive dysfunction and negative symptoms (Wilson & Terry, 2010; Ibrahim & Tamminga, 2011; Geyer et al., 2012). As commented, schizophrenia is a very complex and heterogeneous disease, and, therefore, efforts to model the illness in its entirety are probably unrealistic. However, there are a number of distinct behavioral, structural and molecular components of schizophrenia that can be modeled in animals (Buckley, 2005; Meyer et al., 2005; van Haren et al., 2008; Young et al., 2009). Thus, focusing on specific symptoms of the disorder or modeling changes in neurobiological substrates that are known to be involved in schizophrenia, may represent the most rational approach when trying to find the most appropriate animal model.

Ideal models of schizophrenia should meet some requirements; they should have a good face, construct and predictive validity (**Figure 1.15**).

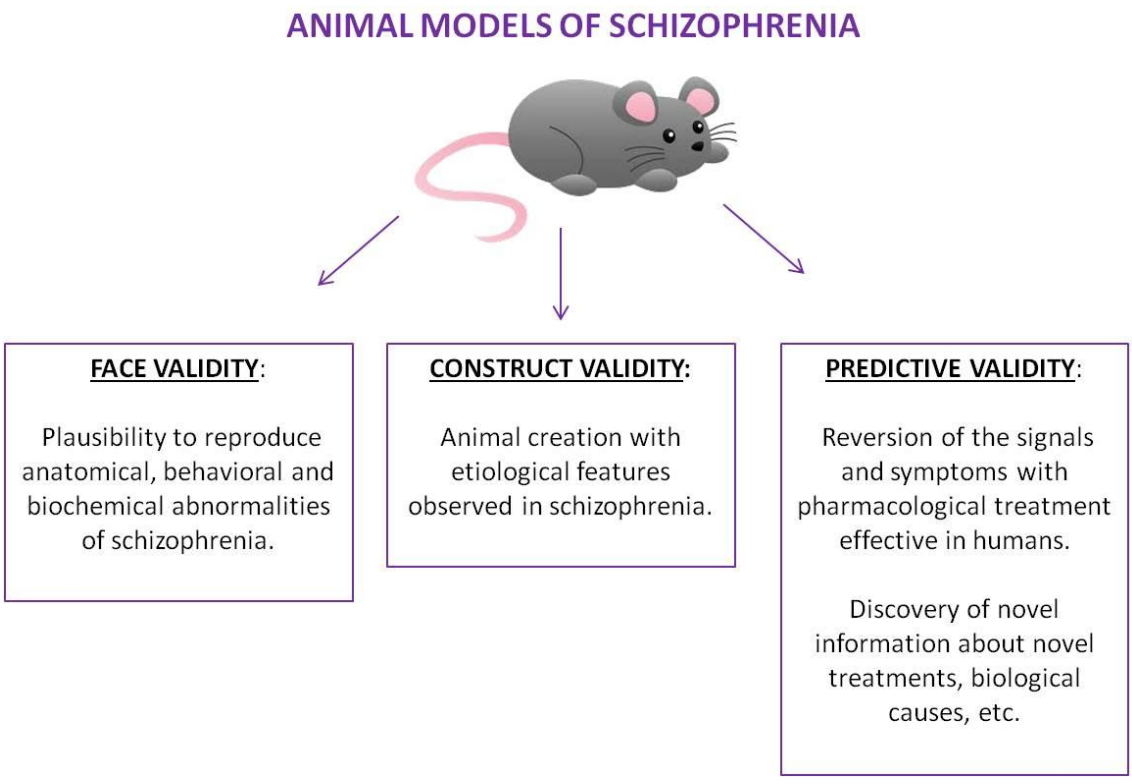

Figure 1.15: Schematic diagram of the three types of validities good animal models should meet.

Based on these premises, the more valid the model turns to be, the greater its utility and significance (Ibrahim & Tamminga, 2011). Although the great diversity of existing animal models and the new ones that are being generated and evaluated during the last years, below are described three large classes of animal models that have, implicitly or explicitly, modeled different aspects of the disorder: genetic models, pharmacological and lesion models, and environmental models.

1.6.1. Genetic models

Genetic factors play an essential role in many psychiatric disorders and several putative candidate genes have been identified as "susceptibility genes" for developing these disorders. Mouse models based on these candidate genes help considerably in understanding the function of a gene and its contribution to disorder pathophysiology regarding cellular pathways, neural circuits and behavior. Despite this, it is important to remember that there are two predominant hypotheses regarding the genetic architecture of complex psychiatric diseases such as schizophrenia: 1) no single gene is necessary or sufficient to cause the disease, 2) common (>1 %) variants in more than one susceptibility gene, each contributing a tiny effect, act in combination to increase the risk of illness (Pritchard & Cox, 2002). In that regard, is logical to think that a mouse model for an individual gene is unlikely to capture the whole complexity of the disorder.

Mostly genetic mouse models have been limited to constitutive or conditional KOs. Even if they do not reproduce the risk alleles and therefore have limited etiological validity, these models should contribute to identify candidate genes and understand their general function, as well as to identify the genetic pathways they participate in. A few examples of these models are the ones described as follows. NRG-1, a leading candidate gene for schizophrenia and the NRG-1-KO mice, showing decreased expression of NMDA receptors, impaired PPI, deficits in latent inhibition (LI) as well as changes in exploratory behavior (Rimer et al., 2005). Related to this, the gene that encodes ErbB-type tyrosine kinase receptor (ErbB), a tyrosine kinase receptor that binds to NRG-1 protein among others, has also been proposed to participate in the pathophysiology of schizophrenia (Chen et al., 2009; Kao et al., 2010). ErbB4 expression in the cortex is particularly abundant among PV-expressing (PV+) fast-spiking interneurons (Fazzari et al., 2010; Neddens et al., 2011) and multiple lines of evidence indicate that PV+ fast-spiking interneurons are predominantly affected in schizophrenia (Curley & Lewis, 2012; Lewis, 2011). In addition, postmortem studies suggest that the number of GABAergic synapses made by these interneurons is reduced in individuals with schizophrenia (Woo et al., 1998; Lewis, 2011). As genetic variation in ErbB4 influences GABA levels in humans (Marenco et al., 2011; Luykx et al., 2012), these observations suggest a plausible link between abnormal ErbB4 signaling and GABAergic function in schizophrenia (Rico & Marin, 2011). In fact, it has been suggested that this mutation in PV+ interneurons impairs the development of synaptic excitatory input and alters the balance between neuronal excitation and inhibition in cortical circuits (Crabtree et al., 2016). Studies with mutant mice with specific loss of ErbB4 from these interneurons showed increased locomotor activity, aberrant social behavior, impaired sociability, poor planning of organized behavior, impaired PPI and WM dysfunction (Del Pino et

al., 2013). Also in this study, the authors observed that deletion of ErbB4 from fast-spiking interneurons caused a partial cell-autonomous disconnection of these neurons from the cortical network that could be interpreted as a "hypo-GABAergic" phenotype. Another model is the conditional KO of the gene encoding the calcineurin-regulatory subunit; these mice show many schizophrenia-related phenotypes such as deficits in WM and PPI (Miyakawa et al., 2003). Another example would be mice deficient in the gene encoding for the Akt1, which have great sensitivity to the disrupting effects of amphetamine on PPI. Actually, there is evidence for a decrease in Akt1 protein levels and substrate phosphorylation levels in the brain of some individuals with schizophrenia (Emamian et al., 2004). Besides, the further analysis confirmed an association between schizophrenia and Akt1 genetic variants, showing that Akt is a key signaling intermediate of D_2Rs, the best-established target of antipsychotic drugs (Beaulieu et al., 2005).

Regarding the dopaminergic system, since the first observations that amphetamine-related compounds could induce schizophrenia-like symptoms in humans and rodents (Snyder, 1972), more in-depth studies have been carried out in the search of more complete genetic models that could provide with more information about the dopaminergic dysregulation. Indeed, Kellendonk (Kellendonk et al., 2006) based on a number of observations linking increased striatal D_2Rs and dopaminergic transmission to the pathophysiology of schizophrenia (Abi-Dargham et al., 1998), created a transgenic mouse line that selectively and transiently overexpressed D_2Rs within the striatum of mice (D_2R-OE). Over time D_2R-OE mice have been analyzed in a battery of behavioral tasks (Kellendonk et al., 2006; Drew et al., 2007; Bach et al., 2008; Ward et al., 2009; Ben Abdallah et al., 2011; Simpson et al., 2011). These mice have been shown to exhibit particular impairments in cognitive tasks dependent of PFC, including deficits in WM and conditioned associative learning (cognitive abilities that are sensitive to cortical lesions in humans and that are impaired in patients with schizophrenia (Bolkan et al., 2016). D_2R upregulation in the striatum of these mice was found to be associated with decreased DA turnover and increased D_1R sensitivity in PFC (Kellendonk et al., 2006). Taking into account the tight relation between D_1R activation and cognition (Goldman-Rakic, 1994; Arnsten et al., 2012) it has been suggested that disrupted cortical D_1R activation may be at the origin of the cognitive phenotype in the mouse. Also, Krabbe and colleagues (Krabbe et al., 2015) discovered a decrease in the burst firing of dopaminergic neurons of the VTA in these D_2R-OE mice; suggesting that abnormal VTA activity may also contribute to impaired cognition in the model, compounding to a decreased cortical D_1R activation; thus, a dopaminergic-dependent cortical hypofunction. Apart from that, D_2R-OE mice showed decreased GABAergic transmission in the cortex (Li et al., 2011), a second cortical abnormality commonly postulated to be present in patients.

In addition to this, the hypothesis of the hypofunction of glutamatergic signaling via NMDA receptors (discussed extensively by Goff & Coyle, 2001) has motivated the creation of genetically engineered mouse models to study this presumed glutamatergic hypofunction. For instance, in one such model, the expression of NR1 was reduced to 5% of endogenous levels in mice (Duman, 2002; Duncan et al., 2002). As it was expected, these mice showed reduced sensitivity to NMDA receptor antagonists and showed a ''schizophrenia-like'' phenotype,

including symptoms as decreased social interactions and hyperlocomotion. In addition, many of these abnormal behaviors were ameliorated with antipsychotic drugs. The usefulness of these models is still questioned, but they may be appropriate instrumental tools for interpreting results obtained from other studies (Arguello & Gogos, 2006).

1.6.2. Pharmacological and lesion models

Pharmacological animal models of schizophrenia are based on the current understanding of the alterations in various neurotransmitter systems. These models generally have some degree of construct validity, although it is extremely limited given the poor understanding of the fundamental basis of thought and cognition. Also, these models suffer from limited face validity. In contrast, regarding predictive validity, even if it is variable, is often good since most pharmacological models involve the administration of drugs that induce or exacerbate schizophrenic symptoms in humans (Costall, 1995; Marcotte et al., 2001; Lipska, 2004).

In animal studies, the administration of amphetamine and related psychostimulants reliably stimulates behavioral alterations such as hyperlocomotion and stereotypy (Kokkinidis & Anisman, 1980; Sharp et al., 1987). Moreover, amphetamine-induced stereotypic behavior can be attenuated by treatment with antipsychotics (Pijnenburg et al., 1975) further supporting the validity of this model. The face validity of pharmacological dopaminergic animal models is also supported by the disruptive effects of DA receptor agonists on PPI (Swerdlow et al., 1994; Swerdlow & Geyer, 1998), a preattentional sensorimotor gating test which is impaired in schizophrenia (Braff & Geyer, 1990; Davis et al., 1991). DA agonist apomorphine can disrupt PPI in humans and rats, mimicking the PPI deficits observed in patients with schizophrenia (Swerdlow et al., 1994). Besides, the administration of antipsychotic drugs can restore PPI function in rats treated with apomorphine (Swerdlow et al., 1994), and this response has been correlated with both clinical antipsychotic potency and D_2R affinity. Finally, PPI can be disrupted in rats by the direct infusion of DA into the nucleus accumbens, an effect which can be also blocked by antipsychotics (Swerdlow et al., 1994). This data supports, at least in some degree, both predictive and construct validity for this model.

Nonparanoid schizophrenia, especially when it includes negative symptoms, can be mimicked by the administration of PCP (Angrist, 1987; Javitt & Zukin, 1991), which appears to act predominantly on glutamatergic NMDA receptors (Lodge, 1987). PCP and other NMDA receptor antagonists induce schizophrenic-like symptoms in healthy subjects and precipitate psychoses in patients with schizophrenia who have stabilized (Javitt & Zukin, 1991; Krystal et al., 1994; Lahti et al., 1995a,b; Malhotra et al., 1996; 1997; Breier et al., 1998). Repeated exposure to PCP has been reported to reduce both basal and evoked DA utilization in the monkey PFC, an effect which persisted even after PCP treatment was stopped (Jentsch et al., 1997). As in the case of DA receptor agonists, PCP administration can disrupt PPI and startle habituation in rats (Geyer et al., 1984; Swerdlow & Geyer, 1998; Yamada et al., 1999). Altered social interactions have also been reported after treatments with PCP (Steinpreis et al., 1994; Sams-Dodd, 1995). Moreover, PCP produces amphetamine-like effects in rodents, including increased locomotor activity, stereotyped movements, circling and ataxia (Javitt & Zukin, 1991;

Snell & Johnson, 1986), and these effects are attenuated by antipsychotics and 6-hydroxydopamine lesions of the mesolimbic DA system (French & Vantini, 1984; Rao et al., 1989). Further, repeated administration of PCP in monkeys also causes deficits in PFC-dependent tasks that can be ameliorated by the atypical antipsychotic clozapine (Jentsch et al., 1997).

The serotoninergic system has also been frequently implicated in schizophrenia (Costall, 1995). The two major classes of psychedelic hallucinogenic drugs, the indoleamines (e.g., LSD) and phenethylamines (e.g., mescaline) (Kulig, 1990; Penington & Fox, 1994; Harrison & Burnet, 1997), are believed to mediate their effects through 5-HT_{2A}Rs (Aghajanian & Marek, 1999). As in the case with dopaminergic and glutamatergic animal models, LSD has been shown to disrupt startle habituation and PPI in humans and rats (Geyer & Braff, 1987). Further, the disruptive effects of PCP on PPI are believed to be mediated through direct stimulation of 5-HT_{2A}Rs (Geyer, 1998; Yamada et al., 1999). Interestingly, both LSD and mescaline have been shown to enhance glutamatergic transmission in rats (Yamada et al., 1999).

An interaction between dopaminergic and GABAergic systems in schizophrenia is supported by the fact that GABA neurons in the middle layers of PFC receive direct synaptic input from DA terminals. They exert inhibitory control over the excitatory output of layer III pyramidal neurons and undergo substantial developmental changes in late adolescence, the typical age of onset for schizophrenia (Weinberger, 1995; Goldman-Rakic & Selemon, 1997; Lewis et al., 1999). Related to this, in animal studies, the $GABA_A$ receptor antagonist picrotoxin has been shown to reduce PPI in rats when injected into the medial PFC (Japha & Koch, 1999). Besides, pretreatment with the DA antagonist haloperidol antagonized this effect, suggesting that blockade of GABA receptors in PFC impairs sensorimotor gating in a DA-dependent manner. However, the lack of any other reported GABA-induced behavioral deficits related to schizophrenic symptoms makes the face and predictive validity of this model difficult to establish (Marcotte et al., 2001).

The neurodevelopmental theory of schizophrenia (Weinberger, 1987; Murray & Lewis, 1987; Weinberger, 1995) postulates that the pathogenic conditions leading to this disorder occur in the middle stage of intrauterine life, long before the formal onset of symptoms (Weinberger, 1987; Roberts, 1991; Bloom, 1993; Feinberg, 1982; Murray et al., 1992; Murray, 1994). Some animal models of schizophrenia, designated as lesion models, are based on the concept of pre- or perinatal insults; suggesting that various obstetric complications (Torrey, 1991; Waltrip et al., 1995; Yolken & Torrey, 1995; Brake et al., 1997; Yamaguchi et al., 1999; Dalman et al., 1999) could result in abnormalities in pruning, cell death, and developmental connectivity (Murray & Lewis, 1987).

In this field, there are some targeted models that have been developed and classified as lesion models. For instance, one model that reflects this possibility is the one that lesions the ventral hippocampus of neonatal rats; the homologous brain region to the most affected portion of the hippocampus in patients (Lipska & Weinberger, 2000). Although this model does not produce behavioral abnormalities in the young pups, during adolescence behaviors related to schizophrenia emerge: increased locomotor response to stress and novelty, supersensitivity to

amphetamine and PPI deficits. Besides, many of these behaviors are reversed with antipsychotic treatment. Similarly, there is another model in which the developing rodent fetus is exposed to a methylating agent at specific time points (Moore et al., 2006) and adult rats show neuroanatomical and behavioral abnormalities such as size reductions in areas as thalamus, hippocampus and PFC, cognitive inflexibility, orofacial dyskinesias, sensorimotor gating deficits, and a post-pubertal supersensitivity to amphetamine.

Lesions of the adult rat PFC result in an enduring hyper-responsiveness to stress (Jaskiw et al., 1990; Jaskiw & Weinberger, 1992), as well as transient increases in locomotor exploration and amphetamine-induced stereotypy (Jaskiw et al., 1990; Whishaw et al., 1992; Braun et al., 1993; Lipska et al., 1995). Moreover, adult rats with PFC lesions show reduced PPI after apomorphine Injections (Swerdlow et al., 1995) and reduced cataleptic response to haloperidol (Lipska et al., 1995). A similar model involves the intracerebroventricular administration of kainic acid (Bardgett et al., 1995; Csernansky et al., 1998), which results in immediate, as well as delayed neuronal loss in the dorsal hippocampus, and has been proposed as an animal model of neurodegeneration that may be comparable to schizophrenia (Csernansky et al., 1998). This intracerebroventricular kainic acid model has been reported to enhance locomotor response to novelty and saline injection, as well as to amphetamine and MK-801 administration (Bardgett et al., 1997). Increased DA receptor binding in the nucleus accumbens has also been reported in this model (Bardgett et al., 1995), similar to that reported after adult hippocampal lesions (Mittleman et al., 1993).

1.6.3. Environmental models

Since the 20th century, along with genetic factors, many environmental causes of schizophrenia and other related disorders have been proposed. Some of them include obstetric complications, malnutrition and viral exposures (Byrne et al., 2007). As it has been mentioned in previous sections, epidemiological studies show increased risks of psychotic disorders between children exposed to influenza and other viruses during the second trimester of pregnancy. Precisely, studies with animal models where pregnant mothers suffer an induction of their immune response during gestation revealed that their offspring suffer from behavioral abnormalities (Meyer et al., 2006a), e.g., impaired PPI and cognitive performance, and increased sensitivity to MK801 (NMDA receptor antagonist) or amphetamine (Shi et al., 2003), to name a few.

Other animal models have demonstrated that maternal infection during pregnancy by viral agents can lead to diverse psychopathologies (Estes & McAllister, 2016b), indicating that these animal models will be critical in determining how this combination of risk factors may give rise to schizophrenia and related disorders. In this study, we will focus specifically on these animal models of immune activation in the mother, and we will describe them in more detail in the following sections.

1.6.3.1. Animal models of maternal infection

Evidence for associations between maternal infection during pregnancy and a higher risk of schizophrenia and related psychiatric disorders in offspring has been growing steadily more compelling, but epidemiology alone cannot establish a causal relationship between MIA and the risk of neurodevelopmental disorders. Thus, this association in humans will probably remain controversial at least into the near future (Estes & McAllister, 2016b). It is complicated to detect and confirm causal relationships as humans are genetically, ecologically and behaviorally heterogeneous and all these can influence susceptibility to disease. Besides, humans can not be subject to invasive experimentation; hence, clinical research is limited in its ability to identify the molecular pathways behind the maternal infection.

It is because of these complications of studying MIA in humans that animal research has become essential for identifying causal mechanisms and developing new diagnostic tools and therapeutics. Indeed, a causal relationship between MIA and autism- and schizophrenia-related behavioral abnormalities has been clearly demonstrated using rodent and, more recently, nonhuman primate animal models (Estes & McAllister, 2016b). These models have provided substantial evidence for a causal relationship between prenatal exposure to numerous infectious and/or immune activating agents and the emergence of multiple brain dysfunctions in adult life (Meyer et al., 2009). Several of the functional deficits induced by prenatal immune challenge, especially in rodents, are implicated in some of the most critical phenotypes of schizophrenia and autism: sensorimotor gating deficiency, abnormalities in selective associative learning, WM impairment, enhanced sensitivity to psychostimulant drugs, and deficits in social behavior. Moreover, some of the behavioral and cognitive deficits induced by in-utero immune challenge in rats and mice can be normalized by acute and/or chronic antipsychotic drug treatment (Borrell et al., 2002; Shi et al., 2003; Zuckerman et al., 2003; Zuckerman & Weiner, 2005; Ozawa et al., 2006; Romero et al., 2007). In addition, parallel morphological and neurochemical analyses in rodents have shown a wide variety of neuroanatomical and neurochemical changes in the adult CNS following prenatal exposure to infection. Some examples of these include pre- and postsynaptic changes in various neurotransmitter systems such as the central DA, GABA, and glutamate systems, together with alterations in neuronal and glial cell number, structure and positioning (Meyer et al., 2009).

1.6.3.1.1. Rodent MIA models

In MIA models, pregnant animals are exposed to immunological manipulation at a specific gestational stage. Then, behavior, brain structure, and function of MIA offspring are compared with those of control offspring. These MIA animal models meet all of the criteria required for validity for disease animal models, such as construct, face and predictive validities.

Each specific MIA model has important advantages and disadvantages. It should be considered that differences in gestational age, type of immunogen, dose, and timing, lead to overlapping and distinct phenotypic signatures that are critical factors in evaluating their use as preclinical models for different psychiatric disorders (Estes & McAllister, 2016b).

1.6.3.1.1.1. Influenza infection

Inasmuch as maternal influenza infection is a well-established risk factor for schizophrenia in humans, characterizing its effects in an animal model was a point of interest.

It has been reported that respiratory infection with human influenza virus in pregnant mice at mid-gestation results in specific histological abnormalities in the hippocampus and cortex of the neonatal offspring (Fatemi et al., 2000), including the pre-synaptic marker SNAP-25, neuronal nitric oxide (NO) synthase, and reelin. Pyramidal cells are more densely packed (Fatemi et al., 2002), which is remindful of schizophrenia. Moreover, adult offspring born to infected mothers show a remarkable abnormality in neuronal migration to layer 2/3 in the cortex; which is similar to that observed when DISC-1 is downregulated in the mouse fetus (Kamiya et al., 2005). In addition to this, a series of behavioral aberrations relevant both to schizophrenia and autism have been reported, such as deficits in social interaction, PPI, open field and novel object recognition test (NORT) (Shi et al., 2003). In another study with a mouse model of influenza infection with A/WSN/33 (N1N1), the offspring of infected mothers showed lower spontaneous locomotor activity, increased head-twitch response to hallucinogens and reduced antipsychotic-like effect of glutamate agonist. Moreover, they found that in PFC of the offspring of infected mothers the 5-HT_{2A}R was up-regulated, but the $mGlu_2$R was down-regulated; suggesting this could be the alteration that caused the behavioral abnormalities observed. In addition, the cortical 5-HT_{2A}R-dependent signaling pathways were altered in the offspring of infected mothers (Moreno et al., 2011). Finally, transcription is also altered in the brains of these offspring, as well as the concentration of 5-HT in the cerebellum (Fatemi et al., 2005; 2008; Winter et al., 2008).

1.6.3.1.1.2. Periodontal bacteria infection

Bacterial infections have recently been associated with schizophrenia (Sørensen et al., 2009), and obstetrical complications caused by such infections increase the risk for schizophrenia (Byrne et al., 2007). Very low birth weight is correlated with perinatal mortality and neonatal morbidity; in fact, between women who give birth prematurely, intrauterine infections are prevalent. Among the microorganisms isolated from the preterm placenta are gram-negative bacteria such as *F. nucleatum* and *P. gingivalis,* that are known to be involved in periodontal disease. Moreover, epidemiological evidence links the periodontal disease with premature delivery (Bobetsis et al., 2006).

1.6.3.1.1.3. Lipopolysaccharide (LPS)

An alternative way to simulate an infection without a real infectious agent is to mimic a bacterial infection by the injection of LPS to pregnant rodents, rabbits or sheep (intrauterine, intraperitoneal (i.p.) or intravenous (i.v.)). LPS is recognized and acts through the Toll-Like Receptor 4 (TLR4), which plays a fundamental role in the recognition of pathogens, and it is capable of producing a cascade of intracellular signaling pathways that result in activation of the mammalian immune system **(Figure 1.16)**. Some of the same behavioral abnormalities seen in the offspring of another MIA model, concretely the polyinosinic:polycytidylic acid

(referred to as Poly (I:C)) model (*further described in next sections*) have been observed in the offspring of LPS-treated mothers (Jonakait, 2007; Meyer et al., 2007; Smith et al., 2007); for instance, increased anxiety, deficits in social interaction and learning, and increased amphetamine-induced locomotion in the adult offspring (Borrell et al., 2002).

Apart from that, also common to the Poly (I:C) model, there have been observations of brain inflammation, as defined by astrogliosis (enhanced glial fibrillary acidic protein staining), altered microglial immunostaining (Jonakait, 2007) and PET imaging (Kannan et al., 2007) in young animals. Actually, animal models based on LPS administration have been used to study the imbalance between anti/pro-inflammatory mediators that probably affects to schizophrenic patients and to test the effects of antipsychotics. In a study where single doses of risperidone were administrated to a mild neuroinflammation model by LPS administration, it was found that risperidone prevented increased inflammatory parameters induced by LPS in brain cortex, such as expression of inflammatory cytokines, interleukin (IL)-1b and tumor necrosis factor α (TNF-α), the activity of the inducible inflammatory enzymes NO synthase and cyclooxygenase, p38 mitogen-activated protein kinase and inflammatory nuclear transcription factor kβ. Furthermore, it also restored anti-inflammatory pathways decreased by LPS challenge (MacDowell et al., 2013). This evidence of inflammatory changes in the brains of offspring of LPS-treated mothers is consistent with the notable findings of immune dysregulation in human autism and schizophrenia disorders.

1.6.3.1.1.4. Poly (I:C) model

Apart from LPS, another well-known alternative to real infection is to induce a maternal anti-viral inflammatory response using Poly (I:C), a synthetic double-stranded RNA (dsRNA), in the absence of a pathogen. Poly (I:C) acts through the TLR3 (**Figure 1.16**), and its injection in pregnant rodents is sufficient to cause all of the behavioral and histological abnormalities shown so far in the offspring of maternal influenza-infected mothers (Shi et al., 2009; Smith et al., 2007) (**Figure 1.17**). Upon binding to the TLR3, Poly (I:C) is capable of stimulating the production and release of many pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α (Fortier et al., 2004; Meyer et al., 2006b; Cunningham et al., 2007). In addition, is a potent inducer of the type I interferons IFN-α and IFN-β. Therefore, the integrity of the placental barrier becomes compromised, allowing the entrance of maternally derived cytokines into the fetal circulation and inducing inflammatory responses in the developing fetus, including the brain. This leads to structural and developmental disturbances associated with various neuropsychiatric diseases such as schizophrenia and depression (Reisinger et al., 2015). Therefore, administration of Poly (I:C) can efficiently mimic the acute phase response to viral infection (Meyer & Feldon, 2012).

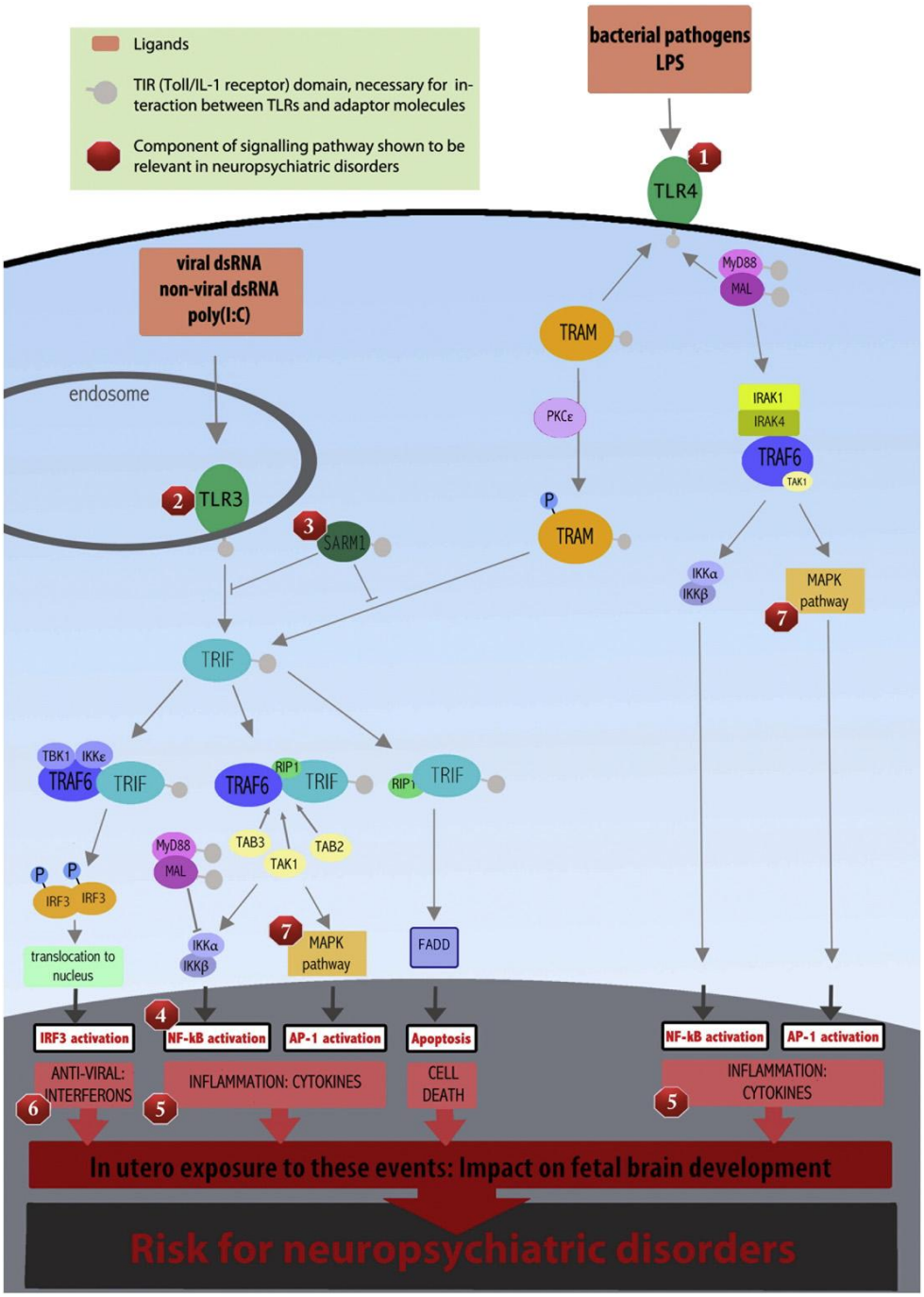

Figure 1.16: Involvement of the TLR3 and TLR4 signaling pathways in neuropsychiatric disorders. Several components and processes of the TLR3 and TLR4 pathways have been implicated in the etiology of neuropsychiatric diseases. Abbreviations: AP-1 (=activator protein 1), DRIIs (=depression-related interferon-induced genes), FADD (=Fas-associated protein with death domain), IKKs (=inhibitors of NF-kB kinase), IRAKs (=interleukin-1 receptor-associated kinase) 1 and 4, IRF3 (=interferon regulatory factor 3), MAL (=MyD88 adaptor like), MAPK (=mitogen-activated protein kinase), MyD88 (=myeloid differentiation primary response gene 88), NF-kB (=nuclear factor kappa-light-chain-enhancer of activated B cells), PKCε (=protein kinase C epsilon), SARM1 (=sterile alpha and TIRmotif containing 1), TAK1 (=transforming growth factor beta-activated kinase-1), TAB (=TAK binding protein) 1 and 3, TLR (Toll-like receptor) 3 and 4; TRAF3 (=TNF receptor-associated factor 3), TRAF6/RIP1 (=TNF receptor-associated factor 6/receptor interacting protein 1), TRAM (=TRIF-related adaptor molecule), TRIF (=TIR-domain-containing adapter-inducing interferon-β). From *Reisinger et al., 2015.*

As in this study, we will focus on a neurochemical and behavioral characterization of an animal model of cognitive deficit in schizophrenia through the use of Poly (I: C) as a stimulating agent of MIA, the characteristics and applications of this model will be described in more detail in the following sections.

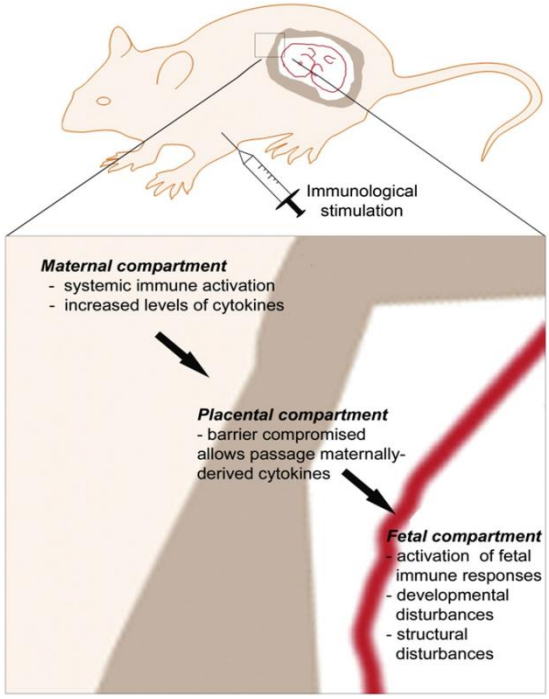

Figure 1.17: Representation of the rodent MIA model: gestational infection is mimicked by immunological stimulation of the gestating dam with immune-stimulating agents such as Poly (I:C). From *Reisinger et al., 2015.*

1.6.4.1.4.1. Evidence regarding the validity of the Poly (I:C) model

As commented previously, there is strong epidemiological evidence supporting the role of MIA in the possible later development of neuropsychiatric diseases (Meyer et al., 2006a). The Poly (I:C) model of MIA possesses a high degree of construct validity and has been repeatedly demonstrated to induce behavioral and neurochemical features related to schizophrenia, autism, and depression in adult offspring (face validity). Moreover, structural and functional aberrations in particular brain regions in adult offspring after Poly (I:C)-assisted MIA overlap with known pathophysiological features of mood and psychotic disorders.

In regard to neurochemical alterations (**Figure 1.18**), Babri et al. (Babri et al., 2014) documented abnormalities in the hypothalamic-pituitary adrenal axis commonly associated with depression-like behavior in offspring after Poly (I:C) MIA. In the mesolimbic pathway, alterations projecting from the VTA to the nucleus accumbens and the amygdala, related to schizophrenia, have been identified in Poly (I:C) MIA offspring (Zuckerman et al., 2003). In addition, prenatal Poly (I:C) is reported to compromise the function of the substantia nigra and striatum (Deleidi et al., 2010). Reduced levels of D_1R and D_2R in the PFC and enhanced TH in

striatal structures (Meyer et al., 2008b) have been reported, and also reduced NMDA receptor expression in hippocampus along with reduced numbers of reelin- and parvalbumin-positive cells in the PFC (Meyer et al., 2008a). Besides this, alterations to proper cortical functioning in adult offspring from Poly(I:C) MIA and neural synchrony disturbances between the hippocampus (Hipp) and PFC, associated with schizophrenia, have been identified in the animal model (Zhang et al., 2012). Moreover, in offspring of Poly (I:C) MIA, compromised concentrations of DA (Bitanihirwe et al., 2010), and 5-HT (Winter et al., 2009) are reported in the nucleus accumbens, hippocampus, and PFC, and changes in levels of GABA are reported in the hippocampus and amygdala (Nyffeler et al., 2006). Structural abnormalities are noted in the hippocampus, the striatum, the PFC and lateral ventricles (Piontkewitz et al., 2012a).

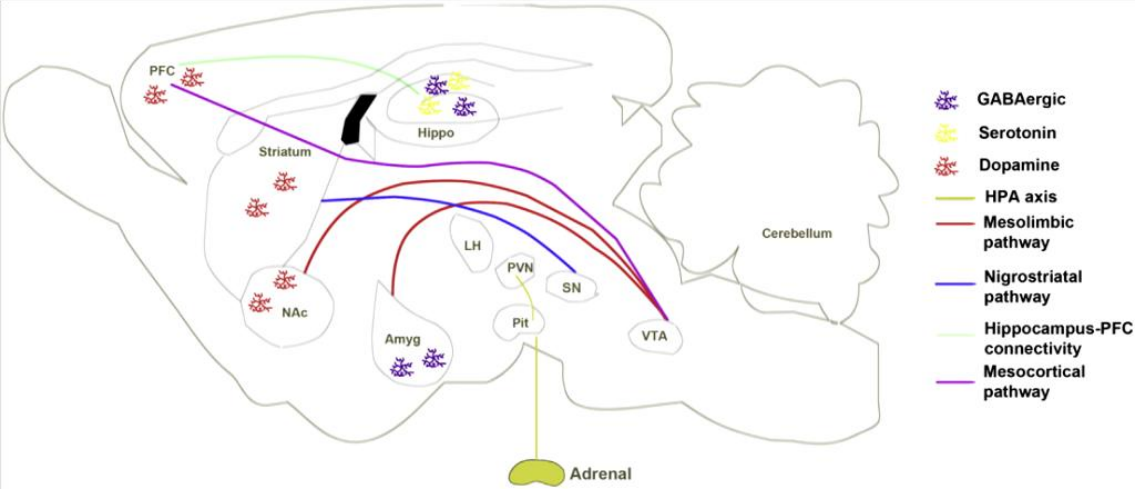

Figure 1.18: Structure and function of brain regions impacted by Poly(I:C)-assisted MIA. Abbreviations: HPA: hypothalamic pituitary adrenal, VTA: ventral tegmental area, NAc: nucleus accumbens, Amyg: amygdala, Pit: pituitary gland, SN: substantia nigra, Hippo: hippocampus, PFC: the prefrontal cortex, GABA: γ-aminobutyric acid, Poly(I:C): polyinosinic:polycytidylic acid, MIA: maternal immune activation, LH: lateral hypothalamus, PVN: paraventricular nucleus of the hypothalamus. From *Reisinger et al., 2015.*

Related to schizophrenia phenotypes and behavioral disturbances, some evidence that has resulted from those studies include deficits in PPI, modulated sensorimotor gating, and decreased LI (related to the attentional control of selective associative learning and WM), have been repeatedly confirmed (Meyer et al., 2009; 2010; Meyer & Feldon, 2012). Recently, evidence for anhedonic deficits (a central manifestation in depressive disorders and a prominent negative symptom of schizophrenia) after prenatal Poly (I:C) has been emerging in rodents models (Khan et al., 2014). Interestingly, enhanced behavioral despair, as assessed by tests related to depression-like phenotypes in rodents, has been so far only documented in mouse Poly (I:C) offspring (Chen et al., 2011; Khan et al., 2014).

Besides all this, the MIA Poly (I:C) model is also being used for testing different drug therapies (Meyer et al., 2010). While it has been previously shown that acute antipsychotic drug administration can block some of the behavioral deficits in MIA models such as influenza and Poly (I:C), some studies (Meyer et al., 2008a; Zuckerman et al., 2003; Ozawa et al., 2006;

Meyer et al., 2010) tested such drugs (haloperidol and clozapine, among others) in immature MIA offspring before the onset of behavioral abnormalities and ventricular enlargement. They demonstrated that treatment for a week, several weeks before behavioral testing, managed to prevent the aberrations and the ventricular enlargement it had been reported previously. Even if it is known that the classic action of these antipsychotics is to block the D_2R, it is worth to highlight that many of them have also been shown to influence cytokine expression, producing increases or decreases in their levels that can be correlated with the severity of some of the symptoms (Pollmacher et al., 2000; Drzyzga et al., 2006). In fact, it is known that chronic risperidone administration up to postnatal day 70 (PND70) prevents structural abnormalities and behavioral deficits of the Poly (I:C) rat offspring, such as enlargement of ventricular volume, impaired neurogenesis, disturbed micro vascularization, loss of parvalbumin-positive hippocampal interneurons and locomotor response to amphetamine (Piontkewitz et al., 2011; Richtand et al., 2011; Piontkewitz et al., 2012b). Further, it has also been described that low doses of paliperidone and risperidone normalize both basal and MK-801 induced increased extracellular glutamate (Roenker et al., 2011). Supporting this even further, in a study where Poly (I:C) offspring young-adults animals (PND60) received chronic paliperidone during 21 consecutive days (MacDowell et al., 2017), the treatment was able to block the neuroinflammatory response by the preservation and activation of endogenous anti-inflammatory mechanisms, such as nuclear factor erythroid 2-related factor 2 and peroxisome-proliferator activated receptor gamma pathways. In addition, paliperidone improved spatial WM deficits showed by the animal model.

As it has been commented before (*section 1.5.*), apart from antipsychotics, other approaches have been proposed for the development of drugs for the treatment of cognitive deficits in schizophrenia, such as the discovery of the POP inhibitors as potential therapeutic tools (Toide et al., 1995; Lopez et al., 2013). Indeed, the Poly (I:C) model has also being used along with other animal models resembling schizophrenia-like behaviors (subchronic PCP and acute MK-801 administration) to test this type of compound. Concretely, IPR19, a novel POP inhibitor, was able to reverse the cognitive deficits of the three animals models in the NORT, T-maze and eight-arm maze; however, it did not manage to modify the impaired PPI response in the Poly (I:C) model (Prades et al., 2017).

All this data strengthen the good predictive validity of the Poly (I:C) MIA model.

1.6.4.1.4.2. Advantages and limitations of the Poly (I:C) model

There are several advantages to using viral-like immunogens such as Poly (I:C) instead of living viral pathogens for the purpose of prenatal immune activation models (Meyer et al., 2009):

- It can be easily handled: unlike viral pathogens, Poly (I:C) can be manipulated without rigorous biosafety precautions.

- The intensity of the cytokine-associated immune response can be easily controlled by appropriate dose-response studies (Shi et al., 2003; Meyer et al., 2005; Cunningham et al., 2007), and will determine the associated maternal response to that stimulation.

- The acute poly (I:C)-induced immunological challenges in rodents are time-limited to a maximum period of 24-48 h depending on the precise dose used (Meyer et al., 2005; Cunningham et al., 2007), facilitating the precise timing of the MIA corresponding to specific periods of fetal development.

- Maternal exposure to Poly (I:C) is capable of altering pro- and anti-inflammatory cytokine levels in the three relevant compartments of the maternal-fetal interface of rodents: the placenta, the amniotic fluid and the fetus, including the brain (Gilmore et al., 2005; Meyer et al., 2006b; 2008b).

Thus, the model takes account of relevant immunological mechanisms suggested being crucial for mediating long-term effects of prenatal infection on the brain and behavioral development (Gilmore & Jarskog, 1997; Meyer et al., 2007; 2009; Patterson, 2009).

Despite its advantages, there are also limitations to the Poly (I:C) MIA paradigm that should be taken into consideration. This model does not readily mimic the precise immunological insults occurring in the human environment; that is, it falls short in modeling the full spectrum of immune responses normally induced by viral exposure because of the limited set of responses elicited (Meyer et al., 2009). Actually, stimulating the maternal immune system administrating a real virus (e.g. influenza) leads to a much wider activation of not only the innate but also the acquired immune system, resembling more closely the situation following real infection during pregnancy in humans (Meyer, 2014).

1.6.4.1.4.3. Important factors to consider when using the Poly (I:C) model

1.6.4.1.4.3.1. The intensity of the immune stimulus

Meyer and collaborators (Meyer & Feldon, 2012) conducted a comprehensive dose-response study with the objective to explore the effect of Poly (I:C)-induced immune challenge at low, middle or high intensity (with 2.5, 5 or 9 mg/kg i.p. to pregnant mice) on gestational day (GD) 9, corresponding to the middle to last first trimester of pregnancy in humans. They reported that some behavioral deficits showed a clear dose-dependent relationship, although others did not (for instance, PPI deficiency and enhanced reaction to systemic amphetamine, emerged in adult mice subjected to prenatal treatment with 5 or 10 mg/kg Poly (I:C), but not at a dose of 2.5 mg/kg). They suggested that there is a threshold of viral-like immune activation which is required to induce long-term brain and behavioral pathology in the offspring.

The dose-dependent nature of prenatal Poly (I:C) goes hand in hand with findings derived from human epidemiological studies suggesting that only a minority of offspring born to an infected mother eventually develop schizophrenia (Fatemi et al., 2005). According to these evidences, maternal (viral-like) infection during pregnancy may enhance the risk of schizophrenia in the offspring only if the prenatal infectious process is associated with relatively strong immunological reactions in the maternal/ fetal compartments, and/or if this early-life immunological insult takes place in conjunction with additional genetic or environmental risk factors implicated in this disease (Meyer & Feldon, 2012).

1.6.4.1.4.3.2. Importance of the immunogenicity of the Poly (I:C) products

Another fundamental factor affecting the robustness and outcome of the Poly (I:C)-based MIA models is the potential source of variability from batch-to-batch variations in the immunogenicity of the Poly (I:C) itself. Harvey and Boksa (Harvey & Boksa, 2012) were first to report product variability in *in-vivo* mouse models and highlighted the necessity to confirm the immunogenicity of different batches of Poly (I:C), even if they originate from the same vendor. It has been recently reported that Poly (I:C) of different molecular weights induced different cytokine responses and sickness behavior in rats (Careaga et al., 2018), implying that the length of dsRNA analog may represent another source of variability in the poly(I:C)-based MIA models. Although these findings derived from investigations using non-pregnant mice or rats (Harvey & Boksa, 2012; Careaga et al., 2018), and pregnancy is known to be associated with considerable hormonal fluctuations and physiological processes affecting maternal immunity (Siiteri & Stites, 1982; Hyde & Schust, 2016), posterior studies have also confirmed these findings in pregnant animals.

Mueller and cols. (Mueller et al., 2019) compared different Poly (I:C) batches from the same vendor in their effectiveness to affect thermoregulation and to stimulate cytokines and chemokines in pregnant mice and their offspring. Consistent with previous reports (Harvey & Boksa, 2012), they found significant batch-to-batch variability in terms of maternal plasma cytokines and chemokines, and marked differences, but not as pronounced, in the placenta and fetal brain. Among this inflammatory mediators, IL-6, IL-17A, and CXCL1 were included, which are believed to be critical mediators of MIA-induced neurodevelopmental pathologies (Choi et al., 2016; Rudolph et al., 2018; Lins et al., 2018). Besides, the varying molecular weights of different Poly (I:C) batches elicited different cytokine and chemokine responses; that is, high molecular weight Poly (I:C) elicited more potent cytokine and chemokine responses in maternal plasma and placenta compared with low molecular weight Poly (I:C). This data suggest that different molecular weights can markedly influence the nature of immune responses. In fact, these diverse responses may occur due to the distinct signaling pathways involved in the process. While low molecular weight dsRNA bind to TLR3, longer frangments of dsRNA with high molecular weight bind to retinoic acid inducible gene I and melanoma differentiation-associated gene 5 receptors (Pichlmair et al., 2009; Zhou et al., 2013). In agreement with this, it was observed that batches with greater enrichment in the high molecular weight dsRNA fragments produced larger cytokine and chemokine responses compared to those products that were less enriched in this type of fragments (Mueller et al., 2019).

It is known that Poly (I:C) administration generally leads to reduced rectal and surface temperature (Cunningham et al., 2007), producing a typical thermal response of hypothermia in mice. Consistent with the cytokine data, batches producing large increases in maternal cytokines and chemokines also induced large decreases in rectal and surface temperature, showing an association between the elicited inflammatory response and hypothermia (Mueller et al., 2019). Another factor that can be affected by the immunogenic variability of the Poly (I:C) products is the spontaneous abortion rate. It has been reported that batches with more

recent release date lead to more pronounce spontaneous abortion rate compared to batches with older release dates; and this correlates with the intensity of the Poly (I:C)-elicited immune response, since products with recent release dates induced stronger immune responses than batches with older release dates (Mueller et al., 2019).

All this data demonstrates that different Poly (I:C) products can induce varying immune responses and that they can differentially affect maternal physiology and pregnancy outcome. Therefore, it is important to highlight the necessity of checking the purity and precise molecular composition of the product to be used to create a MIA model based on Poly (I:C) administration.

1.6.4.1.4.3.3. Importance of prenatal timing

The strength of the association between MIA and enhanced risk of psychiatric disorders is critically influenced by the precise prenatal timing. Numerous retrospective epidemiological studies found that only when the maternal host was infected in the second trimester of a human pregnancy, there was a significant association between MIA and an increased incidence of schizophrenia (Mednick et al., 1988; Stober et al., 2002). Nevertheless, there has been an excessive emphasis on second-trimester infections according to studies using collected and quantifiable serologic samples from epidemiological studies (Brown et al., 2004). The critical relevance of the gestational age during which MIA is induced may relate to both maternal and fetal factors. On the one hand, the immune response in the maternal system significantly varies during the course of pregnancy (Tinsley et al., 2009; Chatterjee et al., 2011), hence determining the nature and intensity of the fetus exposure to inflammatory mediators.

Some studies by Meyer and colleagues (Meyer et al., 2006a; 2008b) found out that maternal Poly (I:C)-induced immunological stimulation at different gestational times precipitates distinct psychopathological and neuropathological symptom clusters in the offspring. Specifically, prenatal Poly (I:C) treatment (at 5 mg/kg, i.v.) on GD9 leads to a pathological profile characterized by suppression in exploratory behavior, abnormalities in selective associative learning (in the form of LI disruption), abolition of the unconditioned stimulus-preexposure effect, impairments in sensorimotor gating (in the form of reduced PPI), enhanced sensitivity to amphetamine, and deficiency in spatial WM when the demand for temporal retention is high (Meyer et al., 2006a,b; 2008a). Otherwise, the same approach but on GD17 leads to a partially overlapping symptom profile involving the emergence of perseverative behavior (in the form of impaired discrimination reversal learning and presence of abnormally persistent LI), deficits in spatial WM and recognition memory even when the demand on temporal retention is low, potentiated response to amphetamine and to the non-competitive NMDA receptor antagonist MK-801, as well as abolition of the unconditioned stimulus-preexposure effect (Meyer et al., 2006a,b; 2008a; Bitanihirwe et al., 2010). Therefore, some symptoms are restricted to the MIA occurring either in early/middle or late gestation (e.g. loss of LI or impairment in reversal learning), while others are common to both situations (e.g. potentiation of amphetamine sensitivity).

Actually, one hypothesis coming from this evidence is that the distinction between early/middle and late pregnancy MIA could capture the positive-negative dichotomy of schizophrenia. It seems that MIA occurring in GD9 (middle/end of the 1st trimester in humans) leads to a variety of symptoms associated with positive symptoms of schizophrenia, with a hyperdopaminergic state in mesocorticolimbic areas (Winter et al., 2009) showing increased sensitivity to dopaminergic agonists, loss of LI effect and abnormal sensorimotor gating. But, MIA occurring in GD17 (middle of the 2nd trimester in humans) induces a marked hypodopaminergic and hypoglutamatergic state in prefrontal cortical and hippocampal areas that are mainly associated (but not limited to) with the negative-cognitive symptoms of schizophrenia (e.g. perseverative behavior, social interaction deficits, anhedonic behavior).

Consequently, the differential effects of early/middle *vs*. late prenatal immune challenge on neurochemical imbalances in the central DA and glutamate systems, captures the suggested relative contributions of bidirectional changes in dopaminergic activity (hyper- *vs*. hypodopaminergia) and impaired glutamatergic signaling to distinct symptom categories in schizophrenia (Knable & Weinberger, 1997; Guillin et al., 2007).

1.6.4.1.4.3.4. The relevance of the postnatal age

Maternal infection during pregnancy induces an immunological lesion to the developing fetal brain, and this lesion is likely to be progressive in nature; affecting postnatal brain development and maturation and leading to structural and functional aberrations that depend on postnatal maturation processes (Meyer et al., 2009).

Indeed, several of the behavioral, cognitive and pharmacological abnormalities induced by prenatal exposure to Poly (I:C) in rodents is depend on post-pubertal maturation processes and only emerge in adult but not in prepubertal subjects (Zuckerman et al., 2003; Zuckerman & Weiner, 2005; Meyer et al., 2006c; Ozawa et al., 2006; Meyer et al., 2008b). This maturational delay is indicative of a progression of pathological symptoms from peri-adolescent to adult life, which is consistent with the post-pubertal onset of psychotic behavior in schizophrenia and related disorders (Weinberger, 1987; Murray et al., 1991; Rapoport et al., 2005; Ross et al., 2006). As an example, using a rat model of chronic prenatal immune activation by the bacterial endotoxin LPS, it has been shown that striatal DA concentrations are significantly decreased in LPS-exposed offspring at peri-adolescent age relative to corresponding control offspring, whilst DA concentrations are significantly enhanced at adult age (Romero et al., 2010). Taken together, it seems clear that maturation-dependent effects need to be taken into consideration in the interpretation of experimental findings resulted from in vivo models of MIA.

1.6.4.1.4.3.5. The relevance of sex

Environmental insults targeting the pregnant maternal host may differentially affect the brain and behavioral development in male and female offspring. Actually, numerous studies provide evidence for critical sex differences in the development of long-term structural and functional brain abnormalities (Weinstock, 2007). The truth is that the majority of studies focused on the

effects of the prenatal immune challenge have only used male offspring in order to avoid possible confounds and interpretative problems arising from hormonal fluctuations in the female offspring (Meyer et al., 2006c; Fortier et al., 2007; Romero et al., 2007; Wolff & Bilkey, 2008).

However, there are some studies which assessed the long-term neurodevelopmental effects of prenatal immune challenge in both sexes, but nothing clear can be concluded from them. The majority of these studies did not reveal marked sex-dependent alterations in brain and behavior (Shi et al., 2003; Zuckerman et al., 2003; Meyer et al., 2005; 2006a,c; Nyffeler et al., 2006; Ozawa et al., 2006; Romero et al., 2010), as opposed to a few studies demonstrating clear sex-dependent effects in at least some brain and behavioral parameters (Meyer et al., 2008b; Schwendener et al., 2009).

Therefore, additional research including male and female offspring is clearly necessary to clarify if male and female subjects may be equally vulnerable to MIA or if there may be important sex-dependent differences.

1.6.4.1.4.3.6. The relevance of gene-environment and environment-environment interactions

Even if the maternal infection is relatively common, most offspring of mothers exposed to infection during pregnancy do not develop severe neurodevelopmental brain disorders such as schizophrenia (Selten et al., 2010). This suggests that, if in utero exposure to infection plays a role in the etiology of these brain disorders, it probably does so by interacting with another factor, including genetic factors.

It should be taken into consideration that there are some genes identified as genetic risk factors of schizophrenia; for instance, promoter polymorphisms of pro-inflammatory (Boin et al., 2001) and anti-inflammatory cytokines (Bocchio Chiavetto et al., 2002), or human leukocyte antigens and alleles (Wright et al., 2001) and that the precise immune-related genetic background of the mother may influence the liability to certain infections or result in an excessive or inappropriate inflammatory response.

Actually, as an example of this hypothesis, it can be mentioned one study carried out by Meyer and colleagues (Meyer et al., 2008a) where they compared the long-term functional consequences of prenatal Poly (I:C) exposure in wild-type mice and transgenic mice constitutively overexpressing the anti-inflammatory cytokine IL-10 in macrophages. The results showed that enhanced IL-10-mediated anti-inflammatory signaling is sufficient to prevent the manifestation of multiple behavioral and pharmacological abnormalities in the adult offspring after the prenatal immune challenge by Poly (I:C). Thus, recent research in mice designed to examine immunological gene-environment interactions has successfully shown that the association between MIA and emergence of schizophrenia-like behavioral and pharmacological dysfunctions is critically influenced by the pro-inflammatory and anti-inflammatory genetic background of the infected host (Meyer & Feldon, 2012).

Additionally, the Poly (I:C) model can be used to identify possible synergisms between prenatal immune challenge and abnormal expression of those genes identified as "candidate genes" or susceptibility factors of schizophrenia (NRG-1, COMT, DISC-1, etc). It could be possible that these genes act only playing minor roles in inflammatory processes, however, aberrant expression of these genes may act synergistically with prenatal infection to increase the risk of long-lasting neurodevelopmental brain disorders (Tsuang, 2000; Ayhan et al., 2009). Among some other findings, the study by Ibi et al., (Ibi et al., 2010) reported additive effects of neonatal Poly (I:C)-induced immune activation treatment and dominant-negative DISC-1 expression, in the precipitation of impaired parvalbumin expression in the medial PFC of adult offspring. This finding is of particular interests because reduced parvalbumin expression is a well-replicated neuropathological finding in schizophrenia (Lewis et al., 2005; Lewis & Gonzalez-Burgos, 2008; Gonzalez-Burgos et al., 2010).

Finally, apart from the gene-environment interaction studies, the Poly (I:C) model also seems to be a useful tool to analyze interactive effects between discrete environmental factors implicated in schizophrenia. Certainly, exposure to stressful situations and/or drugs of abuse during peri-adolescent maturation have often been debated as constituting significant postnatal environmental factors that can increase the risk and/or facilitate the expression of adult mental illness, especially schizophrenia (Corcoran et al., 2003; Phillips et al., 2006). Actually, the susceptibility to such postnatal environments insults can be dependent on vulnerability factors acting during early (fetal and early postnatal) brain development. Therefore, the prenatal immune challenge may then make the brain more vulnerable to peri-adolescence exposure to stress/drugs, facilitating the development of the full spectrum of brain abnormalities relevant to schizophrenia and related disorders (Meyer & Feldon, 2012).

Hence, after reviewing all these features, the rodent Poly (I:C) model of MIA is proposed as a relevant and suitable experimental approach for preclinical drug research and the study of the genetic, molecular and neurochemical mechanisms that give rise to psychotic disorders, providing: 1) feasibility and reproducibility of the experimental management; 2) control over specific stimulation timing and intensity; 3) sensitivity to treatment with available pharmacological treatment; 4) demonstrated interaction with determined genetic and environmental factors; 5) model of disease progression, as opposed to a single-point acute manipulation; 6) developmental testing of novel preventive and symptomatic therapeutic strategies.

1.6.3.1.2. Nonhuman primate MIA models

Although the remarkably strong construct, face and predictive validity for schizophrenia of these rodent models, their potential use to know more about this mental disorder that is so inherently human has remained controversial (Estes & McAllister, 2016b). With the intention to close the gap between humans and rodents, several groups have established rhesus macaque MIA models that display behavioral symptoms of schizophrenia and autism: increased repetitive behaviors, abnormal communication, and impaired social interactions. These symptoms start at weaning and increase in intensity with age (Reisinger et al., 2015;

Machado et al., 2015; Meyer, 2014). Besides, in neonatal nonhuman primate offspring gray and white matter volume is altered in an immunogen-specific manner (Reisinger et al., 2015) causing changes in dendritic arborization (Weir et al., 2015).

A key outstanding question that could be answered thanks to these nonhuman primate models is whether the molecular evidence of MIA found in rodents underlies similar phenotypes to schizophrenia and autism in humans.

1.6.3.1.3. Perspectives of MIA models

Thus, the great promise of MIA models relais in its potential to lead to novel therapeutics and to determine when particular symptoms emerge and are sensitive to the new treatments. If MIA is a disease primer for several CNS disorders, then MIA animal models are critical for determining the molecular pathways that mediate the resulting neuropathology and abnormal behaviors (Estes & McAllister, 2016b). Although the field is still in its infancy, further work seems to promise the identification of new classes of therapies and the characterization of the molecular pathways responsible for these psychiatric disorders.

1.6.4. Animal behavioral tests to evaluate cognitive dimensions altered in schizophrenia

The cognitive deficits characteristic of schizophrenia patients are increasingly recognized as core symptoms of this mental disorder. Reports indicate that cognitive performance is closely correlated with functional outcome experienced by patients, and the lack of effective treatment in this field is discouraging. Thus, more research has turned towards developing drugs to improve cognition in schizophrenia patients and potentially improve global functions such as social interaction and employment (Green, 1996; Floresco et al., 2005; Green, 2006). In response to the lack of effective treatments, the United States National Institute of Mental Health sponsored the MATRICS initiative (Marder et al., 2004) developing a consensus of opinion on the core cognitive deficits suffered by schizophrenia patients. Seven cognitive domains were identified as commonly deficient in schizophrenia patients: attention/vigilance; WM; reasoning and problem solving; processing speed; visual learning and memory; verbal learning and memory; and social cognition (Nuechterlein et al., 2004). Once these domains were identified, a standardized test battery was developed to ensure that future cognitive testing in schizophrenia patients is consistent and comparable across studies (Marder & Fenton, 2004). In 2005, Floresco (Floresco et al., 2005) identified two approaches for developing cognitive paradigms and animal models that mimic the cognitive deficits observed in schizophrenia: first, manipulation of specific systems that are altered in schizophrenic patients using lesions or pharmacological manipulations; and secondly, development of comprehensive models of the disorder with the attempt to identify cognitive deficits in the model that resemble deficits found in schizophrenia.

1.6.4.1. The NORT to test visual learning and memory in rodents

As previously commented, visual learning and memory are part of the list of the cognitive domains considered by the MATRICS to be deficient in schizophrenia. In order to detect and treat this symptomatology, MATRICS identified the Brief Visual Memory Test-Revised (Benedict & Groninger, 1995; Benedict et al., 2012) to assess visual learning and memory in schizophrenia patients. As for preclinical tasks that are accepted and considered appropriate by MATRICS for the study of visual memory and learning in rodents, some tests have been described as appropriate for measuring visual learning and memory, such as maze-based tasks (Morris water maze and Barnes maze) and a non-maze based memory test, the NORT.

The NORT is a recognition memory task that allows the comparison between presented stimuli and previously stored information. Ennaceur and Delacour (Ennaceur & Delacour, 1988) first described the NORT test for rats which was based on the differential exploration of familiar and new objects, and later on, it was minimally modified and adapted to mice (Steckler et al., 1999). This test is a non-rewarded and ethologically relevant paradigm based on rodents' exploratory behavior and innate preference for novelty (Young et al., 2009; Neill et al., 2010; Vogel-Ciernia & Wood, 2014). It has been widely used to study neurobiological mechanisms underlying long-term memory formation and to test episodic memory and possible memory impairments (Barrett et al., 2011; McQuown et al., 2011; Haettig et al., 2011; Vogel-Ciernia & Wood, 2014). In addition, is also often used to screen for memory-enhancing or memory-disrupting compounds (Young et al., 2009). The NORT uses the discrimination index as a measure of spatial and visual learning and memory evocation capacity (Jaiswal et al., 2018). It is currently widely used in lesion and pharmacological studies, animal models of aging, studies comparing rodent strains, as well as for behavioral phenotyping of genetically modified mice (Dere et al., 2007).

Studies have been performed to evaluate the mechanism underlying NORT performance, which can be compared to what is known in humans (Buckner & Wheeler, 2001; Ranganath et al., 2008). Various studies have described the involvement of different brain areas in the processes of visuo-spatial and recognition memory. On the one hand, abundant evidence suggests the involvement of the perirhinal cortex (Winters et al., 2004; Winters & Bussey, 2005) as well as the hippocampus (Clark et al., 2000; Hammond et al., 2004) in NORT performance. Supporting the involvement of the perirhinal cortex, human studies also point to a functional dysregulation of the temporal lobe since schizophrenic patients show decreased volumes in this brain area, where the perirhinal cortex is included (Turetsky et al., 2003; Pihlajamaki et al., 2004). On the other hand, in addition to these areas, the PFC has also been suggested to be involved in NORT performance, especially in object recognition memory at long delays (Nagai et al., 2007). In fact, human studies have shown that PFC is involved in episodic memory and identified the PFC a key component (Rugg et al., 2002; Ofen & Shing, 2013; Foster et al., 2013; Hawco et al., 2013; Wong et al., 2014). The PFC in rodents is considered functionally homologous to the dorsolateral PFC in humans (Preuss, 1995; Farovik et al., 2008; Ongur & Price, 2000), and despite differences in sizes and complexity, many functions attributed to the dorsolateral PFC in humans are equivalent to the PFC in rodents

(Kolb, 1984; Uylings & van Eden, 1990). Lesions to the PFC affect the ability to resolve tasks, and pharmacological interventions in this area have also confirmed the involvement of the PFC in the visual and special memory in rodents (Hannesson et al., 2004; Dere et al., 2006; 2007; Barker et al., 2007; Devito & Eichenbaum, 2011) since is considered important for the integration of object and location information (Devito & Eichenbaum, 2011).

The NORT has been used to evaluate the effects of current antipsychotic treatments and potential cognitive enhancers. As for antipsychotics, mixed effects in the NORT have been reported. On their own, antipsychotics either impair NORT performance or have no effect. For instance, chronic olanzapine or haloperidol disrupted NORT performance and were not able to reverse NORT deficits induced by chronic mild stress (Orsetti et al., 2007). Raclopride, a D_2R antagonist, impaired performance in the NORT (Woolley et al., 2003) as well as chronic oral administration of risperidone and haloperidol (Terry et al., 2007). On the contrary, antipsychotics have also been described to reverse cognitive deficits produced by other pharmacological agents. Subchronic clozapine but not haloperidol, attenuated cognitive impairments detected in PCP administered mice, while acute clozapine and haloperidol had no effects (Hashimoto et al., 2005). Other studies have shown that acute clozapine and risperidone, but not haloperidol, reversed the disruptive effects produced by subchronic PCP and acute MK801 administration on NORT performance (Grayson et al., 2007; Karasawa et al., 2008). Aripiprazole administered acutely or systemically, as well as sertindole, also succeeded to reverse NORT deficits produced by subchronic PCP administration (Nagai et al., 2009; Idris et al., 2010). D_1R antagonist SCH23390 but not D_2R antagonist raclopride, prevented NORT impairments induced by methamphetamine (Kamei et al., 2006). Therefore, in general it can be said that atypical antipsychotics improve performance in pharmacologically induced NORT deficits, while in contrast, typical antipsychotics fail to do so. Regarding the evaluation of potential cognitive enhancers, different types of drugs have been tested in the NORT. The α_7 acetylcholine receptor partial agonist SSR180711 (Pichat et al., 2007) and the full agonist PNU282987 (McLean et al., 2011) have been reported to improve performance in the NORT in rats and mice, while these effects were absent in an α_7 acetylcholine receptor KO mice (Pichat et al., 2007). As for dopaminergic agents, interestingly, D_1R agonist SKF81297 impaired performance after 15 min delay but improved it after a longer delay of 4 h (Hotte et al., 2005; 2006). Regarding glutamate receptor function, D-serine and a glycine transporter inhibitor were shown to reverse MK-801-induced NORT deficits in rats (Karasawa et al., 2008). Also, phosphodiesterase inhibitors as sildenafil and valdenafil showed efficacy in improving NORT performance in rats and mice (Prickaerts et al., 2004; Rutten et al., 2005). Finally, antidepressant fluvoxamine, sometimes used as an adjunctive treatment for schizophrenia, reversed NORT deficits induced by subchronic PCP exposure in mice (Hashimoto et al., 2005).

Hence, the NORT is particularly well suited for preclinical studies of the visual learning and memory domain outlined by MATRICS (Green et al., 2004). Some of the advantages are 1) it is fast and relatively easy to perform; 2) performance varies with the delay interval (e.g., retention interval) and complexity (e.g., number of objects) of the task, allowing assessment of pharmacological compounds as “good” cognitive enhancers or “poor” or cognitive disruptors; 3) it possesses some etiological validity for probing brain regions implicated in the

neurobiology of schizophrenia since antipsychotics and cognitive enhancers improve performance while psychotomimetic drugs impair it; 4) similar aspects of recognition memory can be assessed in humans using the Brief Visual Memory Test, making the NORT very useful as a preclinical tool to screen potential pharmacological treatments for schizophrenia.

1.6.4.2. The 5-CSRTT to test attention/vigilance in rodents

Another cognitive dimension of the seven considered impaired in schizophrenia according to MATRICS is the attention/vigilance domain. Attention can be divided into three subdomains: selective attention, describing the process by which environmental stimuli are chosen for attention; sustained attention, also referred to as vigilance, where attention is focused on particular stimuli for prolonged periods; and divided attention/attentional control, where attention is focused despite distractors, and/or on multiple tasks (Parasuraman, 1998). Schizophrenia patients exhibit impaired attentional processes (Cornblatt & Keilp, 1994), which may reflect the core deficits of the disorder (Chudasama & Robbins, 2004). Many tasks have been developed to assess attention in schizophrenia patients. Between them are included mainly tests such as the Continuous Performance Task (and its updated version of "identical pairs"), and the Wisconsin Card Sorting Task (Beck et al., 1956; Weisbrod et al., 2000; Young et al., 2009). Also in humans, the 5-CSRTT has been used to characterize regional effects of PFC damage (Alexander et al., 2005).

The Cognitive Neuroscience Treatment Research to Improve Cognition aimed to identify animal cognitive paradigms/tasks to be developed for research on the treatment of attention and other cognitive deficits in schizophrenia (Gold et al., 2007). In choosing these tasks, the priority was to identify those with high construct validity, both in terms of the ability of the task to specifically measure the process of interest, and evidence that it recruited the neural systems thought to be critical for that process and impaired in schizophrenia. One of those test chosen was the 5-CSRTT. The 5-CSRTT was first developed by Trevor Robbins and cols. in early 1980 (Carli et al., 1983) to study different aspects of ADHD, and has been suggested to parallel continuous performance testing in humans (Day et al., 2008). It is based on a test of sustained attention or continuous performance of the same name originally developed for humans (Wilkinson, 1963). In addition to the rat and human tasks, versions have been developed for mice (Humby et al., 1999; 2005) and nonhuman primates (Weed et al., 1999). Detailed descriptions of the original task (Robbins, 2002; Bari et al., 2008; Neill et al., 2010; Lustig et al., 2013) and variations of protocols (Bari et al., 2008; Lustig et al., 2013) are provided.

Briefly, the 5-CSRTT trains rodents to report the occurrence and location of brief, visual stimuli presented pseudorandomly across five spatial locations in a horizontal array of apertures. The task can be used to evaluate various aspects of executive function: response accuracy (proportion of correct overall attempted trials) is usually interpreted as a measure of sustained and spatially divided attention; omissions (trials when no response is made) are a putative index of global attentional and motivational processes; premature (responses before stimulus onset) or perseverative responses (extra responses after outcome feedback) are measures of inhibitory control, possibly related to the constructs of impulsivity and compulsivity.

Additionally, response and reward collection latencies relate to processing speed and to motoric or motivational factors, giving insight into the animal's speed of cognitive processing (another one of the seven domains of cognition affected in schizophrenia as outlined by MATRICS) (Young et al., 2009). All of these measures may reflect, to some extent, dissociable cognitive processes, and they have been demonstrated to be sensitive to different pharmacological treatments and the integrity of distinct areas of the rodent PFC (Mar et al., 2013). **Table 1.2** summarizes some of the parameters measured by the 5-CSRTT and their possible interpretation. It is important to note that no single measure reliably predicts attentional performance; the results on all of these measures and the resultant overall interpretation provide more accurately that information (Robbins, 2002; Young et al., 2009).

Table 1.2: Measures more often analized in the 5-CSRTT and domains linked to their interpretation.

5-CRSTT measures	Domain linked to
Accuracy	Attention-selective and sustained
Omissions	Sustained attention, motivation, motoric effects
Premature responses	Impulsivity, motivation
Latency to correct response	Processing speed, motivation, motoric effects

Since the task is motivated by reward, rodents are subjected to food/water restriction before the task (pre-training) to ensure sufficient levels of motivation (Mar et al., 2013). This restriction is continued throughout the course of the experiment. In addition to the food/liquid restriction, pre-training stages also include acclimatization to the operant chambers through stages of increasing complexity in order to get adequate responses during the task.

There are many manipulations and variants of the task given its wide use. These tasks variations include variations of target duration and brightness, reduced temporal predictability (varying the normal fixed 5s default time called inter-trial interval, (ITI), the introduction of white noise distracters interpolated unpredictably in the ITI, changes to the rate of presentation of visual targets (sustained attention), and a number of trials (vigilance paradigm). Recently, (Robinson, 2012) introduced training under unpredictable interreaction time interval to enhance sensitivity to accuracy improvement. The task is constantly being adapted to include possibilities of testing more memory and learning components (Lustig et al., 2013).

Various brain regions have been implicated in a successful performance in the 5-CSRTT. Several studies using excitotoxic agents to lesion-specific areas of the brain have elucidated certain brain regions with direct involvement in the performance of the task. Specifically, the medial PFC appears to be important for attentional processes, particularly the dorsal medial PFC (Muir et al., 1996a; Barbelivien et al., 2001; Passetti et al., 2002), while the lateral medial PFC appears to mediate perseverative behaviors (Passetti et al., 2002). The frontal cortex is important for human attentional performance (Rueckert & Grafman, 1996; Salgado-Pineda et

al., 2003; Ogg et al., 2008) and imaging studies indicate enhanced activation of the frontal lobes during Continuous Performance Test in normal subjects (Salgado-Pineda et al., 2003). Other brain regions that have important significance in the correct performance of the 5-CSRTT include the anterior cingulate cortex, striatum and the thalamus (Muir et al., 1994; 1996a; Robbins, 2002; Chudasama et al., 2003; Chudasama & Robbins, 2004). At the same time, it has been described that excitotoxic lesions of different sectors of the PFC affect the different parameters measured on the task. For instance, accuracy is quite selectively affected by lesions of the dorsal cingulate cortex, whereas more ventral lesions tend to affect measures of response control such as premature responding (Chudasama et al., 2003). In humans, the 5-CSRTT has been used to characterize regional effects of PFC damage (Alexander et al., 2005).

The 5-CSRTT is a behavioral test that captures some of the attentional impairments that may be relevant to schizophrenia. Since it translates to tests of continuous performance frequently employed to evaluate executive performance in patients with schizophrenia in experimental medicine studies, it has several applications. It has been used to study performance in genetically modified mice including a genetic model of AHDH disorder (Davies et al., 2009; Yan et al., 2011) and models of AD (Romberg et al., 2011). Besides, it has also been widely used in pharmaceutical settings and in the evaluation of drug effects relevant to improving sustained attention and controlling impulsivity in preclinical studies, confirming its clinical predictive validity. Actually, Robbins (Robbins, 2002) exhaustively reviewed the literature published until that point. Since then, almost every major pharmacological class of drug has been tested, either systemically or intra-cerebrally. In summary, on accuracy measures, different types of drugs have been reported to enhance performance in the 5-CSRTT. Under often specific protocol conditions, drugs producing enhancement of performance include: D_1R agonist SKF38393 (Granon et al., 2000; Chudasama & Robbins, 2004), the cholinergic agonist donepezil (Romberg et al., 2011), the M_2 muscarinic agonist JWSUSC75IX (Terry et al., 2011), the $\alpha_4\beta_2$ selective nicotinic receptor agonist ABT-594 (Mohler et al., 2010), sertindole (Carli et al., 2011a), aripiprazole (Carli et al., 2011b), clozapine (Amitai et al., 2007), methylphenidate (Paine & Carlezon, 2009; Robinson, 2012), the α_2AR antagonist atipamezole (Jakala et al., 1992; Puumala et al., 1997) and NA reuptake inhibitors such as reboxetine (Liu et al., 2009) and atomoxetine (Robinson, 2012). **Table 1.3** summarizes above mentioned findings.

Table 1.3: Summary table of the most representative drugs that have shown performance changes in the 5-CSRTT.

Drug	Accuracy	Omissions	Premature responses	Latency to correct response
SKF38393 (D_1R agonist)	↑	=	=	↓
Donepezil (cholinergic agonist)	↑	=	=	-
JWSUSC75IX (M_2 muscarinic agonist)	↑	=	=	=
ABT-594 ($\alpha_4\beta_2$ nicotinic receptor agonist)	↑	↓	-	-
Sertindole (antipsychotic)	↑	=	↓	-
Aripiprazole (antipsychotic)	↑	-	-	-
Clozapine (antipsychotic)	↑	=	↓	-
Methylphenidate (NA-DA reuptake inhibitor)	↑	-	-	-
Atomoxetine Reboxetine (NA reuptake inhibitors)	=/↑	-	↓	-
Atipamezole (α_2AR antagonist)	=/↑	-	↑	↓

1.7. α_2-ADRENOCEPTORS IN THE CIAS

1.7.1. α_2-Adrenoceptors: subtypes, distribution, general functions and pharmacology

1.7.1.1. Classification of α_2-adrenoceptor subtypes

This family includes the α_{2A}, $\alpha_{2B,}$ and α_{2C}AR subtypes, which are widely distributed both in the CNS and in the periphery (McCune et al., 1993; Bylund et al., 1994; Scheinin et al., 1994; Nicholas et al., 1996). However, the density of each AR subtype differs greatly in different brain areas and peripheral tissues. Originally, a fourth AR subtype, the α_{2D}, was also described; but today it is accepted that the α_{2A} and the α_{2D}AR subtypes are the same receptors, though they were identified in different species. The α_2ARs are involved in various physiological functions, particularly in the cardiovascular system, as well as in the CNS.

α_2ARs belong to the superfamily of G-protein coupled receptors. They bind to the inhibitory proteins G_i and G_o and decrease AC activity and the formation of cAMP, leading to the inactivation of the PKA. However, α_2ARs also couple to G_s protein and consequently increase AC activity. Indeed, α_2ARs can couple to multiple G-proteins, including G_s (Chabre et al., 1994; Eason et al., 1994; Fraser et al., 1989), G_i and G_o (Cotecchia et al., 1990; Chabre et al., 1994), G_q/G_{11} (Chabre et al., 1994), and G_z (Wong et al., 1992; Jasper et al., 1998). The inhibitory coupling of all three α_2AR subtypes to AC appears to be predominant (Wade et al., 1999), but it depends primarily on the cell type and on the receptor expression level (Chabre et al., 1994). It is suggested that, at low agonist concentration, activation of α_2ARs decreases cellular cAMP level; while at higher agonist concentration, the stimulation of α_2ARs results in increased cAMP level (Jones et al., 1991; Eason et al., 1992; MacDonald et al., 1997; Jasper et al., 1998). The inhibition of the AC is operated via pertussis toxin-sensitive $G_{i/o}$ proteins (Daunt et al., 1997; Pohjanoksa et al., 1997). Other signal transduction systems include inhibition of hyperpolarization/cyclic nucleotide-gated channels (HCNs), G_i protein βγ subunit-mediated activation of inwardly rectifying K^+ currents, phospholipase A_2 stimulation, Na^+/H^+ exchange acceleration or, further on, voltage-dependent Ca^{2+} currents (Bylund et al., 1994; Aantaa et al., 1995; Hieble et al., 1995; Wade et al., 1999).

Heterogeneity of α_2ARs was first characterized by the results obtained with binding assays on human platelets and neonatal rat lung (Timmermans & van Zwieten, 1982). The first two subtypes were defined depending on whether prazosin showed lower and higher affinity, the α_{2A}ARs and the α_{2B}ARs respectively (Bylund, 1985). Later, human colonic cell line (HT29) and neuroblastoma X glioma hybrid cell line (NG108-15) (Bylund et al., 1988) were also shown to have homogeneous α_2AR populations consisting of α_{2A} and α_{2B}AR, respectively (Bylund et al., 1988) and were also present in both human (Petrash & Bylund, 1986) and rat brain (Bylund, 1988). The gene for the α_{2A}AR was cloned from the human (Kobilka et al., 1987) and localized in chromosome 10, while the gene for the α_{2B}AR was in chromosome 2. Actually, antagonists such as ARC239 and BRL41992 (Bylund et al., 1988; Young et al., 1989) showed significant

α_{2B}AR selectivity. Instead, α_{2A}AR selectivity was shown by the partial agonist oxymetazoline, which nevertheless, has a relative affinity for the α_{1A}AR subtype (Lomasney et al., 1991). Analogously, the antagonist BRL44408 and the chloro-derivative BRL44409 were proved to be significantly α_{2A}AR selective (Bylund et al., 1988; Young et al., 1989). The third subtype, α_{2C}AR, was identified through binding studies in the opossum kidney-derived OK cell line (Murphy & Bylund, 1988; Blaxall et al., 1991) and in humans, the coding gene is located on chromosome 4 (Regan et al., 1988). This subtype had also been identified in the human retinoblastoma cell line, Y79 (Gleason & Hieble, 1992). High α_{2C}AR affinity and selectivity were shown by the antagonists BAM1303 and WB4101 (Blaxall et al., 1991). Also, prazosin and ARC239 were endowed with high α_{2C}AR affinity.

The efficacy and ligand selectivity at the α_2ARs could be known by studies of guanosine 5'-O-[gamma-thio]triphosphate ([^{35}S]GTPγS) binding technique, which characterized the intrinsic activity and potency of agonists and antagonists at the cloned mouse $\alpha_{2A/D}$AR and human α_{2A}, α_{2B}, and α_{2C}ARs (Jasper et al., 1998). Adrenaline and NA were full agonists, clonidine and oxymetazoline were partial agonists, and antagonist as rauwolscine did not stimulate [^{35}S]GTPγS incorporation. Although few agonists showed a great deal of selectivity for a single receptor subtype, the rank order of agonist potency compared to NA for selected compounds for the α_{2A}AR was dexmedetomidine > guanabenz > UK-14304 > clonidine > ST-91 > NA. For the α_{2B}-AR, dexmedetomidine > clonidine > guanabenz > NA > ST-91 > UK-14304 and for the α_{2C}AR, dexmedetomidine > NA > UK-14304 > ST-91 ≥ clonidine >> guanabenz. These results are similar to the values reported previously in other functional studies (Eason et al., 1994), suggesting there are very few agonists or antagonist that exhibit subtype selectivity (Jasper et al., 1998).

1.7.1.2. General anatomical distribution of α_2-adrenoceptors

Several studies using different techniques as in situ hybridization, radioligand binding, immunohistochemistry, and electronic microscopy have made possible the identification of the expression pattern and the location of the different α_2AR subtypes.

Different subtypes of α_{2A}AR mRNA have been identified in the periphery. In 1988, the α_{2A}AR subtype was identified in human platelets (Bylund, 1988). Besides, the kidney had transcripts for all three subtypes (Blaxall et al., 1994). More recently, in gastric mucosa of the rat, expression of mRNA of all three subtypes was shown, being the dominant subtype the α_{2A}AR (Gyires et al., 2007). Only one mRNA subtype was detected in several tissues, e.g. aorta and spleen had only α_{2A}AR mRNA, whereas the heart, lung and the liver had only α_{2B}AR mRNA (Makaritsis et al., 1999). All other tissues had two α_2AR subtype transcripts present. In contrast to the rat CNS, which contains predominantly α_{2A} and α_{2C}AR mRNA with little α_{2B}AR mRNA, peripheral tissues contain predominantly α_{2A} and α_{2B}AR mRNA with little α_{2C}AR mRNA (Blaxall et al., 1994).

In addition, α_2AR subtypes are expressed in high densities in the CNS, including several layers of the frontal cortex, cerebellum, striatum, hippocampus, hypothalamus, caudate nucleus, and LC, among other regions (Pazos et al., 1988; Pascual et al., 1992).

1.7.1.3. General functions of the α_2-adrenoceptor subtypes

In general, in the CNS, α-ARs participate in several functions. Both the pharmacological analysis and the studies with genetic alteration of α_2ARs indicate that most of the classical effects of α_2AR agonists are likely to be mediated by the α_{2A}AR subtype (Gyires et al., 2009). Concretely, the antihypertensive and bradycardic effects of the α_2AR agonists were found to be dependent entirely on α_{2A}-AR subtype (MacMillan et al., 1996; Altman et al., 1999). Actually, the α_{2A}AR may be the dominant mediator of hypotensive responses (Civantos Calzada & Aleixandre de Artinano, 2001), although it has been suggested that the α_{2B}AR may also play an important role in these processes. In addition, the α_{2A}AR plays an important role in memory (Arnsten et al., 1999), cortical arousal and attention levels (Timmermans & van Zwieten, 1982; Puolivali et al., 2002), sedation processes (Hunter et al., 1997), motor control responses (Millan et al., 1994), antinociception and thermogenic responses (Bylund, 1985; Millan et al., 1994; Hunter et al., 1997) and analgesia (Hunter et al., 1997; Lakhlani et al., 1997). Apart from that, α_{2A}AR seems to participate significantly in thrombus stabilization (Pozgajova et al., 2006) and anti/proconvulsive effects of α_2AR agonists (Szot et al., 2004). The α_{2A}ARs located presynaptically also seems to modulate several responses in the gastrointestinal tract, including the gastric acid secretion (Blandizzi et al., 1995; Mullner et al., 2001; 2002) or even gastric motility, at least in part, (Gyires et al., 2009; Mullner et al., 2002) in the rat.

There are relatively fewer functions described for the α_{2B}ARs. It appears to play a basic role in eliciting the vasoconstrictor response to α_2AR agonists along with the α_{2A}AR (Link et al., 1996) and in salt-induced hypertension (Makaritsis et al., 1999). Moreover, α_{2B}ARs mediate predominantly contraction of rat pregnant uterus in contrast with α_{2A}- and α_{2C}ARs, which inhibit the contractile response. Centrally located α_{2B}/α_{2C}-like ARs are supposed to mediate the gastroprotective action of α_2AR stimulants (Gyires et al., 2007). Also, α_{2B}ARs seem to participate in the placenta angiogenesis (Kable et al., 2000) and may have a developmental or reproductive role since homozygous α_{2B}-KO mice do not breed well (Link et al., 1996).

The α_{2C}ARs may mediate some peripheral actions too. They are supposed to have a minor role in hypothermic action of α_2AR agonists in mice (Sallinen et al., 1997), and they may participate along with the other α_2ARs, in sexual incentive motivation (Chu & Agmo, 2016). It has also been described its role in motor behavior and memory processes along with the α_{2A}AR (Bjorklund et al., 1999; Tanila et al., 1999). They also seem to mediate venous vasoconstriction (Gavin et al., 1997), and this subtype located in the arterial smooth muscle is likely to be involved in the cold-induced enhancement of α_2AR-induced vasoconstriction (Chotani et al., 2000). In addition, it participates in the endocrine secretion processes along with the α_{2A}AR (Peterhoff et al., 2003). Moreover, it appears to be involved in other CNS processes such as startle reflex, stress response, locomotion and feedback inhibition of adrenal catecholamine release (Kable et al., 2000).

1.7.1.4. Selective compounds of α_2-adrenoceptor subtypes

Although studies on the expression of α_2AR subtypes can reveal where α_2ARs are expressed, it is difficult to establish with total reliability the specific physiological functions related to each of the different α_2AR subtypes. Actually, there is a lack of selective ligands of these α_2AR subtypes, of which only a few have become available for preclinical investigation during the last decade (Crassous et al., 2007; Sallinen et al., 2007; 2013a; Uys et al., 2016). Functional analysis of the role of α_2AR subtypes is based particularly on pharmacological studies. However, the resolution of the functions specific to each α_2AR subtype has been difficult due to the scarcity of sufficiently selective pharmacological tools.

Despite all this, both agonists and antagonists for different α_2AR subtypes have been developed (**Table 1.4**). Clonidine was described as a non-selective α_2AR agonist (Jasper et al., 1998; Li et al., 1999); oxymetazoline, dexmedetomidine, and specially guanfacine were reported to be selective for the α_{2A}AR subtype (Arnsten et al., 1988; Uhlen & Wikberg, 1991; Yoshito Takano, et al., 1992; Uhlen et al., 1995; Ramos & Arnsten, 2007; Wang et al., 2007; Kawaura et al., 2014). ST-91 (2-(2,6-diethylphenylamino)-2-imidazoline hydrochloride), a clonidine analogous, was described as an $\alpha_{2B/C}$AR preferring agonist (Yoshito Takano, et al., 1992), but with certain extra α_{2A}AR agonism (Nazarian et al., 2008). Regarding antagonists, BRL44408 and BLR48962 were selective for the α_{2A}ARs. WB4104, imiloxan, prazosin, and ARC-239 (besides their α_1AR blocking property) were selective for $\alpha_{2B/C}$, while MK-912, rauwolscine, ORM-13070, ORM-10921, and specially JP-1302 showed higher affinity for α_{2C}AR subtypes (Yoshito Takano, et al., 1992; Meana et al., 1996; Civantos Calzada & Aleixandre de Artinano, 2001; Sallinen et al., 2007; 2013a; Uys et al., 2016; Savolainen et al., 2019). Also, yohimbine, atipamezole, and idazoxan probed to be non-selective α_{2A} and $\alpha_{2B/C}$AR antagonists (Winter & Rabin, 1992; Odagaki & Toyoshima, 2008). (For an extensive review, see Civantos Calzada and Aleixandre de Artiñano, 2001).

In addition, the α_2AR subtype selective/preferring agonists and antagonists can bind to other non-adrenergic receptors as well. For instance, binding studies suggested multiple receptor actions of oxymetazoline, probing to be a full and potent agonist at 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D}Rs and a partial agonist at 5-HT_{1C}Rs (Schoeffter & Hoyer, 1991). In addition, the nonselective α_2AR antagonists yohimbine, idazoxan and rauwolscine, the $\alpha_{2B/C}$AR subtype preferring antagonist ARC-239, and the $\alpha_{2A/D}$AR antagonist BRL-44408 can also recognize 5-HT_{1A}Rs (Winter & Rabin, 1992; Meana et al., 1996; Odagaki & Toyoshima, 2008). In contrast, atipamezole, another non-selective α_2AR antagonist, showed an only negligible affinity for 5-HT_{1A}Rs (Winter & Rabin, 1992) (**Table 1.4**).

It should be remembered that in any case, the selectivity of the different ligands *in vivo* is always lower than that shown by the different compounds in binding studies. Therefore functional studies have not permitted to establish a clear differentiation of the function mediated by each α_2AR subtype (Civantos Calzada & Aleixandre de Artinano, 2001). Hence, the possibility of identification of separate physiological roles for different α_2ARs subtypes could improve the design of novel compounds for specific therapeutic goals (Gyires et al., 2009).

Table 1.4: Selective ligands of α_2AR subtypes and additional binding sites.

Compound	Preference for α-AR subtype	Recognition of additional receptors	References
Clonidine	$\alpha_{2A/B/C}$ Agonist		Jasper et al., 1998; Li et al., 1999
Oxymetazoline Dexmedetomidine Guanfacine	α_{2A} Agonist	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}. Partial 5-HT_{1C} (Oxymetazoline)	Arnsten et al., 1988b; Uhlen & Wikberg, 1991;Takano, et al., 1992; Uhlen et al., 1995; Ramos & Arnsten, 2007; Wang et al., 2007; Kawaura et al., 2014; Schoeffter & Hoyer, 1991
ST-91	$\alpha_{2B/C}$ Agonist	α_{2A}	Takano, et al., 1992 Nazarian et al., 2008
BRL44408 BLR48962	α_{2A} Antagonist	5-HT_{1A}(BRL-44408)	Winter & Rabin, 1992; Meana et al., 1996; Odagaki & Toyoshima, 2008
WB-4104, Imiloxan, Prazosin, ARC-239	$\alpha_{2B/C}$ Antagonist	α_1 5-HT_{1A}(ARC-239)	Winter & Rabin, 1992; Meana et al., 1996; Odagaki & Toyoshima, 2008
MK-912, Rauwolscine, ORM-13070, ORM-10921 JP-1302	α_{2C} Antagonist	5-HT_{1A} (Rauwolscine)	Takano, et al., 1992; Civantos Calzada & Aleixandre de Artinano, 2001; Sallinen et al., 2007; Sallinen et al., 2013; Uys et al., 2016; Savolainen et al., 2019)
Yohimbine, Atipamezole Idazoxan	$\alpha_{2A/B/C}$ Antagonist	5-HT_{1A}	Winter & Rabin, 1992; Odagaki & Toyoshima, 2008

1.7.2. α_2-Adrenoceptor subtypes: location and functions in CNS

1.7.2.1. Regional and cellular distribution in the CNS

In the CNS, approximately 90% of CNS α_2ARs are contributed by the α_{2A}AR (Bucheler et al., 2002) and the majority of α_2ARs are postsynaptic (U'Prichard et al., 1979; Pazos, 1988; Wang et al., 1996; Arnsten et al., 2007; Castelli et al., 2016).

In rat, mRNA coding for the α_{2A}AR was found throughout all the brain, especially in the LC but also in the brainstem, cerebral cortex, septum, hypothalamus, hippocampus and amygdala (Sastre & Garcia-Sevilla, 1994; Scheinin et al., 1994; MacDonald & Scheinin, 1995; Pazos, 1988; Lee et al., 1998). α_{2A}AR mRNA is also highly expressed in the DA cells of the VTA and in their axons projecting to the PFC, controlling the mesocortical DA neuron function (Castelli et al., 2016). Likewise, in the raphe nuclei, autoradiography and immunohistochemical studies have also revealed the presence of α_{2A}AR (Unnerstall et al., 1984; Rosin et al., 1996; Talley et al., 1996; Scheibner et al., 2001a). Nevertheless, there are some discrepancies about mRNAs encoding α_{2A}AR in the raphe nuclei. Some studies have not found significant density of mRNAs (McCune et al., 1993; Nicholas et al., 1993), therefore suggesting that the majority of α_2ARs in the raphe nuclei are located in NA terminals functioning as autoreceptors and controlling the release of NA and its effects on 5-HT neurons of this nucleus, while others have reported its

existence (Scheinin et al., 1994). In the rat LC, they are located in catecholaminergic dendrites as well as in astrocytes; therefore they may serve both pre- and postsynaptic functions in this region (Lee et al., 1998). Besides, immunoreactivity for the protein was detected in other rat brain regions like the basal forebrain, nucleus accumbens, diagonal band, and various nuclei of the amygdala (Talley et al., 1996). In the PFC of monkeys, immunoreactivity for the α_{2A}AR was also demonstrated in the cellular soma, dendrites and axon terminals (Aoki et al., 1998). The α_{2A}AR subtype is the most prevalent in the monkey PFC, subtype that has been found both presynaptically on NA terminals, and postsynaptically on dendritic spines of PFC pyramidal cells that receive network input (Aoki et al., 1998; Wang et al., 2007).

The α_{2C}AR subtype has a unique distribution in the brain relative to other ARs. In rodent studies, it has been observed in fewer areas than α_{2A}AR subtype, constituting approximately 10% of the total population of α_2ARs. In situ hybridization studies in rats revealed mRNA of the α_{2C}AR in the olfactory tubercle, cerebral cortex, amygdala and hippocampus and dorsal raphe (McCune et al., 1993). Also, binding and immunohistochemistry studies in rats have revealed that, compared to other brain regions, is most abundantly expressed in the ventral and dorsal striatum, where it is suggested to modulate presynaptic DA release and DA-mediated behaviors. Indeed, the high concentration of mRNA and protein of the α_{2C}AR in the striatum is peculiar given the scarcity of noradrenergic innervations to this brain region. Immunoreactivity for α_{2C}AR was found also in the LC, in the raphe nuclei, in the substantia nigra and in the VTA (Rosin et al., 1996; Scheibner et al., 2001a). On the contrary, the cerebellum is devoid of these receptors. Apart from that, in situ hybridization and autoradiographic studies have also revealed the presence of α_{2C}ARs in the hippocampus in humans, monkeys and rodents (Scheinin et al., 1994; MacDonald et al., 1997; Fagerholm et al., 2008; Bucheler et al., 2002; Finnema et al., 2014).

Regarding α_{2B}ARs, there are confusing data published in relation to its distribution in the CNS. According to MacDonald and Scheinin, the mRNA for α_{2B}ARs can be found only in the thalamus (MacDonald & Scheinin, 1995). However, other studies using in situ hybridization on rat brain showed the presence of the α_{2B}AR mRNA in other brain areas such as the cerebellum, cortex, and hypothalamus (Tavares et al., 1996) and apparently is found mainly postsynaptically (Docherty, 1998; Philipp et al., 2002). Other radioligand binding assays with AGN-209419, a more potent and selective α_{2B}AR antagonist, have reported that the highest density of α_{2B}ARs is found in the thalamus and cortex (Luhrs et al., 2016). In addition, its presence in the spinal cord has also been described, where it seems to mediate the analgesic effect of nitrous oxide (Sawamura et al., 2000). In the human brain, nevertheless, it was found also in striatum and globus pallidus, in contrast with the α_{2A}AR, that was detected in most brain regions (De Vos et al., 1992).

Comparing between rat and mouse brain, the distribution pattern of α_2ARs in the CNS is similar, but it is not so with the amount of expressed receptor. Thus, both in rat and mouse brain, the α_{2A}ARs are highly distributed in LC and cerebral cortex (layers III/IV) (Pazos, 1988; Wang et al., 1996). Autoradiography and radioligand binding experiments indicated that there is a 10-fold higher expression of α_{2A}AR than $\alpha_{2B/C}$AR in mouse brain compared to a higher

expression of α_{2C}AR than α_{2A}AR in rat brain (Link et al., 1992; Wang et al., 1996; Hein et al., 1999; Bunemann et al., 2001).

In humans, the α_{2A}ARs are the ones that are most expressed in PFC along with the α_{2C}AR, but the α_{2A}ARs could represent approximately the 87% of all the α_2ARs (Ordway et al., 1993; Sastre & Garcia-Sevilla, 1994; Grijalba et al., 1996). Besides, in another study in human brain, Erdozain and colleagues (Erdozain et al., 2018a) observed that in the human PFC the great majority of α_{2A}ARs are located postsynaptically (95%) while α_{2C}AR subtype would be distributed more homogeneously in both pre- and postsynaptic locations (60% *vs*. 40%, respectively). That means that the presynaptic α_2AR population could represent approximately 12% of the total α_2AR population, with a similar proportion of α_{2A}AR and α_{2C}AR subtype (4% and 8%, respectively). By contrast, the majority of α_2AR populations would be located postsynaptically (88% of total α_2AR), most of them belonging to the α_{2A}AR subtype (83% α_{2A}AR *vs*. 5% α_{2C}AR of total α_2ARs).

1.7.2.2. The functionality of α_2-adrenoceptors in the CNS: pre *vs.* postsynaptic effect

The α_2ARs located presynaptically in noradrenergic terminals have a dominant role in the inhibition of neurotransmitter release (Starke, 1987; Miller, 1998). It has been described by *in vivo* studies that local administration of α_2AR agonists in terminal noradrenergic areas produces an inhibitory effect on neurotransmitter release at this level (van Veldhuizen et al., 1993; Dalley & Stanford, 1995). It has also been shown that the administration of α_2AR antagonist causes an increase in the extracellular concentration of NA in these areas (Dennis et al., 1987; Thomas & Holman, 1991; van Veldhuizen et al., 1993; Dalley & Stanford, 1995; Wortley et al., 1999). This data indicates that α_2ARs exert an inhibitory tonic effect on NA concentrations.

The presynaptic α_2AR autoreceptor plays an important role in the negative feedback of NA synthesis and release; likewise, presynaptic α_2AR heteroreceptors located on dopaminergic, serotoninergic, glutamatergic, and other terminals regulate the release of these neurotransmitters (Scheibner et al., 2001a; Philipp et al., 2002; Bucheler et al., 2002; Cottingham & Wang, 2012). The α_{2A}AR subtype has a dominant role in the inhibition of neurotransmitter release when located presynaptically, but the α_{2C}AR subtype contributes especially to this function too. In fact, apart from NA, α_{2A} and α_{2C}ARs were shown to be implicated in the inhibition of the release of DA and 5-HT in the CNS (Sallinen et al., 1997; Hein et al., 1999; Bucheler et al., 2002; Trendelenburg et al., 2003). Actually, it has been described by several *in vitro* studies that α_2ARs located on serotonergic terminals inhibit the release of 5-HT (Frankhuyzen & Mulder, 1980; Gothert et al., 1981; Maura et al., 1982; Raiteri et al., 1983; Limberger et al., 1986). Also *in vivo*, local or systemic administration of the agonist clonidine in areas such as the hippocampus or the PFC, showed that α_2ARs exert an inhibitory control on the release of 5-HT in terminal areas (Yoshioka et al., 1992; Tao & Hjorth, 1992; Numazawa et al., 1995; Bel & Artigas, 1996; Mongeau et al., 1997).

Regarding DA, there are several studies claiming that the α_{2C}AR subtype is the main responsible of DA modulation in the striatum. In one study by Zhang and colleagues (Zhang et al., 1999), using antisense oligonucleotide infusions directly into rat striatum, they found that α_{2C}AR is negatively coupled to AC and that this subtype appears to be tonically activated in striatal slices in absence of an exogenously added agonist. In another study using a specific and potent α_{2C}AR antagonist ORM-10921, Sallinen and cols. (Sallinen et al., 2013a) confirmed that DA has a significant role in activating striatal α_{2C}ARs, and it is suggested to modulate DA-mediated behaviors (Bucheler et al., 2002; Sallinen et al., 2013a). Besides, the α_{2C}ARs located in the VTA seem to participate in the modulation of the dopaminergic projections innervating cortical structures and ventral forebrain (mesocorticolimbic pathways) (Rosin et al., 1996; Inyushin et al., 2010). Binding experiments have reported some data including that DA had an affinity for the α_{2C}AR that was 18-fold higher than for the α_{2A}AR.

As previously commented, there are also α_2ARs located at the somatodendritic level in LC. These receptors regulate the electrical activity of NA neurons in the LC (Cedarbaum & Aghajanian, 1978) and therefore modulate NA concentrations in terminal areas of these axons projections (Florin-Lechner et al., 1996). Indeed, it has been proven that the local administration in the LC of α_2AR agonists inhibit the electrical activity of these neurons (Svensson et al., 1975) producing a decrease in the extracellular NA concentration in terminal areas, such as the cingulate cortex (Van Gaalen et al., 1997; Mateo & Meana, 1999), and that the administration of α_2AR antagonists induces an increase in the electrical activity and the extracellular concentration of NA in their projection areas (Cedarbaum & Aghajanian, 1978; Mateo & Meana, 1999; Pudovkina et al., 2001). It has been proposed that this inhibitory effect in the electrical activity of LC neurons is mediated predominantly by the α_{2A}AR (Dennis et al., 1987; Mateo et al., 1998; Callado & Stamford, 1999; Wortley et al., 1999; Fernández-Pastor & Meana, 2002; Ortega et al., 2010). Furthermore, *in vivo* microdialysis and voltammetric techniques have shown that somatodendritic α_2ARs also modulate the release of NA, and equally to terminal areas, the α_{2A}AR subtype seems to be the main responsible in the somatodendritic inhibition of NA release (Callado & Stamford, 1999; Fernández-Pastor & Meana, 2002; Fernández-Pastor, 2003; Ortega et al., 2010).

Postsynaptic activation of α_2ARs, in turn, modulates neuronal excitability *via* regulation of ion channels, including the direct modulation of K^+ voltage-gated channels (KCNQs) and the indirect modulation of HCN channels (Cottingham & Wang, 2012). Specifically, α_{2A}ARs located postsynaptically are likely to increase regional blood flow in PFC and may mediate the beneficial effects of α_{2A}AR agonists in attention deficit hyperactivity disorder (ADHD) (Cho et al., 2008b; Levy, 2008).

NA has the highest affinity for the α_2ARs compared to the α_1ARs and β-ARs (Ramos & Arnsten, 2007)**.** Since α_2ARs can be located both pre- and postsynaptically, their resulting effects will depend on where the mediating receptor is. For instance, α_2AR agonists will enhance noradrenergic function if acting postsynaptically; but it will reduce it if acting at either presynaptic autoreceptors or coeruleal α_2ARs; in this last case by the attenuation of the LC firing (**Figure 1.19**). At low doses of agonist, the presynaptic receptors are stimulated; while at

higher doses postsynaptic actions are activated (Arnsten & Goldman-Rakic, 1985; Arnsten et al., 1988; Arnsten & Cai, 1993). As an example, low doses of clonidine, a non-selective α_2AR agonist, appears to act presynaptically; since such doses produced decreases in NA cell firing of the LC (Svensson et al., 1975), attenuating its release from terminals (Ong et al., 1991). Conversely, low doses of yohimbine, an α_2AR antagonist, facilitated the release of NA from terminals (Ong et al., 1991; Arnsten & Cai, 1993).

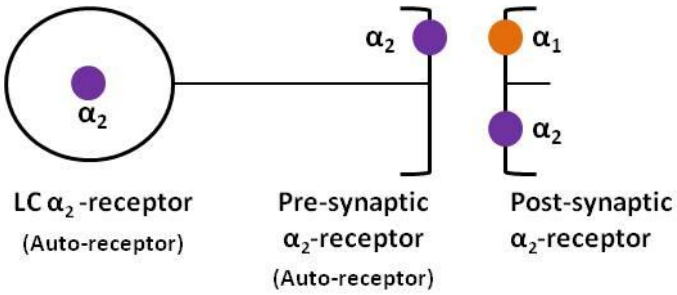

Figure 1.19: Schematic representation of pre- and postsynaptic α_2ARs. Modified from *Coull, 1994.*

As a result of the scarcity of selective ligands of these α_2AR subtypes, of which only a few have become available for preclinical investigation during the last decade (Sallinen et al., 2007; Crassous et al., 2007; Sallinen et al., 2013a; Uys et al., 2016), transgenic mouse models have predominantly been used to shed light on the physiology and pharmacology of the different α_2AR subtypes. Transgenic mouse models employ targeted genetic deletion or overexpression principally of the α_{2A}AR and/or α_{2C}AR to examine the consequence of loss or gain of receptor function (Scheinin et al., 2001). Findings from these transgenic mouse models have suggested distinct and often apparently opposing roles for the α_{2A}AR and α_{2C}AR. Therefore, this problem makes clear the necessity to develop selective ligands for each receptor subtype.

There have been several studies evaluating the different roles of α_2AR subtypes using KO mice. In relation to monoamine synthesis and catabolism, using different α_2AR-KO mice it was found that deletion of α_{2A} and α_{2C}ARs was accompanied by an increased tissue concentration of L-DOPA, DA, and NA compared with WT mice. Further, α_{2A}AR-KO mice presented higher concentration of NA and DA compared with α_{2C}AR-KO mice. Therefore, this data could suggest that the α_{2A}AR may be main α_2AR subtype responsible for the synthesis of NA and DA (Vieira-Coelho et al., 2009). However, there have been some discrepancies concerning this issue. Lahdesmaki and cols. measured monoamine and their metabolites concentrations in brain sections of α_{2A}AR-KO mice. They reported increased NA turnover with augmented MHPG concentrations and increased MHPG/NA ratio in the cortex, striatum, hippocampus, and hypothalamus (Lahdesmaki et al., 2002). However, they did not find any differences between α_{2A}AR-KO and WT mice in the concentrations of DA, 5-HT or their metabolites.

As for monoamine synthesis, Bucheller and cols. (Bucheler et al., 2002) described that both α_{2A} and α_{2C}AR act inhibiting the presynaptic release of NA and DA, in agreement with other studies

that reported these receptor roles in inhibiting NA, DA and other neurotransmitters release such as 5-HT, or acetylcholine (Starke et al., 1989). Concretely, in experiments using α_{2A}AR and α_{2C}AR-KO mice they showed that similar to α_{2A}AR regulating NA release in the brain cortex, DA release from basal ganglia is modulated by both α_{2A} and α_{2C}ARs. This data highlight the importance of the α_{2C}AR as an inhibitory heteroreceptor along with the α_{2A}AR, even if previous studies have shown that the α_{2A}AR is the principal AR subtype involved in the control of DA (Trendelenburg et al., 1994). Bucheler and cols. also showed that the α_{2A}AR operates at a higher speed than the α_{2C}AR in that catecholaminergic release inhibition. Thus, all these data suggest that α_2AR stimulation by DA may contribute to presynaptic regulation of DA exocytosis. As for 5-HT, mice lacking the α_{2A}AR, α_{2C}AR or both α_{2A}/α_{2C}AR showed that in hippocampal and cortical serotonergic nerve terminals, both α_{2A}-ARs and α_{2C}-ARs account for the α_2AR-mediated inhibition of 5-HT release (Scheibner et al., 2001a). Authors of this study also demonstrated that α_{2C}AR-KO mice present lower disinhibition of agonist-induced 5-HT release in hippocampal and occipitoparietal cortex slices compared to α_{2A}AR-KO mice, suggesting the α_{2A}AR as the main α_2AR regulating the 5-HT release and possibly 5-HT synthesis. Furthermore, previous work using mice with altered α_{2C}AR expression indicated a modulatory effect of the α_{2C}AR subtype on *in vivo* turnover of DA and 5-HT, but not NA (Sallinen et al., 1999).

Behavioral studies with α_{2A}AR-KO mice have shown an anxiogenic phenotype in these mice as well as behavioral abnormalities such as altered control of the diurnal pattern of activity, motor coordination impairment, decreased locomotor response to a novel environment, increased immobility in the forced swimming test (FST), and increased PPI levels (Lahdesmaki et al., 2002; Sallinen et al., 2007). Also, the sedative effect of α_2AR agonists is likely to be mediated dominantly by the α_{2A}AR subtype, since no sedative response was developed to the α_{2A}AR agonist dexmedetomidine in α_{2A}AR-KO mice, but it was maintained in the α_{2B} and α_{2C}AR-KO mice (Hunter et al., 1997). Actually, potent hypotensive actions are associated with a relatively higher affinity for α_{2B} and α_{2C}ARs (Arnsten et al., 1988; Arnsten & Leslie, 1991). These data seem consistent with the presence of α_{2B}AR mRNA in the thalamus or the α_{2C}AR mRNA in the nucleus tractus solitarius, the brain regions considered to underlie many of the sedative (Buzsaki et al., 1991) and hypotensive (Reis et al., 1984) actions of α_2AR agonist treatment, respectively.

Even if there is little evidence for the α_{2B}AR function in the CNS, recent data is suggesting the α_{2B}AR involvement compulsivity. Studies with α_{2B}AR-KO mice showed an increased marble-burying behavior than WT mice and enhanced amphetamine-induced stereotyped behavior at lower doses than either α_{2A}AR nor α_{2C}AR-KO mice. Moreover, they exhibited altered locomotor behaviors compared to WT mice, although unaltered PI (Luhrs et al., 2016). In addition, when the selective α_{2B}AR antagonist AGN-209419 combined with amphetamine was administered to α_{2C}AR-KO mice, a marked increase in stereotypy was observed. Therefore, this evidence suggests a compulsive phenotype for the α_{2B}AR-KO mice and a potential role of these receptors in the filtering of incoming sensory information or outgoing motor responses.

In the same way, α_{2C}AR also appears to be related to the modulation of NA-associated behaviors. It seems that this receptor plays a role in motor behavior and possibly in memory processes (Bjorklund et al., 1999; Tanila et al., 1999). It has even been suggested a possible association between the α_{2C}AR subtype and stress-dependent depression (Sallinen et al., 1998; Tanila et al., 1999). In fact, experimental data showed that lack of α_{2C}AR expression was associated with increased amphetamine-induced locomotor activity, startle reactivity, aggression, and activity in the FST (Sallinen et al., 1998; Scheinin et al., 2001; Sallinen et al., 2007). Besides, activation of α_{2C}AR disrupted the execution of spatial and non-spatial search patterns in mice (Bjorklund et al., 2001). This data could suggest that this receptor blockade could be useful for treating stress-related psychiatric disorders (Sallinen et al., 1999). Moreover, an association between the polymorphism of α_{2C}AR gene and ADHD has been suggested (Cho et al., 2008a).

1.7.3. α_2-Adrenoceptors and regulation of PFC activity

The PFC is the most recently evolved region of the brain, subserving our highest cognitive abilities. The cellular networks in the PFC maintain representations of goals and rules and use remembered information to guide attention, actions, and emotion (Arnsten & Pliszka, 2011). It regulates behavior, thought, and emotion using WM (Arnsten & Jin, 2012). The effect of catecholamines (NA especially, but also DA) on arousal, mood, and behavior in the PFC are mediated through interactions with an extensive range of receptors. The α_2ARs play an outstanding role in the functioning of the PFC and mediate the effect of normal, aroused, and stressed NA levels on memory and other cognitive processes (Berridge & Spencer, 2016).

1.7.3.1. Signaling pathways

Pyramidal cells in the PFC are thought to be key processing elements in neuronal networks responsible for complex executive functions such as WM. Through their recurrent synaptic connections, these networks are thought to hold information online in order to guide future behavior (Fuster, 1997; Durstewitz et al., 2000). For this, the suppression of other irrelevant stimuli that may interfere with the active maintenance of this memory trace is required. The ability to maintain such sustained attention is critically dependent on the proper functioning of NA afferents to the PFC (Aston-Jones & Cohen, 2005). The effectiveness of the PFC network connections for these processes relies on noradrenergic stimulation of α_{2A}ARs of this PFC pyramidal cells (Wang et al., 2007). Based on recordings from behaving primates, it has been postulated that NA acts to enhance the signal/noise ratio of PFC neuronal firing during WM tasks by either increasing task-related activity (Li et al., 1999; Wang et al., 2007), or decreasing background activity (Sawaguchi et al., 1990).

Many of the α_{2A}ARs are localized on the dendritic spines of pyramidal neurons, close to HCN channels that control the impact of synaptic inputs on the spine (Wang et al., 2007). HCN channels are integral membrane proteins that serve as nonselective voltage-gated cation channels. They are activated by membrane hyperpolarization, are permeable to Na^+ and K^+, and are constitutively open at voltages near the resting membrane potential. In many cases,

activation is facilitated by direct interaction with cyclic nucleotides, particularly cAMP. The cation current through HCN channels is known as I(h); the opening of HCN channels elicits membrane depolarization toward the threshold for action potential generation, reduces membrane resistance and therefore, the magnitude of excitatory and inhibitory postsynaptic potentials (Benarroch, 2013). HCN channels are known to play important roles in regulating resting membrane potential, synaptic integration and synaptic plasticity (Pape, 1996; Magee, 1999; Nolan et al., 2004; Day et al., 2005).

Given the importance of HCN channels in shaping neural activity, it is not surprising that they are the target of many neuromodulatory systems including DA (Jiang et al., 1993; Rosenkranz & Johnston, 2006), 5-HT (Bobker & Williams, 1989; Pape & McCormick, 1989), and NA (Parkis & Berger, 1997; Yagi & Sumino, 1998). HCN channels are predominantly expressed within the dendrites of pyramidal neurons where they influence the temporal integration of distally generated synaptic inputs (Magee, 1998; Berger et al., 2001; Day et al., 2005). When HCN channels are open, nearby synaptic inputs are diverted and the incoming information escapes, weakening the synaptic connection (**Figure 1.20, Left panel**). Conversely, noradrenergic stimulation of α_{2A}ARs initiates a cascade of chemical events that close these ion channels, strengthening the synaptic connection. These strengthened synaptic connections enable the PFC to regulate more effectively attention, behavior, and emotion (**Figure 1.20, Right panel**). The α_2AR-mediated inhibition of HCN channels produces a hyperpolarization of the resting membrane potential, but significant enhancement in the temporal integration of distally evoked excitatory postsynaptic potentials. The next effect is the suppression of isolated excitatory inputs while enhancing the response to a coherent burst of synaptic activity.

Regarding the mechanism through which α_2AR activation leads to the inhibition of HCN channels, there are different possible explanations. One hypothesis suggests that, as α_2ARs are classically coupled to inhibition of AC via $G_{i/o}$, and since it is known that HCN channels are sensitive to intracellular levels of cAMP, a reduction in cAMP resulting from the inhibition of AC via $G_{i/o}$ could result in a hyperpolarizing shift in activation voltage dependence (Robinson & Siegelbaum, 2003). However, not all studies have supported this first hypothesis (Carr et al., 2007). Actually, a combination of voltage and current clamp studies in acute PFC slices demonstrated that the effects of α_2AR activation by the α_2AR agonist clonidine is mediated by the inhibition of HCN channels through a PLC–PKC linked signaling cascade (Carr et al., 2007). Indeed, HCN currents have also been reported to be inhibited by receptors coupled to increased phosphoinositide turnover and stimulation of PKC (Cathala & Paupardin-Tritsch, 1997). Although α_2ARs are classically coupled to inhibition of AC via $G_{i/o}$, they have also been linked to increased phosphoinositide turnover via $G_{\beta\gamma}$ stimulation of PLC and subsequent activation of PKC (Boehm et al., 1996; Talaia et al., 2006). Besides, supporting this second hypothesis, the effects of clonidine in current-clamp are prevented by either intracellular perfusion with the PLC inhibitor U73122 or pretreatment with the PKC inhibitors chelerythrine chloride or calphostin C (Carr et al., 2007). Further, iontophoresis of an HCN channel blocker ZD7288 enhanced the delay-related firing of PFC neurons during a WM task, similar to the application of the α_2AR agonist guanfacine. In addition, the improvement in WM performance produced by intra-PFC administration of α_2AR agonists (Franowicz & Arnsten, 1999) could be

mimicked by either intra-PFC injections of ZD7288 or RNA_i induced down-regulation of HCN_1 subunit expression (Wang et al., 2007).

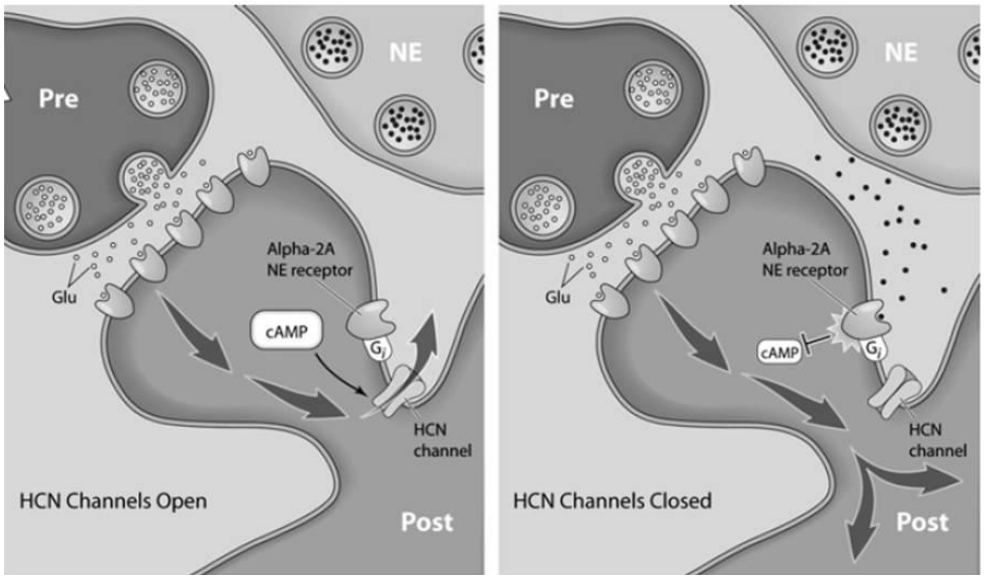

Figure 1.20: Stimulation of postsynaptic α_{2A}ARs on PFC neurons strengthens the functional connections between PFC neurons. **Left panel**: when there is no α_{2A}AR stimulation, cAMP levels are high and HCN channels are open, weakening nearby synaptic inputs and resulting in a decrease in PFC network firing and functionality. **Right panel**: when there is α_{2A}AR stimulation by NA or an agonist, HCN channels are closed increasing the efficacy of network inputs, thus facilitating PFC function. From *Wang et al., 2007.*

1.7.3.2. Neurochemical and physiological functions

For the proper functioning of the PFC, it is important to highlight the relevance of an adequate catecholamine concentration. Indeed, NA and DA release in the PFC is related to arousal state. Low arousal conditions are associated with very low levels of NA cell firing (Foote et al., 1980; Aston-Jones et al., 1999). In contrast, under conditions of alert interest, there is moderate tonic firing with increased phasic firing of NA and DA to relevant stimuli (Finlay et al., 1995; Schultz, 1998; Aston-Jones et al., 1999). Furthermore, under stressful conditions, there are high levels of catecholamine release in PFC (Finlay et al., 1995), which may be the result of the tonic firing of NA neurons (Aston-Jones & Bloom, 1981; Aston-Jones et al., 1999), and DA neurons that respond to aversive events (Matsumoto & Hikosaka, 2009). Thus, the level and timing of catecholamine release in PFC can coordinate the arousal state and PFC function (**Figure 1.21**).

In fact, PFC function results impaired by excessive NA release. High levels of NA release, such as those during a stressful condition, engage α_1ARs that suppress PFC cell firing (Birnbaum et al., 2004). Intracellular recordings from slices of rat PFC neurons suggest that NA may deteriorate PFC function acting at α_1ARs, increasing “noise” on the dendritic stem (Marek &

Aghajanian, 1999). These studies have shown that stimulation of α_1ARs increases non-specific glutamate release from thalamic terminals, which in turn augments excitatory postsynaptic Na^+ currents in the proximal dendritic stem of PFC pyramidal neurons. This increase in background noise may obstruct signal transmission from the dendrite to the soma, therefore impairing PFC function. Furthermore, 5-HT mechanisms may also contribute to this response. Marek and Aghajanian have shown that 5-HT_{2A}R stimulation in the PFC, like α_1AR stimulation, can increase glutamate release and excitatory postsynaptic potentials in the dendritic stem (Marek & Aghajanian, 1999).

Complementary to NA, DA also plays an important role in the PFC, decreasing PFC neuronal activity in response to irrelevant stimuli (Vijayraghavan et al., 2007). In fact, it has been shown that α_2AR agonists and antagonists administered systemically or locally perfused into the PFC markedly decrease and profoundly increase, respectively, DA release in the PFC (Devoto et al., 2001). Besides, there are other mechanisms that may participate in controlling extracellular DA levels in the PFC, namely the NET (Yamamoto & Novotney, 1998), and DA coreleased from noradrenergic terminals (Devoto et al., 2001). In this case, dopaminergic stimulation of D_1Rs opens HCN channels on a set of dendritic spines that receive inputs irrelevant to focused WM and attention. Actually, it is known that D_1Rs and HCN channels colocalize and spatially interact at dendritic spines in monkey layer III dorsolateral PFC (Gamo et al., 2015). As commented previously, the opening of these channels reduces "noisy" input to the neuron and weakens irrelevant network connections, thus enhancing the efficiency of PFC function. It has been demonstrated by in vitro recordings of PFC pyramidal cells that optimal levels of D_1R stimulation can modulate signal from dendrite to soma, enabling transmission of signals by sharpening and strengthening NMDA receptor-mediated connections (Yang & Seamans, 1996; Arnsten, 1997; 2004; Arnsten & Pliszka, 2011), whereas high DA concentrations may activate D_2Rs suppressing NMDA function (Zheng et al., 1999; Marcus et al., 2005). However, reducing these "irrelevant" connections excessively may be harmful in situations that require creative solutions or broad attention. Besides, as it happens with NA, dopaminergic D_1-receptor overstimulation (e.g., under a stressful condition) may lead to disconnection of all network inputs, resulting in a possible halt in cell firing. Hence, PFC neurons require a specific amount of both NA and DA to function optimally (Vijayraghavan et al., 2007; Arnsten, 2009) (**Figure 1.21**).

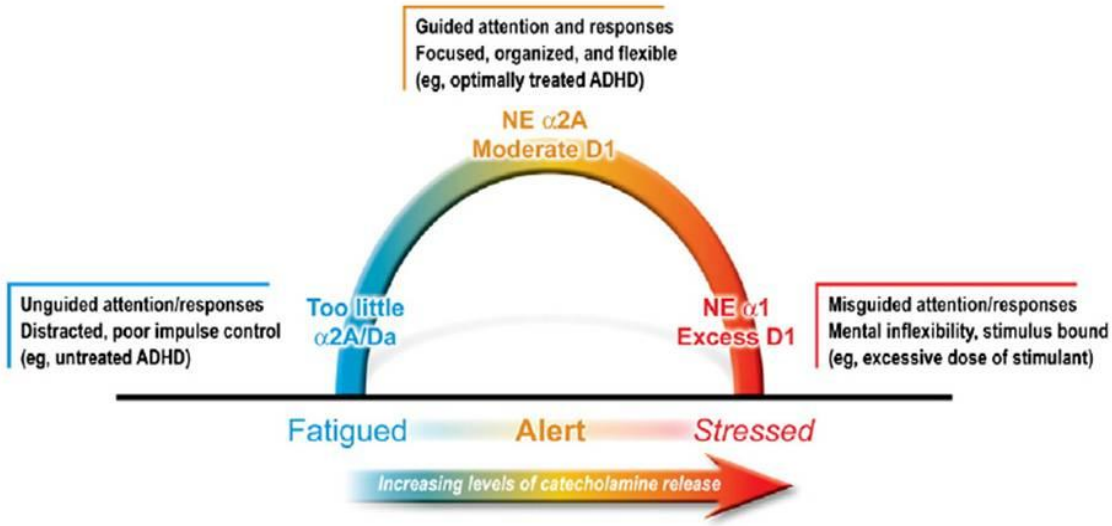

Figure 1.21: PFC function is sensitive to its neurochemical environment: both insufficient and excessive catecholamine release impair PFC function. From *Arnsten & Pliszka, 2011.*

Apart from the effect through α_2ARs located postsynaptically, the presynaptic action at α_2ARs produces an outburst of effects on neurotransmitter negative feedback and regulation that affects neuropsychiatric processes (Neumeister et al., 2006).

The α_{2A}AR and α_{2C}AR are the main autoreceptors involved in presynaptic feedback inhibition of NA, with the α_{2B}AR making no significant contribution to this inhibition (Hein et al., 1999). However, the potency and affinity of NA at the α_{2C}AR is higher than that for the α_{2A}AR (Link et al., 1992; Hein et al., 1999; Bunemann et al., 2001). There is evidence from peripheral and CNS tissue revealing that the α_{2C}AR would inhibit NA release at low endogenous NA concentrations [0.01–0.1 µM (Hein et al., 1999)] conversely to high NA concentrations [0.1–10 µM (Hein et al., 1999)] needed for α_{2A}AR (Hein et al., 1999; Bucheler et al., 2002). Despite this, both the α_{2A}AR, α_{2C}ARs are involved in the presynaptic negative feedback loop on NA release in the cortex, although α_{2C}AR-mediated presynaptic inhibition occurs more slowly than the one mediated by α_{2A}AR (Bucheler et al., 2002).

The proposed differential regulation on NA feedback and pharmacodynamics by the α_{2A}ARs and α_{2C}ARs is shown in **Figure 1.22**. At low endogenous NA concentrations (0.01–0.1 µM), the α_{2C}AR is responsible for inhibition of NA release, while the α_{2A}AR does so but at high endogenous NA concentrations (0.1–10 µM). Furthermore, α_{2C}AR-mediated inhibition of NA release, compared to that of α_{2A}AR-mediated inhibition, is a slower process; although, as commented before, the potency and affinity of NA are higher at the α_{2C}AR than at the α_{2A}AR. Moreover, the α_{2C}AR produces a limited inhibition of NA release (maximum 20–30% in hippocampal tissue) in contrast to the α_{2A}AR (Bucheler et al., 2002). This would suggest that, from a therapeutic perspective, α_{2C}AR modulation would provide a more subtle and targeted effect on NA release. Hence, this limited effect of the α_{2C}AR on NA release may lessen the potential cardiovascular side effects; one of the principal concerns with α_2AR antagonism (Bucheler et al., 2002). It has also been demonstrated that the density of α_{2C}AR, but not the

α_{2A}AR binding sites, increases three weeks after the destruction of NA terminals in the rodent cerebral cortex, which suggests that α_{2C}AR density is regulated by the synaptic availability of NA (Ordway, 1995).

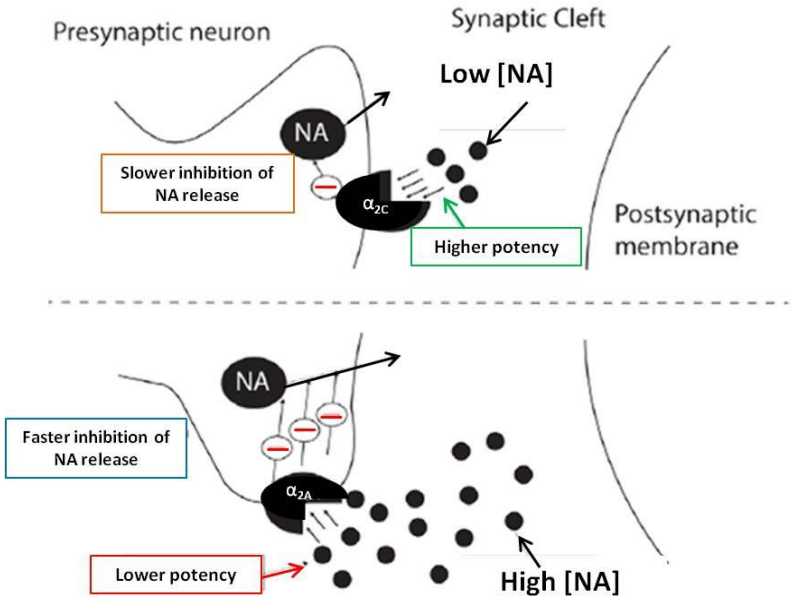

Figure 1.22: Schematic representation of the different presynaptic inhibition processes of NA release by the α_{2C}AR (top panel) and the α_{2A}AR (bottom panel). NA, noradrenaline; Θ, inhibition. Modified from *Uys et al., 2017a.*

1.7.4. α_2-Adrenoceptors and regulation of cognitive functions

1.7.4.1. Actions at α_2-adrenoceptors and improvement of cognitive functions

The stimulation of the cortical postsynaptic α_{2A}AR by NA is critical in the correct functioning of specific cognitive domains such as WM (Arnsten, 2011), which is why α_2AR agonists are successfully used in the treatment of cognitive aspects of diseases coursing with deficiencies in the cognitive function, such as schizophrenia or ADHD, among others (Sallee et al., 2013). Several studies in monkeys and rodents show that α_2AR compounds act directly in the PFC to modulate WM. However, all α_2AR subtypes may not equally contribute to these beneficial effects on mood, psychotic, and cognitive disorders. In fact, findings from transgenic mice studies have revealed different and sometimes opposing roles for the α_{2A}AR and α_{2C}ARs (Hein et al., 1999; Scheinin et al., 2001; Philipp et al., 2002), the two primary α_2AR subtypes involved in the regulation of CNS neurotransmission.

There are numerous studies in rodents, monkeys, and humans all showing that NA has an important beneficial influence on spatial WM performance through its action at α_2ARs.

Besides, these beneficial effects are seen not only in cases where cognitive impairment is present, even in the cases of healthy young monkeys, these α_2AR agonists also improve WM; but higher doses are needed in these cases (Franowicz & Arnsten, 1998). Direct infusions of α_2-AR antagonists (and not α_1 or β) into the dorsolateral PFC produce a delay-related impairment in spatial WM (Li & Mei, 1994), revealing that endogenous NA stimulation of α_2ARs in the PFC is critical to this kind of performance. On the contrary, intra-PFC infusion of α_2AR agonists enhanced WM in either young or aged monkeys (Arnsten, 1997; Mao et al., 1999) and aged rats (Tanila et al., 1996; Ramos et al., 2006). Thus, α_2AR agonists seem to be more potent and more efficient in animals with catecholamine depletion, consistent with actions at postsynaptic receptors (Arnsten & Goldman-Rakic, 1985; Cai et al., 1993). Additionally, the improvements induced by these α_2AR agonists can be reversed with an α_2, but not α_1AR antagonists, and α_2AR antagonists by themselves can impair PFC function; which is consistent with the α_2AR stimulation proposed mechanism (Arnsten & Goldman-Rakic, 1985). In fact, WM for both visuo-spatial (Arnsten et al., 1988) and visuo-feature (Jackson & Buccafusco, 1991) cues is improved by α_2AR agonists, suggesting enhancement of both dorsolateral and ventrolateral PFC function. Importantly, the cognitive-enhancing effects of α_2AR agonists can be completely dissociated from their sedating and hypotensive actions (Arnsten et al., 1988), one of the limitations when using these compounds in clinical therapy.

A pattern that is consistent with drug actions at supersensitive, postsynaptic receptors is that the greater the loss of NA, the lower the dose of $\alpha_2$agonist needed to improve PFC function (Franowicz & Arnsten, 1999). Concretely, the α_{2A}AR may be the most critical subtype for cognitive enhancement (Arnsten et al., 1996). Actually, the α_{2A}AR selective agonist, guanfacine, is the most effective compound in improving WM without marked side effects (Arnsten et al., 1988; Rama et al., 1996). Guanfacine is a selective α_{2A}AR agonist, its selectivity being 47-fold compared to α_{2C}AR and 100-fold compared to α_{2B}AR (Uhlen & Wikberg, 1991; Uhlen et al., 1994; 1995). Compared to the other non-selective α_{2A}AR agonist clonidine, guanfacine is about 10 times weaker in inhibiting the firing of the NA cell bodies in the LC or in decreasing NA release (Engberg & Eriksson, 1991), but is 10–100 times more potent in improving WM in aged monkeys (Arnsten et al., 1988). At the molecular level, guanfacine preferentially binds to postsynaptic α_{2A}ARs (Kawaura et al., 2014). Pyramidal cells in prefrontal regions richly express postsynaptic α_{2A}AR (Aoki et al., 1994), and their stimulation, via inhibition of the cAMP pathway and closing of the HCN channels, leads to increased excitability in prefrontal pyramidal cells and augmented connectivity within prefrontal microcircuits (Wang et al., 2007; Barth et al., 2008). Guanfacine is suggested to exert its positive effects on cognitive functions via these actions on postsynaptic α_{2A}ARs in the dorsolateral PFC (Ramos & Arnsten, 2007; Wang et al., 2007). It is also suggested to suppress glutamatergic synaptic transmission and thereby neural excitability at deeper layers (III/IV) in PFC. Actually, only at high guanfacine concentrations glutamate transmission is affected, explaining an inverted-U-type function of guanfacine (Wang et al., 2007; Yi et al., 2013). Furthermore, at a higher dose, guanfacine can also bind to presynaptic α_{2A}ARs located on NA terminals in the PFC or in the LC terminals that act as inhibitory autoreceptors, thereby decreasing NA release. This may again suggest that high doses of guanfacine could impair cognitive functions (Hassani et al., 2017) and that its

effect on cognition would depend on the dose used. Studies in genetically altered mice emphasize the importance of the α_{2A}AR, since mice with a mutation of this receptor no longer show beneficial effects on WM after guanfacine treatment (Arnsten et al., 1988; Franowicz & Arnsten, 1998), confirming this drug's action at the α_{2A}AR. Moreover, it has been shown that blockade of α_{2A}ARs in the monkey PFC with local infusions of an α_2AR antagonist yohimbine, markedly impairs PFC regulation of attention and behavior, inducing poor impulse control and locomotor hyperactivity (Ma et al., 2003; 2005). In humans, lower activity of DBH, the enzyme that synthesizes NA, is associated with poor sustained attention (Greene et al., 2009), poor executive function (Kieling et al., 2008), and impulsiveness (Hess et al., 2009), demonstrating that endogenous NA is important for proper PFC regulation.

In addition to α_{2A}AR, genetic deletion of the α_{2C}AR subtype, or by extrapolation selective α_{2C}AR antagonism, has been demonstrated to improve memory and cognition in some behavioral test such as the water maze, the 8-arm radial maze, and the NORT. The NORT is a behavioral task that relies on rodent's innate preference to explore novel objects over familiar objects, thereby allowing the measurement of recognition memory and attentional processes (Ennaceur & Delacour, 1988; Antunes & Biala, 2012). The memory processes underlying the NORT rely mainly on the perirhinal cortex and the PFC (Turetsky et al., 2003; Nagai et al., 2007; Wong et al., 2014; Cohen & Stackman, 2015). Furthermore, α_{2C}AR antagonism has been found to benefit neurotrophins and other biomarkers of neuronal resilience associated with cognition (Uys et al., 2016). Uys and collaborators (Uys et al., 2017b) have demonstrated that selective α_{2C}AR antagonism with ORM-10921 markedly improves recognition memory in pathological animal models of schizophrenia (Uys et al., 2016); indeed, benefits of selective α_{2C}AR antagonism on cognitive parameters have been corroborated with studies employing highly selective α_{2C}AR antagonists in animal models of schizophrenia, major depressive disorder, and age-related cognitive impairment (Sallinen et al., 2013a; Uys et al., 2016; 2017b).

It is important to note that, although α_2AR agonists/antagonists can improve PFC performance of tasks that challenge the PFC, they often have little beneficial effects under conditions that do not demand on the PFC. That is, no effect or even an impairment comes from these drugs in relation to spatial reference memory in the hippocampus (Sirvio et al., 1991), visual discrimination memory functions of the inferior temporal cortex (Arnsten & Goldman-Rakic, 1985; Steere & Arnsten, 1997) or the attention shifting functions of the parietal cortex (Witte & Marrocco, 1997). Hence, it could be said that these beneficial effects of the α_2AR agonists are specific to PFC functions.

These α_2AR mechanisms enhancing PFC function are also consistent with imaging studies in monkeys. Monkeys treated with guanfacine prior to performing a spatial WM task presented increased regional cerebral blood flow in the dorsolateral PFC (Avery et al., 2000). Also, guanfacine enhanced WM performance and increased regional cerebral blood flow in the principal sulcus of the PFC, the region essential for this cognitive function (Goldman & Rosvold, 1970). Conversely, in the auditory association cortex (the superior temporal cortex), a region not involved in task performance, guanfacine had no effect on regional cerebral blood flow (Avery et al., 2000). At the cellular level, electrophysiological studies combined with

iontophoretic techniques (which allow neurotransmitters and other chemical agents to be artificially administered very close to living and functioning neurons, and record their activity simultaneously) have also reported similar findings. For instance, iontophoresis of the α_2AR antagonist yohimbine reduced delay-related activity in the PFC neurons of monkeys performing a spatial WM task (Sawaguchi, 1998; Li et al., 1999). However, systemic non-selective α_2AR agonist clonidine administration enhanced delay-related firing in the PFC cells; an effect that was reversed by iontophoretic application of the α_2AR antagonist yohimbine (Li, B. M. et al., 1999). Hence, NA actions at α_2ARs in the PFC have important effects on delay-related firing, which seem to be the neuronal substrate of WM function. It is also possible that the beneficial effects of α_2ARs stimulation occur on dendritic spines as α_{2A}ARs have been localized on the postsynaptic membranes of spines in monkey PFC (Aoki et al., 1994; Arnsten et al., 1996).

As previously described, α_2ARs are commonly coupled to $G_{i/o}$ proteins, inhibiting the AC and cAMP pathways. As activation of this AC/cAMP pathway seems to impair PFC function (Taylor et al., 1999; Ramos et al., 2003), the inhibition of this intracellular signaling pathway may be the mechanism that contributes to the beneficial effects of α_2AR agonists on PFC cognitive function. Indeed, recent evidence demonstrated that treatments that increase cAMP signaling block guanfacine's beneficial effects in both aging rats and monkeys (Ramos et al., 2006). Apart from that, it should be also noted the importance of the α_2AR-mediated inhibition of HCN channels in the PFC, which play a role in strengthening synaptic connections in the PFC. Indeed, clonidine beneficial effects by inhibiting HCN channels in adult rats were completely reversed by the α_2AR antagonist yohimbine and the HCN blocker ZD7288 (Li et al., 2013). Therefore, the improvements produced by α_2AR agonists on WM seem to be mediated via inhibition of the cAMP signaling pathway and/or the inhibition of HCN channels in the pyramidal PFC neurons.

1.7.4.2. Clinical experience with α_2-adrenoceptor compounds

Compared to basic preclinic studies in animals, recent studies with α_2AR agonists in healthy humans and patients with PFC disorders have found surprising similarities. The non-selective α_2AR agonist clonidine usually has mixed effects on PFC functions in healthy young adults, presumably due to competing for pre- *vs.* post-synaptic effects and dose limitations from the sedative and hypotensive side effects (Coull, 1994; Jakala et al., 1999). However, improvement with clonidine has been observed in patients with PFC deficits and alterations in PFC catecholamine concentrations (Mair & McEntee, 1986). Clonidine has also been shown to improve memory recall and performance in schizophrenic patients (Fields et al., 1988), and also spatial WM in Parkinson's disease patients with presumed catecholamine depletion in the PFC (Riekkinen & Riekkinen, 1999a). Riekkinen and cols, (Riekkinen & Riekkinen, 1999b) even demonstrated that clonidine could improve WM in patients with AD.

In imaging studies in humans, low doses of clonidine in normal adults generally show a picture of impaired attention and emerging sedation associated with imaging changes in thalamus and parietal cortex (Coull et al., 1997). This data reinforces the notion that posterior cortex and

most subcortical structures are impaired by α_2AR stimulation. However, a different picture emerges when higher doses of clonidine are given to patients with supposed NA loss, or when guanfacine, a more selective α_2AR agonist, is used. As an example, administration of higher doses of clonidine to Korsakoff's syndrome patients (presenting memory deficits and cerebral atrophy, among other symptoms), increased regional cerebral blood flow in the frontal lobe, and the increased blood flow in the left PFC correlated with improved verbal fluency performance (Moffoot et al., 1994). Clonidine has also been shown to increase the effective connectivity between the LC, parietal cortex and PFC during an attentional task, while decreasing connectivity during rest (Coull et al., 1999). Regarding guanfacine, this compound has been shown to increase regional cerebral blood flow in the frontal lobe of healthy adults when measured by PET imaging (Swartz et al., 2000). These studies reinforce the idea that α_2AR stimulation often impairs the functioning of most brain regions, and that the PFC is exceptional in its beneficial influence from α_{2A}AR stimulation. In contrast to these beneficial effects, blockade of α_2ARs in the PFC of monkeys deteriorate delay-related cell firing and recreates all symptoms of ADHD: poor impulse control (Ma et al., 2003), locomotor hyperactivity (Ma et al., 2005) and impaired WM (Li & Mei, 1994), underlying increased distractibility. From these basic studies, it seems clear that genetic alteration of the enzymes responsible of NA synthesis (DBH) or of the expression and functionality of α_2ARs might contribute to this symptomatology by weakening endogenous NA α_2AR signaling (Arnsten et al., 2007).

Based on the research in animals, guanfacine is now in widespread use for the treatment of a variety of PFC cognitive disorders. There is several clinical evidence suggesting beneficial effects in cognition using guanfacine. It has been shown to be very effective in patients with PFC dysfunction, and also to produce less hypotensive and sedative side effects that, for instance, limit the use of clonidine in clinical therapy. Immediate-release guanfacine was first tested in children with ADHD by Hunt, based on the successful animal data and his previous experience with clonidine (Hunt et al., 1995). Indeed, weaker PFC function is a hallmark of ADHD, particularly deficits in the right inferior PFC that is specialized for inhibiting inappropriate actions (Rubia et al., 1999). Guanfacine helps ADHD patients control their own behavior and inhibit inappropriate distractions (Biederman et al., 2008; Sallee et al., 2009). It also allows inhibition of inappropriate aggressive impulses (Connor et al., 2010), likely through its actions in ventral PFC. In ADHD with prominent PFC dysfunction, guanfacine has proven to be effective in three open-label trials (Chappell et al., 1995; Hunt et al., 1995; Boon-yasidhi et al., 2005), two placebo-controlled trials (Scahill et al., 2001; Taylor & Russo, 2001) where it improved tics and ADHD symptoms, and also showed therapeutic effects regarding performance of PFC tasks such as vigilance, WM and behavioral inhibition (Scahill et al., 2001). Importantly, guanfacine appears to improve the functioning of both lateral (Mao et al., 1999; Wang et al., 2004) and ventromedial (Steere & Arnsten, 1997) PFC circuits, and therefore potentiates regulation of both sensory-motor and emotional responses. In another study with children affected by ADHD, the effects of monotherapies with a psychostimulant D-methylphenidate or guanfacine to combined treatment of both and their effects on cognitive functions were compared. Combined treatment of guanfacine and D-methylphenidate resulted

in greater improvements in WM than placebo or guanfacine alone; but the combined treatment was not superior to D-methylphenidate alone, and did not extend to other cognitive domains (Bilder et al., 2016). Guanfacine seems to shows good tolerability in ADHD. In fact, guanfacine has been marketed since 1986 (Sorkin & Heel, 1986) for the treatment of hypertension and posteriorly for ADHD. Thus, it has established a 30-year safety record of use in adults (McAllister et al., 2004; Arnsten et al., 2007).

Guanfacine may also be useful in treating PFC dysfunction in other disorders besides ADHD. It has been shown to improve WM in patients with a schizotypal disorder with cognitive deficits resembling those in schizophrenia (McClure et al., 2007) and to amend PFC function and metabolism in patients with some forms of epilepsy (Swartz et al., 2000). Moreover, is being proven to treat attentional neglect caused by stroke (Singh-Curry et al., 2011), presumably because of its attention-enhancing effects in normal subjects (Clerkin et al., 2009). In patients with Tourette's syndrome and children with ADHD with tics who often can not take stimulant medications, guanfacine is also used to inhibit inappropriate motor and vocal tics (Scahill et al., 2001). It is also being tested in autism spectrum disorders to treat the disinhibited behaviors that often accompany the social deficits in these disorders (Scahill et al., 2006; McCracken et al., 2010). Furthermore, it has tuned out helpful in patients with schizophrenia (Friedman et al., 1999; McClure et al., 2007), and more recently in subjects with a schizotypal disorder, where it normalized cognitive performance (McClure et al., 2007).

Guanfacine is also being tested in patients with mild traumatic injury in the PFC (McAllister et al., 2004). On the other hand, in a double-blind, placebo-controlled crossover design pilot study of adolescent cannabis users, short-term treatment with guanfacine improved cognitive adverse effects produced by THC consumption (Mathai et al., 2018). Another study with early abstinent cocaine-dependent individuals, the effect of guanfacine over placebo was analyzed and inhibitory control and attentional shifting were measured when performing a battery of neurocognitive tasks. Compared with placebo, the guanfacine group demonstrated attenuated anxiety and negative affect as well as improved performance on selective executive tests. However, guanfacine did not improve strategic WM or peripheral memory (Fox et al., 2015). Therefore, although the preliminary results about the beneficial effects of guanfacine in improving cognitive deficiencies and memory in humans are not yet completely clear, the use of α_2AR agonists such as guanfacine might represent a promising clinical strategy, calling for further testing as a potential treatment of these deficiencies in neuropsychiatric disorders.

1.7.5. Modulation of CIAS by drugs with α_2-adrenoceptor activity

Deficits in PFC cognitive function are now considered a fundamental feature of schizophrenia (Uys et al., 2017a). Much research trying to understand and treat these cognitive impairments has focused on DA since this neurotransmitter exerts effects on WM functions in the PFC through actions at D_1Rs (Brozoski et al., 1979; Sawaguchi & Goldman-Rakic, 1991). Despite the prominence of the DA hypothesis of schizophrenia and the excessive blockade of D_2Rs by antipsychotics giving rise to undesired secondary effects, a hypothesis implicating noradrenergic dysfunction has been widely supported in the literature (Berger, 1981;

Yamamoto & Hornykiewicz, 2004; Grunder et al., 2009; Siuta et al., 2010; Moller et al., 2015; Fitzgerald, 2014). In fact, is well known now that NA has just as powerful influence as DA on PFC cognitive function through actions at α_2ARs (Arnsten & Goldman-Rakic, 1985; Li & Mei, 1994). Similarly to what happens with DA and the inverted U-shape D_1R stimulation, moderate levels of NA enhance PFC functions through actions at postsynaptic α_2ARs, specially α_{2A}ARs, while high levels of NA release, e.g. during stress, impair PFC function through actions at α_1ARs (Arnsten et al., 1999; Birnbaum et al., 1999; Mao et al., 1999). Therefore, the blockade of the α_1AR-mediated pathway (Baldessarini et al., 1992), or the stimulation of α_2ARs and/or D_1Rs (Friedman et al., 2001), have become logical targets for pharmaceutical development.

1.7.5.1. Antipsychotic treatment and CIAS

Cognitive deficits are a core feature of schizophrenia (Buchanan et al., 2011; Nielsen, 2011). These deficits are stable over time and modestly correlated to negative symptoms, and positive symptoms only being correlated in a minor degree to cognitive deficits (Szoke et al., 2008; Mohamed et al., 2008; Dominguez Mde et al., 2009). Results of preclinical and clinical investigations suggest that the DA system is affected, to varying degrees, by all antipsychotic medications (Arnt & Skarsfeldt, 1998; Meltzer, 1991). Data on the general effect of FGAs on cognition in patients with schizophrenia is poor, as most studies have used haloperidol as the comparator. FGAs are generally associated with deficits in WM, processing speed, and motor skills, perhaps due to a higher affinity for the D_2R or because anticholinergic drugs are often used in combination with FGAs (Hill et al., 2010). A high affinity for the cholinergic and the histaminergic receptors is also seen in low-potency FGAs, with antagonistic cholinergic effects being correlated with decreases in attention, memory, and executive functions (Minzenberg et al., 2004; Hill et al., 2010).

Acute effects of FGAs include both blockade of pre- and postsynaptic DA receptors, and increased firing rate and synthesis of presynaptic DA-containing neurons that comprise the major DA pathways (Arnt, 1998). Chronic effects of FGAs, similar to acute effects of these agents, involve a high degree of postsynaptic DA receptor antagonism (Arnt, 1998). Manipulations that decrease DA function through either inhibition of presynaptic release (LeMoal et al., 1976; Simon et al., 1980) or postsynaptic blockade of DA receptors, (Levin & Gunne, 1989; Hoskins et al., 1991; Sawaguchi & Goldman-Rakic, 1991) have been demonstrated to impair cognitive function in animal models. More importantly, several lines of evidence also suggest that factors associated with reduced DA function in striatal and cortical brain regions lead to impairments in the cognitive function of humans (Javoy-Agid & Agid, 1980; Scatton et al., 1983). For instance, in idiopathic Parkinson's disease, TH is decreased in DA neurons projecting from VTA (in addition to nigrostriatal pathways), and cortical DA levels are reduced (Javoy-Agid & Agid, 1980; Scatton et al., 1983). Non-demented patients with idiopathic Parkinson's disease experience cognitive abnormalities, an observation that may be related to decreases in DA function (Brown & Marsden, 1990). Also, individuals exposed to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exhibit cognitive changes similar to those seen in Parkinson's disease or schizophrenia (Stern et al., 1990). By PET studies, Volkow et al. (Volkow et al., 1998)

demonstrated an association between decreased DA activity in striatal regions of the brain with age and both cognitive and motor performance in healthy individuals. The D_2R density was also correlated with cognitive dysfunction in most tasks involving frontal brain regions. All these data suggest that changes in DA activity in the brain are associated with declines in cognitive functions of frontal brain regions. To the degree that cognitive function is dependent on the integrity of DA brain systems, chronic FGA treatment could impair cognitive performance by decreasing both pre- and postsynaptic DA activity (Byerly et al., 2001).

In contrast to FGAs, newer antipsychotics appear less likely to alter DA functions that underlie potential cognitive processes (Maixner et al., 1999). SGAs receptor profiles are more diverse, and the group is more heterogeneous than FGAs (Correll, 2010). However, each of these agents manifests different effects on DA systems (Bymaster et al., 1997; Arnt, 1998). Clozapine is an effective SGA nearly devoid of extrapyramidal symptoms (Kane et al., 1988; 1993; Parkinson Study Group, 1999). This agent blocks approximately 50% of D_2Rs at clinically effective doses (Kapur et al., 1999; Nordstrom et al., 1995), conversely to FGAs that generally block > 70% of D_2Rs. Compared to D_1R and D_2Rs, it is far more potent antagonist of the D_4R. Related to the potential cognitive advantages of clozapine versus FGAs, antagonism of D_1R (Sawaguchi & Goldman-Rakic, 1991; Arnsten et al., 1994; Didriksen, 1995) and D_2R (Hoskins et al., 1991; Didriksen, 1995; Skarsfeldt, 1996) receptors appears to be negatively related to cognitive function in animal models, while antagonism of D_4R may be less likely to cause cognitive impairment (Skarsfeldt, 1996; Jentsch et al., 1999). Quetiapine is a weak D_2R antagonist which D_2R occupancy is approximate 44%. At clinically effective doses, risperidone, olanzapine, and ziprasidone generally produce a blockade of > 70% of D_2R, a proportion similar to FGAs.

Some newer antipsychotics also differ markedly from traditional antipsychotics by selectively inhibiting mesolimbic DA projection fibers with relative “light” effects on nigrostriatal and mesocortical DA pathways (Arnt & Skarsfeldt, 1998), the desired profile for these agents that would make them less likely than FGAs to impair the cognitive function. In a review of the relative selectivity of the SGAs for DA limbic pathways, Arnt and Skarsfeldt (Arnt & Skarsfeldt, 1998) described the effects of these agents in selectively inhibiting limbic projection fibers as follows: clozapine > olanzapine > quetiapine > ziprasidone = risperidone > FGAs; where greater selectivity is expected to provide a more advantageous cognitive profile. Thus, theoretically, agents with fewer DA receptor antagonist effects and the ability to moderate DA in striatal regions and/or enhance DA in frontal cortical regions (e.g. clozapine and quetiapine) would theoretically provide cognitive advantages over antipsychotic agents with higher degrees of DA receptor antagonism and/or less limbic selectivity (e.g. risperidone).

Early, smaller, clinical studies suggested that SGAs could reduce cognitive impairment in patients with schizophrenia, but newer, larger, clinical studies have only shown modest to negligible effects in both chronic and first-episode patients (Keefe et al., 2007; Davidson et al., 2009; Hill et al., 2010). In fact, a meta-analysis evaluated available data on studies with SGAs between 1980 and 2013, with patients diagnosed with schizophrenia and a minimum study duration of eight weeks (Nielsen et al., 2015). They showed decreased verbal WM in patients

with clozapine, olanzapine, quetiapine, and FGA treatment compared to ziprasidone, but positive effects of sertindole on executive function compared to clozapine, olanzapine, ziprasidone, and FGAs. In addition, clozapine and olanzapine had positive effects on verbal fluency. But overall, the authors reported that their study did not show any general differences between SGAs and FGAs regarding cognitive functions (Nielsen et al., 2015). Therefore, the need for agents that achieve greater effectiveness in this field and lack of side effects are urgently needed.

1.7.5.2. Antipsychotics and α_2-adrenoceptors

The clinical potency of antipsychotic compounds is related to the affinity and blockade of the D_2R (Seeman et al., 1976; Creese et al., 1976; Kapur et al., 2000). To some extent, above their efficacy against positive symptoms, SGAs compared to FGAs are reported to show "improved" efficacy against negative and cognitive symptoms of schizophrenia (King, 1994; Sharma, 1999) and against the induction of extrapyramidal side effects (Leucht et al., 1999; Grunder et al., 2009). The more recently developed SGAs affect several neurotransmitter systems, including the cholinergic, noradrenergic, serotoninergic, histaminergic and dopaminergic systems (Schotte et al., 1996). Improvement or deterioration of cognitive function induced by antipsychotic treatment is probably related to these auxiliary receptor activities (Keefe et al., 1999; Meltzer & McGurk, 1999).

Almost all atypical antipsychotics (e.g. clozapine, quetiapine, risperidone, and asenapine) display moderate to potent levels of α_2AR antagonism, which has been suggested to contribute to the "atypical" profile of these drugs (Kalkman & Loetscher, 2003; Shahid et al., 2009) participating in the stabilization of dysregulated DA activity (Svensson, 2003). More importantly, cognitive parameters are also influenced by α_2AR modulation with α_2AR antagonism, especially the α_{2C}AR, strategy that has been shown to improve attentional, verbal, and episodic memory deficits (Coull et al., 1996). Clozapine, in particular, shows a prominent α_{2C}AR/ D_2R, as well as α_{2C}/α_{2A}AR selectivity (Kalkman & Loetscher, 2003; Shahid et al., 2009). Surprisingly olanzapine, which has a very similar structure compared to clozapine, including a polyvalent radioligand binding profile, strongly differs from clozapine with regards to the affinity for α_2ARs (Schotte et al., 1996). Apparently, the mechanism responsible for the improvement achieved by clozapine compared to other antipsychotics could be attributed to this action as an α_2AR antagonist, which enhances DA output from the PFC, and this DA stimulates postsynaptic D_1Rs; facilitating NMDA receptor-mediated transmission in the PFC (Marcus et al., 2005). Although there are mixed results, it has been found that most SGAs show higher α_{2C}AR antagonist activity than α_{2A}AR antagonist activity. Additionally, SGAs asenapine and lurasidone both present a potent α_{2C}AR binding affinity (Shahid et al., 2009; Ishibashi et al., 2010). Indeed, a pharmacological profile constituting a higher α_2AR/ D_2R binding ratio, and specifically a higher α_{2C}AR/D_2R selectivity ratio (Kalkman & Loetscher, 2003; Shahid et al., 2009), has been suggested to mediate the improved antipsychotic efficacy on cognitive function of drugs like clozapine.

Interestingly, the α_2AR antagonism approach has already been suggested to be a promising strategy to treat neuropsychiatric disorders. Previous studies have revealed the marked involvement of noradrenergic fibers in the reuptake/co-release of DA in the PFC, showing that noradrenergic drugs can enhance DA output in the PFC (Carboni et al., 1990; Devoto et al., 2001; 2005; Devoto & Flore, 2006; Masana et al., 2011). There are studies showing that noradrenergic drugs can augment DA in the PFC (but not in the nucleus accumbens) and that this DA enhancement on PFC can be produced by reboxetine (a selective NET inhibitor), but not by a selective DAT inhibitor GBR12909 (Masana et al., 2011). Moreover, extracellular DA in the PFC seems to be sensitive to the α_2AR blockade with RX821002, as observed with NA output in the PFC (Mateo et al., 1998; Sacchetti et al., 1999; Ortega et al., 2010). Based on these observations about the selective effect of noradrenergic drugs on the mesocortical DA enhancement, combinations of NET inhibitors (such as reboxetine) and α_2AR antagonists (such as mirtazapine and/or RX821002) have demonstrated to potentiate the selective increase of DA (and NA) output in the PFC (Masana et al., 2011; 2012b). In agreement with this, Yamauchi and cols. (Yamauchi et al., 2012) reported that the α_2AR antagonist mirtazapine augmented the effect of a selective NA reuptake inhibitor milnacipran on extracellular monoamines in the PFC. Thus, the α_2AR blockade strategy especially when combined with other drugs, might be a useful approach to selectively increase catecholamine concentrations in the PFC. Therefore, it could contribute to improving cognitive dysfunction in patients with disorders associated with a hypoactive DA function in the PFC, such as schizophrenia.

1.7.5.3. α_2-Adrenoceptor compounds in CIAS

In order to understand the objectives of this work and after having reviewed and emphasized the role of α_2ARs in cognitive processes of the PFC, it is worth making a brief summary of the evidence that has led to suggest and support the idea that these receptors are potentially good targets in the search for new effective compounds to treat cognitive impairment in schizophrenia.

1.7.5.3.1. α_{2A}-Adrenoceptor stimulation for the treatment of cognitive disorders in schizophrenia

The first groundbreaking studies of the PFC role in cognition began in the 1930s at Yale School of Medicine. John Fulton together with Carlyle Jacobsen examined the effects of specific brain lesions on behavior in primates, resulting in their most important discovery: the dorsolateral region of the PFC is essential for abstract thought (Jacobsen, 1936). He found that monkeys with bilateral lesions to the dorsolateral PFC, but not other cortical lesions, were markedly impaired on problems requiring WM and that this impairment was permanent (Jacobsen, 1936). Subsequent studies conducted by Karl Pribram and Mortimer Mishkin showed the importance of PFC for resisting distraction (Grueninger & Pribram, 1969) and the key roles of the sensory association cortices for object perception (Pribram & Mishkin, 1955).

Later, Patricia Goldman-Rakic continued studying the neurobiology of thought and part of her work showed that columns of pyramidal cells in deep layer III of the PFC excite to each other to

maintain firing necessary for proper cognitive processes (Goldman-Rakic, 1995). These layer III microcircuits are the ones that expand most in primate evolution (Elston, 2003) and are the focus of neuropil loss in schizophrenia (Arnsten, 2011). She also made the landmark discovery that DA inputs onto PFC neurons have a critical modulatory influence on dorsolateral PFC spatial WM function and that depletion of DA (Brozoski et al., 1979) or blockade of D_1Rs (Sawaguchi & Goldman-Rakic, 1991) in the dorsolateral PFC produced spatial WM deficits as profound as ablation of the cortex itself. This was the first indication that the dorsolateral PFC was absolutely dependent on the correct neurochemical environment. Arnsten and Goldman-Rakic then discovered that very high levels of DA release, as occurs during stress (Deutch & Roth, 1990) or with drugs of abuse (Bradberry & Rubino, 2004), was as detrimental to WM function as was too little DA, the known as DA "inverted U" shape effect (Arnsten et al., 1988; Arnsten & Goldman-Rakic, 1998; Arnsten, 2000; Vijayraghavan et al., 2007). Apart from DA, they also reported that NA is equally important for proper PFC function via its actions at post-synaptic α_{2A}ARs (Arnsten & Goldman-Rakic, 1985) and that this receptor may be especially liable as a therapeutic target.

Actually, when both scientists studied the cognitive-enhancing effects of DA agonist in aged monkeys with naturally occurring DA depletion, the compound that produced the most dramatic improvement in their cognitive performance was not a dopaminergic drug, but clonidine, an α_2AR agonist. Following the clonidine administration, the aged monkeys were almost asleep and yet, they could perform almost perfectly (Arnsten & Goldman-Rakic, 1985). These beneficial cognitive effects arose from actions at postsynaptic α_2ARs in the dorsolateral PFC, and not due to the expected presynaptic effects on LC neurons (Arnsten & Goldman-Rakic, 1985). Indeed, the destruction of the presynaptic sites only made clonidine's effects more potent (Arnsten & Goldman-Rakic, 1985; Arnsten & Cai, 1993). Subsequent research revealed that the α_{2A}AR subtype was essential for these actions (Franowicz et al., 2002) and that the α_{2A}AR agonist guanfacine can improve WM (Arnsten et al., 1988; Rama et al., 1996), attention regulation (Arnsten & Contant, 1992; O'Neill et al., 2000), and behavioral inhibition (Steere & Arnsten, 1997) independent of its sedative actions. As it was mentioned previously, guanfacine is more selective for the α_{2A}AR subtype than is clonidine, which also binds with high affinity to α_{2B}AR, α_{2C}AR, and imidazoline receptors (van Zwieten & Chalmers, 1994; Uhlen et al., 1995). Guanfacine is weaker than clonidine in producing hypotension and sedation (Arnsten et al., 1988) and it is less effective in reducing LC firing and NA release in the brain (Engberg & Eriksson, 1991). However, guanfacine is more potent than clonidine in enhancing WM functioning in the PFC of aged monkeys, suggesting greater efficacy at postsynaptic sites in PFC (Arnsten et al., 1988). In fact, the PFC is the site of the beneficial drug action, as when infused directly into the rat or monkey PFC (Wang et al., 2004; Arnsten & Li, 2005; Ramos et al., 2006) is capable of improving cognition performance. Conversely, dorsolateral PFC α_{2A}AR blockade markedly impairs WM and behavioral inhibition (Ma et al., 2003; 2005), reducing persistent neuronal firing (Li et al., 1999; Wang et al., 2007). Therefore, NA stimulation of α_{2A}ARs is essential for PFC regulation of behavior, thought, and emotion.

Further research identified the molecular basis of guanfacine's enhancing effects in dorsolateral PFC. As it has been commented before, α_{2A}ARs are coupled to $G_{i/o}$ proteins that

inhibit cAMP signaling. It is known that cAMP actions on ion channels dynamically alter the strength of PFC network connections (Arnsten et al., 2010). The pyramidal cell microcircuits in layer III of dorsolateral PFC interconnect on dendritic spines via NMDA receptor synapses, exciting each other to keep information "in mind" (Arnsten et al., 2010). Immunoelectron microscopy has revealed α_{2A}ARs on these spines, situated next to ion channels that can gate network connections (Wang et al., 2007). Between these channels are included the HCN channels opened by cAMP signaling, and KCNQ channels opened indirectly by cAMP activation of PKA. Opening of these channels by high cAMP signaling (stress exposure or α_{2A}AR blockade), weakens PFC network connections and reduces persistent firing (**Figure 1.23**). In contrast, NA or guanfacine stimulation of α_{2A}ARs on PFC spines strengthens PFC network connections by inhibiting cAMP signaling, closing HCN channels, and increasing delay-related firing (Wang et al., 2007) (**Figure 1.23**). Besides, these enhancing effects of guanfacine on PFC persistent firing (Wang et al., 2007) and WM performance (Ramos et al., 2006) can be reversed by the cAMP analog Sp-cAMP. Guanfacine has also been shown to restore persistent firing in the aged monkey dorsolateral PFC via inhibition of cAMP-HCN or KCNQ channel signaling (Wang et al., 2011a). Studies with young monkeys with WM impairment induced by local PFC (Arnsten & Goldman-Rakic, 1985) or global (Cai et al., 1993) catecholamine depletion showed that they were highly improved by systemic treatment with guanfacine; and this effect was also shown in aged monkeys (Arnsten & Goldman-Rakic, 1985; Arnsten et al., 1988; Rama et al., 1996) and rats with catecholamine loss caused by age (Carlson et al., 1992; Ramos et al., 2006).

Taken together, this data confirms that guanfacine inhibits cAMP opening of K^+ channels on spines, which increases PFC network firing and strengthens PFC cognitive control of behavior. Therefore, the significant role of α_2ARs, and especially the α_{2A}AR in the treatment of deficits associated with cognitive impairment becomes evident.

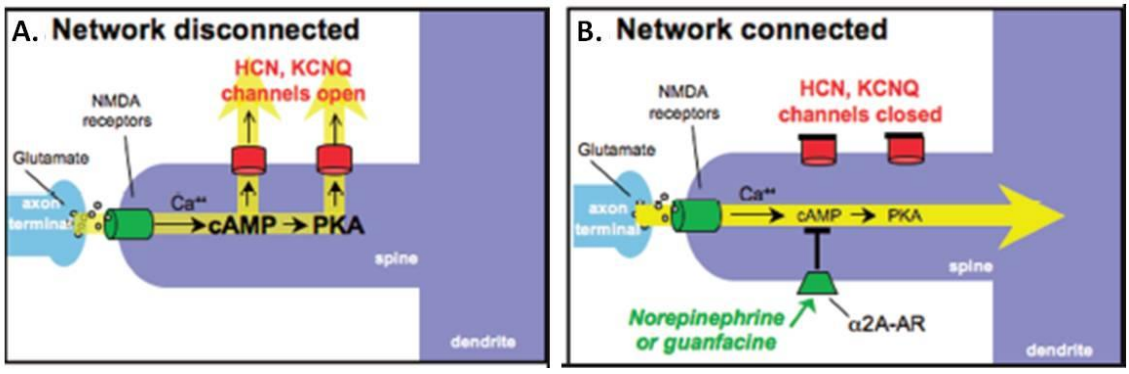

Figure 1.23: cAMP-K^+ channel signaling mechanisms in spines dynamically weaken synaptic efficacy and gate out network inputs to the neuron. **A**: cAMP directly opens HCN channels, while cAMP activation of PKA signaling increases the open state of KCNQ channels. **B**: NA or guanfacine stimulation of α_{2A}ARs on spines inhibits cAMP production and closes HCN and KCNQ channels, strengthening network connectivity, increasing neuronal PFC firing, and thus improving PFC regulation of behavior, thought and emotion. Modified from *Arnsten & Jin, 2012.*

1.7.5.3.2. α_{2C}-Adrenoceptor blockade for the treatment of cognitive disorders in schizophrenia

In addition to the role of α_{2A}ARs and their stimulation by selective drugs like guanfacine as a strategy for treating cognitive function, it should not be forgotten another potential strategy consisting of the selective blockade of the α_{2C}ARs.

As it has been already described in previous sections, there is numerous evidence considering the important role of the α_{2C}AR antagonism in managing disorders accompanied by cognitive dysfunction. It should be remembered that, compared to α_{2A}ARs, the expression and distribution of the α_{2C}ARs is more restricted to specific areas such as the striatum, basal ganglia, hippocampus, olfactory tubercle and cerebral cortex (Ordway et al., 1993; Nicholas et al., 1993; Scheinin et al., 1994; Rosin et al., 1996; Wang et al., 1996; Holmberg et al., 1999), constituting approximately 10% of the total population of α_2ARs (Bucheler et al., 2002). The dense expression in the striatum has distinctive importance when striatal dysfunction in schizophrenia is considered, especially its intricate connection to frontal cortical cognitive deficits (Simpson et al., 2010). Even if the majority of the α_2ARs in the PFC are α_{2A}ARs, and the α_{2C}ARs represent only 13% of the total population (Ordway et al., 1993; Grijalba et al., 1996), from this proportion the great majority are located presynaptically in human PFC (Erdozain et al., 2018a). More importantly, the α_{2C}AR population located presynaptically plays an important role, along with the α_{2A}AR, in the inhibition of NA release and negative feedback. This AR subtype is also involved in the inhibition of the release of DA and 5-HT (Sallinen et al., 1997; Hein et al., 1999; Bucheler et al., 2002; Trendelenburg et al., 2003) and it seems that the α_{2C}AR subtype is the main adrenergic modulator of the firing of DA cells in the VTA (Rosin et al., 1996; Inyushin et al., 2010). Since the PFC, striatum, and hippocampus have noradrenergic and dopaminergic terminals expressing auto- and hetero- α_{2C}ARs (Scheinin et al., 1994; Holmberg et al., 2003), and since these areas are implicated in the pathophysiology of schizophrenia, the α_{2C}-ARs become a potentially beneficial pharmacological approach.

Experimental data with mice also supports the α_{2C}AR antagonism strategy. Actually, benefits of selective α_{2C}AR antagonism on cognitive parameters have been demonstrated with studies employing highly selective α_{2C}AR antagonists in animal models of schizophrenia, and age-related cognitive impairment (Sallinen et al., 2013a,b; Uys et al., 2016; 2017a). Lack of α_{2C}AR expression was associated with increased startle reactivity, aggression, locomotor activity induced by amphetamine, and elevated activity in the FST (Sallinen et al., 1998; 2007; Scheinin et al., 2001). Moreover, the selective α_{2C}AR antagonist ORM-12741 showed improved effects on NMDA-antagonist-induced disruptions in WM and spatial learning, navigation, and memory in rodents (Sallinen et al., 2013b). Besides, associations between genetic polymorphism of the α_{2C}ARs and certain aspects of psychotic disorders have been described (Rivero et al., 2016).

Interestingly, it has been demonstrated that JP-1302 and ORM-10921, selective α_{2C}AR antagonists, exert antipsychotic-like properties by the reversion of PPI impairment induced by PCP in mice (Sallinen et al., 2007; 2013a). Also, it has been reported that the α_2AR antagonist

idazoxan is able to improve cognitive domains such as verbal fluency, attention, and memory in patients with frontal lobe dementia (Sahakian et al., 1994; Coull et al., 1996).

Previous studies have reported that combinations of the α_2AR antagonist idazoxan with raclopride, a D_2/D_3R antagonist, enhanced its antipsychotic-like effects, increased DA efflux in the PFC and facilitated the NMDA receptor-mediated glutamatergic transmission in cortical areas (Hertel et al., 1999; Marcus et al., 2005; Wadenberg et al., 2007). The addition of idazoxan to both low doses of FGAs (haloperidol) and SGAs (olanzapine and risperidone) resulted in a potentiation of the antipsychotic effects, an increased DA efflux in the PFC and an augmented cortical NMDA receptor-mediated neurotransmission (Wadenberg et al., 2007; Marcus et al., 2010b). This NMDA receptor-mediated neurotransmission is precisely believed to be DA-dependent and mediated by D_1R (Chen & Yang, 2002; Ninan & Wang, 2003; Marcus et al., 2005), which goes in agreement with the increased availability of DA in the PFC followed by the treatment. Similarly, the addition of the NET inhibitor reboxetine to a sub-effective dose of olanzapine also resulted in enhanced antipsychotic effect, augmented DA outflow in the PFC and NMDA receptor-mediated neurotransmission (Marcus et al., 2010a). Based on these observations, it has been proposed that the addition of an α_{2C}AR antagonist to the antipsychotic treatment could not only enhance the antipsychotic-like properties but also reduce its dose thus decreasing the risk of extrapyramidal effects, and improve the negative and cognitive symptomatology associated with schizophrenia.

Thus, selective stimulation of postsynaptic α_{2A}ARs and/or selective blockade of presynaptic α_{2C}ARs could provide useful and effective therapeutic strategies for the treatment of cognitive impairment associated with schizophrenia.

2. AIMS

There is extensive evidence supporting the involvement of the noradrenergic system and the α_2ARs in the pathophysiology of schizophrenia. Based on the observations that the antipsychotics that seem to have any positive effects against the cognitive symptoms of schizophrenia have also actions at the level of α_2ARs, these receptors are being proposed as potentially effective therapeutic strategies for the treatment of cognitive deficits of this disease.

Thus, the present work had two main general objectives. The first aim was to make a neurochemical and behavioral characterization of an animal model of MIA by the administration of Poly (I:C), a synthetic analog of a dsRNA capable of eliciting a maternal immune response. Then, the second general objective was to study the effects of compounds with α_2AR activity on the cognitive performance of the offspring of the MIA model. With these purposes, the specific aims of this present work were:

1. The quantification of monoamine (NA, DA, and 5-HT) brain tissue concentrations in different brain areas of the offspring of Poly (I:C) mice in comparison with their controls.

2. The evaluation of the protein expression of different components of the dopaminergic and noradrenergic systems in the cerebral cortex and striatum of the offspring of Poly (I:C) mice and their controls.

3. The *in vivo* neurochemical characterization of the extracellular monoamine concentrations in brain cortex and striatum of Poly (I:C) animals and their controls.

4. The *in vivo* evaluation of the cognitive status of the offspring of Poly (I:C) animals and their controls by the NORT and the 5-CSRTT behavioral tests.

5. The study of the effects of the selective α_{2A}AR agonist and selective α_{2C}AR antagonist on the cognitive performance of Poly (I:C) mice and their controls.

6. The study of the effect of the selective α_{2A}AR agonist and selective α_{2C}AR antagonist on the monoamine release in the cortex and striatum of Poly (I:C) animals and their controls.

7. The selective targeting of noradrenergic neurons originating from the LC that project to the PFC using different optogenetic approaches in mice.

3. MATERIALS AND METHODS

3.1. ANIMALS

3.1.1. CD1 swiss mice and C57BL/6J mice

The majority of the experiments with mice collected in this work were carried out in the facilities of the University of the Basque Country (UPV/EHU), but some of them were performed in the facilities of the Department of Anesthesiology in the Center of Clinical Pharmacology at the Washington University School of Medicine in St. Louis, MO, USA. In all cases and for both strains, animals used for all the experiments were male.

Regarding the animals used for the experiments in the University of the Basque Country (UPV/EHU), wild-type Hsd: ICR (CD1) mice and wild-type C57BL/6J mice with a weight of 25-30 g were used. On a 12 h light/dark cycle, they were housed (5 per cage) at room temperature (22-25 °C) and 65-70% humidity, with access to food and water *ad libitum*. Animals were obtained from the Animal Facility of the University of the Basque Country (UPV/EHU), Leioa, (Spain). The animal husbandry, housing, and all experimental procedures were carried out in the facilities of the University of the Basque Country (UPV/EHU). The C57BL/6J strain of animals was used only for the cognitive characterization by the 5-CSRTT experiments.

Animal care and experimental protocols with these two strains of animals were executed in accordance with the principles of animal care established by the European Directive for the Protection of Vertebrate Animals used for experimental and other scientific purposes (European Union Directive 2010/63/UE) in accordance with European Ethical Standards (6106/10-EEC), as well as in agreement with the Spanish legislation which regulates the welfare of animals used in experimentation and other scientific purposes (Royal Decree 53/2013). Also, all the experimental protocols were approved by the Committee of Ethics for Animal Experimentation of the University of the Basque Country (UPV/EHU) (CEBA/402/2015/MEANA MARTÍNEZ CEEA).

Animals used for the experiments at the Washington University School of Medicine in St. Louis were adult (25–35 g) male wild-type CD1 mice. They were group-housed, given access to food pellets and water *ad libitum*, and maintained on a 12:12-hr light/dark cycle (lights on at 6:00 a.m.). All procedures were approved by the Animal Care and Use Committee of Washington University in St. Louis and conformed to NIH guidelines.

3.1.2. MIA model: the Poly (I:C) model

3.1.2.1. The methodology of the MIA model by Poly (I:C)

For the purpose of this study, we generated a MIA model in wild-type CD1 and C57BL/6J mice. This MIA is based on prenatal treatment with the inflammatory agent Poly (I:C) (=polyriboinosinic-polyribocytidylic acid), a synthetic analog of dsRNA (**Figure 3.1**).

In this prenatal Poly (I:C) model, pregnant mouse dams are exposed to the immunological manipulation at a specific gestational stage, and the brain and behavioral consequences of the prenatal immunological manipulation are then compared in the resulting offspring relative to offspring born to vehicle-treated control mothers (Meyer et al., 2009). Poly (I:C) is a

commercially available synthetic analog of dsRNA. dsRNA is generated during viral infection as a replication intermediate for single-stranded RNA or as a by-product of symmetrical transcription in DNA viruses (Takeuchi & Akira, 2007). It is recognized as foreign by the mammalian immune system through the transmembrane protein TLR3 (Meyer et al., 2009). TLRs are a class of pathogen recognition receptors that recognize invariant structures present on and/or associated with virulent pathogens (**Figure 3.1**). Upon binding to TLRs, dsRNA or its synthetic analog poly(I:C), stimulates the production and release of many pro-inflammatory cytokines, including IL-1b, IL-6, and TNF-α (Fortier et al., 2004; Meyer et al., 2006a). In addition, Poly(I:C) is a potent inducer of the type I interferons IFN-a and IFN-b. Administration of Poly(I:C) can therefore efficiently mimic the acute phase response to viral infection (Meyer & Feldon, 2012).

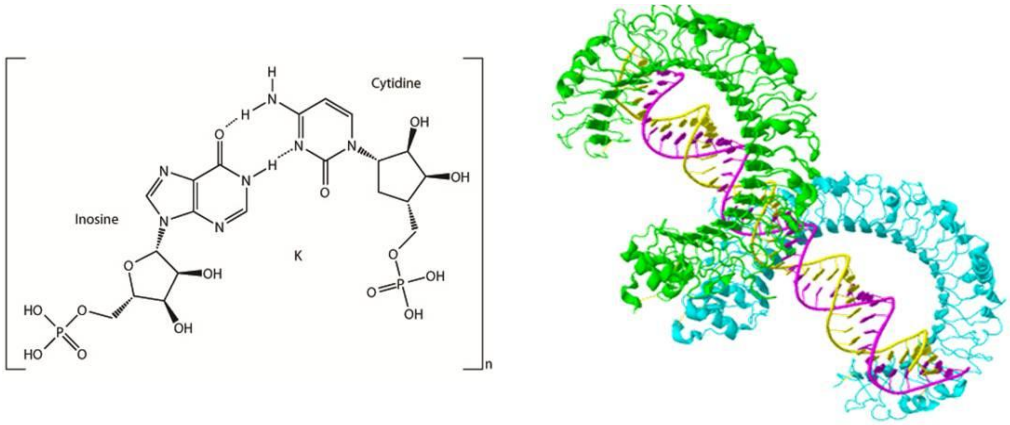

Figure 3.1: Left side: Schematic structure of Poly (I:C). Right side: Glycosylated mouse TLR3 dimer complex with dsRNA. Abbreviations: TLR 3: Toll-like receptor 3, sdRNA: double-stranded RNA.

3.1.2.2. Poly (I:C) protocol

In our laboratory, we designed a protocol for the MIA paradigm by the administration of Poly (I:C) or saline on the GD9.5. **Table 3.1** describes animals included in each experimental technique and from which female they originated.

Table 3.1: Summary table of animals intended for each experimental technique and the number of treated females they originated from. "Treated ♀" column indicates the number of females administrated with saline/Poly (I:C). "Offspring" column indicates the total number of pups from those females. "Used ♂" column indicates the number of males used and represented in graphs in each experimental technique.

Technique	SALINE			POLY (I:C)		
	Treated ♀	Offspring	Used ♂	Treated ♀	Offspring	Used ♂
Tissue monoamines	2	13	7	2	15	7
Western Blot	2	13	6	2	12	7
NORT (basal)	3	26	9	4	27	15
5-CSRTT (C57BL/6J strain)	11	56	26 (10) *	7	36	18 (15) *
Microdialysis (PFC)	26	114	50	28	120	51
Microdialysis (Striatum)	8	35	19	9	40	19
NORT (α_2AR)	20	93	40	24	101	45
Microdialysis (α_2AR)	10	50	23	12	33	19
Optogenetics	-	-	15 (1st approach) 8 (2nd approach) 18 (3rd approach)	-	-	-

*Number in brackets: total number of animals that finally reached the criterion and therefore are represented in graphs. Number not in brackets: the total number of animals that started the experiment.

The Poly (I:C) protocol consisted of the following steps (**Figure 3.2**):

- Depending on the demand, we gather some cages with one male and two females in each cage, with the aim that they come together to procreate.
- The next day, around 8 am, females are checked for vaginal plugs. In case there is not a vaginal plug, males and females stay together until the checking time the following day. If there is a vaginal plug, those females are separated from the male and housed in different cages depending on the treatment they will receive (saline (vehicle) or Poly (I:C)) and in groups of maximum three females together. That day would be considered the GD0.5, and until the time of administration of the drug/vehicle, the animals will be supervised to assure the correct development of the pregnancy.
- On the GD9.5, pregnant dams will receive either 7.5 mg/kg i.p. of Poly (I:C), which will be dissolved in saline, or 5 ml/kg i.p. of saline.

- In approximately 21 days (3 weeks), pups will be born and they will be looked after until they are 3 weeks old. At that time, they will be weaned, separated from their mothers and housed in cages depending on the sex and the treatment received.
- These mice will be ready for experiments when they are 8 weeks (2 months) old.

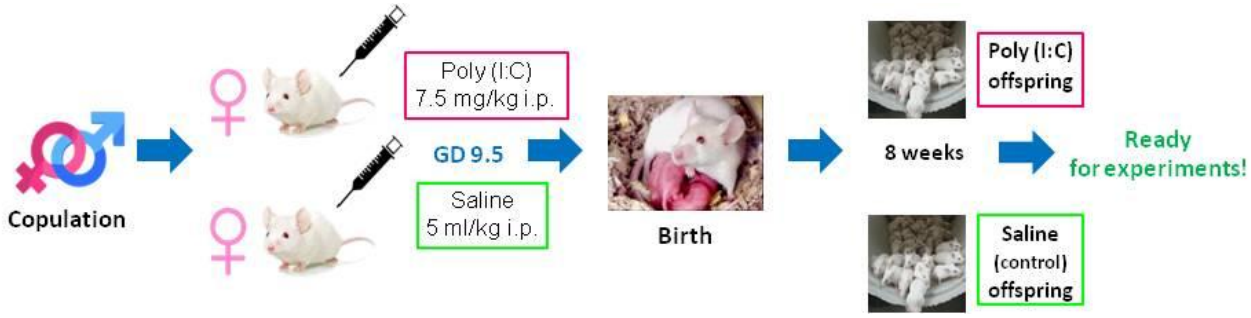

Figure 3.2: Schematic representation of the Poly (I:C) protocol carried out in our CD1 and C57BL/6J mice.

3.2. MATERIALS

3.2.1. Drugs

- Amphetamine (*alpha-methyl-phenethylamine*): catecholamine reuptake blocker and inducer of catecholamine release (Sigma Aldrich®, St. Louis, MO, USA). Batch: 058K3350

- Dopamine *(2-(3,4-Dihydroxyphenyl)ethylamine hydrochloride):* neurotransmitter (Sigma Aldrich®, St. Louis, MO, USA). Batch: 83H2512.

- Guanfacine chlorhydrate: selective α_{2A}AR agonist (Sandoz, Barcelona, Spain). Batch: 2K10324.

- Isoflurane (*1-chloro-2,2,2-trifluoromethyl difluoromethyl ether*): general inhaled anesthetic drug. Two-pore K^+ channel activator. It is also a very widely used anesthetic for *in vivo* animal research and for *in vitro* studies on anesthesia mechanisms (IsoFlo®, ESTEVE VETERINARIA, Barcelona, Spain). Batch: 50016XN.

- JP-1302 dihydrochloride *(N-[4-(4-Methyl-1-piperazinyl)phenyl]-9-acridinamine dihydrochloride):* selective α_{2C}AR antagonist (Tocris®, Ellisville, MO, USA). Batch: 1A/77518.

- MK-801 hydrogen maleate (*5S,10R)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine hydrogen maleate)* or *Dizocilpine:* non-competitive antagonist of the NMDA receptor, glutamate receptor (Sigma Aldrich®, St. Louis, MO, USA). Batch: 022M4616V.

- MK-912 hydrochloride *(2S,12bS)-1',3'-dimethylspiro[1,3,4,6,7,12b-hexahydro-[1]benzofuro[2,3-a]quinolizine-2,4'-1,3-diazinane]-2'-one;hydrochloride):* selective α_{2C}AR antagonist (Sigma Aldrich®, St. Louis, MO, USA). Batch: 056K4717.

- Noradrenaline (*L-(−)-Noradrenaline (+)-bitartrate salt monohydrate*): neurotransmitter (Sigma Aldrich®, St. Louis, MO, USA). Batch: SLBC1882V.

- Poly (I:C) *(polyriboinosinic-polyribocytidylic acid):* a synthetic analog of dsRNA (Sigma Aldrich®, St. Louis, MO, USA). Batch: 095M4049V.

- Serotonin (*3-(2-Aminoethyl)-5-hydroxyindole hydrochloride; 5-HT; 5-Hydroxy-tryptamine hydrochloride*): neurotransmitter (Sigma Aldrich®, St. Louis, MO, USA). Batch: SLBV1958.

3.2.2. Gases

- Oxygen (O_2): (Carburos Metálicos, Barcelona, Spain).

3.2.3. Antibodies

Primary antibodies used for Western Blot experiments were:

- Anti-Dopamine Transporter Antibody (Rat) MAB369 (Merck Millipore®, Darmstadt, Germany). Batch: LV1652261.

- Anti-Tyrosine Hydroxylase Antibody (Mouse) SC25269 (Santa Cruz Biotechnology®, Dallas, TX, USA). Batch: G1917.

- Anti-Tyrosine Hydroxylase Antibody (Rabbit) AB41528 (Abcam®, Cambridge, MA, USA). Batch: GR226873-1.

- Anti-Norepinephrine Transporter Antibody (mouse) (Mab Technologies®, Neenah, WI, USA). Batch: NET05-2.

- Anti-Dopamine Receptor D1 Antibody (mouse) AB78021 (Abcam®, Cambridge, MA, USA). Batch: GR284017-3.

- Anti-Dopamine Receptor D2 Antibody (mouse) SC5303 (Santa Cruz Biotechnology®, Dallas, TX, USA). Batch: H0417.

- Anti-β-actin antibody (mouse) A1978 (Sigma Aldrich®, St. Louis, MO, USA). Batch: 065M4837-V.

- Anti-β-actin antibody (rabbit) AB8227 (Abcam®, Cambridge, MA, USA). Batch: GR3251791-1.

Secondary antibodies used for Western Blot experiments were:

- Dylight 800 anti-rat (Goat) SA5-10024 (Invitrogen, Waltham, MA, USA). Batch: SJ2457471.

- Alexa Fluor 680 anti-mouse (Goat) A21057 (Invitrogen, Waltham, MA, USA). Batch: 1752100.

- Dylight 800 anti-rabbit (Donkey) 611-745-127 (Rockland Instruments, Pottstown, PA, USA). Batch: 33207.

Antibodies used for immunohistochemistry were:

- Anti-Tyrosine Hydroxylase Antibody (Chicken) TYH (Aves Labs Inc., David, CA, USA). Batch: TYH88967984.

- Anti-Norepinephrine Transporter Antibody (Mouse) 1447-NET (PhosphoSolutions, Aurora, CO, USA). Batch: CH417g.

- Alexa Fluor 594 Anti-chicken IgG (Goat) (Invitrogen, Waltham, MA, USA). Batch: A11042.

- Alexa Fluor 633 Anti-chicken IgG (Goat) (Invitrogen, Waltham, MA, USA). Batch: A21103.

- Alexa-Fluor 594 Anti-mouse (Goat) (Invitrogen, Waltham, MA, USA). Batch: A11032.

- Alexa-Fluor 633 Anti-mouse (Goat) (Invitrogen, Waltham, MA, USA). Batch: A21052.

- Nissl blue 435/455 (Neurotrace) (Invitrogen, Waltham, MA, USA). Batch: N21479.

3.2.4. Reagents

The following two figures summarize the reagents used for all the experiments and their providers (**Figure 3.3**):

REAGENT	PROVIDER
Ammonium persulfate (APS)	**Bio-Rad Laboratories** (Hercules, CA, USA)
Bradford protein analysis method	
N, N, N, N'-tetramethylethylenediamine (TEMED)	
Laemmli Buffer	
Acrylamide 30% -bisacrylamide 0.8%	**National Diagnostics** Atlanta, GA, USA
Antipain	**Sigma Aldrich®** (St. Louis, MO, USA)
Aprotinin	
Bromophenol blue	
Bovine albumin (BSA)	
β-mercaptoethanol	
1,4-Dithiothreitol (DTT)	
Glycerol	
Glycine	
Igepal	
Ponceau S reagent	
Protease inhibitor cocktail	
Sodium Dodecyl Sulfate (SDS)	
Sodium vanadate	
Sodium fluoride	
Sodium azide	
Sodium octyl sulfate	
Sodium deoxycholate	
Tris (2-amino-2-hydroxymethyl-1,3-propanediol) HCl	
Tritón™ X-100	
Tween 20	

REAGENT	PROVIDER
Citric acid monohydrate, 99.5%	**Thermo Fisher Scientific** (Waltham, MA, USA)
Ethylenediaminetetraacetic acid, disodium salt dihydrate, +99%	
1-Octanesulfonic acid, sodium salt, HPLC grade	
Calcium chloride dihydrate	**Merck Millipore** (Darmstadt, Germany)
Ethylenediaminetetraacetic acid disodium salt (EDTA)	
Magnesium chloride hexahydrate	
Paraformaldehyde	
Potassium Chloride	
Sodium dihydrogen phosphate monohydrate	
Sodium chloride	
Sodium hydroxide	
Sodium hydroxide solution	
Perchloric acid 60%	**Probus S.A.** (Badalona, Spain)
Acetonitrile	**Panreac Química S.A.U.** (Barcelona, Spain)
Glacial Acetic Acid (HPLC)	
Ortho-Phosphoric acid 85%	
Methanol	**Carlo Erba Reagents** (Val de Reouil, France)

Figure 3.3: Summary tables of the reagents used for the experiments as well as their providers.

3.2.5. Viruses

- AAV5-Ef1α-DIO-eYFP: adeno-associated virus serotype 5, allow Cre-dependent expression of an enhanced yellow fluorescent protein (eYFP) via the double-floxed inverse orientation (DIO) reading frame approach, under the eukaryotic translation elongation factor 1 α (Ef1α) promoter. It is the promoter of an isoform of the eEF-1 complex α subunit, a ubiquitously expressed elongation factor protein. It is known to be responsible for the enzymatic delivery of aminoacyl transfer RNAs to the ribosome for the protein synthesis. (Addgene.org, Watertown, MA, USA).
- AAVrg-Ef1α-mCherry-IRES-Cre: adeno-associated retrograde virus expressing Cre recombinase and a cytoplasmatic red fluorescent protein (mcherry), that are separated by an internal ribosome entry site (IRES), and both expressed under the Ef1α promoter. (Addgene.org, Watertown, MA, USA)
- AAV9-rTH-PI-Cre-SV40: adeno-associated anterograde virus serotype 9, expressing cre recombinase under a fragment of the promoter of TH and a terminator sequence (SV40). (Addgene.org, Watertown, MA, USA)
- AAVrg-PGK-Cre: adeno-associated retrograde virus expressing Cre-recombinase by the promoter phosphoglycerate kinase (PGK), an enzyme necessary in every cell for glycolysis. (Addgene.org, Watertown, MA, USA)

3.3. DRUG ADMINISTRATION

3.3.1. Systemic administration

Systemic administration of the drugs of interest was performed by acute i.p. injection using injection volumes ranging from 0.15 to 0.2 ml. Dissolution procedures for each drug are described below:

- Amphetamine: it was dissolved in saline solution (0.9% NaCl) and administered at a dose of 2.5 mg/kg.
- Guanfacine chlorhydrate, JP-1302 dihydrochloride, and MK-912 hydrochloride: they were dissolved in saline solution (0.9% NaCl) in agitation for a few minutes (min) in a sonic bath. They were administered at a dose of 0.1 mg/kg for guanfacine and 0.05 mg/kg for JP-1302 and MK-912, respectively. For microdialysis experiments, these drugs were administered after collecting the basal samples and for the behavioral experiments, they were administered 30-45 min before the trial.
- MK-801 hydrogen maleate: it was dissolved in saline solution (0.9% NaCl) and administered at a dose of 0.5 mg/kg.
- Poly (I:C): it was dissolved in saline solution (0.9% NaCl) in agitation for a few mins in a sonic bath. It was administered at a dose of 7.5 mg/kg at GD9.5 in pregnant dams.

3.3.2. Local administration

One of the main advantages of brain microdialysis is the possibility to administer different substances locally through a dialysis probe located in a specific brain region, allowing us to observe their effect when they act directly on that specific brain nucleus. Two important aspects to take into account are: first, the pore size of the dialysis membrane (6 (kDa)); and second, the pH of the solution containing the drug, which must be in the physiological range between 7-7.4. Drugs administered locally through the microdialysis probe were dissolved in artificial cerebrospinal fluid (aCSF) whose composition was: 148 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$ with pH of 7.4, adjusted with 1 mM K_2HPO_4.

In the case of the microdialysis experiments administering locally hiperK^+ aCSF with a concentration of 50 mM ([K^+] = 50 mM), the original composition of the normal aCSF was modified, reducing Na^+ concentration to maintain the isotonicity of the solution. Thus, the hiperK^+ aCSF had the following composition: 100.68 mM NaCl, 50 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$ with a pH of 7.4 adjusted with 1 mM K_2HPO_4.

In contrast, for non-net flow microdialysis experiments where different solutions of the neurotransmitter of interest (NA or DA) were randomly perfused through the probe, the composition of the aCSF included 0.25 mM ascorbic acid as an antioxidant, in order to avoid processes of degradation of the different monoamines during the experiment. The pH was adjusted to be between 7.0-7.2 with NaOH 0.5 N.

3.4. MONOAMINE CONCENTRATION ANALYSIS IN MOUSE BRAIN TISSUE

3.4.1. Extraction of brain areas and tissue processing

Mice were decapitated by cervical dislocation and brains quickly removed for dissection. The hypothalamus, cerebellum, striatum, hippocampus, left and right cortex were dissected, weighted and processed fresh on the same day.

Tissue samples were placed in glass tubes and homogenized in cold buffer containing 0.1N perchloric acid with 100 µM ethylenediamine tetraacetic acid (EDTA) (15 µl/mg of tissue) using a T-10 basic Ultra-Turrax® (IKA, Staufen, Germany) and trying to get the same level of homogenization in all the samples. During the homogenization, the tubes containing the sample and the vehicle were maintained in contact with ice the maximum time possible. Then, the tubes were centrifuged at 20817 RCF/ G-force for 15 min at 4 °C. Subsequently, the supernatants were filtered using Costar Spin-X™ Centrifuge filters 0.22 µm (Sigma-Aldrich, St. Louis, MO, USA) at 1000 RCF/ G-force during 5 min at 4 °C. From the obtained final filtrates, 40 µl of each sample was separated for the immediate monoamine analysis with high-performance liquid chromatography (HPLC) equipment coupled to an electrochemical detector. In addition, 1 ml aliquots of the 1/100 dilution of the samples were prepared for further analysis; separating 10 µl of the original filtrate and adding 990 µl of the buffer. The remaining volumes of the samples were stored at -80 °C for future analysis.

3.4.2. Chromatographic analysis of monoamine concentration

3.4.2.1. Fundamentals of the HPLC/UHPLC technique

Chromatography is a technique commonly used for separating mixtures of substances into their individual components on the basis of their molecular structure and molecular composition. This involves a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Sample components that display stronger interactions with the stationary phase will move more slowly through the column than components with weaker interactions. This difference in relative affinities of the molecules for the mobile and the stationary phase, and therefore the difference in rates, causes the separation of various components. The retention time (RT) is a measure of the time taken for a solute to pass through the chromatography column. It is calculated as the time from injection to detection and it is specific for a substance under the same environmental conditions.

The sort of chromatography carried out in this study was reversed phase HPLC/ultra high-performance liquid chromatography (UHPLC). In this case, the column is filled with long hydrocarbon chains (C1-C18) attached to the surface of silica particles, which makes the stationary phase non-polar. A polar solvent is used, combining a specific range of salts to create the appropriate electrochemical conditions to detect the substances of interest passing through the column. There is a strong attraction between the polar solvent and polar molecules in the mixture being passed through the column. However, there is not as much

attraction between the hydrocarbon chains in the stationary phase and the polar molecules in the solution. Polar molecules in the mixture, therefore, spend most of their time moving with the solvent. Non-polar chemical groups present in the compounds in the mixture tend to form attractions with the hydrocarbon groups because of the van der Waals dispersion forces.

The detection system coupled to the chromatographic equipment depends on the characteristics of the analytes of study. Electrochemical detection is based on potential difference resulting from the oxidation-reduction reaction of the analytes induced by the electrodes adjusted to a specific electrochemical potential. This difference in potentials is directly proportional to the concentration of the analyte. Subsequently, a chromatogram is obtained, which is the record of the chromatographic separation, where each peak corresponds to a specific *redox* component in the sample.

3.4.2.2. Chromatographic equipment

The HPLC equipment used for chromatographic detection of tissue monoamines consisted of (**Figure 3.4**):

- Degasser system for the mobile phase that avoids air entrance (Agilent HP 1100 Series, Santa Clara, CA, USA).
- A quaternary pump to push the mobile phase at a constant rate through the system (Agilent HP 1100 Series, Santa Clara, CA, USA).
- Refrigerated autosampler (Agilent HP 1100 Series, Santa Clara, CA, USA).
- A chromatographic column, which contains a stationary phase (Antec Scientific, Zoeterwoude, The Netherlands).
- A thermostat that allows keeping column temperature constant (Agilent HP 1100 Series, Santa Clara, CA, USA).
- Electrochemical detector Decade II (Antec Scientific, Zoeterwoude, The Netherlands).
- Dual channel interface: it converts the analog signal from analytical instruments to digital data and transmits it to the host computer (control station) for further processing (Agilent 35900E, Santa Clara, CA, USA).
- A control station to obtain chromatograms (DELL Optiplex 760, Round Rock, TX, USA).

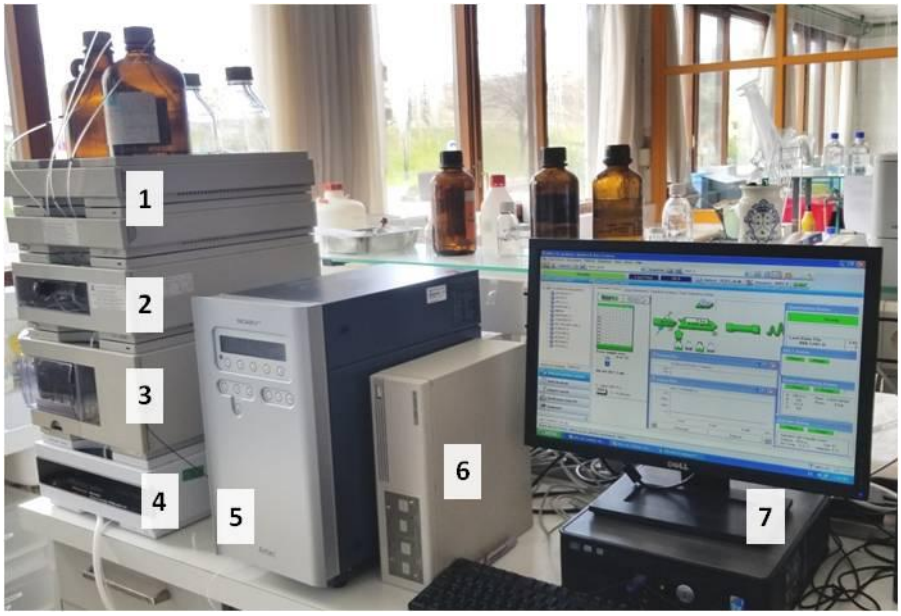

Figure 3.4: HPLC equipment coupled to an electrochemical detector. 1) Degasser; 2) Quaternary pump; 3) Autosampler; 4) Chromatographic column; 5) Electrochemical detector; 6) Dual Channel Interface; 7) Control station.

3.4.2.3. Monoamine detection

Samples (30 µl) were injected by a refrigerated autosampler (Agilent HP 1100 Series, Santa Clara, CA, USA), and analyzed in separate assays for tissue content of DA and 5-HT or for NA. First, the original sample filtrates were injected (no diluted), followed by the 1/100 diluted samples. The injection volume for chromatographic analysis was 30 µl.

The mobile phase used for detection and measure of DA and 5-HT was composed of: 50 mM phosphoric acid (Panreac Química S.A.U. Barcelona, Spain), 0.1 mM EDTA (Merck, Darmstadt, Germany), 8 mM NaCl (Merck), 500 mg/l sodium octyl sulfate (Sigma Aldrich, St. Louis, MO, USA), and methanol (12%) (Carlo Erba Reagents, Val de Reouil, France). The pH 6.0 was adjusted with a sodium hydroxide solution 6 N (Merck, Darmstadt, Germany). This mobile phase was filtered to avoid impurities, passing it through a filter - pore size of 0.45 µm diameter (Millipore, Billerica, MA, USA), with the help of a vacuum pump. For detection and measure of NA, the same mobile phase components were used but the proportion of methanol was reduced from 12% to 5% in order to be able to correctly detect and quantify its corresponding chromatographic signal.

The mobile phase flowed through the system at a 0.2 ml/min rate. To detect and quantify monoamine concentrations, a VT-03 (Antec Scientific, Zoeterwoude, The Netherlands) electrochemical cell was used. *Redox* potential was 0.30 V, and ALF-215, 2.1 x 150 mm, C18 (Antec Scientific, Zoeterwoude, The Netherlands) chromatographic column was used. The oven temperature was 35 °C. Specifically, for detection and quantification of DA and 5-HT, sample running time was fixed at 30 min with a 1 nA output range (**Figure 3.5**). In the case of NA, the output range would vary between 2 nA or 5 nA depending on the brain region (**Figure 3.6**).

The peak areas of NA, DA, and 5-HT were integrated by a Chemstation plus software (Hewlett Packard Ltd., Morain, OH, USA). Tissue monoamine concentration was determined using a linear regression analysis of the peak heights obtained from a range of standards of known concentration (0.1, 0.5, 1, 3, 5, 10, 100 nM) and expressed as nM/mg tissue.

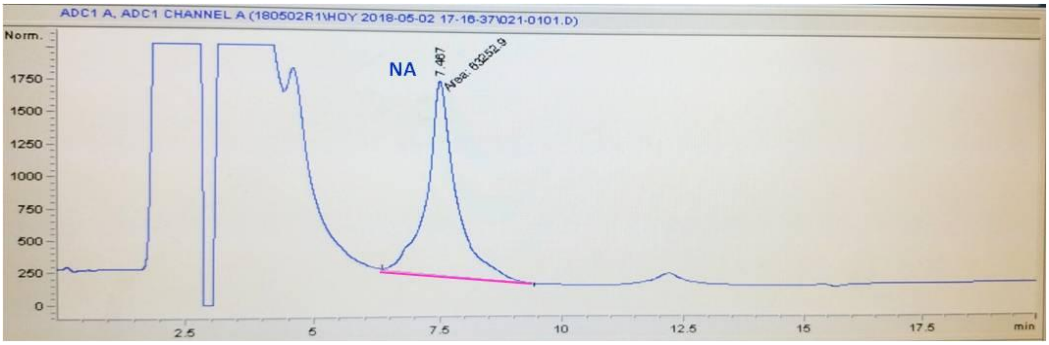

Figure 3.5: Representative chromatogram of NA quantification (RT: 7.467 min) in a sample of the cerebellum of a saline mouse (output range 2 nA) from the HPLC system.

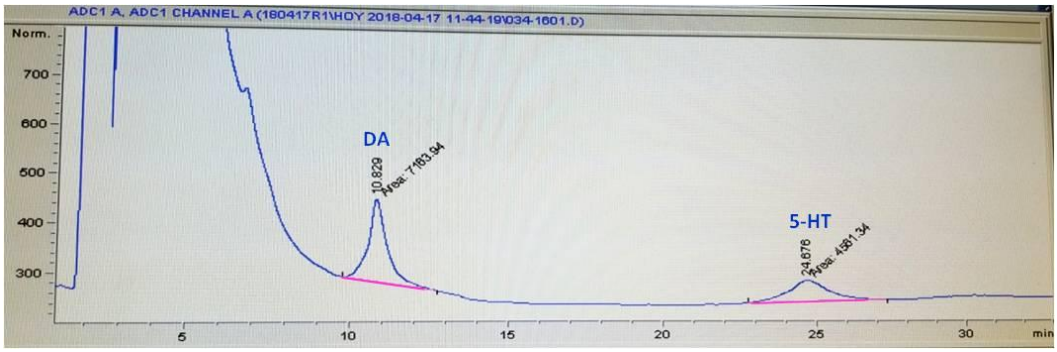

Figure 3.6: Representative chromatogram of DA and 5-HT quantification (RT: 10.829, 24.676 min) in a sample of the hippocampus of a saline mouse (output range 1 nA) from the HPLC system.

3.4.3. Statistical analysis

The statistical analysis was performed using GraphPad Prism™ v5 software (GraphPad Software Inc., CA, USA). For each monoamine and region studied, data are expressed as mean ± SEM (standard error of the mean). The statistical comparison between means was made by the Student's *t*-test. The level of statistical significance was set at $p<0.05$.

3.5. PROTEIN EXPRESSION IN BRAIN TISSUE BY WESTERN BLOT

Western blot is a broadly used technique for the detection and identification of proteins by means of antibodies. The process is divided into various steps: electrophoresis separation in gel by molecular weight of the proteins present in the sample; protein transfer from the gel to nitrocellulose membranes, which immobilizes the proteins and makes them accessible to the antibodies; and exposition of the nitrocellulose membrane to a solution containing the antibodies that recognize and bind specifically the target protein.

In this study, the immunoreactive density of different components of the dopaminergic and noradrenergic systems was quantified in the brain cortex and striatum of saline and Poly (I:C) mice. Simultaneously, the density of the cytoskeletal protein β-actin was also measured and used as a loading control to normalize the signal of interest. The β-actin protein is one of the six isoforms that actin presents and it is constitutively expressed in eukaryotic cells. It participates in cellular structure, integrity, and mobility. It has a molecular weight of 42 kDa and is a very conserved protein between species and throughout evolution. In addition, it presents little variability in response to different experimental manipulations. Therefore, it is used as a loading control in different techniques such as the Western Blot (Gilsbach et al., 2006; Greer et al., 2010).

3.5.1. Sample preparation

The samples used in the western blot experiments were a total homogenate of the cerebral cortex and a total homogenate of the striatum of saline and Poly (I:C) mice. After brain extraction, these areas were dissected and stored at -80 °C until their use.

For obtaining the total homogenates, hundred milligrams of brain tissue was homogenized in 1 ml of cold Tris-sucrose buffer (0.32 M sucrose in 5 mM Tris-HCl, pH 7.4) supplemented with protease and phosphatase inhibitors (50 µL/g of Sigma protease inhibitor Cocktail, 5 mM Na_3VO_4, and 10 mM NaF) using a mechanical tissue homogenizer at 4 °C.

Once the total homogenates were prepared, we proceeded to quantify the amount of proteins present in the samples with the Bradford method (Bradford, 1976). Briefly, 200 µl of cold Bradford protein assay reagent (Bio-Rad Laboratories, California, USA), diluted 1:5 in MilliQ water, was added to 10 µl of protein suspension. After incubation at room temperature for 5 min, absorbance at 595 nm wavelength was determined in a plate spectrophotometer (ELx808™ Absorbance Microplate Reader, Biotek™ Instruments, Vermont, USA). Sample protein content was measured in triplicates in a 1:20 dilution. Protein values were extrapolated from the linear regression of a standard line carried out with BSA between 0 and 0.7 mg/ml, following the same procedure described above. Linear regression analysis and extrapolation of the data were performed with GraphPad Prism™ v5 software (GraphPad Software Inc., California, USA). Finally, samples were diluted in order to obtain the same final concentration for all the samples of the same brain region (4 µg/µl for the cortex and 2.14 µg/µl for the striatum) and aliquots of 400 µl of sample were stored at -70 °C until use.

The day of the experiment, 400 µl aliquots of the total homogenates were defrosted and combined with 80 µl 6X homemade Laemmli sample buffer (12% SDS, 0.06% bromophenol

blue, 47% glycerol, 0.5M Tris HCl) and 48 µl of dithiothreitol (DTT) 1M (Sigma, MO, USA) obtaining a different final protein content depending on the brain region (cortex final concentration of the samples resulted in 3 µg/µl and for the striatum 1.62 µg/µl). Then the mixtures were denatured at 95 °C for 5 min. The samples of saline and Poly (I:C) mice were processed in parallel on the same day.

3.5.2. Electrophoresis in polyacrylamide gels

The first step consists of the separation of the solubilized proteins present in the sample according to their molecular weight using polyacrylamide gels under denaturing conditions. This process is known as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis.

Polyacrylamide gels are formed by the polymerization of acrylamide and bis-acrylamide in the presence of an initiator and a catalyst. TEMED (N, N, N, N'-tetramethylethylenediamine) was used as an initiator and persulphate ion ($S_2O_8^-$), which was added in the form of ammonium persulfate, was used as a catalyst. These two compounds determine the speed at which the gel polymerizes.

The electrophoresis is a discontinuous process, since the proteins migrate, in the first place, through what is known as “stacking gel”, causing their accumulation in the front. The stacking gel is prepared with 5% of the polyacrylamide, 125 mM Tris HCl, 0.1% SDS, 0.07% prostate specific antigen (PSA) (freshly prepared each experiment day), and 0.14 % TEMED pH 6.8. However, protein separation begins when the migration front reaches what is called the “running gel”. This gel is prepared in a 10% acrylamide-bisacrylamide concentration in a solution of 0.37 M Tris HCl, 0.1% SDS, 0.07% PSA and 0.07% TEMED, pH 8.8. A comb from Teflon™ was inserted in each stacking gel in order to form the 15 lanes where the samples run.

In these experiments, the samples were loaded on 15-lane gels, sized 6 x 8 cm each. The loading volume of the samples in each lane depended on the brain region (since the sample concentration was different) and the protein of study (**Table 3.2**) (prior experiments were performed in order to determine this). The first lane of each gel was loaded with a suspension of a commercial molecular weight marker (10-250 kDa, Precision Plus Protein™, Dual Color Standards, Bio-Rad). It consists of a set of proteins of known molecular weight that are separated and transferred with the rest of the samples and allow to determine the molecular weight of band of interest. The rest of the lanes were loaded with the corresponding volume of the samples of the two different groups (six saline mice and seven Poly (I:C) mice). The gel loading sketch is shown in **Figure 3.7**.

Table 3.2: Loading volume of the samples for the western blot experiments depending on the brain region.

Protein of study	Loaded volume (µl) Cortex	Loaded volume (µl) Striatum
Dopamine Transporter (DAT)	25 (75 µg)	15 (24.3 µg)
Tyrosine hydroxylase (TH)	20 (60 µg)	15 (24.3 µg)
Noradrenaline Transporter (NET)	15 (45 µg)	15 (24.3 µg)
Dopamine D_1 receptor (D_1R)	15 (45 µg)	15 (24.3 µg)
Dopamine D_2 receptor (D_2R)	15 (45 µg)	15 (24.3 µg)
Molecular weight marker (MW)	2	2

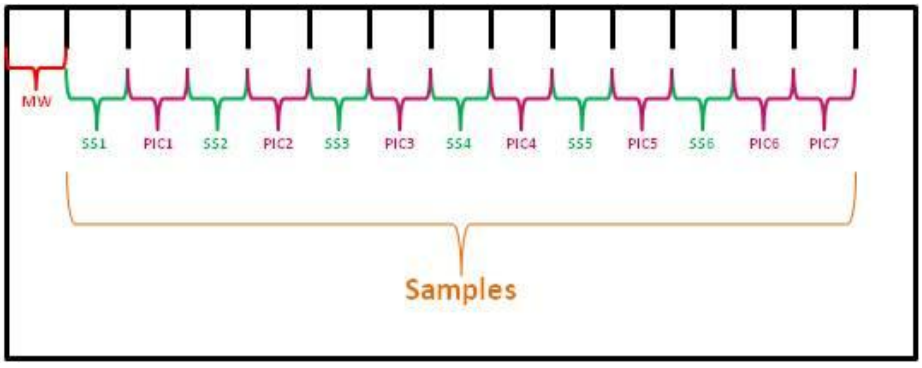

Figure 3.7: Gel loading sketch of an example of a western blot experiment. MW: molecular weight marker. SS: saline mice. PIC: Poly (I:C) mice.

The electrophoresis was carried out in a buffer consisting of 25 mM Tris HCl, 192 mM glycine and 0.1% SDS pH 8.3. Initially and during 30 min approximately, a 60 V tension was applied to concentrate the proteins in the "stacking gel", followed by a 120-130 V tension during running on the "running gel". Running was stopped when the electrophoresis front reached the end of the gel, approximately 150 min later.

3.5.3. Transference

The next step was the transference or electroblotting, a process in which the proteins are transferred from the gel to a nitrocellulose membrane (PROTAN™ BA85, Schleicher & Schuell GmbH, Dassel, Germany) by applying an electric field of 0.3 A per tank during 60 min. The transference cassettes were prepared as indicated by the provider (Bio-Rad Laboratories, California, USA). The procedure started by stacking successively within the cassettes, in order: a flat sponge, three Whatman™ chromatography papers soaked in transfer buffer (GE Healthcare, Buckinghamshire, UK), the gel, the membrane in direct contact with the gel, another three chromatographic papers and finally, another flat sponge (**Figure 3.8**). The sponges were an important component in order to keep the system joined and under pressure in the transfer cassette. Transfer buffer consisted of 25 mM Tris HCl, 192 mM glycine, 20% methanol, pH 8.3. The electric field applied perpendicular to the plane allowed the proteins to migrate from the gel to the surface of the membrane, being strongly adhered, so that the resulting membrane was an exact copy of the protein pattern present in the polyacrylamide gel.

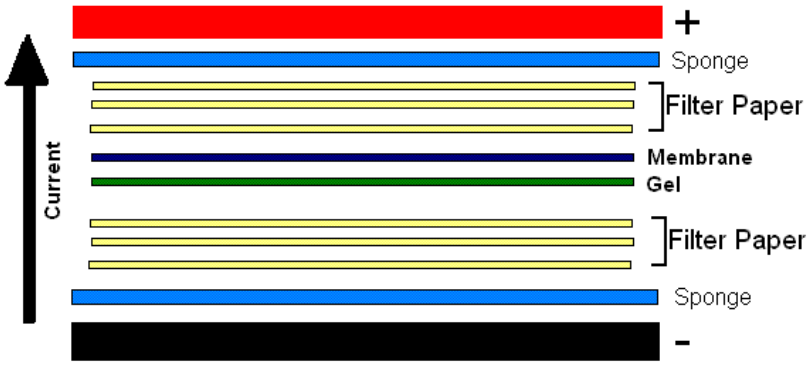

Figure 3.8: Representative image of the different components of a transfer cassette.

3.5.4. Blockage of the membrane

Once the transference had finished, the membranes were dyed with Ponceau S solution (0.5% Ponceau S red, 1% acetic acid) in order to check that the proteins had been transferred properly into the membrane. Afterwards, nitrocellulose membranes were rinsed in phosphate-buffered saline solution (PBS) (137 mM NaCl, 2.7 mM KCl, 12 mM Na_2HPO_4, 1.38 mM KH_2PO_4, pH 7.4) and incubated 1 h at room temperature in a blocking solution (5% non-fat dry milk in PBS) to reduce non-specific binding of the antibodies and the background signal. This process is necessary because the membrane has high affinity for proteins, which is an advantage during the transference process. However, subsequent processes involve incubation of antibodies that are also proteins that should specifically bind the proteins against which they were designed. For this reason, a common stage in all the immunodetection processes is the membrane blockade, preventing non-specific binding of the detection system.

3.5.5. Incubation with the antibodies

After 1 h of the blockade, nitrocellulose membranes were incubated overnight at 4 °C in the blocking solution, supplemented with 0.1% Tween 20 and containing both the appropriate primary antibodies against the proteins of study. In addition, in order to avoid cross-talk reactions, β-actin was detected either by cutting the membranes or by reincubating the following day the membranes that had already been previously incubated with the primary antibody corresponding to the protein of interest (**Table 3.3).**

Table 3.3: Experimental conditions in the western blot experiments for the overnight incubation with the primary antibodies.

Protein	Primary antibody	Dilution	Provider
Dopamine Transporter (DAT)	Anti-Dopamine Transporter Antibody (Rat) MAB369	1:500	Millipore
Tyrosine hydroxylase (TH)	Anti-Tyrosine Hydroxylase Antibody (Mouse) SC25269	1:500	St. Cruz
Tyrosine hydroxylase (TH)	Anti-Tyrosine Hydroxylase Antibody (Rabbit) AB41528	1:500	Abcam
Noradrenaline Transporter (NET)	Anti-Norepinephrine Transporter Antibody (mouse) NET05-2	1:1000	MabTechnologies
Dopamine D_1 receptor (D_1R)	Anti-Dopamine Receptor D1 Antibody (mouse) AB78021	1:500	Abcam
Dopamine D_2 receptor (D_2R)	Anti-Dopamine Receptor D2 Antibody (mouse) SC5303	1:500	St. Cruz
Beta actin	Anti-β-actin antibody (mouse) A1978	1:200,000	Sigma-Aldrich
Beta actin	Anti-β-actin antibody (rabbit) AB8227	1:10,000	Abcam

Regarding the use of the different antibodies in this study, it is important to remark the necessity of characterizing the specificity of each antibody, since it is essential that the selected antibody specifically recognizes the band of the protein against which it has been designed. To carry out this characterization, different procedures can be used, including their validation with KO animals and the use of blocking peptides as a negative control. However, it is important to consider that antibodies against membrane proteins, especially of GPCRs, usually have a very nonspecific band pattern. In many cases, the strategies designed to characterize their specificity are not available or are not successful. Therefore, in those cases, the specificity defined by the manufacturer, and that based on the expected molecular weight for the protein of interest is considered.

In our case, the DAT antibody (*Millipore,* MAB369) from was selected because our team previously validated its specificity, using DAT-KO animals, where the signal disappeared

(Erdozain et al., 2018b). Likewise, the specificity of the selected NET antibody (*MabTechnologies,* NET05-2) had previously been shown in KO mice for this transporter (Matthies et al., 2009). The specificity of the D1 antibody (*Abcam,* AB78021) was also verified in striatal D_1-KO tissue (Keeler et al., 2016).

For the D_2R immunoblotting, we did not find any antibody validated in KO mice, so the selection of the antibody (*St Cruz* SC5303) was driven from the elevated numbers of papers using it (Goodman et al., 2012; Luessen et al., 2016). In any case, we confirmed that the protein was visualized at molecular weight predicted by the provider.

For the TH, since we did not find a KO-validated antibody, we used two different antibodies to see whether the results were similar: one from *Abcam* (AB41528), and one from *St. Cruz* (SC25269). The first TH antibody (*Abcam*, AB41528) had been previously used by our team and validated with the blocking peptide, which completely erased the signal (Itziar Gil de la Pisa, Doctoral Thesis, 2013). As for the second TH antibody (*St.Cruz,* SC25269), it was selected since it had been previously reported its specificity in another MIA model in mice (Deslauriers et al., 2013).

Next day, the excess of the primary antibody was removed by washing the nitrocellulose membrane with PBS supplemented with 0.1% Tween 20. These washing processes are important to eliminate excess unbound reagents and to reduce the background noise. Subsequently, the fluorescent secondary antibodies that specifically recognized the primary antibodies were incubated simultaneously in the blocking solution at room temperature for 1 h (**Table 3.4**). Finally, the nitrocellulose membranes were washed from an excess of secondary antibodies with PBS.

Table 3.4: Experimental conditions in the western blot experiments for incubation with the secondary antibodies.

Protein	Secondary antibody	Dilution	Provider
Dopamine Transporter (DAT)	Dylight 800 anti-rat (Goat) SA5-10024	1:5000	Invitrogen
Tyrosine hydroxylase (TH) with St. Cruz antibody	Alexa Fluor 680 anti-mouse (Goat) A21057	1:5000	Invitrogen
Tyrosine hydroxylase (TH) with Abcam antibody	Dylight 800 anti-rabbit (Donkey) 611-745-127	1:5000	Rockland
Noradrenaline Transporter (NET)	Alexa Fluor 680 anti-mouse (Goat) A21057	1:5000	Invitrogen
Dopamine D_1 receptor (D_1R)	Alexa Fluor 680 anti-mouse (Goat) A21057	1:5000	Invitrogen
Dopamine D_2 receptor (D_2R)	Alexa Fluor 680 anti-mouse (Goat) A21057	1:5000	Invitrogen
Beta actin (mouse)	Alexa Fluor 680 anti-mouse (Goat) A21057	1:5000	Invitrogen
Beta actin (rabbit)	Dylight 800 anti-rabbit (Donkey) 611-745-127	1:5000	Rockland

3.5.6. Quantification of the immunoreactive signal

Nitrocellulose membranes incubated with fluorescent conjugated secondary antibodies were detected and quantified using the Odyssey infrared imaging system (LI-COR Biosciences, Nebraska, USA). The image of the Dylight 800 conjugated antibody was captured at 800 nm, visualized in green, while the Alexa Fluor 680 conjugated one was detected at 680 nm and visualized in red. Signal was expressed as integrated intensity.

The immunoreactivity values of the different target proteins of the study were normalized for the amount of β-actin and given as a percentage of actin-normalized immunoreactivity. Finally, a mean value of all the percentages of the mice belonging to the same experimental group was used as a final estimate and expressed with respect to the saline mice, considered as controls (100%).

3.5.7. Statistical analysis

The analyses were carried out with GraphPad Prism™ v5 software and results are expressed as means ± SEM. The statistical comparison between means was made by the Student's *t-test*. The level of significance was taken as $p<0.05$.

3.6. THE NOVEL OBJECT RECOGNITION TEST (NORT)

In rodents, NORT has become a benchmark task for assessing recognition memory (Broadbent et al., 2009). Recognition memory refers to the ability to judge a previously encountered item as familiar (Squire et al., 2007). Tasks that assess recognition memory (and object recognition memory, in particular) have become increasingly useful tools for basic and preclinical research investigating the neural basis of memory (Winters et al., 2008).

This test was performed always in the same room with the same experimental conditions:

- Light source: the room was equipped with a set of fluorescent ceiling lights with an automatic system that allowed to adjust the lighting conditions to be appropriate for the experiment (see *light measurement*), as the standard fluorescent ceiling lights are too bright for this task.

- Light measurement: a light-meter was used to measure the light intensity of the room. This intensity was set up to be between 35-50 LUX. This value was checked every day before starting the trial. The measurement was taken by placing the lux-meter in the center of the cage where the test was going to be performed.

- Experimental box: the box where the trials were performed consisted of a square-shaped wooden box (45 x 45 x 40 cm), of light brown color and made by a non-porous and odorless material. The material used for the box is a key factor to be taken into account since it is intended to prevent the box from taking odors from mice excrements and urine and also to facilitate its posterior cleaning (**Figure 3.9**).

- Recording camera: the recording camera (Sony Color Video Camera SSC-G118, AC 220-240V-50 Hz, Sony Europe Limited, Surrey, UK) was located on the roof, just above the experimental box where the trials were carried out. To achieve a better capture of the movements of the animal, the experimental box was placed on top of a cart with 4 legs. This way, the distance between the camera and the area to be recorded was reduced, thus, achieving a better and clearer image of what it was happening inside the box (**Figure 3.9**).

- Objects: object choice is potentially one of the most important and underappreciated aspects of conducting successful NORT protocol (Vogel-Ciernia & Wood, 2014). Since this task relies on rodents' innate preference for novelty, all objects should meet the following criteria: 1) do not produce a fear response, 2) are adequately explored during a testing session (at least 3 seconds per object), 3) the two objects have an equal innate preference and can be discriminated. Objects were chosen to be matched for size and be easily cleanable. In this study, the objects presented to be explored were plastic objects that varied in color and size (width = 7.6-8.9 cm; height = 7.5-12.7 cm) **(Figure 3.24)**. Four identical copies of each object were available (Mega Bloks-8327, Mattel, Hawthorne, California, USA). The objects were situated 9 cm apart approximately and secured to the floor of the cage by scotch tape, to prevent animals from moving them during exploration.

Figure 3.9: Materials used for the NORT test: experimental box (left side); recording camera (center); objects (right side).

3.6.1. The NORT

This test consists of 4 consecutive days of experimental trials: the first day of habituation, two days of training and the last day of testing. Each day, before starting the session, animals were brought to the experimental room at least 30-45 min before the trial, allowing them to acclimatize to the experimental conditions.

3.6.1.1. Habituation

The first day of the test consists of the habituation to the experimental box. Each animal was placed in the center of the box with no objects at all, and it was allowed to freely explore the box for 10 min. After this time, animals were returned to their cage and the feces and/or urine removed. The box was cleaned with wet paper towels and well dried before starting the next trial with the next mouse.

3.6.1.2. Training

The second and third day of the protocol are exactly the same. They consist of training the animal to recognize the objects. For that purpose, each animal was placed in the center of the box with two identical objects located in opposite corners of the box, separated 9 cm approximately one from the other. Animals were allowed to freely explore the objects for 10 min. Equally to the habituation phase, after that time, animals were returned to their cage and the box cleaned and dried properly.

This procedure is repeated exactly the same the third day (training day 2).

3.6.1.3. Testing

The fourth and last day of the protocol is the “test day”. For testing the animals, one of the two identical objects used during the training phase (familiar object) is replaced with a new different object (the novel object), and located in the same spot as the one before. Thus, there is a familiar object and a novel object in the box now. Then, animals were placed in the center

of the box and allowed to explore both objects for 10 min. Object exploration was scored when the mouse's nose was within 2 cm of the object and the vibrissae were moving towards it (Broadbent et al., 2009). Object exploration was not scored when the animal used the object to rear upward with the nose facing the ceiling (**Figure 3.10**).

Data such as time spent with the familiar object, the novel object, and both familiar and novel objects (i.e. the total exploration time) were obtained manually and the preference for the novel object was calculated by the discrimination index, using the formula [(*time devoted to the novel object – time devoted to the familiar object) / (time devoted to the novel object + time devoted to the familiar object*)]. This is the parameter for the evaluation of cognitive impairment. The object served as novel object and the left/right position of the novel object were counter-balanced within each group.

Besides, when the effect of the α_2AR compounds wanted to be tested on the performance of the saline and Poly (I:C) mice during the NORT, the protocol was carried out exactly as described previously, with the only difference on the fourth day of the trial (the testing day). That day, the drug (either guanfacine or MK-912), was administered (0.1 mg/kg i.p. or 0.05 mg/kg i.p., respectively) to each of the animals 30-45 min before starting the test.

Figure 3.10: Representative picture of the mouse exploring the objects during the "testing day" session.

3.6.2. Statistical analysis

Data from the evaluation of the cognitive basal status and the potential cognitive impairment of the saline and Poly (I:C) mice were analyzed using GraphPad Prism™ v5 software (GraphPad Software Inc., CA, USA).

In the case of the cognitive basal status evaluation, data are expressed in bars as mean ± SEM and a Student's unpaired *t*-test was used for the comparison between saline and Poly (I:C) mice; considering to be statistically significant when $p<0.05$. In the case of the data from the evaluation of the effect of the α_2AR compounds on the NORT, data is also expressed in bars as mean ± SEM and a two-way analysis of variance (ANOVA) followed by Bonferroni *post-hoc* test was used in the statistical analysis.

3.7. THE 5-CHOICE SERIAL REACTION TIME TASK (5-CSRTT)

Although the importance of cognitive dysfunction as a target for decreasing and preventing psychiatric morbidity has been repeatedly emphasized, few pharmacotherapeutic options have been developed (Green, 1996; 2006; Holthausen et al., 2007; Keefe et al., 2007; Weiss et al., 2002). Besides, little is known about the mechanisms supporting the existing pharmacological treatments and their relation to the clinical outcome (Insel & Wang, 2010; Insel et al., 2013). The absence of data connecting mechanistic action to therapeutic efficacy remains a severe shortcoming, as such knowledge is crucial for developing more refined agents and establishing novel therapeutic avenues (Hvoslef-Eide et al., 2016).

One reason for this lack of success could be the mismatch between how cognitive functions are evaluated in experimental animals and humans. In humans, for assessing different cognitive domains, batteries of visual touchscreen-based tasks are usually used, facilitating between-task comparison and reducing confounds (Sahakian & Owen, 1992; Levaux et al., 2007; Barnett et al., 2010). However, in rodents, comprehensive cognitive batteries are rarely employed and different cognitive domains are assessed by tasks that typically vary widely in the nature of stimuli, responses, reinforcers, and environment. In addition, these tasks often have no resemblance to the tests used in humans, reducing the likelihood of rodent and human studies evaluating comparable cognitive functions, hence compromising the efficacy of translation (Hvoslef-Eide et al., 2016).

In this study we use a touchscreen-based cognitive testing method, which has the potential to achieve more accurate, efficient, and reproducible phenotyping of rodents, helping bridge the “translational divide” between animal and human studies of cognition (Hvoslef-Eide et al., 2016). The tasks have high translational face validity which minimizes the methodological differences, enabling back- and forward- translational opportunities and improving the likelihood of neurocognitive validity (Romberg et al., 2011; Talpos & Steckler, 2013).

3.7.1. The rodent 5-CSRTT

The rodent 5-CSRTT has been a predominant method for modeling attentional and inhibitory functions in rodents, and its translational utility is discussed at length elsewhere (Lustig et al., 2013; Robbins, 2002; Young et al., 2009). In this task, the animal is required to simultaneously monitor a number of potential stimulus inputs across a delay before responding within an appropriate time frame to a single, unpredictable stimulus location signaled by the brief onset of a light source. The task allows the identification of a behavioral profile based on a range of indices—including accuracy, omissions, premature responses, perseverative responses, and latency measures (Robbins, 2002). There is a clear advantage of using the touchscreen as opposed to the 9-hole version of the 5-CSRTT, which was first developed in a 9-hole operant chamber where animals responded in a nose-poke hole following the onset of an LED. The 5-CSRTT adds the potential to include in a single task a full battery of tests targeting a range of cognitive constructs. Furthermore, the recent use of touchscreen 4-CSRTT in human studies (Voon et al., 2014; Worbe et al., 2014) facilitates comparison between future work and the extensive rodent literature generated using the 5-CSRTT.

3.7.1.1. Animals

For these specific experiments, C57BL/6J mice were used. These animals went through the Poly (I:C) protocol described in *section 3.1.2.2*. For this characterization, the experiments were carried out only in males (15 males from the 26 mice of the total saline group, and 10 males from the 18 mice of the total Poly (I:C) group were able to reach baseline conditions).

Animal housing, distribution, and husbandry are described before (*section 3.1.1*).

3.7.1.2. Apparatus

Training and testing were conducted in a touchscreen-operant apparatus with four sound and light-attenuated operant boxes (Campden Instruments, LTD., Leicester, UK). Each box contained the following parts (**Figure 3.11**):

- Training trapezoid-shaped chamber (1): where the mouse is placed to perform the task. Placed within wooden insulated boxes (40 x 34 x 42 cm).
- A house light and a tone generator (2): it illuminates/makes a sound depending on the election made by the mouse.
- A liquid reward delivery system (3): It consists of a channeling system through a tube that carries the reward by a peristaltic pump so that it can be collected by the animal.
- A reward magazine (4): where mice could start trials and collect the liquid reward (10 % condensed milk (CM)), delivered by a peristaltic pump.
- A recording camera (5): installed on the ceiling of the box, retransmits live what the animal does through a screen connected to it.
- A touch-sensitive screen (6): an infrared touchscreen monitor (24.5 x 18.5 cm) where mice could elicit nose-poke responses.

There were two beams inside the chamber; one responsible for quantifying the locomotor activity of the animal, and the other one capable of detecting and quantifying the head entries to the reward magazine located in the narrowest part of the trapezoid chamber. The visual stimuli presented to the animal consisted of a white square, and it was displayed in a touch-sensitive screen on the opposite side of the chamber (6). Just in front of the touch screen was placed an acrylic black mask (7) containing five equally shaped (4 x 4 cm each) and distanced (3.5 cm from the mask's sides, 1.5 cm from the steel floor) apertures.

ABET II software (Campden Instruments, Ltd., Leicester, UK) and Whisker Server software (Cambridge University Technical Services Ltd., Cambridge, UK) were the software systems controlling the chambers' operations and responsible for recording, collecting and generating the raw data necessary for further data analysis.

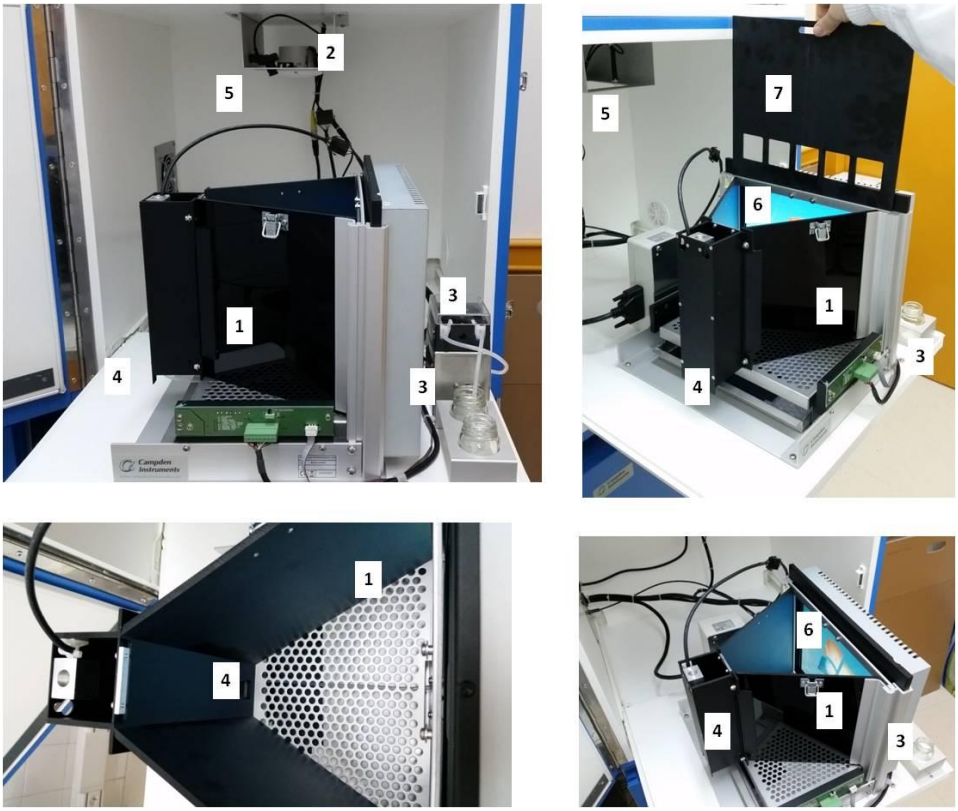

Figure 3.11: Description of the different parts of one of the touchscreen-operant boxes.

3.7.1.3. 5-CSRTT procedure

The 5-CSRTT is divided into 3 different phases: pre-training, training to baseline, and testing. All mice were trained as described by the Instruction Manual of the system (Basic Tax Protocol-89543CAM 5-Choice Serial Reaction Time Task with Cambridge Amendment for Mouse Touch Screen Systems and ABET II).

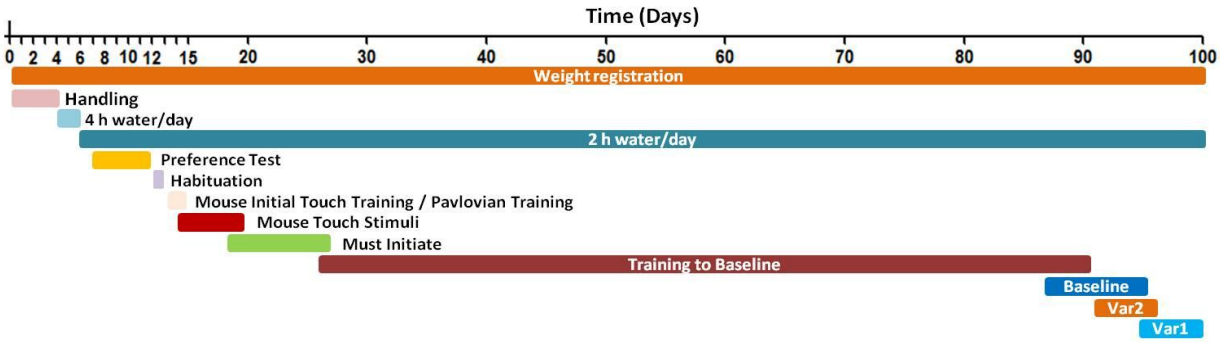

Figure 3.12: Schematic representation of the different phases of the 5-CSRTT and their duration.

3.7.1.3.1. Pre-training phase

3.7.1.3.1.1. Handling and water deprivation

This part if the first step of the pre-training phase. Before the experiments started, all the mice were allowed to acclimate to researchers for 2 weeks and identified by tail marking (handling). After that, a liquid deprivation protocol was initiated, in order to increase the motivation of the animals to what later would be their liquid reward / reinforce during the test (10 % CM in water) (Nestle™, Vevey, Switzerland). Motivation is a critical determinant of performance in this task and water deprivation allows greater control of this variable. Although only access to water is limited and they had free access to food, their food consumption will also decrease because of the tendency of animals to eat only when they can drink (**Figure 3.12**).

Therefore, we monitored the weight of these animals during the whole experiment. If an animal's weight fell more than 10 % from an average of the previous two days, then the animal should be put onto free water until the body weight increased.

Liquid deprivation protocol started by removing the water bottles on the evening of the first day. During the next two days, animals had access to water only for 4 h (20 h of deprivation). After checking that the body weights had not fallen more than 10 % from the calculated average, the access to water was reduced to 2 h a day (22 h of deprivation). From this moment onwards, body weights are monitored daily, and this 22 h of deprivation would be maintained until the end of the experiment.

3.7.1.3.1.2. Preference-test: habituation to reinforcement

The reinforce of liquid reward for the animals in the task was 10 % CM in water. With the objective to habituate the mice to this reward and detect possible pre-existing differences in their preferences, a CM vs water preference-test was carried out. This test was performed after 7 days of stable body weight (**Figure 3.12**).

The preference test takes place over 5 days. Animals are placed into cages (similar to the ones they are housed in) where 2 small plastic bowls (10 mm high, 20 mm diameter and maximum volume of 3 ml) are placed approximately 50 mm from the rear wall. During the first and second days, both bowls are filled with normal tap water. Then, on days 3, 4 and 5, one bowl is filled with water and the other is filled with 10 % CM, alternating the sides daily. Bowls are weighed before and after each session, and the volumes consumed of each solution are calculated. The mice are allowed to stay and drink from the bowls for 10 min in each session.

3.7.1.3.1.3. Habituation

This is the first stage in which the animals finally have contact with the operant box. For the habituation, the mouse is left in the chamber for a 10 min session, with all the lights turned off. There is no stimulus or reward presentation during the session. The activity of the mouse inside the chamber is monitored by the software (**Figure 3.12**).

3.7.1.3.1.4. Mouse Initial Touch Training / Pavlovian Training

In this initial stage, the trials start with the light stimulus (a white square) displayed randomly in one of the 5 windows of the screen and only in one window at a time. The position is chosen pseudo-randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. After a delay of 30 s, the image is removed and the liquid reward is delivered to the reward magazine, accompanied by illumination of the tray light and a tone, to facilitate the learning process.

If the mouse touches the window where the stimulus is displayed during the time it is present on the screen, the image will be removed, a tone will be played and a triple volume of liquid reward will be delivered to the reward magazine.

The mice will perform this phase during one day, and their activity will be monitored by the software. All of them will pass to the following stage of the pretraining (**Figure 3.12**).

3.7.1.3.1.5. Must Touch Stimuli

The trial starts with the stimulus displayed in one of the 5 windows randomly, and it is not removed until the mouse touches ("nose-pokes") the stimulus on the screen. After that, there would be a liquid reward delivery to the magazine, accompanied by illumination of the tray light and the tone. If the mouse nose-pokes in the other 4 blank windows there is no response.

The criterion to pass to the following phase if to complete 30 trials in a 60 min session during two consecutive days (**Figure 3.12**).

3.7.1.3.1.6. Must Initiate

In this stage, the trial starts with free delivery of reward and the tray light turned on. The mouse must nose-poke and exit the reward magazine before a stimulus is displayed randomly on the screen in one of the 5 windows. Entries to collect the reward turn off the tray light and after ITI of 5 s, the tray light is illuminated again.

The following trial will start when the mouse nose-pokes again in the reward magazine and exits, and the next light stimulus is displayed on the screen.

The criterion to pass to the following phase is to complete 30 trials in a 60 min session, during two consecutive days (**Figure 3.12**).

3.7.1.3.2. Training to baseline

This phase is composed of different sessions where mice are trained to reach what is called the "baseline" point; the moment when the animals have gained a capacity to respond to the stimulus in a certain amount of time, and it is considered the point of the training when they are ready to be tested.

This part of the training is composed of eight sessions (from session 9 to session 16). As a session is overcome, the difficulty of the task increases as the stimulus duration (SD) and the limited hold (LH) decrease in each session.

As previously described, for each session the trial starts with first delivery of the reward and the mouse must nose-poke and exist the reward magazine. After the exit, a default delay of 5 s ITI begins, and the end of which the stimulus is presented in one of the five windows of the screen. The mouse must be able to respond to the stimulus within a period of time, a period in which the stimulus is extinguished, the LH (**Figure 3.13**).

If the mouse nose-pokes correctly, touching at the location where the stimulus was presented, the reward will be delivered accompanied by illumination of the tray light and a tone. Then, the animal will collect the reward entering the magazine. When exiting, the ITI will begin (default 5 s), after which the tray light will turn on again, ready for the next trial when the mouse would enter and exit the magazine again (**Figure 3.13**).

If the mouse makes an incorrect response (nose-poking in a location other than where the stimulus was presented) or makes no response at all (an omission), it will cause a time out (TO, default 5 s). In this situation, the house light will be turned on during that 5 s (contrary to the default state) and then it will be turned off again. After a delay of 5 s, the tray light will be illuminated again, and the next trial will be ready to be started by the mouse entering the reward magazine. It can also happen that the mouse touches one of the windows during the delay of 5 s prior to the emergence of the stimulus. That is considered a premature response, and it will also cause a TO (**Figure 3.13**).

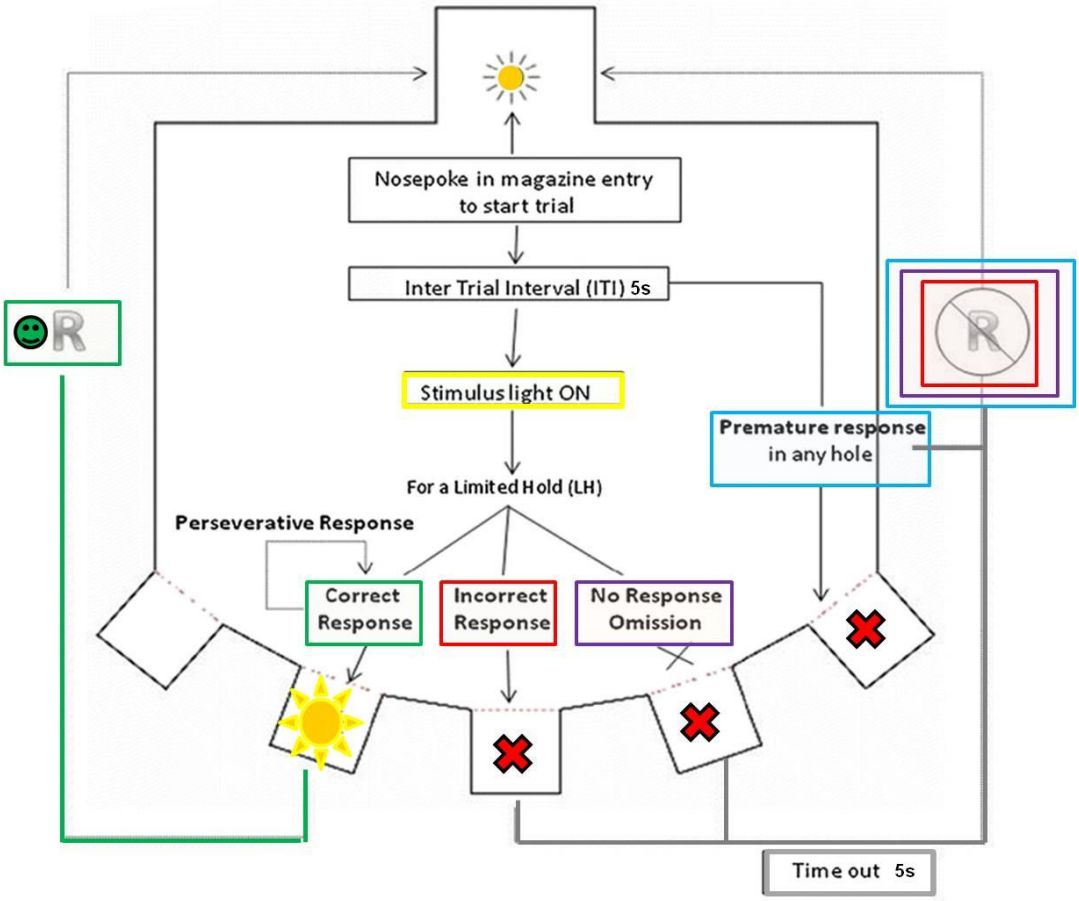

Figure 3.13: Schematic representation of a trial in a 5-CRSTT operant box. Modified from *(Sanchez-Roige et al., 2012)*.

The difficulty in this training to baseline phase is that the SD on the screen is progressively decreasing from 32 to 1.4 s, and the LH period goes from 37 to 6.4 s (**Table 3.5; Figure 3.12**). The mice were given one training session per day and were trained for 5-7 days a week until baseline performance was reached (SD= 1.4 s). Each session had to be repeated until the mouse achieves the following criterion on two consecutive days:

- ≥80 % Accuracy: [(Number of correct trials / Total number of trials responded to (correct and incorrect) x 100]
- ≤ 20 % Omissions: [(Number of trials missed / number of trials presented) x 100].

Table 3.5: Descriptive table showing the SD and the LH period of each session.

Session	Session 9	Session 10	Session 11	Session 12	Session 13	Session 14	Session 15	Session 16
Stimulus duration (SD)	32 s	16 s	8 s	4 s	2 s	1.8 s	1.6 s	1.4 s
Limited Hold (LH)	37 s	21 s	13 s	9 s	7 s	6.8 s	6.6 s	6.4 s

3.7.1.3.3. Testing schedules / Task manipulations

Once the performance of the mice reached the baseline (SD = 1.4 s) achieving the mentioned criterion for at least 2 consecutive days, the attentional function could be further assessed by the manipulation of the task's basic parameters (**Figure 3.12**). In this study, two different schedules were designed to evaluate the cognitive flexibility of these animals:

- For the first manipulation, it was decided to enforce a burst of a distractor white noise during the delay time (the time between the mouse exits the reward magazine and the stimulus is presented on the screen), within the objective of distracting the animals from their task. This variation of the task was called **"Variation 2".** The burst of distracting white noise would appear at 0.5, 2.5, 4.5, 5 s or "not at all" (the default is 5 s), in random order (**Figure 3.12**).
- For the second manipulation, called "**Variation 1**", it was decided to alter the duration of the delay time (default 5 s), with the objective to increase the attentional charge by disrupting the temporal predictability of the stimulus onset. In this case, the delay period would be shortened to 2, 3, 4 or 5 s, and it would be set randomly (**Figure 3.12**).

The rest of the parameters of each session would be the baseline parameters; SD = 1.4 s and the LH = 6.4 s, keeping the rest of the parameters equal.

3.7.1.3.4. Experimental procedure

Each day of the experiment, and during the whole experiment, mice were allowed to acclimatize to the experimental room 1 h prior to the training/test. All sessions were carried out between 8 am and 3 pm. Each mouse performed the task always in the same operant box

that was assigned randomly at the beginning of the experiment, and both saline and PIC animals performed in all the operant boxes.

3.7.1.3.4.1. Cleaning procedure

The liquid reward (10 % CM in water) is quite sticky and the feeding tubes need to be cleaned after each operant box session. The pump is provided with a prime override switch to make this process easier.

For the cleaning procedure the following steps were followed:

- For each operant box, distilled water was placed in the reservoir. The feed-tube was disconnected from the reward magazine and placed in a collecting vessel.
- The pump was then switched on allowing it to pump water through the tubes for at least 30 s or until the tubing appeared to be completely clean.

Next day, prior to starting the session, the feeder had to be primed to ensure that the reward was being delivered to the reward magazine.

Regarding the cleaning of the boxes, between sessions and before starting with a different animal, urine and feces were removed from the boxes, cleaned with wet paper and then dried. At the end of the day, the surfaces of all the boxes were also washed with diluted ethanol. Once a week, modules and operating boxes were disconnected and a deeper cleaning was carried out.

3.7.1.3.5. Statistical analysis

All data was compiled and analyzed using the ABET II Software, while statistical analysis was performed using GraphPad Prism™ v5 (GraphPad Software Inc., CA, USA).

Results are expressed as the mean ± SEM of n individual animals. For statistical analysis, two-way ANOVA for repeated measures followed by Bonferroni's *post-hoc* test between saline and Poly (I:C) animals was assessed. *F* values are expressed as $F_{\text{treatment}}$ (F_{tr}) expresses the ability of the treatment to exert an effect, F_{time} (F_t) expresses differences between groups across time and, $F_{\text{interaction}}$ (F_i) expresses the differences between the two groups of treatment across time. Results were considered statistically significant when $p<0.05$.

3.8. CEREBRAL MICRODIALYSIS IN FREELY MOVING AWAKE ANIMALS

Cerebral microdialysis in awake animals is a technique that allows us to estimate the concentrations of neurotransmitters, as well as their metabolites or other substances present in the extracellular space, through the implantation of a probe in a specific brain region. This technique is based on the passive diffusion of low molecular weight compounds (neurotransmitters in this case) through a porous membrane from the compartment with the highest concentration (the extracellular space) to the less concentrated compartment (the dialysis probe, perfused with a buffer solution at physiological pH and that does not contain neurotransmitters).

In this project, the dialysate samples obtained from the probe outlet were analyzed using UHPLC with electrochemical detection for the quantification of oxidizable molecules recovered from the extracellular space.

3.8.1. Microdialysis probe fabrication

The probes used for the microdialysis experiments were handmade in the laboratory and for their manufacture, the following materials were used:

- One 25-G steel tube (0.3 mm internal diameter x 0.5 mm external diameter), 2 cm length (CIBERTEC S.A., Madrid, Spain).
- Two 27-G steel tubes (0.2 mm internal diameter x 0.4 mm external diameter), 0.7 cm length (CIBERTEC S.A., Madrid, Spain).
- One 2.5 cm length and one 4.5 cm length silica tube (Composite Metal Services Ltd, Southampton, United Kingdom).
- Two polyethylene tubes (0.28 mm internal diameter x 0.61 external diameter) (BD Intramedics, New Jersey, USA).
- 6-kDa cut off cuprophan membrane 0.25 mm diameter (Enka AG, Wuppertal, Germany).
- Araldite adhesive® (Huntsman Advanced Materials, The Woodlands, TX, USA).
- Loctite® gel (Loctite Spain S.A., Madrid, Spain).
- TAB 2000 Dental cement (Kerr®, Orange, CA, USA).
- Low melting point thermoplastic adhesive.

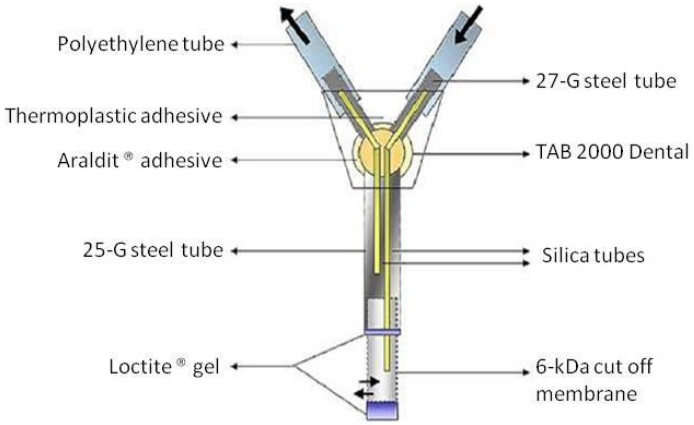

Figure 3.14: Representative image of a microdialysis probe. Modified from *M. Victoria Puig Velasco, 2004, Doctoral Thesis, University of Barcelona, IDIBAP.*

Probes consisted of the assembly of silica piping systems covered by steel tubes, which provide the formers with robustness and resistance (**Figure 3.14**). This "Y" shape system was fixed with Araldite adhesive and TAB 2000 dental cement at the insertion point. The sequence for the construction of the probes is described in **Figure 3.15**.

Two mm length of the silica tube leaned out from the bottom of the probe. This length depends on the animal species and implantation area. A dialysis membrane surrounded the external silica and was assembled to the bottom of the probe using Loctite® gel. The other end of the membrane was sealed with Loctite® gel, leaving a space of 1 mm from the silica tube. Input and output steel tubes were connected to a polyethylene cable in each side and glued with a thermoplastic low melting point adhesive (**Figure 3.15**).

The 6-kDa pore size membrane enables the exchange of small molecules substances, excluding proteins and big compounds. Therefore, sample purification was not necessary before chromatographic analysis.

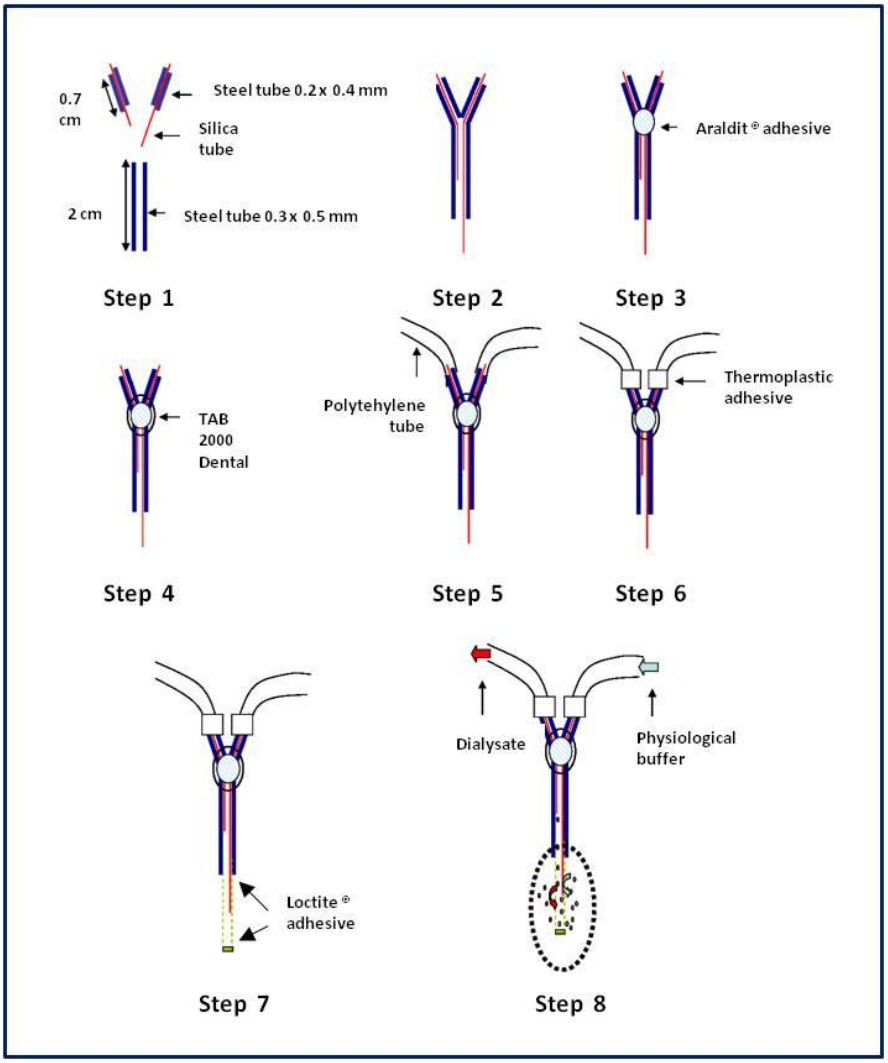

Figure 3.15: Assembly sequence for the construction of the microdialysis probes. First, the different parts are assembled (steps 1 and 2) and then they are fixed (steps 3 and 4). Subsequently, the inlet and outlet polyethylene tubes are added and sealed (steps 5 and 6). Finally, the dialysis membrane is placed and fixed (steps 7 and 8) (*Modified from J.E. Ortega Calvo, 2007, Doctoral Thesis, University of the Basque Country (UPV/EHU)*).

3.8.2. Stereotaxic surgery

3.8.2.1. Anesthesia

The advantages of the use of inhalation agents are that the procedure is precise, rapidly adjustable, safe and effective. Moreover, the postoperative recovery is rapid and less complicated than with injectable anesthetics.

Animals were anesthetized using a CA-ECA20 Anesthesia Trolley System (CIBERTEC, Madrid, Spain) equipment (**Figure 3.16**), composed of:

- Flowmeter: to adjust the medical oxygen flow rate.

- Vaporizer: a chamber where the volatile liquid anesthetic is deposited. Gas flux coming from the flowmeter flows through the vaporizer and the anesthetic is added to this flux in the desired proportion.
- O_2 emergency valve: when pressed, allows oxygen to flow directly without the anesthetic agent.
- Safety pressure valve: avoids exceeding the maximum system working pressure.
- Manifold: composed of three individual connection valves in order to provide up to three accessories which can be connected simultaneously (induction boxes, anesthetic masks, etc).
- Manometer: it displays the gas pressure rate into the system.
- Evacuation system with a fluid absorbent canister: active vacuum to eliminate excesses of the anesthetic gas.

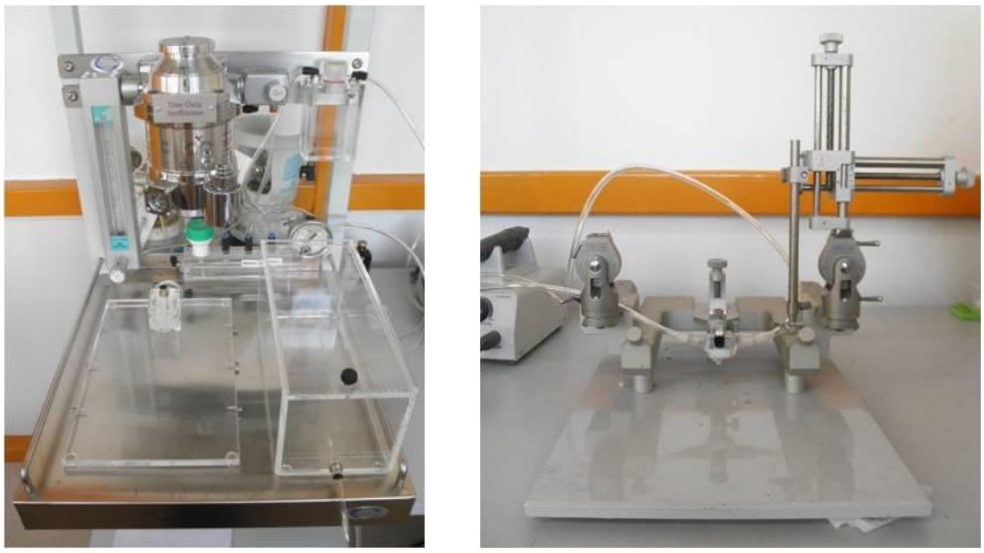

Figure 3.16: Left image: inhalation anesthesia system. Right side: mouse mask for anesthesia maintenance coupled to the inhalation anesthesia system shown on the left.

Animals were anesthetized with isoflurane. This volatile drug possesses general anesthetic properties and was delivered to animals via inhalation. Mice were placed in an induction chamber with a concentration of 4% of isoflurane until they were completely anesthetized. Afterwards, mice were placed in a stereotaxic frame and coupled to a mouse mask where anesthesia maintenance was provided (1.5-2% concentration of isoflurane). Breathing rate was visually controlled by the researcher during the entire procedure.

3.8.2.2. Stereotaxic surgery equipment

Surgery was performed using a Kopf™ stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The system has two arms to allow accurate probe positioning in specific brain areas by following three coordinates: anterior-posterior (A/P), medial-lateral (M/L) - both relative to bregma -, and dorso-ventral (D/V) - relative to skull surface -. Head immobilization was assured by adjustment of the skull inserting two earbars in both ears, model 957 18° (David Kopf

Instruments, Tujunga, CA, USA). The stereotaxic frame is connected to the anesthesia system - for anesthesia maintenance - by a mask where snout is adapted (**Figure 3.16 and 3.17**).

Figure 3.17: Stereotaxic surgery equipment.

3.8.2.3. Microdialysis probes implantation

Animals were anesthetized in an induction chamber breathing isoflurane (4%) for 3-4 min until they were completely anesthetized. Unconscious mice were then placed on the stereotaxic frame under a constant flow of isoflurane (2%) for anesthesia maintenance. To avoid hypothermia, animals laid over a heating pad during the whole surgery.

Once the head was horizontally immobilized - *bregma* and *lambda* in the same plane -, a sagittal 1 cm long incision was performed to expose the skull surface. A surgical microscope (OPMI 99, Carl Zeiss, Oberkochen, Germany) was necessary to gain precision in the implantation. Firstly, the skull was drilled twice, one hole - 1 mm approx. - was for the probe implantation and the other one - 0.5 up to 1 mm - was for the anchor screw placement, to aid fixation to the skull with the help of dental cement. These orifices were milled using a steel burr connected to an electric turbine (Elco 5118, Leobersdorf, Austria). Prior to probe implantation, meninges were carefully removed to allow vertical insertion of the probe.

Intra-cerebral probes, with 2-mm membrane length, were stereotaxically implanted in two brain regions depending on the experiment of interest: the PFC or the striatum of saline and Poly (I:C) mice. Implantation coordinates were selected following the guidelines set by Franklin and Paxinos atlas (2008) and strain-comparative atlas by Hof and co-workers (2000). Implantation coordinates in PFC were as follows (**Figure 3.18**):

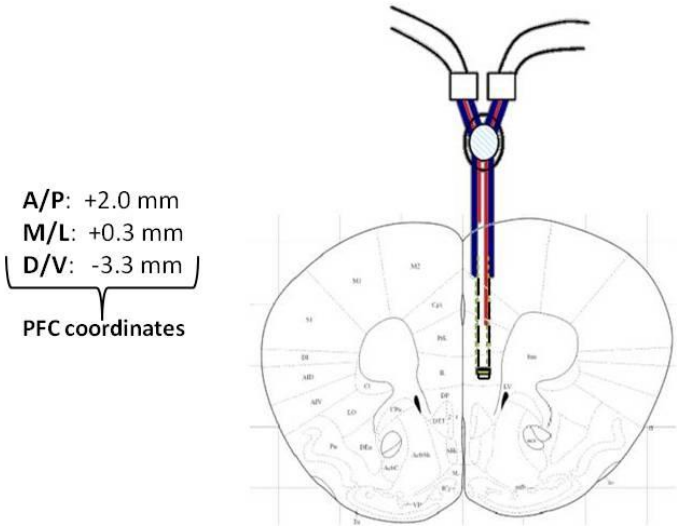

Figure 3.18: Representative diagram of the location of the microdialysis probe in the PFC of mice.

In the case of the striatum, the implantation coordinates were (**Figure 3.19**):

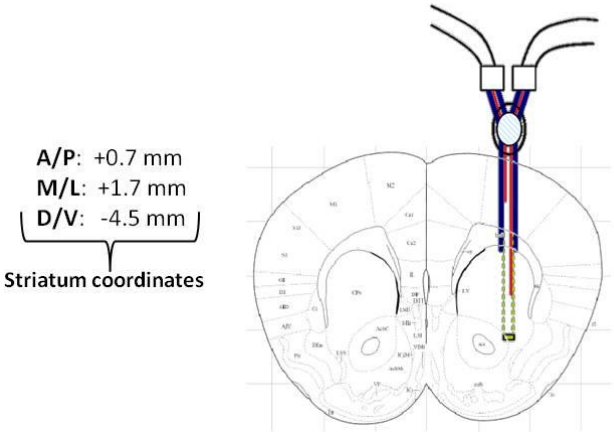

Figure 3.19: Representative diagram of the location of the microdialysis probe in the striatum of mice.

Bregma was the reference point to obtain AP and L coordinates, meanwhile skull surface was for DV coordinate. The aCSF solution was steadily perfused through the probe during the insertion time. This way, strength, and firmness were gained in the probe trying to avoid breakage of the dialysis membrane and facilitating a vertical inlet to ensure accurate implantation. Once implanted, probes were fixed in place with an anchor screw, a plastic housing as reinforcement and dental cement (**Figure 3.20**). Once the surgery was completed, the proper functioning of the probes was checked out and the ends of the polyethylene tubes were sealed.

Figure 3.20: Left image: Image of the implantation of a microdialysis probe and anchor screw. Right image: Image of mouse placement and skull fixation during surgery.

After surgery, animals were left to recover for at least 18 h, housed in single cages provided with free access to water and food.

The necessity of this recovery is due to the fact that the implantation of the probe generates damage to the neuronal tissue, mainly affecting blood flow, the blood-brain barrier (BBB) and the processes of neurotransmission. These disturbances, as well as damages to neuronal morphology in the vicinity of the dialysis tube and BBB integrity, become normal when allowing animals to recover between 10 and 24 h post-implantation (de Lange et al., 1997; Imperato & Di Chiara, 1984). The extension of the recovery period beyond 3 days may give interpretation problems due to the developing gliosis in the surroundings of the probe, increasing the risk of reducing dialysis and subsequently neurotransmitters detection. Taking all these into account, the microdialysis procedures were performed the following two days after recovery. Probes placement was histologically verified at the end of the experiments by staining the membrane with Fast Green solution.

3.8.3. Collection of the dialysate samples

3.8.3.1. Microdialysis equipment for awake mice

The brain microdialysis technique in awake animals requires complex equipment capable of maintaining a constant flow through the implanted probes while the animal moves freely through the cage. Sampling was carried out using BASi equipment (BASi, West Lafayette, IN, USA), which allowed a constant flow rate throughout the probes (**Figure 3.21**). The equipment consisted of the following components:

- Methacrylate cage covered by sawdust, where the animal remained throughout the whole experimental process (MD-1570, BASi, West Lafayette, IN, USA).

- Precision pump (MD-1001, BASi, West Lafayette, IN, USA) which allowed to work at constant flow rates from 0.1 to 100 µl/min with two separate syringes.

- Syringe exchanger or switch (MD-1508, BASi, West Lafayette, IN, USA) that allows manual switching between up to three perfusion syringes and a microdialysis probe.

This makes it possible to change different solutions instantaneously without any risk of introducing air bubbles into the microdialysis probe.

- Refrigerated microvolume sample collector (MD-1201, BASi, West Lafayette, IN, USA) which kept the samples refrigerated at 4 °C from their collection until their transfer to the analytical equipment.

All these components were interconnected by a red tubing system (PEEK-tubing, BASi, West Lafayette, IN, USA) to protect monoamines from light deterioration, as well as by adaptors (Microdialysis Tubing Connectors BASi, West Lafayette, IN, USA). Raturn Microdialysis Stand-Alone System for use with mice (MD-1409 BASi, West Lafayette, IN, USA) allowed animals to freely move in the cage, as well as to record the frequency and duration of rotational movements and elevations.

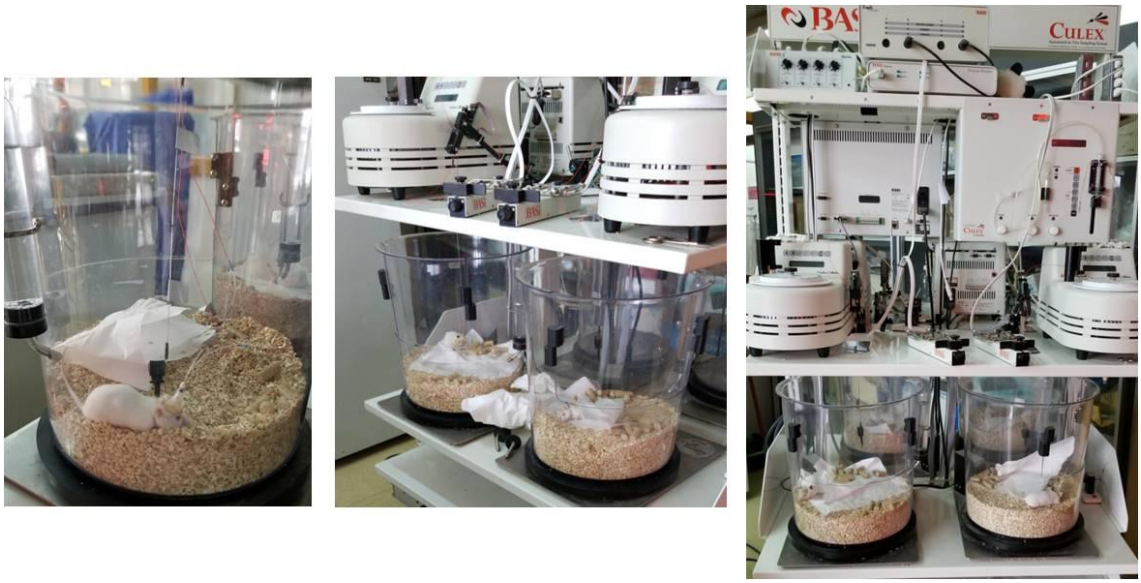

Figure 3.21: Left side: Swiss strain mouse during microdialysis experiment. Middle and right sides: BASi equipment during a microdialysis experiment.

3.8.3.2. Conventional microdialysis

After the recovery period, probes were connected to the system and the aCSF solution was perfused at a rate of 1 µl/min through the microdialysis probes incessantly during the entire experiment. The aCSF driven by the precision pump flowed through the system from the inlet tube until reaching the dialysis membrane, where osmotic balance is produced. Dialysate fluid was pushed into the outlet tube and collected into a microvial that was kept refrigerated. Before starting the collection of the experiment samples, a period of 1 h of stabilization was left. Subsequently, samples were collected every 35 min.

In all the cases, the first 5-6 samples were used to establish monoamine baseline concentrations, although for the calculation of the mean of basal values, only the last two samples were taken into consideration. This mean of the basal values was considered 100%, and the pharmacological effect was analyzed using that basal value as the reference.

Afterward, the drug of interest was administrated i.p. Following the injection of the drug, between 7 and 11 additional experimental samples were collected (**Figure 3.22**).

In the case of the experiments that required local administration in PFC, after the collection of the basal samples (5-6 samples), the drug was perfused locally for 105 min. Once this time had passed, aCSF was perfused again through the probes, and another 7 samples were collected (**Figure 3.23**). Each time the perfusing syringe was changed (between aCSF and the drug), we waited for 10 min before collecting the next sample, allowing the arrival of the exchange solution to the dialysis area of the probe.

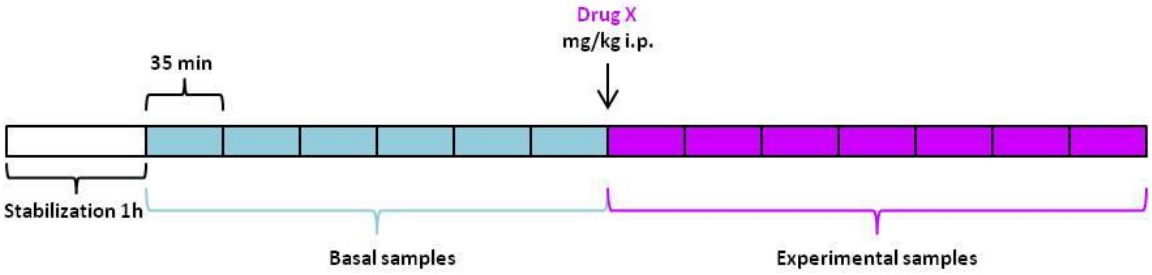

Figure 3.22: Representative scheme of a systemic administration of drug or saline in mice.

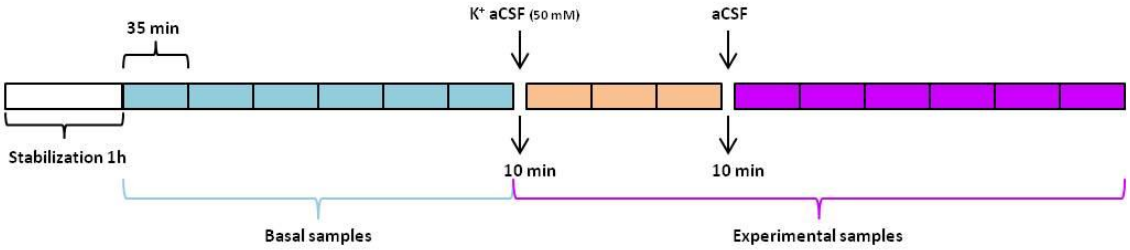

Figure 3.23: Representative scheme of the local administration in the PFC of K^+ aCSF (50 mM) in mice.

In all cases, the implanted microdialysis probe was perfused throughout the whole experiment with aCSF and the diffusion of the substances occurred in favor of concentration gradient at the level of the dialysis membrane. It is important to keep in mind that the transport of substances through the membrane is a slow process and, therefore, the concentration reached in the perfusion fluid does not correspond exactly to what is present in the extracellular space. In order to know the real extracellular concentration of the substances from the concentration obtained in the dialysate, it is necessary to calculate the recovery percentage of the probe with respect to the substance of interest through *in vitro* studies. This parameter depends on the perfusion flow, the pore size of the membrane, and the molecular weight of the molecule of interest. *In vitro* studies carried out in this laboratory have determined that the recovery percentage of the probe has values of 15-20% of the actual concentration of the neurotransmitters of interest when using a microdialysis membrane of 2 mm in length (*data not shown*). Results presented in this project are not corrected by this degree of recovery.

Nevertheless, non-net flow microdialysis experiments were performed to be able to calculate the actual, and non-relative, basal concentrations of neurotransmitter present in the extracellular space of the area in which the probe was implanted; the PFC and the striatum, in our case.

3.8.3.3. Non-net flow microdialysis

After the recovery period of the stereotaxic surgery, the implanted probes were connected to the system and the perfusion of the aCSF started. In this case, the composition of the aCSF included 0.25 mM ascorbic acid as an antioxidant, in order to avoid processes of degradation of the different monoamines during the experiment. The pH was adjusted between 7.0-7.2 with NaOH 0.5 N. The perfusion flow was set at 1 µl/min and samples were collected every 35 min, as in conventional microdialysis experiments. After 1 h of stabilization, the experiment started collecting between 1-3 samples that were used neither in the analysis nor in the subsequent interpretation of the results, but this procedure allowed to guarantee the stabilization of the neurotransmitter concentrations in the specific brain area.

Then, the non-net flow microdialysis began. Different solutions of the neurotransmitter of interest (NA or DA) were randomly perfused through the probe at concentrations of 0, 5, 10 and 20 nM (C_{in}). This process was performed in both saline and Poly (I:C) animals for each neurotransmitter. When changing the concentration solutions, 70 min were allowed to elapse before starting to collect a sample, since it is considered the necessary time to reach a steady or equilibrium state. Once this state was reached, two samples were collected for each of the administered concentrations and three samples after the final administration; the ones that were used to evaluate the neurotransmitter concentratios in the dialysate (C_{out}) (**Figure 3.24**).

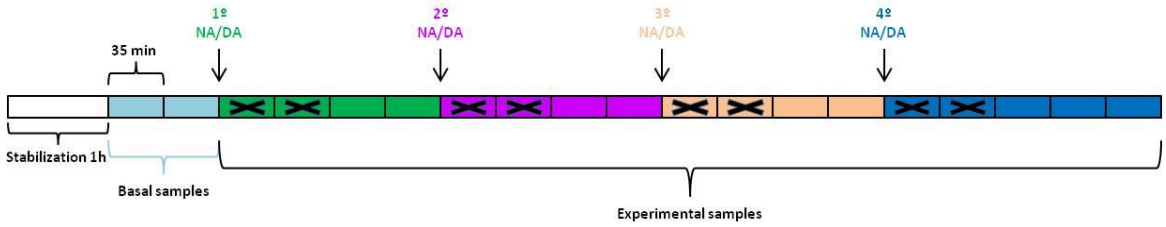

Figure 3.24: Representative diagram of the non-net microdialysis technique procedure.

3.8.4. Chromatographic analysis of the samples

3.8.4.1. Chromatographic equipment

The equipment used for chromatographic analysis of the microdialysis experiments in mice (except the non-net microdialysis experiments' samples, which were analyzed with the HPLC system described in *section 3.4.2.2*) was an ALEXYS™ UHPLC Neurotransmitter Analyzer (Antec Scientific, Zoeterwoude, The Netherlands). It consisted of (**Figure 3.25**):

- Degasser (OR 110, Antec Scientific, Zoeterwoude, The Netherlands), double-channel degasser with an integrated pulse snubber.
- Two pumps (LC 110s, Antec Scientific, Zoeterwoude, The Netherlands), able to support up to 700 bars of pressure.

- A double loop refrigerated autosampler (AS 110, Antec Scientific, Zoeterwoude, The Netherlands), able to effectively inject low volumes. This injector is able to inject simultaneously the same sample into two separate chromatographic columns.
- Two chromatographic columns (Waters, Milford, MA, USA). 1 x 100 mm; C18, 1.7 µm; with the stationary phase.
- Electrochemical detector (DECADE Elite, Antec Scientific, Zoeterwoude, The Netherlands) with high sensitivity and integrated thermostat. It holds two electrochemical detection cells (SENCELL™, Antec Scientific, Zoeterwoude, The Netherlands).
- Control station (Hewlett-Packard computer, Palo Alto, CA, USA) with CLARITY software (DataApex, Prague, The Czech Republic).

Figure 3.25: UHPLC equipment coupled to two electrochemical detectors. 1) Degasser; 2) Pump; 3) Autosampler; 4) Chromatographic columns; 5) Electrochemical detector; 6) Control station.

3.8.4.2. Monoamine detection

Samples with a final volume of 40 µl (35 µl dialysate + 5 µl acetic acid (0.1 M)) were placed in the AS 110 autosampler. The injection volume in this UHPLC equipment was 13 µl. The mobile phase conditions used allowed the detection of NA, DA, and 5-HT in each of the samples. This mobile phase was composed of 100 mM phosphoric acid, 100 mM citric acid, 0.1 mM EDTA, 950-1500 mg/l 1-octanesulfonic acid (OSA), 5% v/v acetonitrile, and Milli-Q® water. The pH was adjusted up to 6.0 with 50% NaOH/45% KOH solution. The mobile phase was degassed for 10-15 min in a sonic bath.

The mobile phase flowed through the system at a rate of 0.075 ml/min. The flow cell SenCell (Antec Scientific, Zoeterwoude, The Netherlands), with a *redox* potential of 0.46 V, and an Acquity UPLC BEH C18, 1.7 µm, 1 x 100 mm (Waters, Milford, MA, USA) chromatographic column, were able to separate and detect NA, DA and 5-HT neurotransmitters (**Figure 3.26** and **Figure 3.27**). The oven temperature was 37 °C.

NA, DA, and 5-HT peaks were integrated using the Clarity software (DataApex, Prague, Czech Republic). A calibration line was calculated by the integration of the peak areas from 8 standard concentration solutions of NA, DA, and 5-HT in aCSF (0.025, 0.05, 0.1, 0.5, 1, 3, 5, 10 nM).

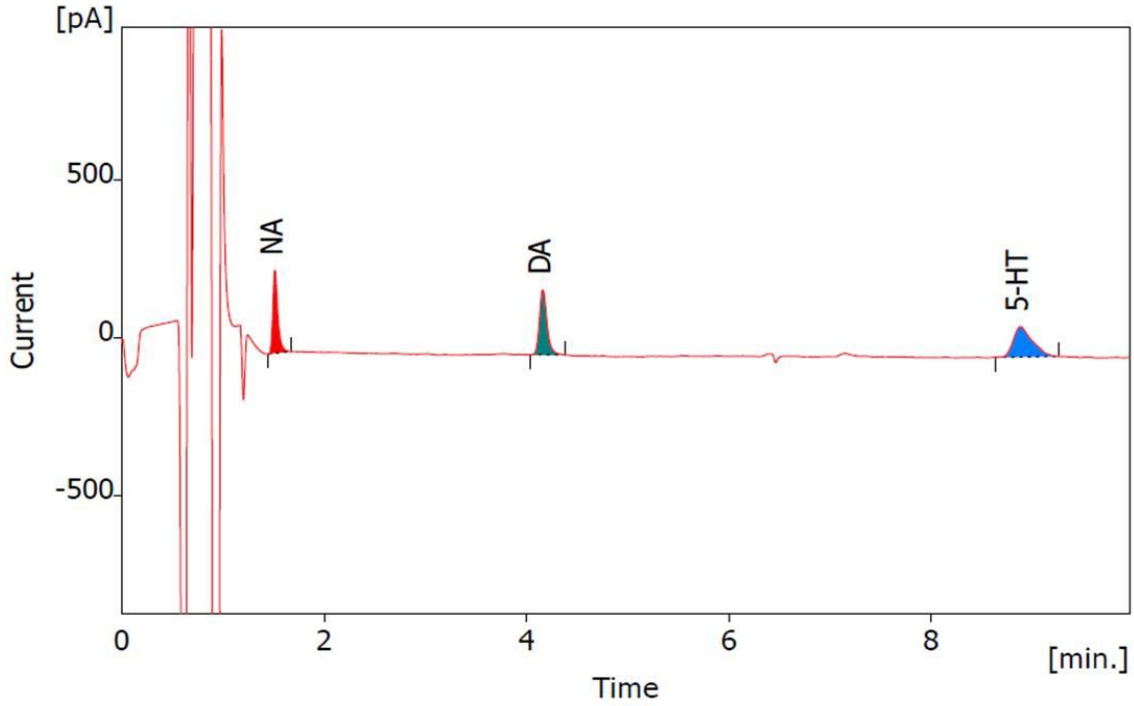

Figure 3.26: Representative chromatogram of NA (RT: 1.66 min), DA (RT: 4.25 min), and 5-HT (RT: 9.07 min) in a 3 nM standard sample from the UHPLC system.

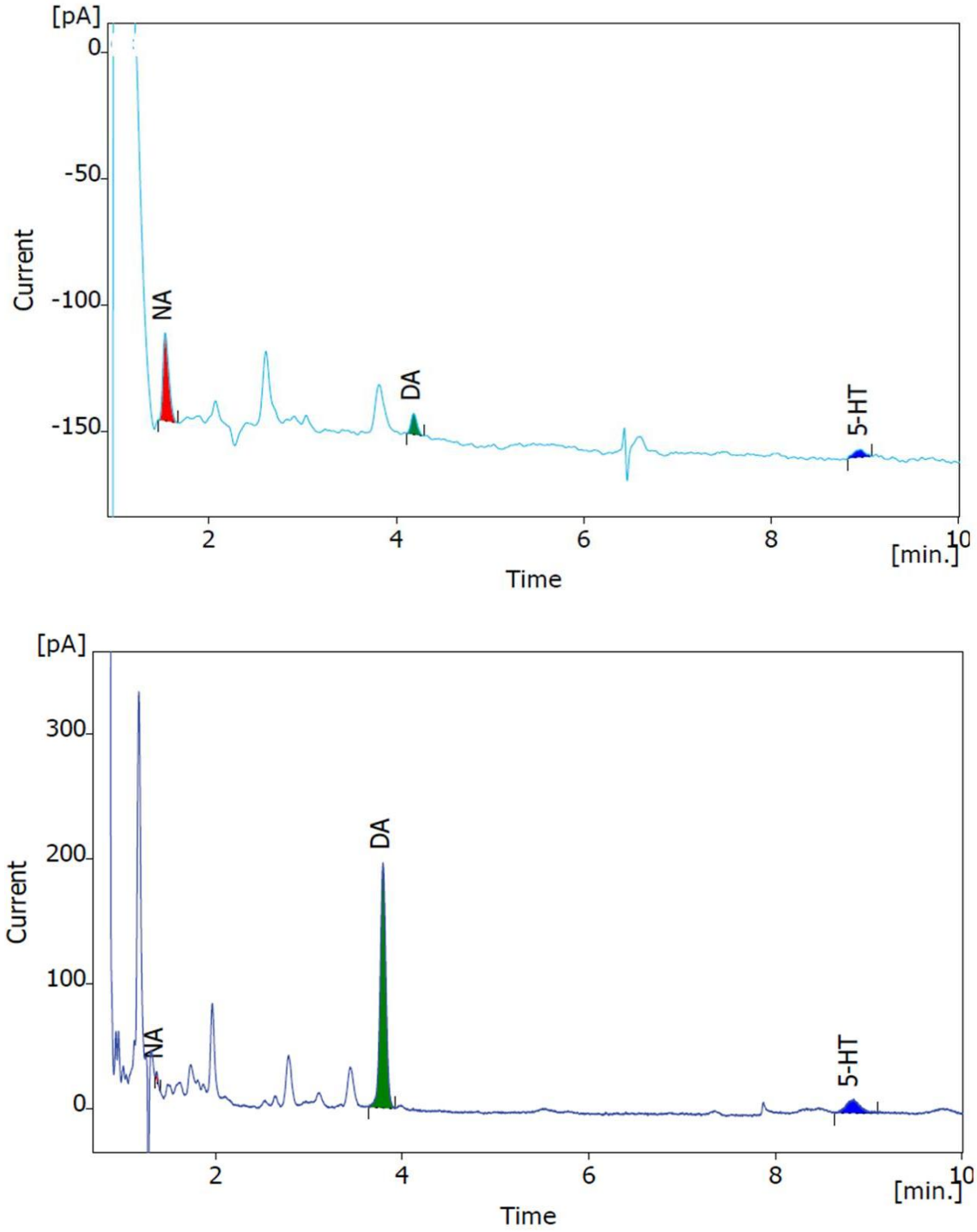

Figure 3.27: Upper panel: representative chromatogram of NA (RT: 1.62 min), DA (RT: 4.25 min), and 5-HT (RT: 9.03 min) in a basal sample of mouse PFC from the UHPLC system. Lower panel: representative chromatogram of NA (RT: 1.36 min), DA (RT: 3.79 min), and 5-HT (RT: 9.08 min) in a basal sample of mouse striatum from the UHPLC system.

3.8.5. Statistical analysis

All the graphs and statistical analysis were carried out using GraphPad Prism™ v5 software (GraphPad Software, San Diego, CA, USA).

3.8.5.1. Conventional microdialysis

In order to establish the monoamine (NA, DA, and 5-HT) basal concentrations, the first 6 sample fractions were collected in mice; from these only the last 2 samples were taken into consideration to calculate the mean basal values, and were considered the 100%. The effect triggered by the administration of the drug of interest was calculated and represented as the percentage of these basal values (baseline).

To analyze the difference between the basal values of the saline and Poly (I:C) groups, a two-tailed unpaired Student's *t*-test was used.

Also, to analyze the effect produced on the basal concentrations in each of the groups in response to a determined drug administration, one-way ANOVA of repeated measures was used, followed by the Dunnett's *post hoc* test to analyze the effect of the drug over time. For this analysis, the basal values and the samples after the administration of the drug were taken into consideration.

Two-way repeated-measures ANOVA, with treatment (saline *vs*. PIC) groups as the independent variables and time as the repeated-measure factor, was used to evaluate whether an interaction between these two variables on the dependent variable existed. Bonferroni's *post hoc* test was employed for individual sample comparison. *F* values are expressed as $F_{\text{treatment}}$ (F_{tr}) expresses the ability of the treatment to exert an effect, F_{time} (F_t) expresses differences across time and, $F_{\text{interaction}}$ (F_i) expresses the differences between the two groups of treatment across time. For this analysis, the basal values and the samples after the administration of the drug were taken into consideration.

Finally, the areas under the curve (AUC) were calculated from the drug administration time point until the end of the assay. The net area AUCs were calculated and differences between the groups were assessed by a two-tailed unpaired Student's *t*-test.

In all cases, results were represented as mean values ± SEM. The minimum statistical significance was set at $p<0.05$.

3.8.5.2. Non-net flow microdialysis

In a graphical representation of the non-net flow microdialysis results, the difference between the neurotransmitter concentration applied through the probe (C_{in}) and the concentration of neurotransmitter present in the dialysate after the determined concentration perfusion (C_{in}-C_{out}) is represented (**Figure 3.28**). C_{out} was obtained by calculating the mean value of the neurotransmitter concentrations present in the two samples collected for each administered standard pattern. By means of a linear regression of the obtained data, the extracellular concentrations of the neurotransmitter and the extraction fraction (*Ed*) were calculated individually for each animal in each of the areas of study.

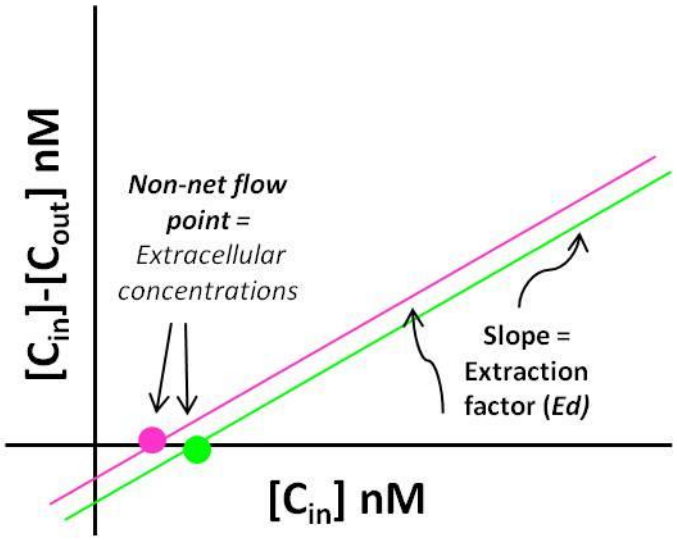

Figure 3.28: Example of a graphid representantion of the non-net flow microdialysis results.

The value of C_{in} to which C_{in}-C_{out} = 0 corresponds to the conditions of equilibrium state and, therefore, to the concentration of the neurotransmitter in the extracellular fluid. It is known as a *non-net flow point*. On the other hand, by means of this linear regression, we also obtained the value of the slope of the line or *Ed*, considered a direct function of the clearance of the neurotransmitter from the extracellular space.

For the neurotransmitter concentration in the extracellular fluid and for the *Ed* values, the results are represented as the mean values of each group ± SEM The analysis of the comparison of both parameters in the two groups was carried out by a two-tailed unpaired Student's *t*-test.

3.9. CELL-TYPE SELECTIVE TARGETING OF THE LOCUS COERULEUS-PREFRONTAL CORTEX (LC-PFC) PROJECTION IN MICE

All the experiments collected in this section were carried out in the facilities of the Department of Anesthesiology in the Center of Clinical Pharmacology at the Washington University School of Medicine in St. Louis, MO, USA.

3.9.1. Viral injections and optogenetic approach

3.9.1.1. Viral preparation

Plasmids were obtained from and packaged into viruses by Addgene (Addgene.org, Watertown, MA, USA). Subsequently, aliquots of 5 µl were made and they were kept frozen at -80 °C until their use. In particular, we used viruses encoding AAV5-Ef1α-DIO-eYFP [final titer 5 x 10^{12} vg/ml], AAVrg-Ef1 α-mCherry-IRES-Cre ([final titer 2 x 10^{13} vg/ml], AAV9-rTH-PI-Cre-SV40 [final titer 2 x 10^{13} vg/ml] and AAVrg-PGK-Cre [final titer 2 x 10^{13} vg/ml].

3.9.1.2. Stereotaxic surgery

For surgery, animals were anesthetized in an induction chamber (4% isoflurane) and placed in a stereotaxic frame where they were maintained at 1%-2% isoflurane throughout the procedure. A craniotomy was performed and mice were injected with AAV5-Ef1α-DIO-eYFP or AAV9-rTH-PI-Cre-SV40 into the LC (stereotaxic coordinates from bregma: -5.45 mm AP, +1.1 mm ML, and -3.75 mm DV) or with AAVrg-Ef1 α-mCherry-IRES-Cre or AAVrg-PGK-Cre into the PFC (stereotaxic coordinates from bregma: +2.0 mm AP, +0.3 mm ML, and -2.25 mm DV) (**Figure 3.29**).

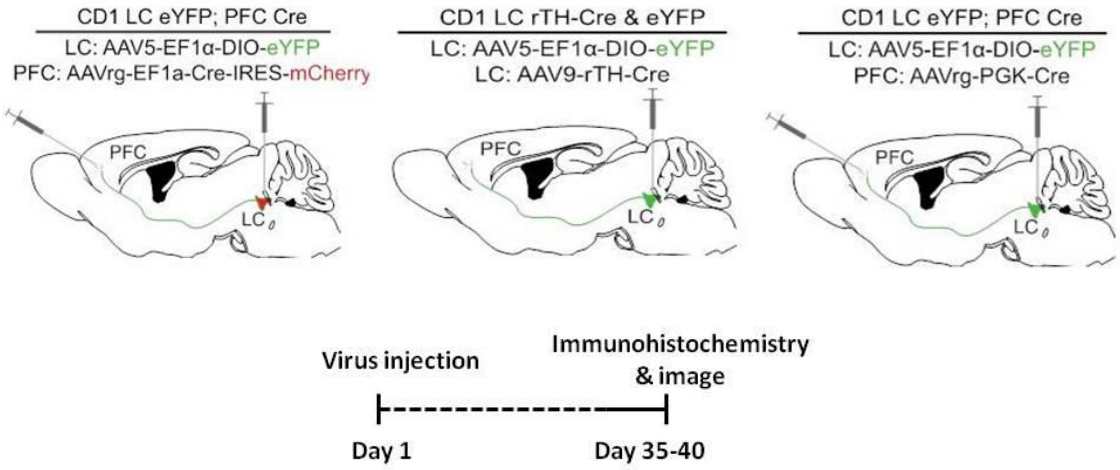

Figure 3.29: Schematic cartoon representations of the different strategies to target the LC-PFC circuitry and temporal stimation for posterior analysis

These viruses were delivered with a Hamilton syringe (65457-02 0.5 µL, Neuros Model 7000.5 KH, point style 4, Reno, Nevada, USA) at a rate of 50 nL/min for a total volume of 300 nL in the LC and 250 nL in the PFC. The syringe remained in place for 5 min post infusion before being slowly removed. Animals were sutured (Ethicon 4-0 x 18”, Nylon suture, McKesson Corporations, San Francisco, CA, USA) and allowed to recover for 3-6 weeks prior to perfusion for histological examination, to permit adequate time for expression and distribution of the virus.

3.9.2. Immunohistochemistry protocol

Immunohistochemistry is a microscopy-based technique for visualizing cellular components, for instance, proteins or other macromolecules in tissue samples. The strength of this technique is the intuitive visual output that reveals the presence and localization of the target protein in the context of different cell types, biological states, and/or subcellular localization within complex tissues.

This technique involves the detection of epitopes expressed by a single protein-target within a tissue sample using a "primary antibody" capable of binding those epitopes with high specificity. After the epitope-antibody binding event, a "secondary antibody" capable of binding the primary antibody with high specificity is added. The secondary antibody is coupled to a reporter molecule and after the antibody-antibody binding event, a chemical substrate is added which reacts with the reporter molecule to produce a colored precipitate at the site of the whole epitope-antibody complex.

The microscope used for this technique is the epifluorescence microscope, which operation is represented in **Figure 3.30**. The light of the excitation wavelength illuminates the specimen through the objective lens. The fluorescence emitted by the specimen is focused on the detector by the same objective that is used for the excitation, which for greater resolution will need an objective lens with a higher numerical aperture. Since most of the excitation light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light. Therefore, the epifluorescence method gives a high signal-to-noise ratio. The dichroic beamsplitter acts as a wavelength-specific filter, transmitting fluoresced light through to the eyepiece or detector, but reflecting any remaining excitation light back towards the source. For our experiments, we used the Leica DMC2900 digital microscope (Leica, Wetzlar, Germany).

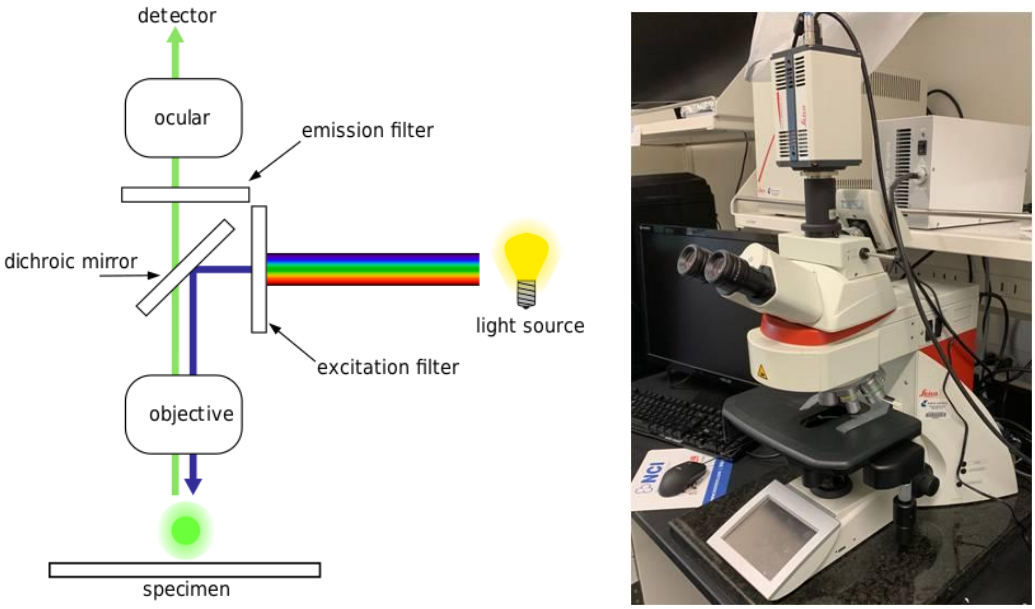

Figure 3.30: Left side: Schematic representation of the functioning of an epifluorescence microscope. Right side: Picture of the Leica DMC2900 microscope used for the imaging.

3.9.2.1. Tissue fixation

Preparation of the sample is important to preserve tissue morphology, architecture, and antigenicity of target epitopes. The advantage of directly perfusing fixative through the circulatory system is that the chemical can quickly reach every corner of the organism using the natural vascular network.

Immunohistochemistry was performed as described (McCall et al., 2015; 2017). Prior to surgery, mice were completely anesthetized via i.p. injection (0.1-0.2 ml/mouse) of 100 mg/kg ketamine/5 mg/kg xylazine/1 mg/kg acepromazine cocktail (Ketaset/Anased/Acepromazine; McKesson Corporation, CA, USA). A 4-5 cm lateral incision along the entire length of the rib cage was made to expose the pleural cavity. Carefully, any tissue connecting it to the heart was trimmed. The tip of the sternum was clamped with the hemostat and the hemostat was placed over the head. A perfusion needle was inserted through the left ventricle into the ascending aorta, without reaching the aortic arch. A hemostat was used to clamp the heart in order to secure the needle and prevent leakage. After making an incision to the right atrium to create as large an outlet as possible without damaging descending aorta, animals were perfused with PBS for a few min to remove all the blood in the vascular system. Subsequently, the fixation was performed using a freshly prepared solution of 4% paraformaldehyde (PFA) in PBS (42.58 g Na_2HPO_4 and 13.79 g $NaH_2PO_4 \cdot H_2O$ in 1 L of water).

Afterwards, the brain was removed from the skull and the fixation process was extended by incubation with 2 ml of 4% PFA for an additional 24 h at 4 °C. After this time, PFA was replaced by 2 ml of 30% sucrose solution (30 g sucrose, 5 µl sodium azide dissolved in 100 ml PBS at pH 7.4) for at least 24 h at 4 °C, until its precipitation. Once precipitated, the brain was stored at 4 °C until sectioning.

3.9.2.2. Brain sectioning

Brains were embedded in a cryogenic gel (Tissue-Tek, Radnor, PA, USA) on mounting plates for sectioning with a sliding microtome (Leica SM 2000R, Leica Microsystems Inc, Buffalo Grove, IL, USA). Coronal slices of 35 µm-thick were sectioned and preserved in 1 ml PBS (1x) at 4 °C to perform immunofluorescence assays in 12-well plates.

3.9.2.3. Immunofluorescence

For processing, the 35 µm brain sections were washed three times in PBS and blocked in PBS containing 0.5% Triton X-100% and 5% normal goat serum with agitation, to reduce nonspecific binding and to permeabilize the cell membrane to antibodies. LC sections were then incubated with the chicken anti-TH antibody (1:1000, Aves Labs, Tigard, OR) and PFC sections with the mouse anti-NET antibody (1:2000, PhosphoSolutions, Aurora, CO, USA) for approximately 16 h at 4 °C and covered from light.

Following overnight incubation, sections were washed three times in PBS and then incubated for 2 h at room temperature: LC sections with goat anti-chicken Alexa Fluor 594 or 633 (1:1000, Invitrogen, Carlsbad, CA, USA) and PFC sections with goat anti-mouse Alexa Fluor 594 or 633 (1:500, Invitrogen, Carlsbad, CA, USA). Then, there were washed three times in PBS and followed by three 10 min rinses in phosphate buffer. In some cases, a 1 h incubation with fluorescent Nissl stain (1:400, Neurotrace, Invitrogen, Carlsbad, CA, USA) was conducted between the PBS and phosphate buffer rinses. Finally, they were mounted on glass slides with Hard-set Vectashield (Vector Labs, Burlingame, CA, USA) (RRID: AB_2336787) for microscopy. All the sections were imaged on an epifluorescent microscope (Leica DM6). Gain and exposure time were constant throughout, and all the image groups were processed at the same time.

Table 3.6: Experimental conditions in the immunohistochemistry experiments for the incubation with primary and secondary antibodies.

Antibody	Species	Dilution	Source
TH (TYH)	Chicken	1:1000	Aves Labs Inc.
NET (1447-NET)	Mouse	1:2000	PhosphoSolutions
Alexa Fluor 594 Anti-chicken IgG	Goat	1:1000	Invitrogen
Alexa Fluor 633 Anti-chicken IgG	Goat	1:1000	Invitrogen
Alexa-Fluor 594 Anti-mouse	Goat	1:500	Invitrogen
Alexa-Fluor 633 Anti-mouse	Goat	1:500	Invitrogen
Nissl blue 435/455	Neurotrace	1:400	Invitrogen

4. RESULTS

4.1. NEUROCHEMICAL, FUNCTIONAL AND BEHAVIORAL CHARACTERIZATION OF AN ANIMAL MODEL OF MIA BY THE ADMINISTRATION OF POLY (I:C)

4.1.1. Monoamine concentration in brain tissue of saline and Poly (I:C) mice

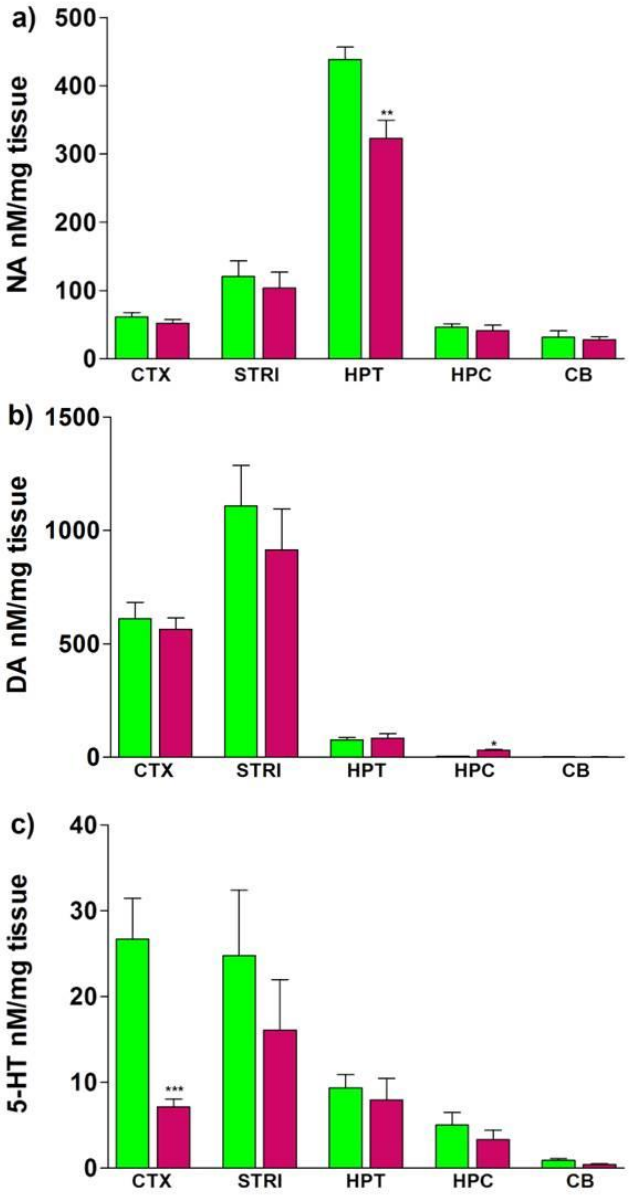

Figure 4.1: Mean values ± SEM (in nM/mg tissue) of (a) NA, (b) DA, (c) 5-HT in the cortex (CTX), striatum (STRI), hypothalamus (HPT), hippocampus (HPC) and cerebellum (CB) of adult saline offspring (■) and Poly (I:C) mice (■). For individual regions, statistical significance levels are denoted by ** p<0.05, ** p<0.01, *** p<0.001*, based on Student's *t*-tests.

4.1.1.1. Tissular brain NA concentrations

NA was detectable in all brain areas examined: high concentrations of NA were found in the hypothalamus followed by the striatum, and relatively intermediate concentrations of NA were observed in the cortex, the hippocampus and finally in the cerebellum (**Figure 4.1**). The Poly (I:C) exposure, compared with the saline group, decreased tissue NA content significantly in the hypothalamus (*t=3.55, p=0.004)*, but not in any of the other regions examined (**Figure 4.2, Figure 4.1**).

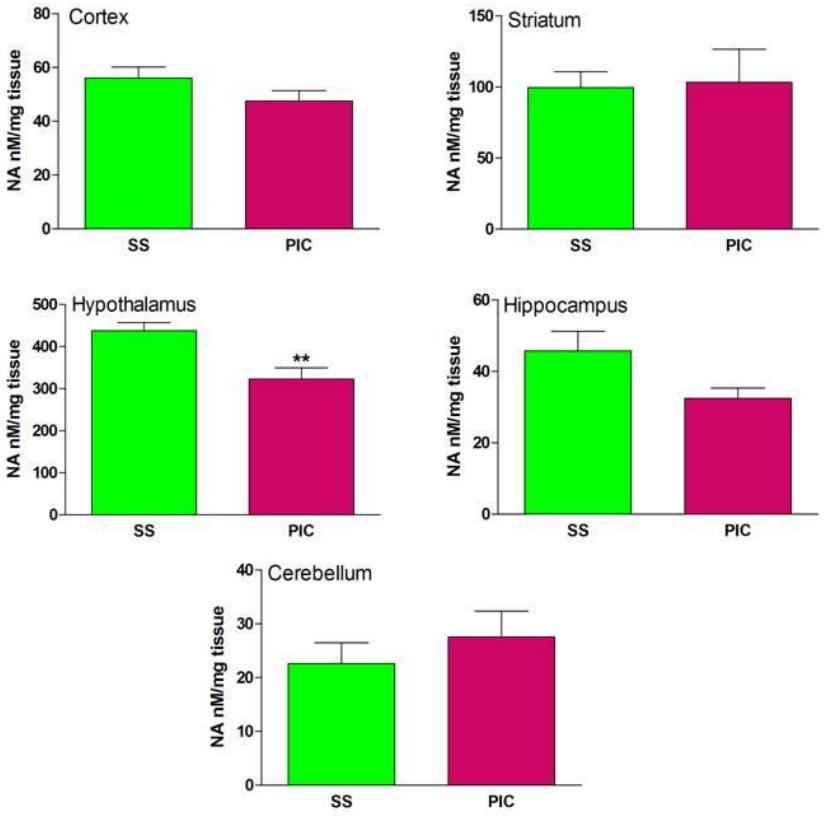

Figure 4.2: Mean values ± SEM (in nM/mg tissue) of NA in the brain cortex (SS n=6, PIC n=6), striatum (SS n=6, PIC n=7), hypothalamus (SS n=7, PIC n=7), hippocampus (SS n=7, PIC n=6) and cerebellum (SS n=6, PIC n=7) of adult saline offspring (■) and Poly (I:C) mice (■). Statistical significance levels are denoted by ** *p<0.01,* based on Student's *t*-tests.

4.1.1.2. Tissular brain DA concentrations

DA was measurable in all brain areas, but in the cerebellum, DA concentrations were almost undetectable compared with the other measured brain areas (**Figure 4.1**). As expected, very high concentrations of DA were found in the striatum, followed by the cortex, hypothalamus, hippocampus, and cerebellum. In this case, Poly (I:C) exposure significantly increased basal DA tissue content in the hippocampus *(t=7.9, p<0.0001)*, but no other significant differences were found in the rest of the brain areas examined (**Figure 4.3, Figure 4.1**).

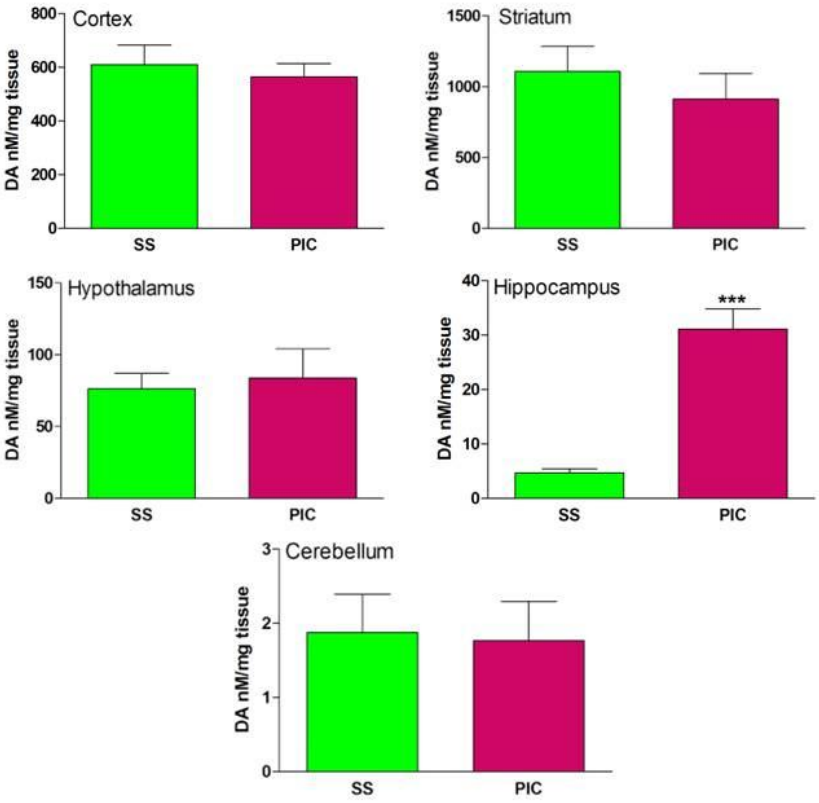

Figure 4.3: Mean values ± SEM (in nM/mg tissue) of DA in the brain cortex (SS n=7, PIC n=7), striatum (SS n=7, PIC n=7), hypothalamus (SS n=7, PIC n=5), hippocampus (SS n=5, PIC n=4) and cerebellum (SS n=5, PIC n=5) of adult saline offspring (■) and Poly (I:C) mice (■). Statistical significance levels are denoted by *** *p<0.001,* based on Student's *t*-tests.

4.1.1.3. Tissular brain 5-HT concentrations

In the case of 5-HT, it was measurable in all brain regions studied, although very low concentrations were found in the cerebellum compared to the other areas (**Figure 4.1**). However, relatively high concentrations of 5-HT were found in the striatum, cortex, and hypothalamus, followed by the hippocampus. Specifically, in the brain cortex, Poly (I:C) mice showed decreased 5-HT tissue concentrations (*t=4.04, p<0.0004)* compared to saline mice. Eventually, no other significant differences were found in the rest of the examined brain areas (**Figure 4.4**, **Figure 4.1**).

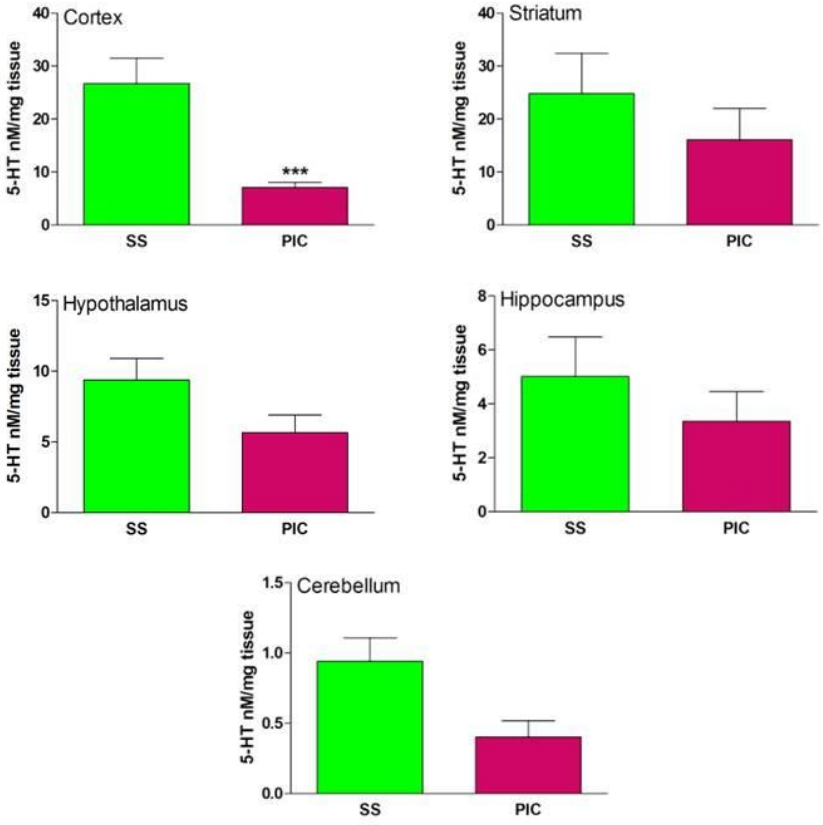

Figure 4.4: Mean values ± SEM (in nM/mg tissue) of 5-HT in the brain cortex (SS n=7, PIC n=7), striatum (SS n=7, PIC n=7), hypothalamus (SS n=6, PIC n=6), hippocampus (SS n=6, PIC n=6) and cerebellum (SS n=3, PIC n=2) of adult saline offspring (■) and Poly (I:C) mice (■). Statistical significance levels are denoted by **** p<0.001*, based on Student's *t*-tests.

4.1.2. Protein expression in brain tissue of saline and Poly (I:C) mice

The protein expression was evaluated by Western-Blot in the two brain areas of interest: the PFC and the striatum. Since we wanted to process the samples of each mouse individually, we decided to perform all the experiments in the total homogenate, to be able to have enough amount of tissue for all the tests and to have all the proteins measured in the same fraction. Thus, the samples used for the experiments were:

- A total homogenate of the brain cortex of saline mice (n=6) and Poly (I:C) mice (n=7).
- A total homogenate of the striatum of saline mice (n=6) and Poly (I:C) mice (n=7).

4.1.2.1. Protein expression in the brain cortex of saline and Poly (I:C) mice

4.1.2.1.1. DA Transporter (DAT)

The band we visualized for the DAT migrated at approximately 70-80 kDa, which matched the molecular weight predicted by the provider *Millipore* (70-85 kDa).

The quantification of the immunoreactivity of the DAT protein in the cortex demonstrated a significant increase in the Poly (I:C) mice *(t=2.33, p=0.040)* compared to saline mice (**Figure 4.5**). DAT expression levels were 100.0±7.69% for the saline mice (n=6) and 166.1±25.28% for the Poly (I:C) mice (n=7).

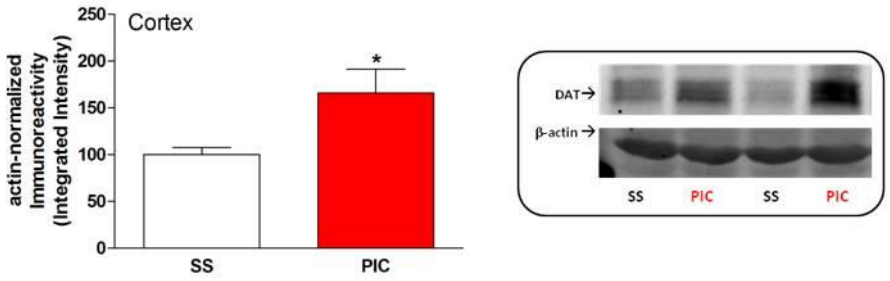

Figure 4.5: Western blot analysis for the DAT expression in brain cortex of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the actin-normalized immunoreactivity levels as a percentage of the saline mice as the controls (100%). Significance levels are denoted by **p<0.05,* based on Student's *t*-tests.

4.1.2.1.2. Noradrenaline Transporter (NET)

The band we visualized for the noradrenaline transporter (NET) migrated in the membranes at approximately 70 kDa, and it matched the molecular weight predicted by the provider *MabTechnologies* (50-75 kDa).

The quantification of the immunoreactivity of the NET protein in the cortex showed no significant differences between the Poly (I:C) mice and the saline mice (*t=0.19, p=0.854*)

(**Figure 4.6**). NET expression levels were 100.0±5.92% for the saline mice (n=6) and 101.7±6.85% for the Poly (I:C) mice (n=7).

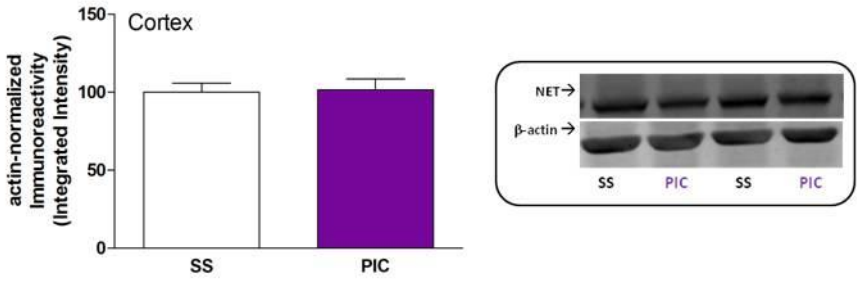

Figure 4.6: Western blot analysis of the noradrenaline transporter (NET) expression in the brain cortex of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the actin-normalized immunoreactivity levels as a percentage of the saline mice as the controls (100%).

4.1.2.1.3. Tyrosine hydroxylase (TH)

For the TH we used two different antibodies; one from the provider *St. Cruz* (SC25269) with a predicted molecular weight band of 55-60 kDa, and another one from the provider *Abcam* (AB41528), with a predicted molecular weight of 55-65 kDa. This way, we could validate the results obtained with each one of them, in both cortex and striatum.

4.1.2.1.3.1. *St. Cruz* (SC25269) antibody

The predicted molecular weight band (55-60 kDa) matched the band we observed at approximately 55 kDa.

No significant differences were detected in the quantification of the immunoreactivity of the TH protein labeled with the *St. Cruz* antibody in the cortex between the saline mice and the Poly (I:C) mice (*t=0.06, p=0.955*) (**Figure 4.7**). TH expression levels were 99.97±20.34% for the saline mice (n=6) and 101.9±24.97% for the Poly (I:C) mice (n=7).

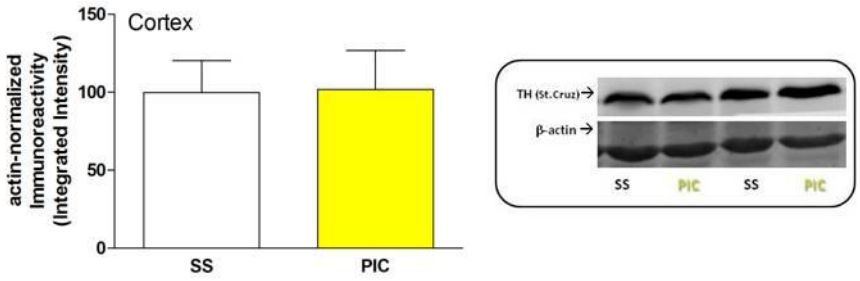

Figure 4.7: Western blot analysis of the TH expression measured with the *St. Cruz* antibody (sc25269) in the brain cortex of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%).

4.1.2.1.3.2. *Abcam* (AB41528) antibody

The predicted molecular weight band (55-65 kDa) matched the band we observed at approximately 60 kDa.

As observed with the *St. Cruz* TH antibody, no statistical differences were observed with the TH levels measured with the antibody from the provider *Abcam* between the saline mice and the Poly (I:C) mice *(t=0.24, p=0.817)* (**Figure 4.8**). TH expression levels were 100.0±16.22% for the saline mice (n=6) and 108.0±27.92% for the Poly (I:C) mice (n=7).

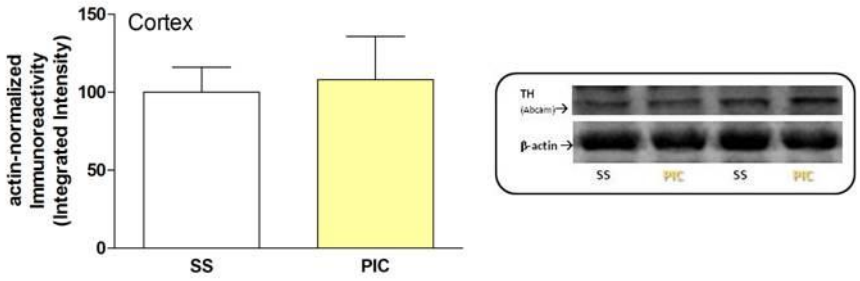

Figure 4.8: Western blot analysis of the TH expression measured with the *Abcam* antibody (ab41528) in brain cortex of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%).

4.1.2.1.4. Dopamine D_1 receptor (D_1R)

For the D_1R, the predicted molecular weight band reported by the provider was 50 kDa. In our experiments, we could see one thick band at 50 kDa but also another thinner band at approximately 60-65 kDa. According to the provider information, the specific band for that antibody was the one that appeared at 50 kDa. However, they also reported the presence of some other nonspecific bands for that antibody, whose identity they were not sure of. For this reason, we decided to quantify this band at 50 kDa.

The quantification of the immunoreactivity of the D_1R protein in the cortex showed no significant differences between the Poly (I:C) mice and the saline mice (*t=1.23, p=0.220*), although there was a trend to an increase (**Figure 4.9**). D_1R expression levels were 100.0±8.84% for the saline mice (n=6) and 121.6±13.35% for the Poly (I:C) mice (n=7).

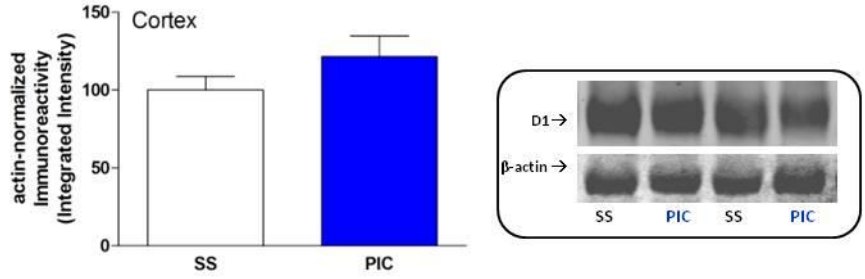

Figure 4.9: Western blot analysis of the D_1R expression in the brain cortex of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%).

4.1.2.1.5. Dopamine D_2 receptor (D_2R)

As for the D_2R, the predicted molecular weight band reported by the provider was 48-51 kDa. Similarly to the D_1R, in our experiments, we could observe this predicted band but also another band at 60-65 kDa. Following the provider information and confirming the specific band for the D_2R should appear at 48-51 kDa, we quantified this band.

As with D_1R, the quantification of the immunoreactivity of the D_2R protein in the cortex showed no significant differences between the Poly (I:C) mice and the saline mice (*t=1.53, p=0.155*), although there was also a trend to an increase in this case (**Figure 4.10**). D_2R expression levels were 100.0±9.44% for the saline mice (n=6) and 127.8±14.72% for the Poly (I:C) mice (n=7).

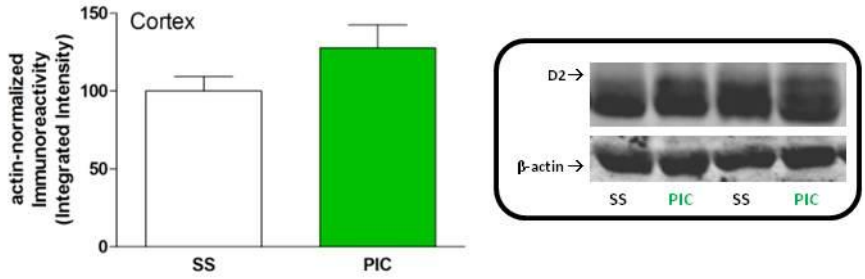

Figure 4.10: Western blot analysis of the D_2R expression in the brain cortex of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%)
.

4.1.2.2. Protein expression in the brain striatum of saline and Poly (I:C) mice

4.1.2.2.1. DA Transporter (DAT)

Opposed to what we could observe in the cortex, the quantification of the immunoreactivity of the DAT protein in the striatum showed a significant decrease in the Poly (I:C) mice *(t=4.19, p=0.001)* compared to saline mice (**Figure 4.11**). DAT expression levels in the striatum were 100.1±9.97% for the saline mice (n=6) and 54.33±5.46% for the Poly (I:C) mice (n=7).

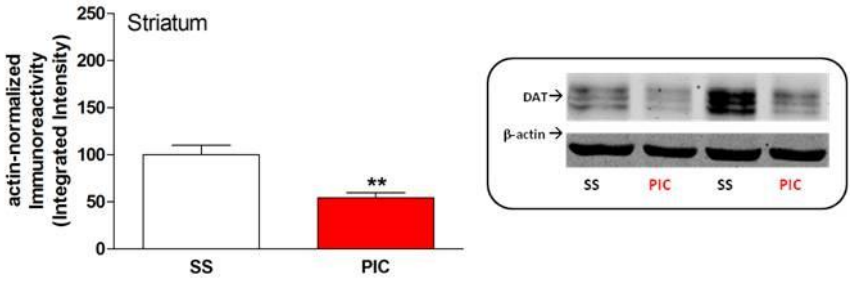

Figure 4.11: Western blot analysis of the DAT expression in brain striatum of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%). Significance levels are denoted by ***p<0.01,* based on Student's *t*-tests.

4.1.2.2.2. Noradrenaline Transporter (NET)

The quantification of the immunoreactivity of the NET protein in the striatum showed no significant differences between the Poly (I:C) mice and the saline mice (*t=0.38, p=0.711*) (**Figure 4.12**). NET expression levels in the striatum were 100.0±12.49% for the saline mice (n=6) and 94.26±9.01% for the Poly (I:C) mice (n=7).

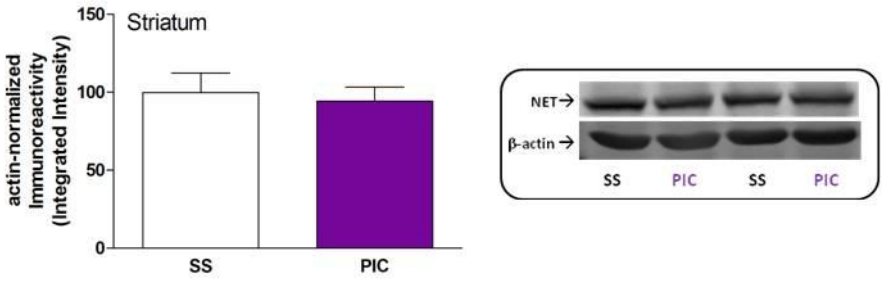

Figure 4.12: Western blot analysis of the noradrenaline transporter (NET) expression in the brain striatum of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%).

4.1.2.2.3. Tyrosine Hydroxylase (TH)

4.1.2.2.3.1. *St. Cruz* (SC25269) antibody

A statistical decrease was detected in the quantification of the immunoreactivity of the TH protein labeled with the *St. Cruz* antibody in the striatum in the Poly (I:C) mice (*t=4.52, p=0.0009*) compared with the saline mice (**Figure 4.13**). TH expression levels were 100.0±8.15% for the saline mice (n=6) and 60.57±4.13% for the Poly (I:C) mice (n=7).

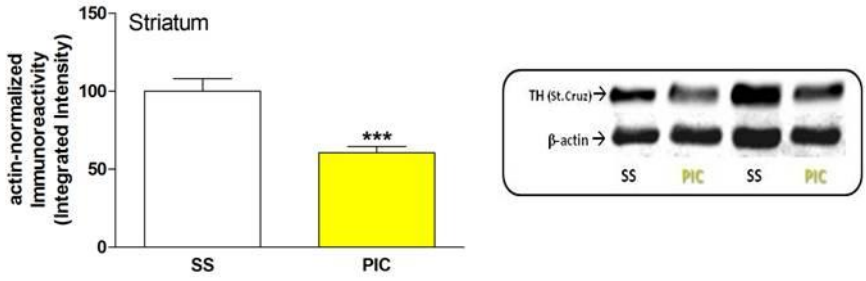

Figure 4.13: Western blot analysis of the TH expression measured with the *St. Cruz* antibody (sc25269) in the brain striatum of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%). Significance levels are denoted by ****p<0.001,* based on Student's *t*-tests.

4.1.2.2.3.2. *Abcam* (AB41528) antibody

Following the same trend as observed with the *St. Cruz* antibody, the quantification of the immunoreactivity of the TH protein labeled with the *Abcam* antibody showed a statistically significant decrease in the Poly (I:C) mice (**Figure 4.14**) *(t=2.52, p=0.028)*. TH expression levels were 100.0±13.40% for the saline mice (n=6) and 65.20±5.72% for the Poly (I:C) mice (n=7).

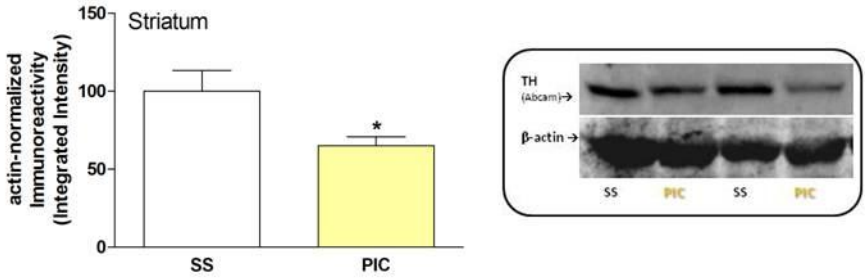

Figure 4.14: Western blot analysis of the TH expression measured with the *Abcam* antibody (ab41528) in brain striatum of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%). Significance levels are denoted by **p<0.05,* based on Student's *t*-tests.

4.1.2.2.4. Dopamine D_1 receptor (D_1R)

In the striatum, there were no significant differences in the immunoreactivity of the D_1R protein between the Poly (I:C) mice and the saline mice (*t=0.46, p=0.657*) (**Figure 4.15**). D_1R expression levels were 100.0±8.33% for the saline mice (n=6) and 106.4±10.69% for the Poly (I:C) mice (n=7).

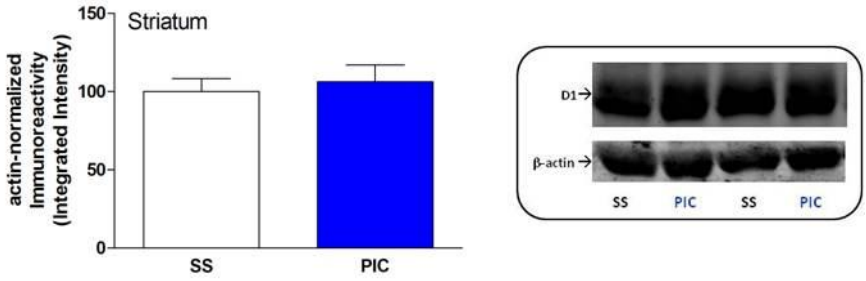

Figure 4.15: Western blot analysis of the D_1R expression in the brain striatum of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%).

4.1.2.2.5. Dopamine D_2 receptor (D_2R)

Also in the case of the D_2R, no significant differences were observed in the quantification of the immunoreactivity of the D_2R protein in the striatum between the Poly (I:C) mice and the saline mice (*t=1.53, p=0.155*) (**Figure 4.16**). D_2R expression levels were 100.0±9.44% for the saline mice (n=6) and 127.8±14.72% for the Poly (I:C) mice (n=7).

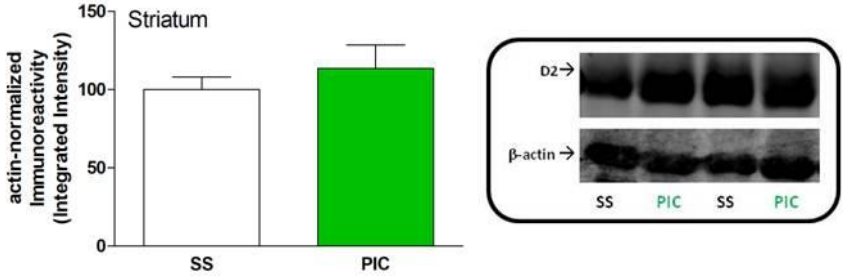

Figure 4.16: Western blot analysis of the D_2R expression in the brain striatum of adult saline offspring mice (n=6) and Poly (I:C) mice (n=7). Data shown are mean ± SEM values of 3-5 independent experiments and express the corrected immunoreactivity levels as a percentage of the saline mice as the controls (100%).

4.1.3. Cognitive status of saline and Poly (I:C) mice by the NORT

The NORT is a behavioral task which uses spatial novelty and delayed non-matching to evaluate time spent with a novel object versus a familiar object, as a measure of spatial and visual learning and memory evocation capacity (Jaiswal et al., 2018).

The day of the test, both saline and Poly (I:C) mice spent a similar time exploring the familiar object, with no differences between groups (*t=0.305, p=0.763*) (**Figure 4.17-A**). In the case of the novel object, a lower although no significant trend of the time devoted to the novel object was observed in the Poly (I:C) mice compared to the saline mice (*t=1.73, p=0.096*) (**Figure 4.17-B**). Thus, the total time both groups of mice devoted to the exploration of both the familiar and the novel objects was not different (*t=1.32, p=0.198*) even if the Poly (I:C) mice showed an inclination to a shorter exploration time than saline mice (**Figure 4.17-C**).

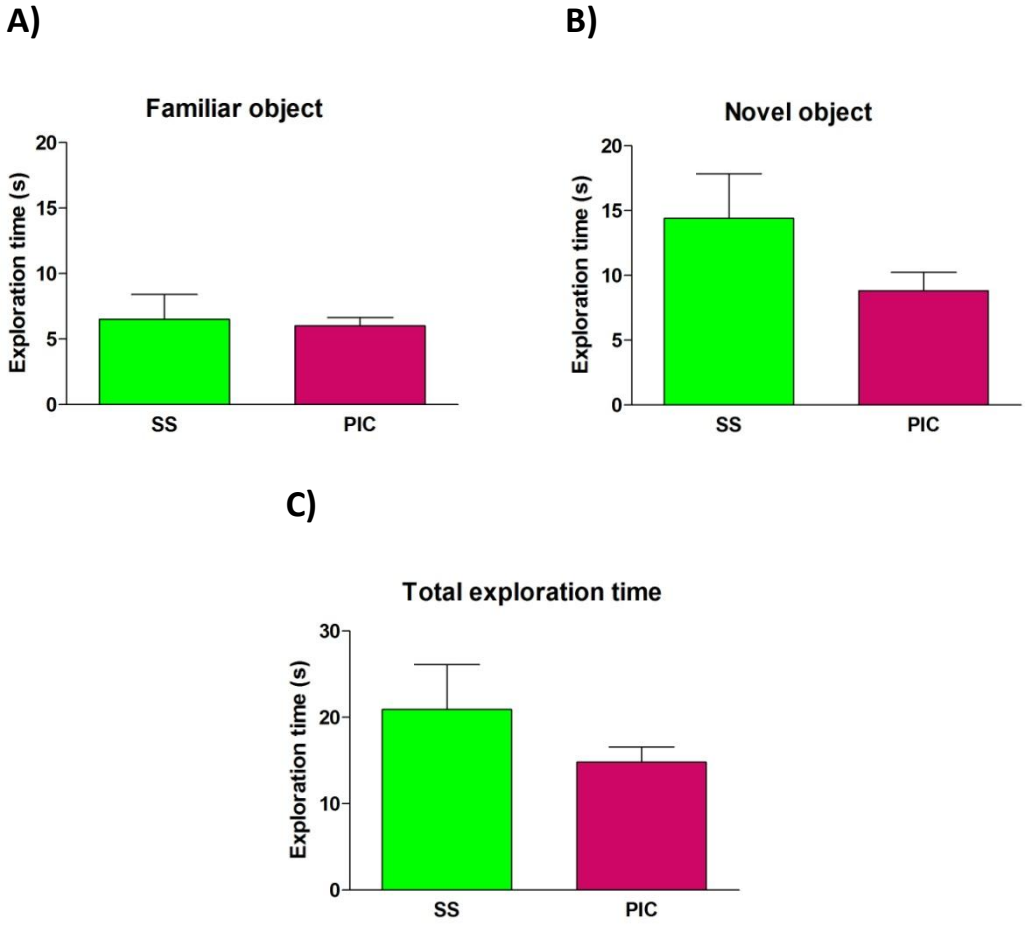

Figure 4.17: **A)** Representation of the exploration time (in s) devoted to the familiar object; **B)** Representation of the exploration time (in s) devoted to the novel object and **C)** Representation of the total exploration time (in s) of both familiar and novel objects by the saline mice (n=9) and the Poly (I:C) mice (n=15) during the NORT. Bars are a representation of the mean ± SEM values.

As a parameter of the potential cognitive status, the discrimination index was calculated as it follows: *([time devoted to novel object minus time devoted to familiar object]/ [time devoted to novel object plus time devoted to familiar object])*. In this case, Poly (I:C) mice presented a lower discrimination index (*t=2.30, p=0.031*) compared to the saline mice (**Figure 4.18**).

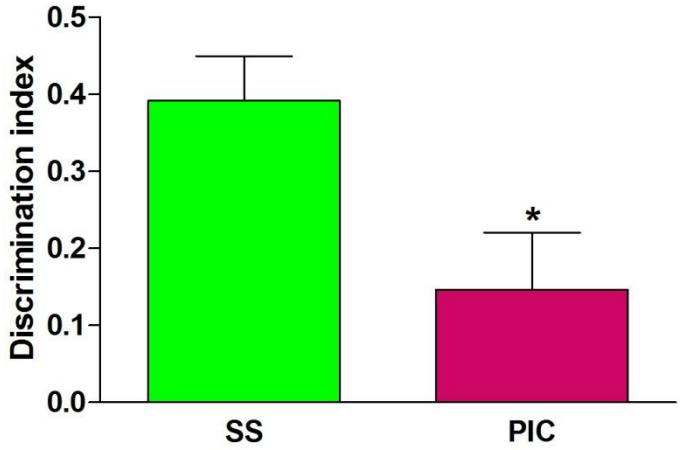

Figure 4.18: Representation of the discrimination index of saline (n=9) and Poly (I:C) (n=15) mice as the parameter for the evaluation of cognitive status. Bars are a representation of the mean ± SEM values.*$p<0.05$, Student's unpaired t-test *vs*. saline group.

4.1.4. Cognitive status of saline and Poly (I:C) mice by the 5-CSRTT

The 5-CSRTT is a validated test to evaluate, among other abilities, sustained attention and cognitive flexibility. It requires a pre-training and training periods of the animals through different phases of increasing difficulty until they reach a baseline state, considered the moment when they "know" how to perform the task. After that, they can be tested with distinct manipulations of the basic task.

4.1.4.1. Pre-training phase

4.1.4.1.1. Weight registration

The 5-CSRTT involves water deprivation to motivate the mice to look for the liquid reward. This water deprivation protocol starts even before the task itself, thus is very important to monitor the weight of each animal during the whole experiment (see **Figure 3.12** for chronogram). The weight of the animals was not different in any time during the task, even if the statistical analysis by the two-way ANOVA showed statistical differences in the weight between saline (n=15) and Poly (I:C) mice (n=10) across the time ($F_t[104,2392]=67.04$, $p<0.0001$; $F_{tr}[1,2392]=0.316$, $p=0.579$; $F_i[104,2392]=3.033$, $p<0.0001$; $n=25$). This is due to the fact that the growth slope of each group is different; that is, they have a distinct growth rate over time. It is important to note that although the existence of this difference at the beginning of the pre-training, by the time the training of the task began, the weight of both groups was equal (**Figure 4.19**).

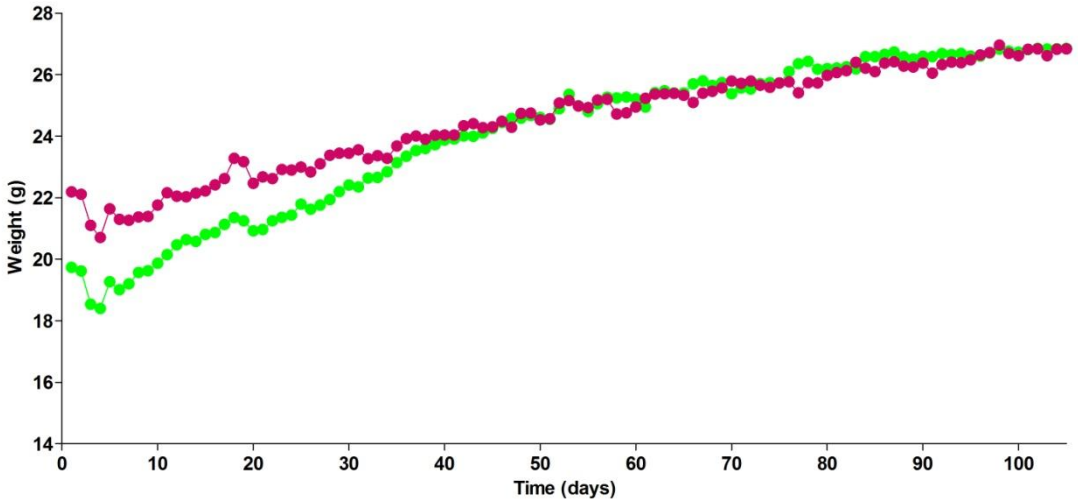

Figure 4.19: Graphic representation of the weight registration of both saline (•) (n=15) and Poly (I:C) (•) (n=10) mice throughout the experiment. Points are representations of the mean values.

4.1.4.1.2. Preference-Test

With the aim to habituate the mice to the liquid reward (10 % CM in water) and detect the possibility of pre-existing differences that could influence their behavior based on the reward, a CM vs. water preference-test was conducted daily and continued until the two groups of mice showed a preference for the CM (**Figure 3.12**). During the preference test, the total consumed volume increases each day in both groups of mice across time, until they consume

the same volume of liquid on day 12th (**Figure 4.20-A)**. The two-way ANOVA showed a significant increase of consumed volume ($F_t[4,92]=12.04$, $p<0.0001$), and light differences between saline and Poly (I:C) groups ($F_{tr}[1,92]=0.887$, $p=0.036$, $n=25$). However, the *post*-hoc analysis demonstrated the absence of differences in the total consumed volume at day 12th ($t=0.65$, $p>0.05$).

The preference for the CM compared to water increased from day 10th to day 12th (**Figure 4.20-B**) ($F_t[2,46]=8.538$, $p=0.0007$) without significant differences between saline and Poly (I:C) mice ($F_{tr}[1,46]=2.449$, $p=0.1313$; $F_i[2,46]=1.133$, $p=0.3309$; $n=25$). On day 12th, the preference for CM in the consumed volume was 77.81±8.1% in saline mice and 70.32±8.9% in Poly (I:C) mice (**Figure 4.20-B**). All this data indicated that both groups of mice were ready to start a training task based on reward behavior. No differences in weight, palatability (preference for CM) and/or food needs seem to be observed between saline and Poly (I:C) mice.

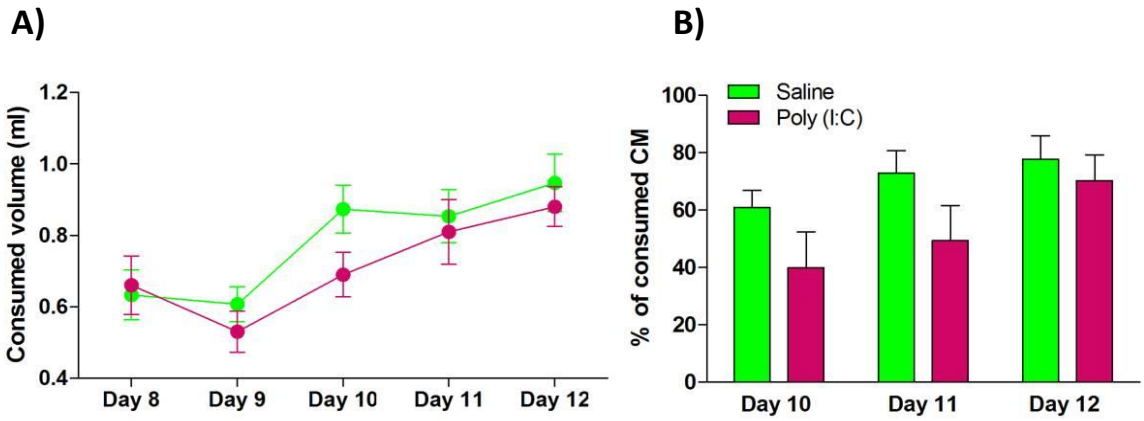

Figure 4.20: **A)** Graphic representation of the total consumed volume of liquid (in ml) during the preference-test by the saline (•) (n=15) and Poly (I:C) (•) (n=10) mice. **B)** Graphic representation of the percentage of CM consumed by the saline (n=15) and Poly (I:C) mice (n=10) related to the total consumed volume. Points and bars are representations of the mean ± SEM values.

4.1.4.1.3. Must Touch Stimuli and Must Initiate training

After the preference-test, pre-training of the animals continued with the Must Touch Stimuli phase followed by the Must Initiate phase. The animals need to meet the criterion to pass to the following phase (completing 30 trials in a 60 min session during two consecutive days) and we monitored the days both groups of animals spent in each of the phases (**Figure 3.12**). There were no statistical differences between them in the time spent in any of the phases (**Figure 4.21-A** and **4.21-B**). These data suggest that none of the two groups of mice suffers motor, visual or any other type of sensorial impairment that could affect them negatively to perform the following tasks in the next phases.

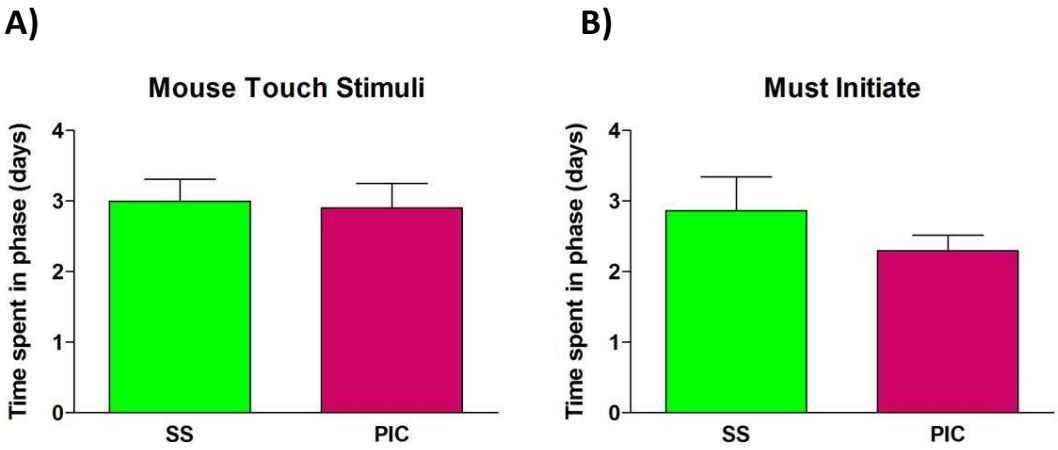

Figure 4.21: **A)** Graphic representation of the time spent in the Mouse Touch Stimuli phase by the saline (n=15) and Poly (I:C) (n=10) mice. **B)** Graphic representation of the time spent in the Must Initiate phase by the saline (n=15) and Poly (I:C) (n=10) mice. Bars are representations of the mean ± SEM values.

4.1.4.2. Training to baseline

Once the pre-training is completed and the mice are habituated to the apparatus and to the liquid reward, the training to baseline phase starts (**Figure 3.12**). From this moment onwards, mice would have to overcome a total of eight sessions, reaching in each of them the established criterion to pass to the following session, taking into account that the difficulty of each one increases. It was considered that the animals had achieved the baseline conditions when they had been able to get to the last session (SD= 1.4 s) and meet the criterion to pass it during two consecutive days (≥80 % of accuracy and ≤20 % of omissions).

In regard to the accuracy percentage, both groups of mice needed to meet the criterion of ≥80 % of accuracy during two consecutive days in all the sessions for a specific SD to be able to pass to the next session. As it can be observed in (**Figure 4.22-A**), both saline and Poly (I:C) mice manage to reach and exceed 80% of accuracy and keep it throughout the whole task. The two-way ANOVA revealed a rapid increase of accuracy without statistical differences between saline and Poly (I:C) ($F_t[7,161]=74.75$, $p<0.0001$; $F_{tr}[1,161]=0.274$, $p=0.605$; $F_i[7,161]=1.443$, $p=0.192$; $n=25$).

As to the percentage of omissions, the criterion to fulfill was to achieve ≤20 % of omissions for each session during two consecutive days. In **Figure 4.22-B** can be observed that during the first sessions, as the difficulty augments, the % of omissions increased in both groups of mice. However, at a certain point, the number of omissions stabilizes and remains unchanged until the end of the task in both saline and Poly (I:C) mice. The two-way ANOVA revealed this progressive increase of omissions and showed no statistical differences between saline and Poly (I:C) mice ($F_t[7,161]=54.89$, $p<0.0001$; $F_{tr}[1,161]=0.183$, $p=0.673$; $F_i[7,161]=0.914$, $p=0.4974$; $n=25$).

In addition to this, we also analyzed the time each group of animals spent in each of the sessions for a specific SD, and likewise, there were no differences between the two groups ($F_t[7,196]=34.88$, $p<0.0001$; $F_{tr}[1,196]=0.182$, $p=0.188$; $F_i[7,196]=1.210$, $p=0.2987$; $n=25$) (**Figure 4.22-C**), suggesting that Poly (I:C) mice do not need more time than saline mice to be able to reach the next session.

Taking into account these similar data between saline and Poly (I:C) mice, it could be suggested that Poly (I:C) exposure would not affect the capacity to process spatial information and reward task in these animals.

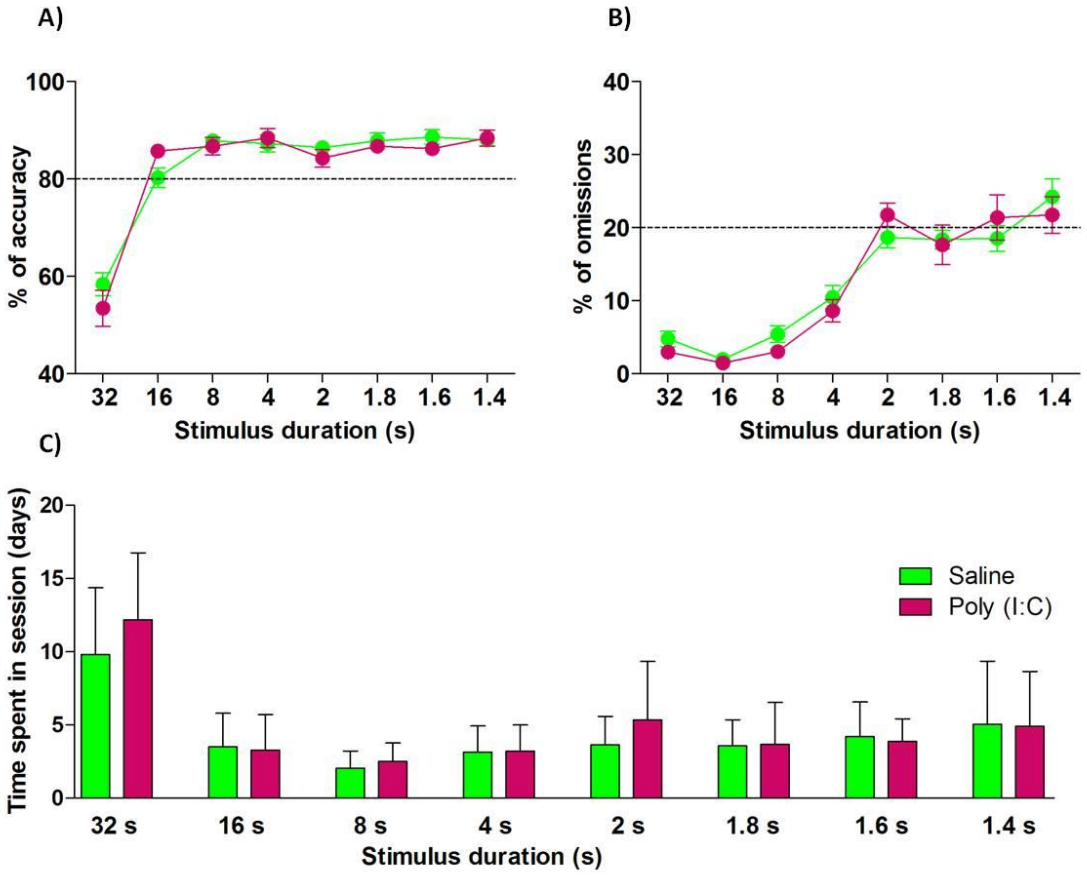

Figure 4.22: **A)** Graphic representation of the % of accuracy in each of the sessions of saline (•) (n=15) and Poly (I:C) (•) (n=10) mice for a specific SD. **B)** Graphic representation of the % of omissions in each session of saline (•) (n=15) and Poly (I:C) (•) (n=10) mice for a specific SD. **C)** Graphic representation of the time spent (days) in each of the phases of saline (•) (n=15) and Poly (I:C) (•) (n=10) mice. Points and bars are representations of the mean ± SEM values.

4.1.4.3. Testing schedules / Task manipulations

Once animals had reached the baseline conditions achieving the established criteria, our next aim was to further assess the attentional function of these mice. For that, the basic 5-CSRTT task was modified to present the mice two different schedules that would allow us to evaluate their cognitive flexibility: "Variation 2" was intended to distract the animals from their task by presenting a distractor noise during the delay time. "Variation 1" aimed to increase the attentional charge by altering the duration of the delay time, making it shorter, variable in duration and offered randomly. These variations were introduced sequentially, first "Variation 2" and secondly, "Variation 1".

For each variation, the percentage of accuracy, percentage of omissions, percentage of premature responses (when the mouse touched the screen during the delay of 5 s prior to the emergence of the stimulus) and latency to correct response (the time it took them to respond

correctly to the stimulus) were measured and compared to baseline conditions (parameters unaltered).

First, regarding the accuracy, neither the appearance of the distractor noise ("Variation 2") nor the alteration of the delay ("Variation 1") altered the % of the accuracy of any of the groups of mice. In fact, the two-way ANOVA did not show neither statistical intra-group, nor inter-group differences for any of the variations compared to baseline conditions between saline and Poly (I:C) animals *(F_t[2,56]=0.725, p=0.489; F_{tr}[1,56]=0.914, p=0.3471; F_i[2,56]=0.772, p=0.467; n=25)* (**Figure 4.23-A**).

As for the omissions, "Variation 2" (distractor noise) did not modify the % of omissions performed by saline or Poly (I:C) mice. However, the posterior introduction of "Variation 1" (alteration of the delay) did modify the behavior of the mice *(F_t[2,56]=0.720, p=0.491; F_{tr}[1,56]=2.570, p=0.1202; F_i[2,56]=7.066, p=0.002; n=25)*. In fact, saline mice reduced the % of omissions during "Variation 1" compared to baseline conditions (*t=3.15, p<0.01*), and this reduction was significantly lower in "Variation 1" compared to "Variation 2" (*t=2.86, p<0.05*), suggesting control mice are able to learn as time goes by and decrease their omissions even if the complexity of the task augments (**Figure 4.23-B**). However, this did not happen in the case of Poly (I:C) mice, that maintained their % of omissions equal in both "Variation 2" and "Variation 1". In fact, there were also differences in the performance of saline *vs.* Poly (I:C) mice in "Variation 1". As can be observed in **Figure 4.23-B**, Poly (I:C) mice made significantly higher % of omissions compared with saline mice (*t=3.89, p<0.001*) during this task manipulation, suggesting that these mice are not able to adapt to the more complex requirements of the task, thus lacking cognitive flexibility.

In the case of the % of premature responses, according to the two-way ANOVA, there were no inter-group differences between the performance of saline and Poly (I:C) mice, but there were intra-group differences (*F_t[2,56]=19.09, p<0.0001; F_{tr}[1,56]=0.134, p=0.7168; F_i[2,56]=0.585, p=0.560; n=25).* Regarding saline mice, the initially introduced manipulation ("Variation 2") did not modify their number of premature responses; however, during "Variation 1", they reduced their % of premature responses compared to baseline (*t=3.33, p<0.01*) and this reduction was significantly lower in "Variation 1" compared to "Variation 2" (*t=2.88, p<0.05*). These data suggest that saline mice were able to learn during the process and decrease their premature responses across time (**Figure 4.23-C**). In the case of Poly (I:C) mice, a similar trend is shown in **Figure 4.23-C**. Although in "Variation 2" they do not behave differently compared to baseline, they reduced their premature responses in "Variation 1" compared with baseline conditions (*t=4.59, p<0.001*), and this reduction in "Variation 1" was significantly lower compared with the number of premature responses made previously in "Variation 2" (*t=4.26, p<0.001*). Therefore, these mice can also improve their performance across time as controls, and it could be said that the Poly (I:C) exposure does not result in increased impulsivity.

Finally, when analyzing the time needed by each group to make a correct response (latency to correct response), neither "Variation 2" nor "Variation 1" modified the latency to correct response in saline mice. This demonstrates that these mice, as a result of the learning process and even if the complexity of the task increases, they do not need more time to respond correctly (**Figure 4.23-D**). However, there were differences between saline and Poly (I:C) mice

in their performance ($F_t[2,56]=0.902$, $p=0.411$; $F_{tr}[1,56]=2.038$, $p=0.164$; $F_i[2,56]=6.057$, $p=0.004$; $n=25$). Poly (I:C) mice, even if the first manipulation of the task ("Variation 2") did not modify their behavior, the posterior introduction of "Variation 1" produced an increase in their latency to correct response compared to baseline ($t=2.35$, $p<0.05$); and this increase was also statistically higher compared to the first manipulation "Variation 2" ($t=3.23$, $p<0.01$). Moreover, Poly (I:C) mice showed an increase in the latency to correct response in "Variation 1" compared to saline mice ($t=3.30$, $p<0.01$), demonstrating that, as the complexity of the task augment, they need more time to adapt and be able to perform correctly the established criterion (**Figure 4.23-D**).

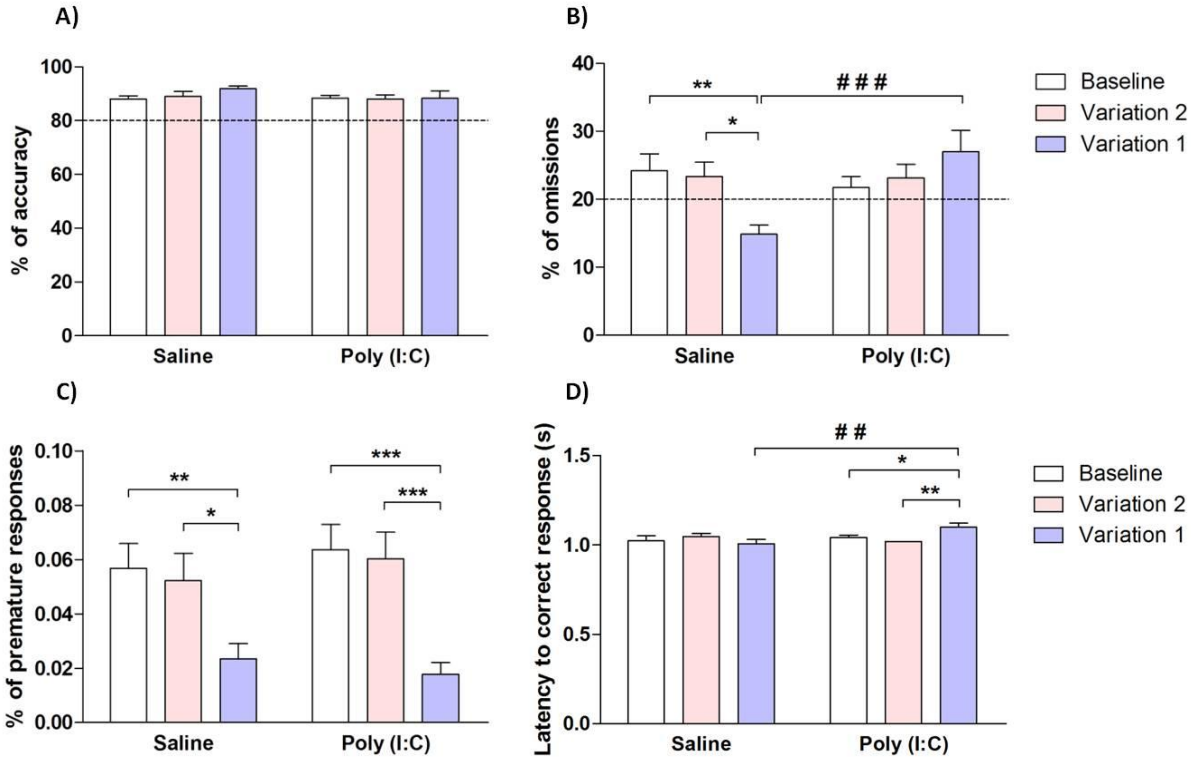

Figure 4.23: Graphic representation of the % of accuracy **(A)**, % of omissions **(B)**, % of premature responses **(C)** and latency to correct response **(D)** of saline (n=15) and Poly (I:C) (n=10) mice in baseline conditions compared to "Variation 2" (distractor noise) and "Variation 1" (alteration of the delay) of the task. Bars are representations of the mean ± SEM values. Intra-group differences: *$p<0.05$, **$p<0.01$, ***$p<0.001$. Inter-group differences: $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, Two-way ANOVA followed by Bonferroni *post-hoc* test.

4.1.5. Characterization of extracellular monoamine concentrations in awake saline and Poly (I:C) mice by microdialysis

4.1.5.1. Extracellular monoamine concentrations in saline and Poly (I:C) mice

4.1.5.1.1. Extracellular monoamine concentrations in the PFC

4.1.5.1.1.1. NA basal concentrations and *Ed* in the PFC by the non-net flow microdialysis technique

There were no statistically significant differences between the NA basal values presented by the Poly (I:C) mice (1.85±0.747 nM) and the respective saline group (2.20±0.469 nM) (*t=0.38, p=0.7102*) (**Figure 4.24** and **Figure 4.25-A**).

The same situation is applicable to the *Ed*, with no statistically significant differences between groups (*t=0.24, p=0.8134*). Poly (I:C) mice presented a mean value of 0.62±0.076 and the saline group 0.65±0.095, respectively (**Figure 4.24** and **Figure 4.25-B**).

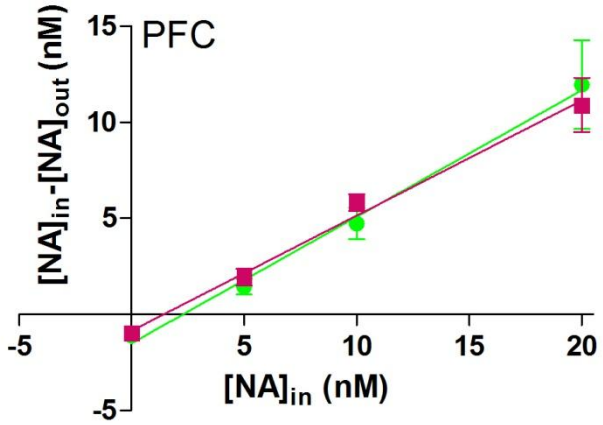

Figure 4.24: Graphic representation of the study of non-net flow cerebral microdialysis technique of NA in PFC of saline (n=7) (●) and Poly (I:C) (n=8) (■) mice.

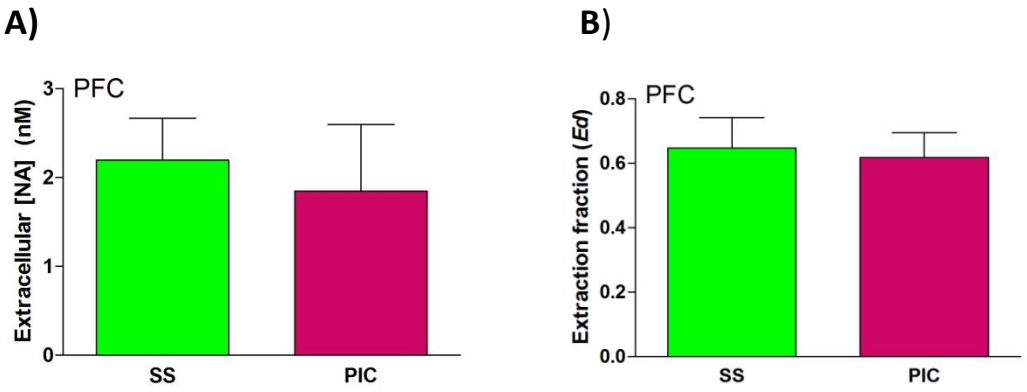

Figure 4.25: **A)** Graphic representation of extracellular NA concentrations in the PFC of saline (n=7) and Poly (I:C) mice (n=8) as indicated by the point of non-net flow microdialysis. **B)** Graphic representation of the *Ed in vivo* of the PFC of saline (n=7) and Poly (I:C) mice (n=8). Bars are a representation of the mean ± SEM values.

4.1.5.1.1.2. DA basal concentrations and *Ed* in the PFC by the non-net flow microdialysis technique

There was a statistically significant decrease in the DA basal values presented by the Poly (I:C) mice (0.63±0.11 nM) compared with the saline group (1.65±0.301 nM) (*t=3.17, p=0.0068*) (**Figure 4.26** and **Figure 4.27-A**).

However, regarding the *Ed*, there were no statistically significant differences between groups (*t=0.24, p=0.8134*). Poly (I:C) mice presented a value of 0.52±0.044 and the saline group 0.55±0.049 (**Figure 4.26** and **Figure 4.27-B**).

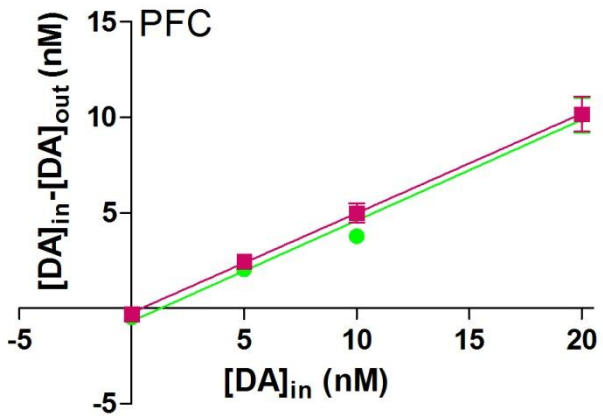

Figure 4.26: Graphic representation of the study of non-net flow cerebral microdialysis technique of DA in PFC of saline (n=9) (●) and Poly (I:C) (n=11) (■) mice.

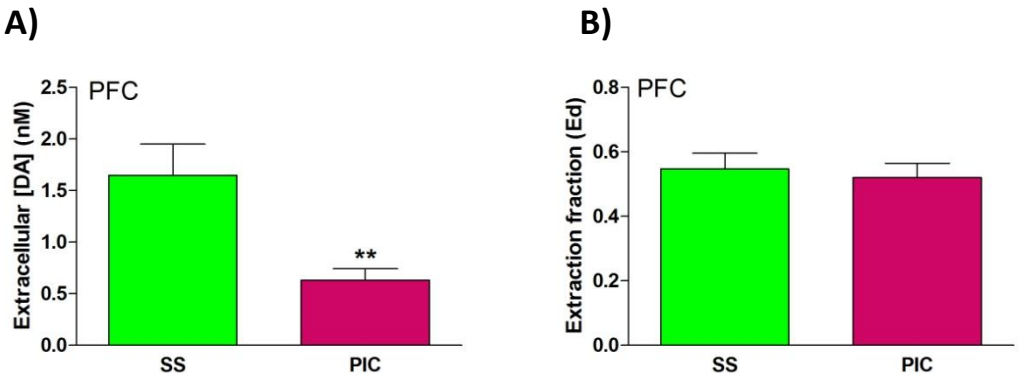

Figure 4.27: **A)** Graphic representation of extracellular DA concentrations in the PFC of saline (n=8) and Poly (I:C) mice (n=8) as indicated by the point of non-net flow microdialysis. **B)** Graphic representation of the *Ed in vivo* in the PFC of saline (n=9) and Poly (I:C) mice (n=11). Bars are a representation of the mean ± SEM values.***p<0.01,* Student's unpaired *t*-test vs saline group.

4.1.5.1.1.3. K^+-evoked release of monoamines in the PFC by the local administration of hiperK^+ aCSF (50 mM)

4.1.5.1.1.3.1. Effect of the administration of hiperK^+ aCSF in the release of NA in PFC

Extracellular basal concentrations of NA in the PFC of the saline mice were 0.086±0.01 nM (n=18) and 0.087±0.009 nM for the Poly (I:C) mice (n=23). There were no statistically significant differences between groups (*t=0.07, p=0.9441*).

The local administration in PFC of hiperK^+ aCSF (50 mM) induced a significant increase in NA concentration in saline mice (maximal effect (Emax)=611.22±59%; p<0.001 vs basal conditions). This maximum effect was reached after 70 min from the beginning of hyperK^+ perfusion (*F[10,50]=22.64, p<0.0001, n=6)* (**Figure 4.28-A**). For the Poly (I:C) mice, the local administration in PFC of hiperK^+ aCSF also increased NA concentrations (Emax=545.72±17%; p<0.001 vs basal conditions) and the maximum effect occurred after 105 min from the beginning of the perfusion (*F[10,40]=25.80, p<0.0001, n=5)*. Two-way ANOVA for repeated measures revealed significant differences between groups *(F_t[10,90]=36.27, p<0.0001; F_{tr}[1,90]=1.841, p=0.208; F_i[10,90]=8.686, p<0.0001; n=11).*

The analysis of the AUC of the curves that represent the effect of the hyperK^+ perfusion did not show statistical differences between groups (*t=1.51, p=0.166*) (**Figure 4.28-B**).

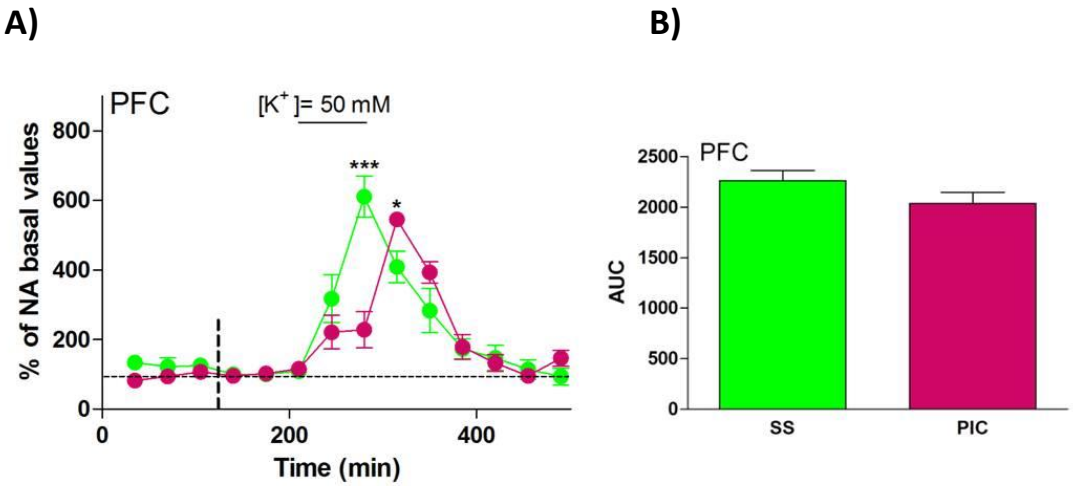

Figure 4.28: **A)** Graphic representation of the effect of the local administration in PFC of hiperK$^+$ aCSF (50 mM) during 105 min on extracellular concentrations of NA in PFC of saline (•) (n=6) and Poly (I:C) (•) mice (n=5). The upper horizontal continuous line indicates the time interval of administration of hiperK$^+$ aCSF. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **p<0.05, ***p<0.001*, Two-way ANOVA followed by Bonferroni *post-hoc* test. **B)** Graphic representation of the AUC of the effect of the local administration of hiperK$^+$ aCSF on extracellular concentrations of NA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.1.5.1.1.3.2. Effect of the administration of hiperK$^+$ aCSF in the release of DA in PFC

Extracellular basal concentrations of DA in the PFC were 0.28±0.04 nM (n=26) for saline mice and for the Poly (I:C) mice were 0.42±0.06 nM (n=26). Similarly to NA, there were no significant differences between both groups (*t=1.64, p=0.106*).

The local administration in PFC of hiperK$^+$ aCSF (50 mM) provoked a significant increase in DA concentration in saline mice (Emax=757.01±43%; p<0.001 vs. basal conditions) (**Figure 4.29-A**). This maximum effect occurred after 70 min from the beginning of the hiperK$^+$ perfusion (*F[10,50]=49.39, p<0.0001, n=6)*. For the Poly (I:C) mice, the local administration in PFC of hiperK$^+$ aCSF also increased DA concentrations significantly (Emax=663.09±121%; p<0.001 vs. basal conditions) and this maximum effect was reached after 105 min from the beginning of the perfusion (*F[10,40]=9.952, p<0.0001, n=5)*. Two-way ANOVA for repeated measures revealed significant differences between groups *(F_t[10,90]=43.33, p<0.0001; F_{tr}[1,90]=1.224, p=0.2972; F_i[10,90]=5.916, p<0.0001; n=11)*.

The analysis of the AUC of the curves representing the effect of the hiperK$^+$ perfusion did not reveal statistical differences between groups (*t=1.23, p=0.249*) (**Figure 4.29-B**).

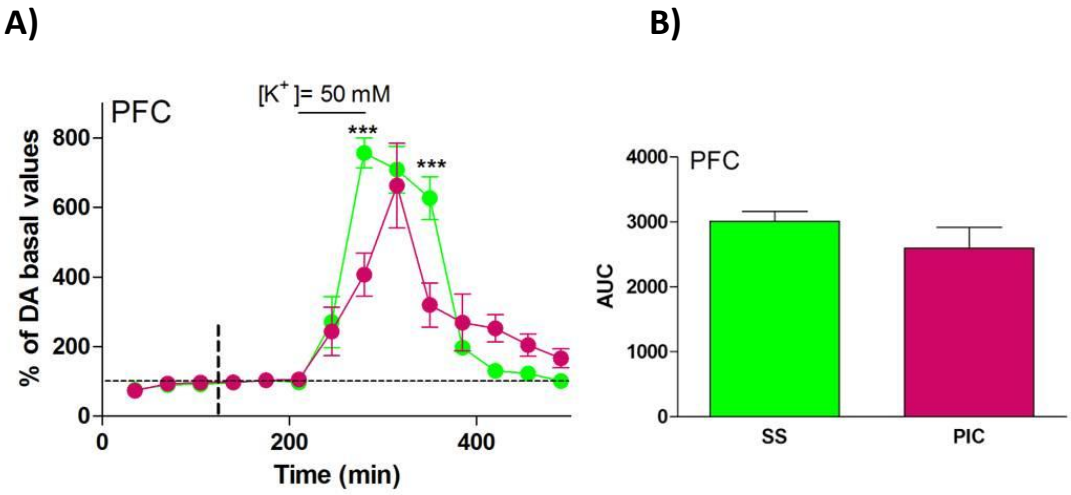

Figure 4.29: **A)** Graphic representation of the effect of the local administration in PFC of hiperK$^+$ aCSF (50 mM) during 105 min on extracellular concentrations of DA in PFC of saline (●) (n=6) and Poly (I:C) (●) mice (n=5). The upper horizontal continuous line indicates the time interval of administration of hiperK$^+$ aCSF. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. ****p<0.001*, Two-way ANOVA followed by Bonferroni *post-hoc* test. **B)** Graphic representation of the AUC of the effect of the local administration of hiperK$^+$ aCSF on extracellular concentrations of DA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.1.5.1.1.3.3. Effect of the administration of hiperK$^+$ aCSF in the release of 5-HT in PFC

In the PFC, extracellular concentrations of 5-HT in PFC of saline mice were 0.13±0.01 nM (n=25) and 0.098±0.01 nM (n=26) for the Poly (I:C) mice. Neither in this case, there were no statistical differences between the two groups (t=*1.81, p=0.076*).

In saline mice, local administration in PFC of hiperK$^+$ aCSF (50 mM) increased significantly 5-HT concentrations (Emax=251.83±28%, p<0.001 vs. basal conditions). This maximum effect was reached after 70 min from the beginning of the hiperK$^+$ perfusion (*F[10,50]=22.17, p<0.0001, n=6)* (**Figure 4.30-A**). Also in the case of the Poly (I:C) mice, 5-HT concentrations increased significantly in PFC after the hiperK$^+$ perfusion, with a maximum effect of 363.86±44%, p<0.001 vs. basal conditions, occurring after 70 min from the beginning of the hiperK$^+$ perfusion (*F[10,40]=26.84, p<0.0001, n=5)*. Two-way ANOVA for repeated measures revealed significant differences between groups (F_t*[10,90]=50.07; p<0.0001;* F_{tr}*[1,90]=20.37; p=0.0015;* F_i*[10,90]=4.829; p<0.0001; n=11).*

The analysis of the AUC of the curves showed statistical differences between the two groups (*t=4.47, p=0.001*), showing higher values of 5-HT in the Poly)I:C) group of mice (**Figure 4.30-B**).

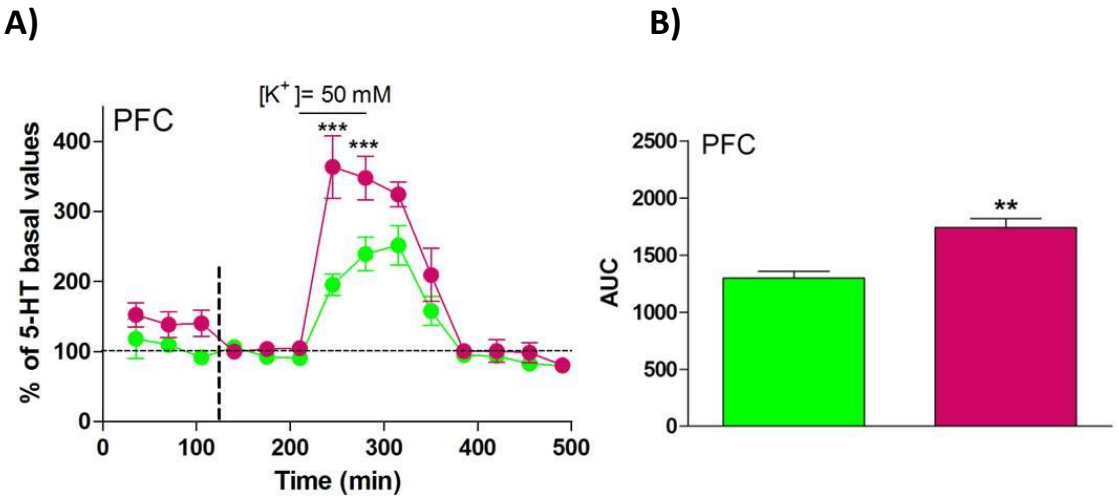

Figure 4.30: **A)** Graphic representation of the effect of the local administration in PFC of hiperK$^+$ aCSF (50 mM) during 105 min on extracellular concentrations of 5-HT in PFC of saline (•) (n=6) and Poly (I:C) (•) mice (n=5). The upper horizontal continuous line indicates the time interval of administration of hiperK$^+$ aCSF. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. ****p<0.001*, Two-way ANOVA followed by Bonferroni *post-hoc* test. **B)** Graphic representation of the AUC of the effect of the local administration of hiperK$^+$ aCSF on extracellular concentrations of 5-HT in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.**p<0.01*, Student's unpaired t-test vs. saline group.

4.1.5.1.1.4. Effect of the systemic administration of amphetamine 2.5 mg/kg i.p. on monoamine concentration in PFC

4.1.5.1.1.4.1. Effect of the systemic administration of amphetamine on NA concentrations in the PFC

Systemic administration of amphetamine (2.5 mg/kg i.p.) induced a significant increase of NA concentration in PFC of saline mice (Emax=518.10±33%; p<0.001 vs. basal conditions). The maximum effect was reached 105 min after the injection of the drug (*F[8,160]=32.49, p<0.0001, n=21)* (**Figure 4.31-A**). In the PFC of Poly (I:C) mice, the systemic administration of amphetamine also increased significantly NA concentrations (*F[8,168]=22.34, p<0.0001, n=22),* with a maximum effect of 397.41±43% (p<0.001 vs. basal conditions) reached 140 min after the injection. The two-way ANOVA for repeated measures revealed statistically significant differences between both groups (F_t*[8,328]=52.07; p<0.0001;* F_{tr}*[1,328]=4.322; p=0.044;* F_i*[8,398]=5.727; p<0.0001; n=43*).

The analysis of the AUC of the curves representing the effect of amphetamine also revealed differences between the two groups (*t=2.28, p=0.027*), showing lower values of NA response to amphetamine in the Poly (I:C) mice compared with the saline mice (**Figure 4.31-B**).

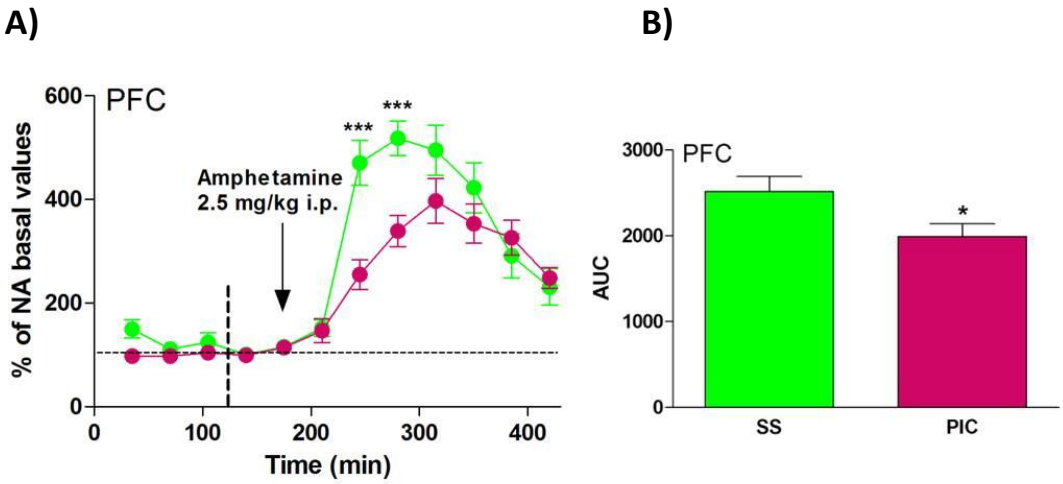

Figure 4.31: **A)** Graphic representation of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on NA extracellular concentrations in PFC of saline (•) (n=21) and Poly (I:C) (•) mice (n=22). The vertical arrow indicates the time of administration of amphetamine. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values**.** ****p<0.001*, Two-way ANOVA followed by Bonferroni *post-hoc* test. **B)** Graphic representation of the AUC of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on extracellular concentrations of NA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.**p<0.05*, Student's unpaired t-test vs. saline group.

4.1.5.1.1.4.2. Effect of the systemic administration of amphetamine on DA concentrations in the PFC

Systemic administration of amphetamine (2.5 mg/kg i.p.) provoked a significant increase of DA concentration in PFC of saline mice (Emax=190.95±16%; p<0.001 vs. basal conditions) (**Figure 4.32-A**). This maximum effect occurred 105 min after injecting the drug (*F[8,168]=15.84, p<0.0001, n=22)*. As for the Poly (I:C) mice, the systemic administration of amphetamine also increased significantly DA concentrations in the PFC (*F[8,160]=7.237, p<0.0001, n=21)*, reaching a maximum effect of 149.81±14% (p<0.001 vs. basal conditions) 105 min after the injection. The two-way ANOVA for repeated measures showed significant differences between groups (F_t*[8,328]=22.01, p<0.0001;* F_{tr}*[1,328]=4.507, p=0.040;* F_i*[8,328]=2.319, p=0.020; n=43*).

The analysis of the AUC of the curves of the effect of the drug also showed a statistically lower response of DA to amphetamine in the Poly (I:C) mice (*t=2.04, p=0.047*) compared to the saline mice (**Figure 4.32-B**).

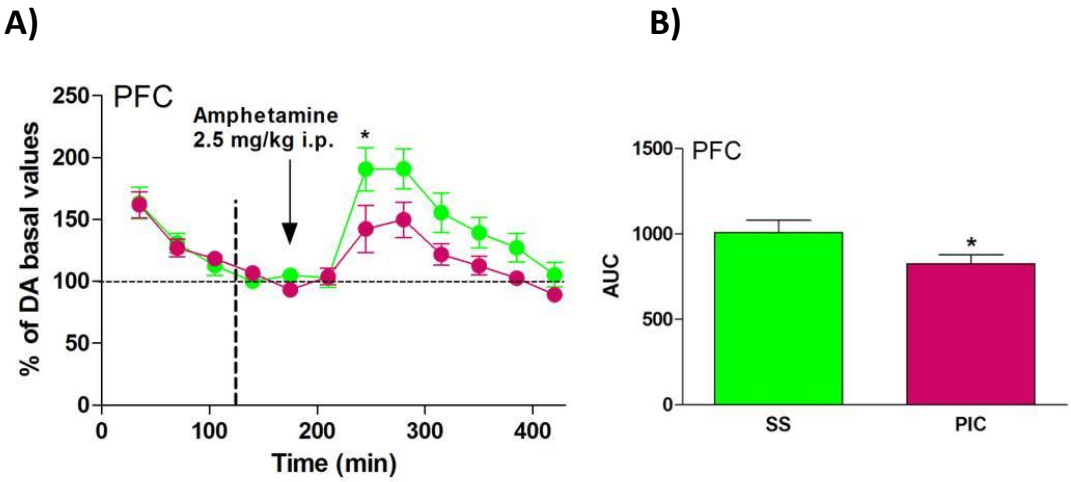

Figure 4.32: **A)** Graphic representation of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on DA extracellular concentrations in PFC of saline (•) (n=22) and Poly (I:C) (•) mice (n=21). The vertical arrow indicates the time of administration of amphetamine. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **p<0.05*, Two-way ANOVA followed by Bonferroni *post-hoc* test. **B)** Graphic representation of the AUC of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on extracellular concentrations of DA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.**p<0.05*, Student's unpaired t-test vs. saline group.

4.1.5.1.1.4.3. Effect of the systemic administration of amphetamine on 5-HT concentrations in the PFC

Finally, after systemic administration of amphetamine (2.5 mg/kg i.p.), 5-HT concentrations in the PFC increased in a significant manner in saline mice (Emax=194.40±15%; p<0.001 vs. basal conditions) obtaining the maximum 5-HT concentration 35 min after injecting the drug (*F[8,168]=14.31, p<0.0001, n=22)* (**Figure 4.33-A**). Regarding the Poly (I:C) mice, a significant increment of 5-HT concentrations was also observed in PFC (Emax=213.03±21%; p<0.001 vs. basal conditions) and in this case, the maximum effect was reached 70 min after the injection of amphetamine (*F[8,168]=6.397, p<0.0001, n=22)*. Two-way ANOVA for repeated measures also revealed significant differences between saline and Poly (I:C) mice (F_t*[8,336]=14.39; p<0.0001;* F_{tr}*[1,336]=3.612; p=0.064;* F_i*[8,336]=3.147; p=0.0019; n=44)*.

The analysis of the AUC revealed no significant differences between groups (*t=1.81, p=0.076*) (**Figure 4.33-B**).

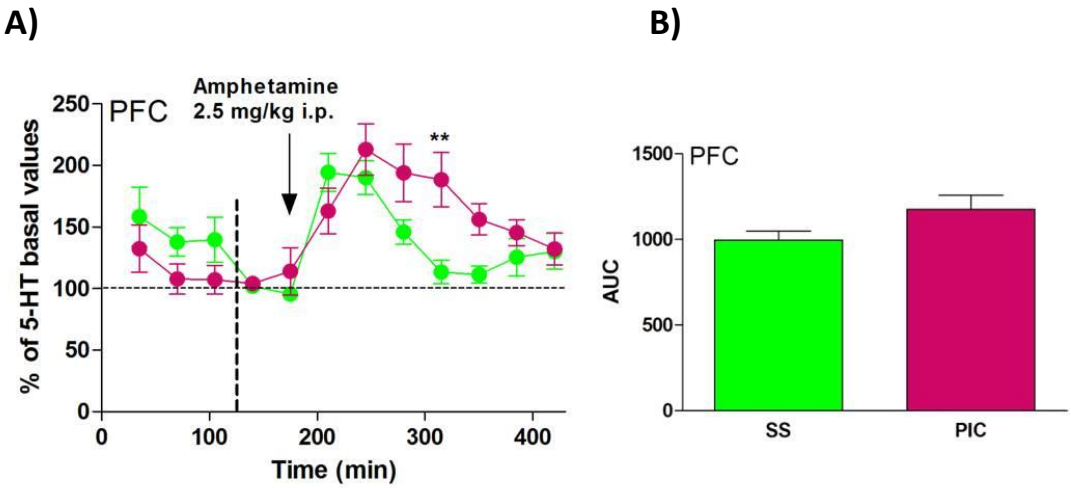

Figure 4.33: **A)** Graphic representation of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on 5-HT extracellular concentrations in PFC of saline (•) (n=22) and Poly (I:C) (•) mice (n=22). The vertical arrow indicates the time of administration of amphetamine. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. ***p<0.01*, Two-way ANOVA followed by Bonferroni *post-hoc* test. **B)** Graphic representation of the AUC of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on extracellular concentrations of 5-HT in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.1.5.1.1.5. Effect of the systemic administration of the NMDA receptor antagonist MK-801 0.5 mg/kg i.p. on monoamine concentrations in PFC

4.1.5.1.1.5.1. Effect of the systemic administration of MK-801 on NA concentrations in PFC

The systemic administration of the NMDA receptor antagonist MK-801 (0.5 mg/kg i.p.) produced a significant increment of NA concentration in the PFC of saline mice (Emax=193.53±10%; p<0.001 vs. basal conditions) (**Figure 4.34-A**). The highest effect on NA concentrations was reached 35 min after the injection *(F[12,48]=7.758, p<0.0001, n=5)*. As for the Poly (I:C) mice, the NMDA receptor antagonist was also capable of increasing NA concentration in the PFC significantly, producing a maximum effect of 181.01±13% (p<0.05 vs. basal conditions) 70 min after the administration of the drug (*F[12,48]=1.133, p<0.0001, n=5).* Two-way ANOVA for repeated measures did not reveal significant differences between groups *(F_t[12,96]=12.21; p<0.0001; F_{tr}[1,96]=2.196; p=0.1767; F_i[12,96]=1.485; p=0.1431; n=10*).

Also, the analysis of AUC of the curves of the effect of MK-801 did not show statistical differences between groups (*t=1.007, p=0.343*) (**Figure 4.34-B**).

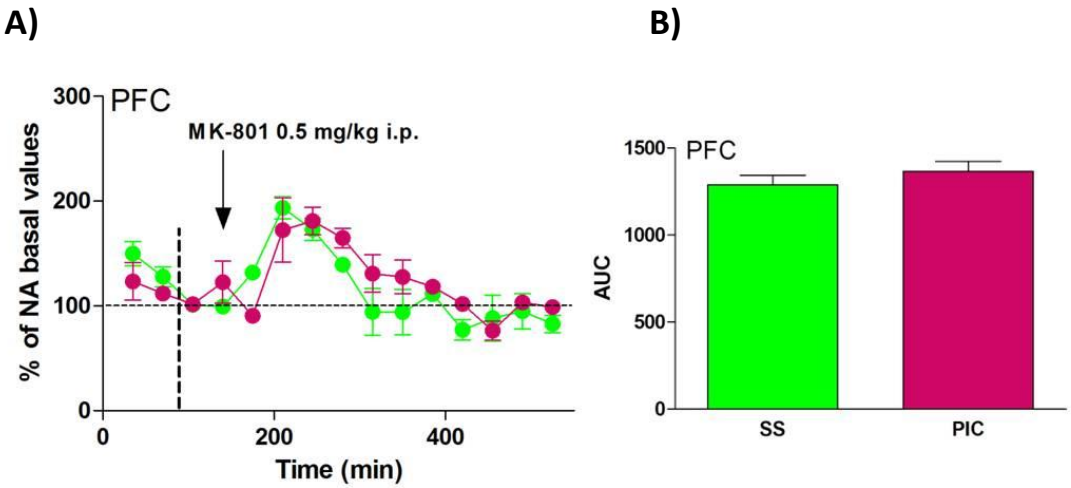

Figure 4.34: **A)** Graphic representation of the effect of systemic administration of the NMDA receptor antagonist MK-801 0.5 mg/kg i.p. on NA extracellular concentration in PFC of saline (•) (n=5) and Poly (I:C) (•) mice (n=5). The vertical arrow indicates the time of administration of MK-801. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **B)** Graphic representation of the AUC of the effect of systemic administration of MK-801 0.5 mg/kg i.p. on extracellular concentration of NA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.1.5.1.1.5.2. Effect of the systemic administration of MK-801 on DA concentrations in PFC

Systemic administration of the NMDA receptor antagonist (MK-801 0.5 mg/kg i.p.) produced a significant increase in DA concentrations in the PFC of saline mice (Emax=242.51±34%; p<0.001 vs. basal conditions) (**Figure 4.35-A**), reaching the maximum effect 105 min after the injection (*F[12,60]=11.05, p<0.0001, n=6)*. Regarding the Poly (I:C) mice, DA concentration was also increased significantly by MK-801, with a maximum effect of 221.09±20% (p<0.01 vs. basal conditions) (*F[12,48]=5.881, p<0.0001, n=5*) obtained 105 min after the injection of the drug. Two-way ANOVA for repeated measures showed no significant differences between groups (F_t*[12,108]=14.81; p<0.0001;* F_{tr}*[1,108]=0.498; p=0.498;* F_i*[12,108]=1.368; p=0.192; n=11)*.

The statistical analysis of the AUC of the curves did not reveal differences between the two groups (*t=0.80, p=0.443*) (**Figure 4.35-B**).

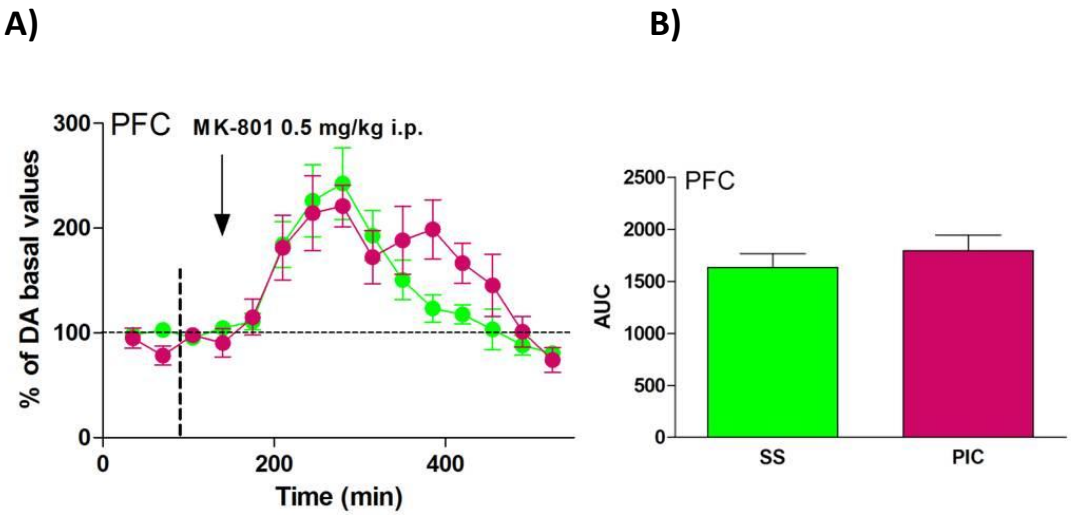

Figure 4.35: **A)** Graphic representation of the effect of systemic administration of the NMDA receptor antagonist MK-801 0.5 mg/kg i.p. on DA extracellular concentration in PFC of saline (•) (n=6) and Poly (I:C) (•) mice (n=5). The vertical arrow indicates the time of administration of MK-801. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **B)** Graphic representation of the AUC of the effect of systemic administration of MK-801 0.5 mg/kg i.p. on extracellular concentration of DA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.1.5.1.1.5.3. Effect of the systemic administration of MK-801 on 5-HT concentrations in PFC

5-HT concentration in PFC of saline mice rose significantly after the injection of the NMDA receptor antagonist (MK-801 0.5 mg/kg i.p.) (Emax=188.15±20%; p<0.001 vs. basal conditions) (**Figure 4.36-A**), reaching the maximum effect 140 min after the injection of the drug (*F[12,60]=1.955; p<0.0001, n=6*). Regarding Poly (I:C) mice, the systemic administration of the drug also increased 5-HT concentrations in the PFC significantly, with a maximum effect of 194.08±32% (p<0.05 vs. basal conditions) reached 70 min after the injection (*F[12,48]=4.103; p=0.0017, n=5).* Two-way ANOVA for repeated measures did not reveal significant differences between the two groups (F_t*[12,108]=8.835; p<0.0001;* F_{tr}*[1,108]=0.765, p=0.404;* F_i*[12,108]=1.785, p=0.060; n=11*).

The analysis of the AUC of the curves showed no differences between the groups (*t=0.95, p=0.368*) (**Figure 4.36-B**).

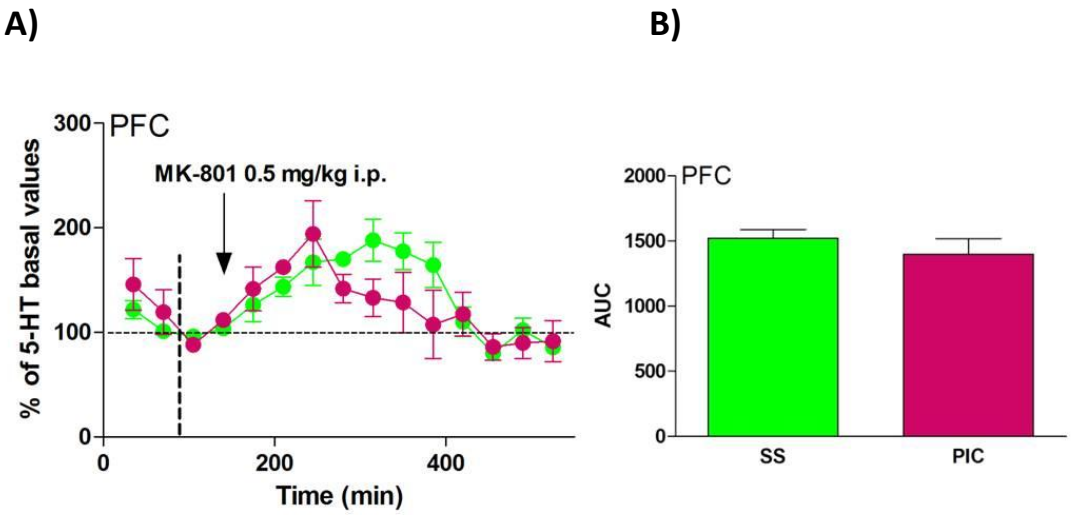

Figure 4.36: **A)** Graphic representation of the effect of systemic administration of the NMDA receptor antagonist MK-801 0.5 mg/kg i.p. on 5-HT extracellular concentration in PFC of saline (●) (n=6) and Poly (I:C) (●) mice (n=5). The vertical arrow indicates the time of administration of MK-801. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **B)** Graphic representation of the AUC of the effect of systemic administration of MK-801 0.5 mg/kg i.p. on extracellular concentration of 5-HT in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.1.5.1.2. Extracellular monoamine concentrations in the striatum

4.1.5.1.2.1. DA basal concentrations and *Ed* by the non-net flow microdialysis technique

In the case the striatum, there was a statistically significant decrease in the DA basal concentrations presented by the Poly (I:C) mice (7.60±2.60 nM) compared with the saline mice (16.0±2.89 nM) (*t=2.17, p=0.0468*) (**Figure 4.37** and **Figure 4.38-A**).

The *Ed* showed significantly lower values for the saline mice (0.25±0.016) compared with the Poly (I:C) mice (0.46±0.077) (*t=2.40, p=0.031*) (**Figure 4.37** and **Figure 4.38-B**).

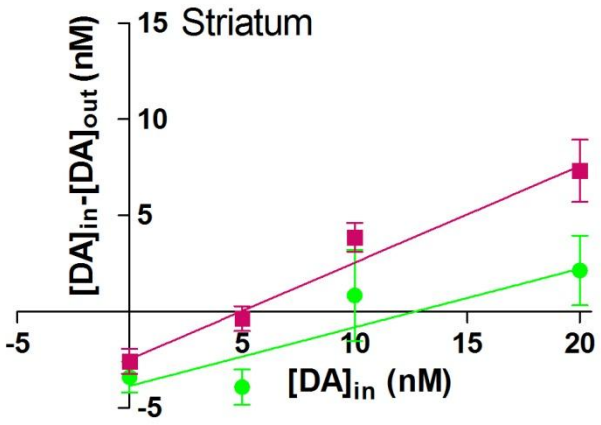

Figure 4.37: Graphic representation of the study of non-net flow cerebral microdialysis technique of DA in the striatum of saline (n=9) (●) and Poly (I:C) (n=10) (■) mice.

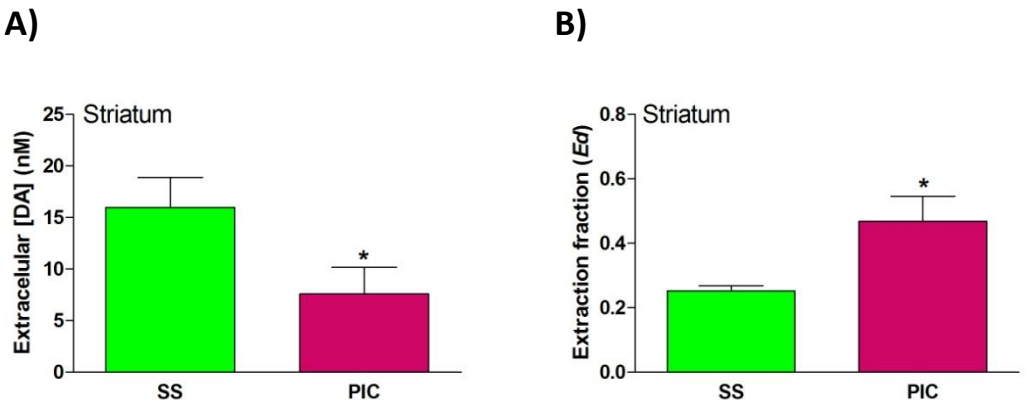

Figure 4.38: **A)** Graphic representation of extracellular DA concentrations in the striatum of saline (n=8) and Poly (I:C) mice (n=9) as indicated by the point of non-net flow microdialysis. **B)** Graphic representation of the *Ed in vivo* in the striatum of saline (n=7) and Poly (I:C) mice (n=9). Bars are a representation of the mean ± SEM values. **p<0.05*, Student's unpaired t-test vs. saline group.

4.1.5.1.2.2. Effect of the systemic administration of amphetamine 2.5 mg/kg i.p. on monoamine concentrations in the striatum

4.1.5.1.2.2.1. Effect of the systemic administration of amphetamine on NA concentrations in the striatum

Extracellular basal concentrations of NA in the striatum of the saline mice were 0.07±0.01 nM (n=13) and 0.10±0.01 nM for the Poly (I:C) mice (n=11). There were no statistically significant differences between groups (*t=1.34, p=0.1931*).

Systemic administration of amphetamine (2.5 mg/kg i.p.) induced a significant increase in NA concentration in the striatum of saline mice (Emax=253.52±37%; p<0.001 v.s basal conditions). The maximum effect was reached 70 min after the injection of the drug (*F[8,48]=6.082, p<0.0001, n=7)* (**Figure 4.39-A**). In the striatum of Poly (I:C) mice, the systemic administration of amphetamine also increased significantly NA concentrations (*F[8,40]=19.50, p<0.0001, n=6),* with a maximum effect of 323.42±29% (p<0.001 vs. basal conditions) reached 70 min after the injection. The two-way ANOVA for repeated measures revealed statistically significant differences between both groups *(F_t[8,88]=18.25, p<0.0001; F_{tr}[1,88]=9.884, p=0.009; F_i[8,88]=3.201, p=0.003; n=13)*.

The analysis of the AUC of each one of the curves representing the effect of amphetamine showed a statistically significant increase in NA concentrations in the Poly (I:C) mice (*t=2.87, p= 0.015*) compared with the saline mice (**Figure 4.39-B**).

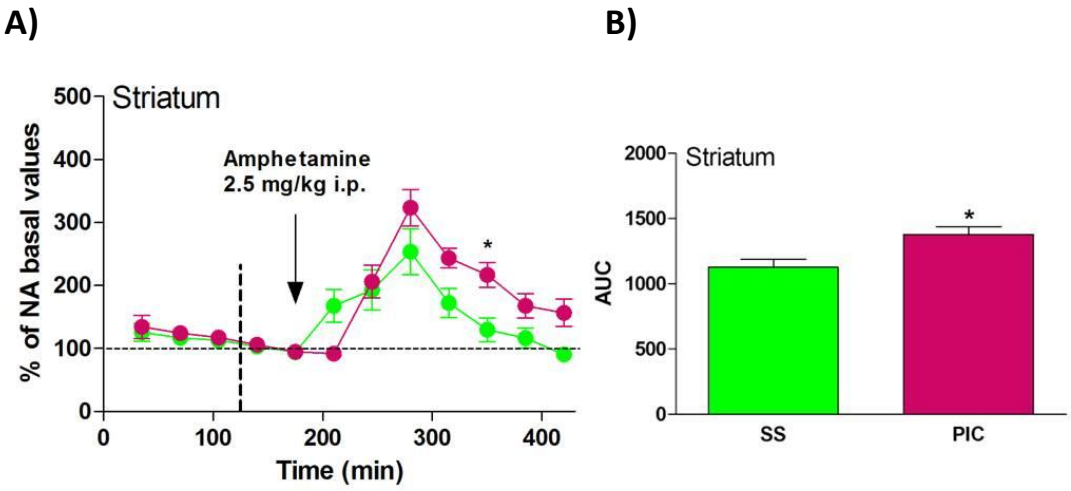

Figure 4.39: **A)** Graphic representation of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on NA extracellular concentration in the striatum of saline (•) (n=7) and Poly (I:C) (•) mice (n=6). The vertical arrow indicates the time of administration of amphetamine. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **p<0.05*, Two-way ANOVA followed by Bonferroni *post-hoc* test. **B)** Graphic representation of the AUC of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on extracellular concentration of NA in the striatum of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.**p<0.05*, Student's unpaired t-test vs. saline group.

4.1.5.1.2.2.2. Effect of the systemic administration of amphetamine on DA concentrations in the striatum

In the case of DA in the striatum, saline mice presented extracellular DA concentrations of 5.36±0.65 nM (n=20) and the Poly (I:C) mice 4.73±0.78 nM (n=18), respectively. There were no statistical differences between the two groups (*t=0.62, p=0.5384*).

Systemic administration of amphetamine (2.5 mg/kg i.p.) increased significantly DA concentrations in the striatum of saline mice (Emax=401.94±48% p<0.001 vs. basal conditions). The maximum effect occurred 70 min after injecting the drug (*F[8,72]=27.28, p<0.0001, n=10)* (**Figure 4.40-A**). In the case of Poly (I:C) mice, systemic administration of amphetamine also increased significantly DA concentration in the striatum (*F[8,64]=42.78, p<0.0001, n=9)* with a maximum effect of 412.10±34% (p<0.001 vs. basal conditions) reached 105 min after the injection. The two-way ANOVA showed significant differences between groups (F_t*[8,136]=59.73; p<0.0001;* F_{tr}*[1,136]=1.403; p=0.253;* F_i*[8,136]=3.740; p=0.0006; n=19*).

The analysis of the AUC of the curves did not reveal differences between groups (*t=1.09, p=0.287*) (**Figure 4.40-B**).

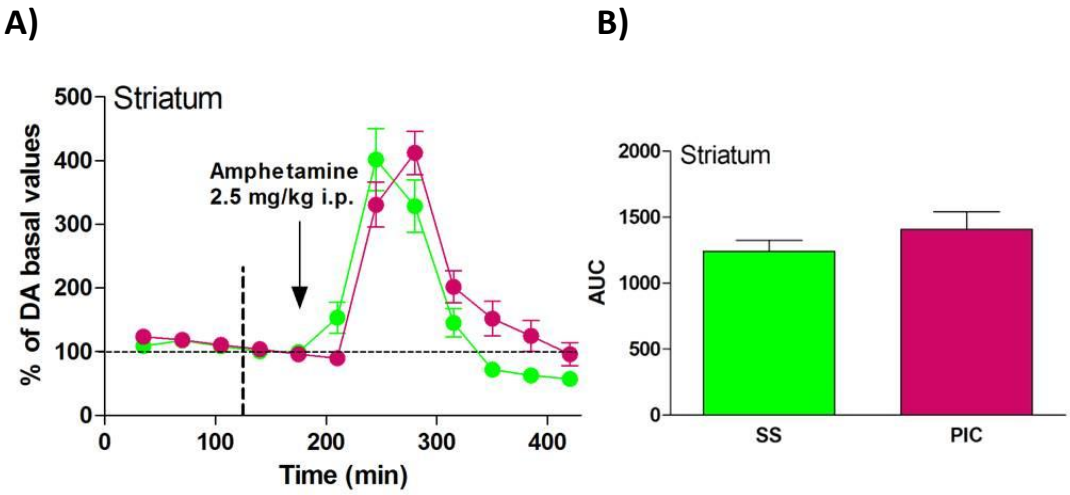

Figure 4.40: **A)** Graphic representation of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on DA extracellular concentration in the striatum of saline (•) mice (n=10) and Poly (I:C) (•) mice (n=9). The vertical arrow indicates the time of administration of amphetamine. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **B)** Graphic representation of the AUC of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on extracellular concentration of DA in the striatum of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.1.5.1.2.2.3. Effect of the systemic administration of amphetamine on 5-HT concentrations in the striatum

Regarding 5-HT, the basal values in the striatum for the saline mice were 0.26±0.03 nM (n=20) and 0.22±0.03 nM for the Poly (I:C) mice (n=18). No statistical differences were found between groups (t=*0.91, p=0.367*).

Amphetamine (2.5 mg/kg i.p.) administered systemically increased 5-HT in the striatum of the saline mice in a significant manner (Emax=407.75±47% p<0.001 vs. basal conditions), with the maximum effect obtained 70 min after the injection (*F[8,72]=24.65; p<0.0001, n=10*) (**Figure 4.41-A**). For the Poly (I:C) mice, amphetamine administered systemically (2.5 mg/kg i.p.) also increased significantly 5-HT in the striatum, with a maximum effect of 318.88±33% (p<0.001 vs. basal conditions), reached 70 min after the injection (*F[8,64]=13.04, p<0.0001, n=9*). The two-way ANOVA did not reveal significant differences between groups (F_t*[8,136]=35.94; p<0.0001;* F_{tr}*[1,136]=0.027; p=0.871;* F_i*[8,136]=1.341; p=0.228; n=19*).

The analysis of the AUC of the curves reflecting the effect of amphetamine did not show differences between groups (*t=0.19, p=0.846*) (**Figure 4.41-B**).

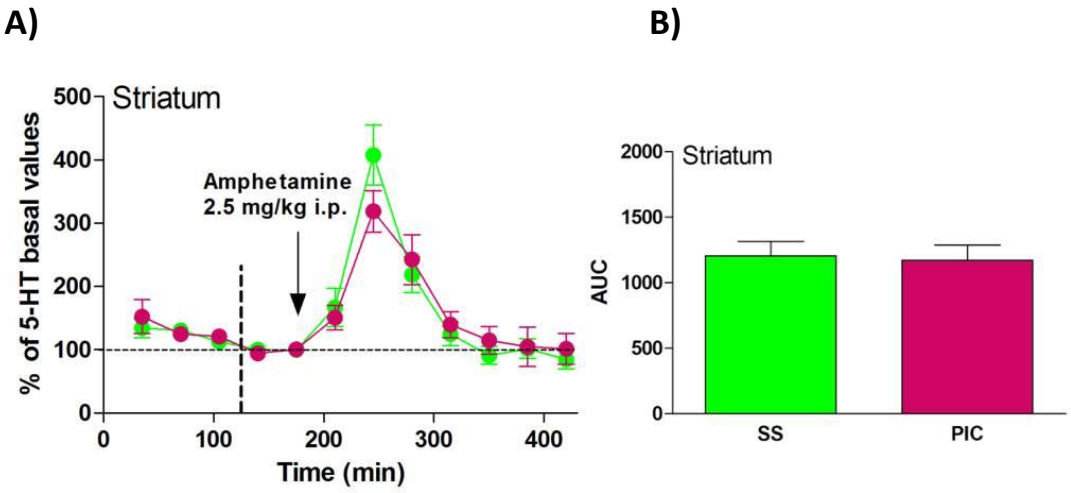

Figure 4.41: **A)** Graphic representation of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on 5-HT extracellular concentration in the striatum of saline (•) mice (n=10) and Poly (I:C) (•) mice (n=9). The vertical arrow indicates the time of administration of amphetamine. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **B)** Graphic representation of the AUC of the effect of systemic administration of amphetamine 2.5 mg/kg i.p. on extracellular concentration of 5-HT in the striatum of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.1.6. Cell-type selective targeting of the LC-PFC projection in mice

With the objective of targeting the noradrenergic neurons originating from the LC that project to the PFC in mice, it was decided to try three different approaches. Then, it was selected the one considered worked best for further behavioral experiments in these mice.

The first approach consisted of an injection of a retrograde virus expressing Cre recombinase and a cytoplasmic red fluorescent protein, mCherry, (AAVrg-Ef1α-mCherry-IRES-Cre) into the PFC and a Cre-dependent reporter (AAV5-DIO-Ef1α-eYFP) in the LC (**Figure 4.42-A**).

The retrograde virus injected in the PFC (mCherry-IRES-Cre) was expected to be able to travel backward to LC cell bodies (through the LC fibers that project to PFC). A Cre-dependent reporter was also injected in the LC (eYFP), which would only be able to express in the cells if there is Cre.

From the images below it can be observed that there is an expression of the retrograde virus locally in the PFC (**Figure 4.42-C**) but there is no expression in the LC (**Figure 4.42-B**), suggesting that the retrograde virus was not able to travel and infect the cells in the LC as expected.

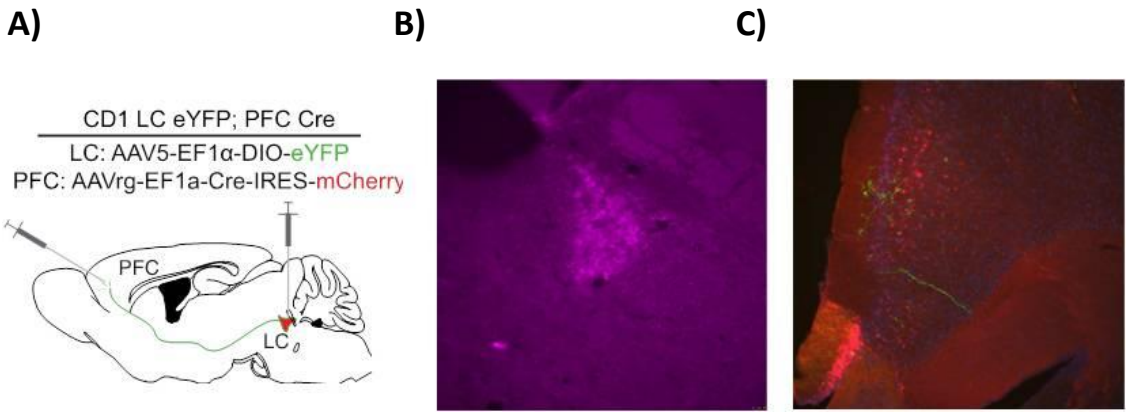

Figure 4.42: **A)** Cartoon depicting the first strategy to target the LC-PFC circuitry: AAVrg-Ef1α-mCherry-IRES-Cre (AAV Retrograde) injection in the PFC and AAV5-DIO-Ef1α-eYFP (*cre*-dependent reporter) injection in the LC. **B)** Representative image (selected from 10 injected mice) shows no viral expression in the LC (absence of green fibers). **C)** Representative image (selected from 5 injected mice) shows a local expression of the retrograde virus in PFC (red).

As the first attempt was unsuccessful, for the second approach, an anterograde virus expressing Cre under a fragment of the promoter for TH, the rate-limiting enzyme necessary for catecholamine synthesis (AAV9.rTH.PI.Cre.SV40) was injected in the LC, in combination with the Cre-dependent reporter (AAV5-DIO-Ef1α-eYFP) (**Figure 4.43-A**). In this approach, the anterograde virus was expected to travel forward to the noradrenergic terminals in the PFC, targeting the circuit of interest. **Figure 4.43-B** shows there is a viral expression in the LC, but this expression is not restricted to this area; it is also present in the areas all over around the LC. That could mean that the expression observed in the PFC (**Figure 4.43-C**) may be originated from areas that are not the LC specifically. Thus, this approach was also not selective for the LC-PFC circuit.

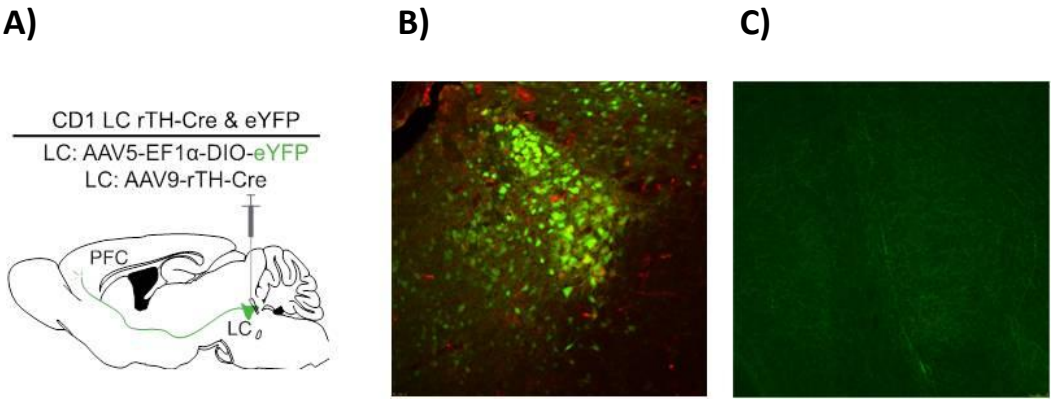

Figure 4.43: **A)** Cartoon representing the second strategy to target the LC-PFC circuitry: AAV9.rTH.PI.Cre.SV40 in combination with the AAV5-DIO-Ef1α-eYFP (Cre-dependent reporter) injection in the LC. **B)** Representative image (selected from 4 injected mice) shows viral expression in the LC (red) but also in the surrounding areas (green). **C)** Representative image (selected from 4 injected mice) shows expression in the PFC that may not be exclusively from the LC neurons (green).

Finally, another approach that was similar to the first one was attempted. It consisted of injecting a different Cre-retrograde virus (AAVrg-PGK-Cre) in the PFC and the Cre-dependent reporter (AAV5-DIO-Ef1α-eYFP) in the LC (**Figure 4.44-A**). As with the first approach, the Cre virus was expected to travel retrograde to the LC. There, in the presence of the Cre-dependent reporter injected locally in LC, it should enable expression only in the noradrenergic cells projecting to the PFC. **Figure 4.44-B** shows viral expression specifically in the LC neurons only and **Figure 4.44-C** depicts the PFC with the projections coming exclusively from the LC.

Finally, this was the approach that was chosen since it best enabled cell-type selectivity of this projection in CD1 mice.

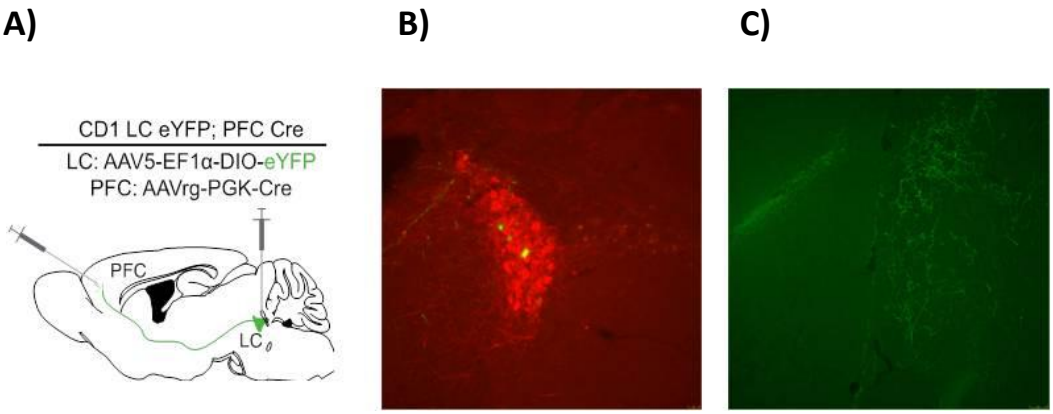

Figure 4.44: **A)** Cartoon representing the third (and selected) strategy to target the LC-PFC circuitry: AAVrg-PGK-Cre (AAV Retrograde) injection in the PFC and AAV5-DIO-Ef1α-eYFP (*cre*-dependent reporter) injection in the LC. **B)** Representative image (selected from 10 injected mice) shows viral expression specifically in the noradrenergic neurons of the LC (green and red cells). **C)** Representative image (selected from 8 injected mice) shows expression in the PFC from the projections originated in the LC (green).

4.2. EFFECT OF α_2-ADRENOCEPTOR COMPOUNDS IN THE COGNITIVE STATUS OF AN ANIMAL MODEL OF MIA BY THE ADMINISTRATION OF POLY (I:C)

4.2.1. Effect of α_2-adrenoceptor compounds on the performance of saline and Poly (I:C) mice in the NORT

4.2.1.1. Effect of the systemic administration of different doses of the α_{2A}-adrenoceptor agonist guanfacine on the performance of saline mice

Before conducting the definitive behavioral experiments, we decided to do a dose-response pilot study to find out the most appropriate dose of guanfacine to exert an effect while avoiding one of its most common side effects, sedation. Different doses of guanfacine were administered to saline (control) mice and analyzed the total exploration time of the objects during the NORT. As shown in **Figure 4.45**, compared to vehicle administration, a dose of 0.3 mg/kg i.p. of guanfacine produced a significant decrease in their exploration time. However, a dose of 0.1 mg/kg i.p. of guanfacine did not reduce their exploration time compared to vehicle administration. The statistical analysis by the one-way ANOVA revealed statistical differences between the doses used *(F[2,21]= 9.150, p=0.0014, n=24)*. Besides, Dunnet *post-hoc* test showed statistically significant differences between the administration of vehicle and the dose of 0.3 mg/kg i.p. of guanfacine *(p<0.01)*.

Therefore, a dose of 0.1 mg/kg i.p. of guanfacine was chosen for definitive behavioral experiments.

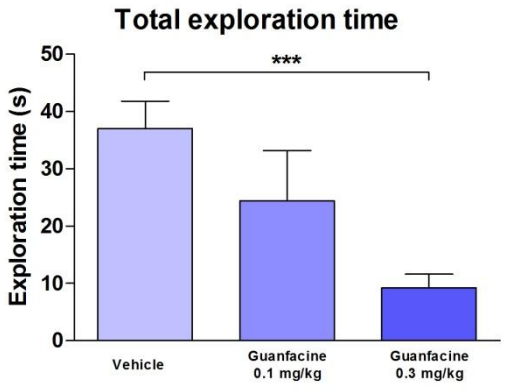

Figure 4.45: Representation of the total exploration time (s) of the guanfacine dose-response pilot study where control mice received vehicle (n=12), guanfacine 0.1 mg/kg i.p. (n=4) and guanfacine 0.3 mg/kg i.p. (n=8) during the NORT. Bars are a representation of the mean ± SEM values. ****p<0.001*, One-way ANOVA followed by Dunnet *post-hoc* test vs. vehicle.

4.2.1.2. Effect of the systemic administration of the α_{2A}-adrenoceptor agonist guanfacine on the performance of saline and Poly (I:C) mice

When evaluating the time saline and Poly (I:C) mice spent exploring the familiar object, the statistical analysis by the two-way ANOVA showed significant differences between saline and Poly (I:C) groups in the response to guanfacine ($F_{G/V}[1,29]=1.584$, $p=0.218$; $F_{PIC/SS}[1,29]=3.081$, $p=0.089$; $F_i[1,29]=4.796$, $p=0.036$). This analysis followed by Bonferroni *post-hoc* test showed: first, there were differences between Poly (I:C) mice that received vehicle *vs.* guanfacine ($t=2.55$, $p<0.05$), suggesting that guanfacine reduced the time they spent exploring the familiar object; and second, between saline *vs.* Poly (I:C) mice when receiving guanfacine ($t=2.67$, $p<0.05$), suggesting that guanfacine reduced the time Poly (I:C) mice, but not the saline mice, spent exploring the object (**Figure 4.46-A**). With regard to the time spent exploring the novel object, the two-way ANOVA did not reveal statistical differences between groups in the response to guanfacine ($F_{G/V}[1,29]=1.994$, $p=0.168$; $F_i[1,29]=0.003$, $p=0.956$). Nevertheless, the exploration time of the novel object was significantly lower in the two groups of Poly (I:C) mice compared with the saline mice ($F_{PIC/SS}[1,29]=7.58$, $p=0.010$) (**Figure 4.46-B**).

As to the total exploration time devoted to both novel and familiar objects, the two-way ANOVA did not show statistical differences between the two groups of mice in the response to guanfacine ($F_{G/V}[1,29]=2.112$, $p=0.157$; $F_i[1,29]=1.033$, $p=0.3179$). However, it did reveal a different behavior between saline and Poly (IC) mice, with a trend to reduce the total exploration time of the Poly (I:C) mice ($F_{PIC/SS}[1,29]=6.353$, $p=0.017$). Moreover, this analysis followed by Bonferroni *post-hoc* test showed a statistical reduction in the total exploration time devoted by the Poly (I:C) mice when receiving guanfacine, compared to saline mice ($t=2.39$, $p<0.05$) (**Figure 4.46-C**).

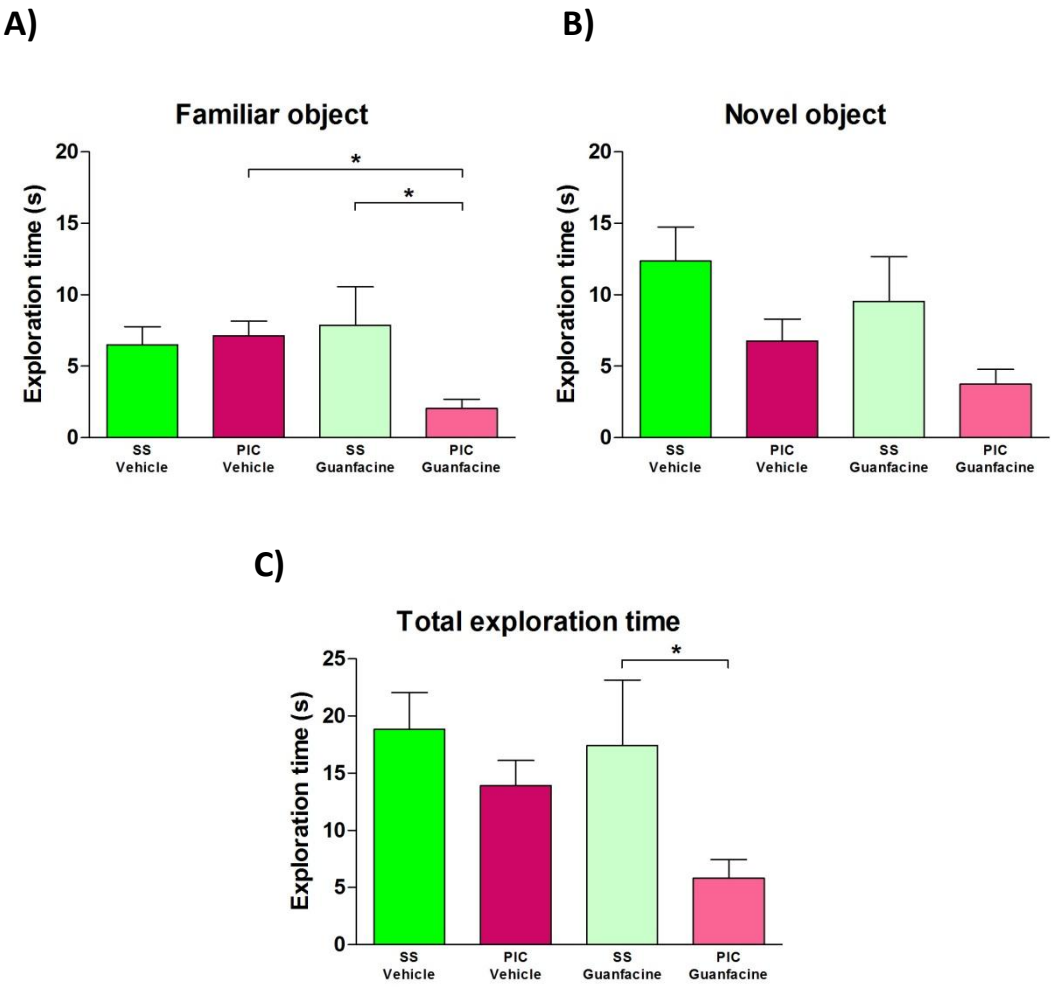

Figure 4.46: **A)** Representation of the exploration time devoted to the familiar object; **B)** Representation of the exploration time devoted to the novel object and **C)** Representation of the total exploration time of both familiar and novel objects, of the saline mice treated with vehicle (n=8), Poly (I:C) mice treated with vehicle (n=10), saline mice treated with guanfacine 0.1 mg/kg i.p. (n=7) and Poly (I:C) mice treated with guanfacine 0.1 mg/kg i.p. (n=8) during the NORT. Bars are a representation of the mean ± SEM values. **p<0.05*, Two-way ANOVA followed by Bonferroni *post-hoc* test.

As previously shown in the evaluation of the basal cognitive status of saline and Poly (I:C) mice by the NORT (see *section 4.1.3.*), in this group of animals, Poly (I:C) mice injected with vehicle also showed a lower discrimination index compared with the correspondent saline mice injected with vehicle; therefore, demonstrating cognitive impairment (**Figure 4.47**). However, when Poly (I:C) mice were injected systemically with the α_{2A}AR agonist guanfacine (0.1 mg/kg i.p.), the lower discrimination index value of these mice was reversed. The two-way ANOVA showed significant differences between saline and Poly (I:C) mice in the response to guanfacine ($F_{G/V}[1,29]=0.887$, $p=0.353$; $F_{PIC/SS}[1,29]=0.951$, $p=0.337$; $F_i[1,29]=10.61$, $p=0.003$). Specifically, this analysis followed by Bonferroni *post-hoc* test revealed statistical differences in two cases: first, between saline *vs.* Poly (I:C) mice when they received vehicle ($t=3.13$, $p<0.01$), thus corroborating again the data shown previously (see *section 4.1.3.*); and also when Poly (I:C) mice received guanfacine *vs*. vehicle ($t=3.11$, $p<0.01$), demonstrating that guanfacine was able to reverse the cognitive deficits shown by Poly (I:C) mice. In addition, Bonferroni *post-hoc* test showed no differences between the saline mice that received vehicle *vs.* guanfacine

(*t=1.57, p>0.05*), confirming that guanfacine did not have a significant effect on the performance of saline mice in the NORT (**Figure 4.47**).

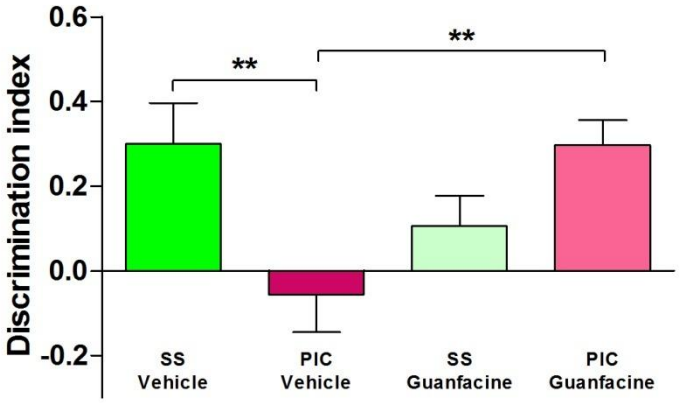

Figure 4.47: Representation of the discrimination index as the parameter for evaluation of the cognitive status of saline mice administered with vehicle (n=8), Poly (I:C) mice administered with vehicle (n=10), saline mice administered with guanfacine 0.1 mg/kg i.p. (n=7) and Poly (I:C) mice administered with guanfacine 0.1 mg/kg i.p. (n=8). Bars are a representation of the mean ± SEM values. ***p<0.01*, Two-way ANOVA followed by Bonferroni *post*-hoc test.

4.2.1.3. Effect of the systemic administration of the α_{2C}-adrenoceptor antagonist MK-912 on the performance of saline and Poly (I:C) mice

When we evaluated the time the two groups of mice spent exploring the familiar object, the statistical analysis by the two-way ANOVA did not reveal differences between groups ($F_{MK/V}$*[1,48]=0.063, p=0.802;* $F_{PIC/SS}$*[1,48]=0.325, p=0.571;* F_i*[1,48]=0.045, p=0.832)* (**Figure 4.48-A**). With regard to the time spent exploring the novel object, the two-way ANOVA showed statistical differences between saline and Poly (I:C) mice ($F_{MK/V}$*[1,48]=0.667, p=0.418;* $F_{PIC/SS}$*[1,48]=2.61, p=0.112;* F_i*[1,48]=5.099, p=0.028)*. Bonferroni *post-hoc* test revealed a statistically significant reduction of the exploration time of the novel object by the Poly (I:C) mice when receiving vehicle (t=*2.847, p<0.05*) compared to saline mice; supporting once again, the minor exploration time of the novel object by the Poly (I:C) mice (**Figure 4.48-B**).

Regarding the total exploration time devoted to both novel and familiar objects, the two-way ANOVA did not show differences between groups ($F_{MK/V}$*[1,49]=0.198, p=0.659;* $F_{PIC/SS}$*[1,49]=1.801, p=0.186;* F_i*[1,49]=2.611, p=0.112*) (**Figure 4.48-C**). In this case, the trend to a reduction of the total exploration time of the Poly (I:C) mice when receiving MK-912 was not observed, contrary to what happened when receiving guanfacine.

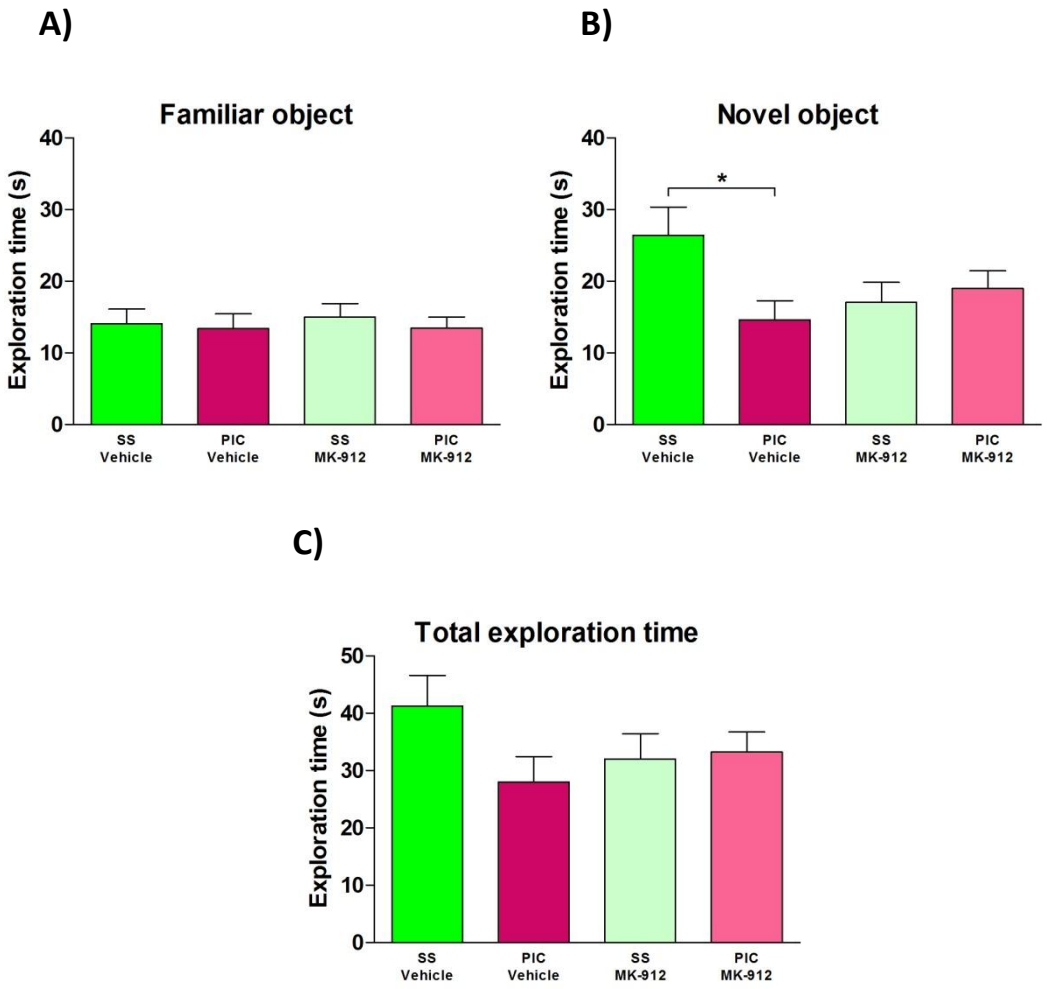

Figure 4.48: **A)** Representation of the exploration time devoted to the familiar object; **B)** Representation of the exploration time devoted to the novel object and **C)** Representation of the total exploration time of both familiar and novel objects, of the saline mice treated with vehicle (n=13), Poly (I:C) mice treated with vehicle (n=15), saline mice treated with MK-912 0.05 mg/kg i.p. (n=12) and Poly (I:C) mice treated with MK-912 0.05 mg/kg i.p. (n=12) during the NORT. Bars are a representation of the mean ± SEM values. *$p<0.05$, Two-way ANOVA followed by Bonferroni *post-hoc* test.

In the same way that it has been previously demonstrated, the Poly (I:C) mice showed a lower discrimination index than the saline animals when receiving vehicle (**Figure 4.49**). The statistical analysis by the two-way ANOVA showed significant differences between the groups in the response to MK-912 ($F_{MK/V}[1,48]=0.469$, $p=0.496$; $F_{PIC/SS}[1,48]=1.690$, $p=0.199$; $F_i[1,48]=12.15$, $p=0.001$), and Bonferroni *post-hoc* test revealed statistical differences: first, between saline *vs.* Poly (I:C) mice when receiving vehicle ($t=3.51$, $p<0.01$), once more confirming the cognitive deficits of these mice; and secondly, differences between saline mice when receiving vehicle *vs*. MK-912 ($t=2.90$, $p<0.05$), suggesting a potential negative effect of this drug on the performance of saline mice. Interestingly, as happened with guanfacine, systemic administration of MK-912 (0.05 mg/kg i.p.) did reverse this lower value of discrimination index of Poly (I:C) mice, very close to reaching statistical significance ($t=2.01$, $p=0.055$) (**Figure 4.49**).

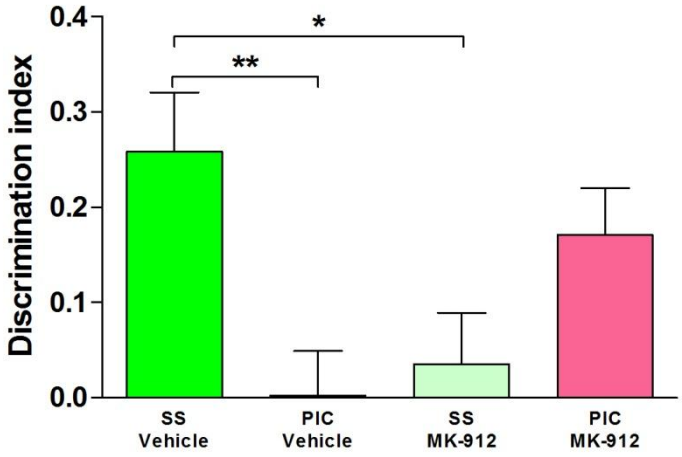

Figure 4.49: Representation of the discrimination index as the parameter for evaluation of the cognitive status of the saline mice treated with vehicle (n=13), Poly (I:C) mice treated with vehicle (n=15), saline mice treated with MK-912 0.05 mg/kg i.p. (n=12) and the Poly (I:C) mice treated with MK-912 0.05 mg/kg i.p. (n=12) during the NORT. Bars are a representation of the mean ± SEM values. *$p<0.05$, **$p<0.01$, Two-way ANOVA followed by Bonferroni *post-hoc* test.

4.2.2. Effect of α_2-adrenoceptor compounds on monoamine concentrations in the PFC of saline and Poly (I:C) mice

4.2.2.1. Effect of the systemic administration of the α_{2A}-adrenoceptor agonist guanfacine

4.2.2.1.1. Effect of the systemic administration of guanfacine on NA concentrations in PFC

Systemic administration of guanfacine (0.1 mg/kg i.p.) provoked a transient increase in NA concentrations in the PFC of saline mice (Emax=136.72±11%, p<0.01 *vs*. basal conditions). The maximum effect occurred 35 min after the injection of the drug (*F[8,80]=2.854, p=0.0077, n=11)* (**Figure 4.50-A**). Regarding Poly (I:C) mice, systemic administration of guanfacine (0.1 mg/kg i.p.) slightly elevated NA concentrations in PFC with a maximum effect of 115.26±6% (*p=ns vs*. basal conditions) reached also 35 min after the injection. However, this increase was not significant in this case (*F[8,72]=1.340, p=0.238, n=10)*. Two-way ANOVA for repeated measures did not show significant differences between saline and Poly (I:C) mice (F_t*[8,152]=3.474, p=0.001;* F_{tr}*[1,152]=0.01421, p=0.906;* F_i*[8,152]=1.195, p=0.305; n=21)*.

Besides, the analysis of the AUC of each one of the curves that represent the effect of the drug determined that there were no statistical differences between both groups of mice (*t=0.02, p=0.983*) (**Figure 4.50-B**).

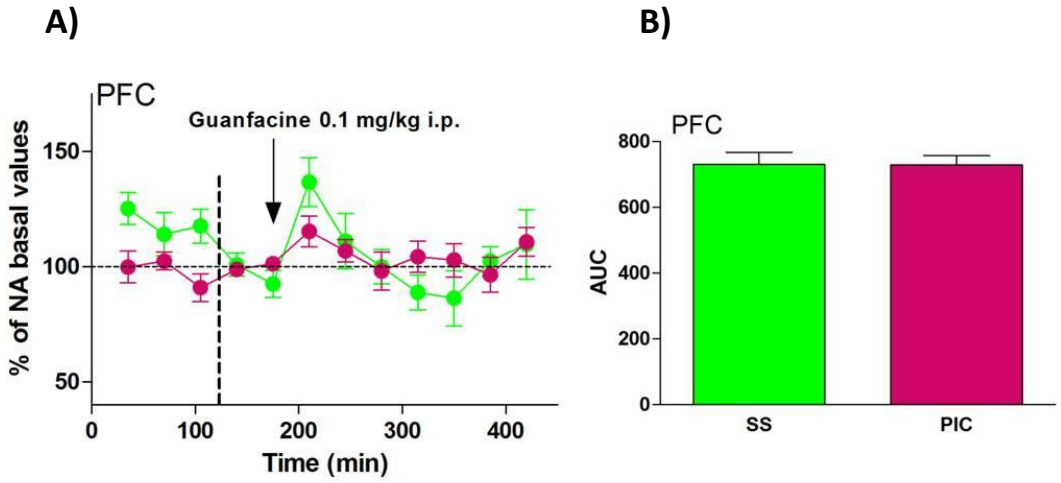

Figure 4.50: **A)** Graphic representation of the effect of systemic administration of guanfacine (0.1 mg/kg i.p.) on NA extracellular concentration in PFC of saline (•) (n=11) and Poly (I:C) (•) mice (n=10). The vertical arrow indicates the time of administration of guanfacine. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **B)** Graphic representation of the AUC of the effect of systemic administration of guanfacine (0.1 mg/kg i.p.) on extracellular concentration of NA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.2.2.1.2. Effect of the systemic administration of guanfacine on DA concentrations in PFC

Systemic administration of guanfacine (0.1 mg/kg i.p.) increased DA concentrations momentaneously in the PFC of saline mice (Emax=126.83±12%, *p=ns* vs. basal conditions), reaching the maximum effect 35 min after the injection (*F[8,80]=6.892, p=0.0031, n=11)* and showing a trend to decrease as the experiment progressed (**Figure 4.51-A**). In the case of Poly (I:C) mice, systemic guanfacine did not increase DA concentrations significantly (Emax=100.14±10%, *p=ns* vs. basal conditions); in fact, it showed a tendency to decrease DA concentration as time passed (*F[8,72]=3.097, p=0.0047, n=10)* (**Figure 4.51-A**). Two-way ANOVA for repeated measures did not reveal significant differences between the two groups of mice (F_t*[8,152]=4.845, p<0.0001;* F_{tr}*[1,152]=0.222, p=0.643;* F_i*[8,152]=1.336, p=0.229; n=21)*.

Similarly, the analysis of the AUC did not show differences between saline and Poly (I:C) mice either (*t=0.642, p=0.529*) (**Figure 4.51-B**).

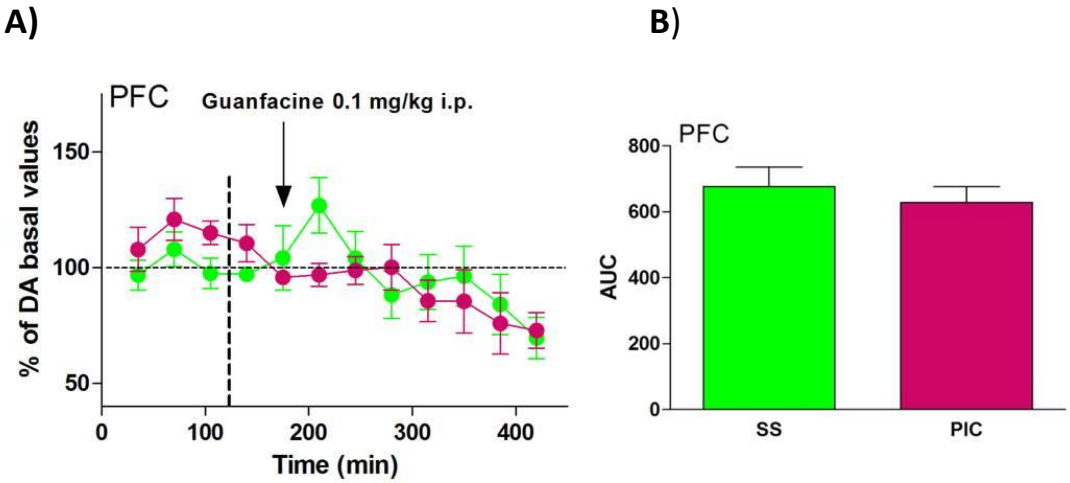

Figure 4.51: **A)** Graphic representation of the effect of systemic administration of guanfacine (0.1mg/kg i.p.) on DA extracellular concentration in PFC of saline (•) (n=11) and Poly (I:C) (•) mice (n=10). The vertical arrow indicates the time of administration of guanfacine. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **B)** Graphic representation of the AUC of the effect of systemic administration of guanfacine (0.1 mg/kg i.p.) on extracellular concentration of DA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.2.2.1.3. Effect of the systemic administration of guanfacine on 5-HT concentrations in PFC

Regarding cortical 5-HT concentration, systemic administration of guanfacine (0.1 mg/kg i.p.) did not produce a statistically significant difference of 5-HT concentrations in saline mice (Emax=119.83±5%, *p=ns* vs. basal conditions) reached 35 min after the injection of the drug (*F[8,80]=1.942, p=0.0648, n=11*) (**Figure 4.52-A**). As for the Poly (I:C) group, systemic guanfacine modified 5-HT concentrations in a statistically significant manner during the experiment (*F[8,72]=2.412, p=0.0229, n=10*), with the maximum increase in 5-HT concentration occurring 70 min after the injection (Emax= 113.03±12%, *p=ns* vs. basal

conditions) and showing a tendency to decrease the neurotransmitter concentrations by the end of the experiment. Despite this, the two-way ANOVA for repeated measures did not show significant differences between the two groups of mice ($F_t[8,152]=2.946$, $p=0.0043$; $F_{tr}[1,152]=0.857$, $p=0.366$; $F_i[8,152]=1.486$, $p=0.166$; $n=21$).

In the same way, the analysis of the AUC of the curves was not different between the groups ($t=0.579$, $p=0.569$) (**Figure 4.52-B**).

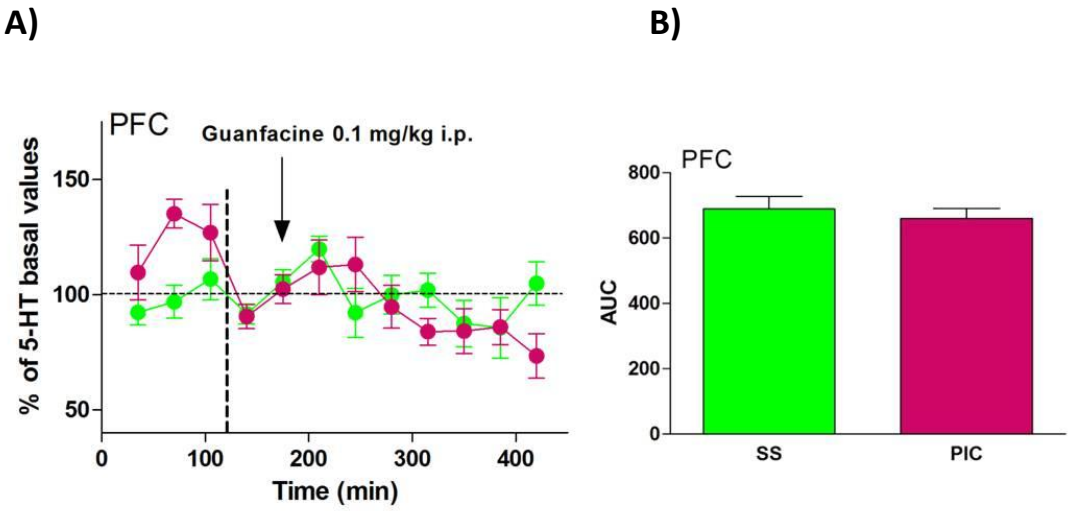

Figure 4.52: **A)** Graphic representation of the effect of systemic administration of guanfacine (0.1 mg/kg i.p.) on 5-HT extracellular concentration in PFC of saline (•) (n=11) and Poly (I:C) (•) mice (n=10). The vertical arrow indicates the time of administration of guanfacine. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Data points are representations of the mean ± SEM values and are expressed as percentages of basal values. **B)** Graphic representation of the AUC of the effect of systemic administration of guanfacine (0.1 mg/kg i.p.) on extracellular concentration of 5-HT in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.2.2.2. Effect of the systemic administration of the α_{2C}-adrenoceptor antagonist MK-912

4.2.2.2.1. Effect of the systemic administration of MK-912 on NA concentrations in PFC

Systemic administration of the α_{2C}AR antagonist MK-912 (0.05 mg/kg i.p.) produced an increase in NA concentration in the PFC of saline mice (Emax=113.36±6%, $p<0.05$ vs. basal conditions), reaching the maximum effect 70 min after the injection. The statistical analysis by the one-way ANOVA showed a significant effect exerted by the drug ($F[8,88]=3.635$, $p=0.001$, $n=12$) on these mice (**Figure 4.53-A**). For the Poly (I:C) mice, the maximum effect exerted by systemic MK-912 on cortical NA concentrations was 111.44±6% ($p=ns$ vs. basal conditions). In this case, the analysis by the one-way ANOVA did not reveal a significant effect of the drug ($F[8,64]=1.391$, $p=0.217$, $n=9$). The two-way ANOVA did not show statistical differences between groups either ($F_t[8,152]=3.398$, $p=0.001$; $F_{tr}[1,152]=0.000037$, $p=0.995$; $F_i[8,152]=1.403$, $p=0.199$; $n=21$).

In addition, the analysis of the AUC of the effect exerted by MK-912 was not different between groups either (*t=0.171, p=0.866*) (**Figure 4.53-B**).

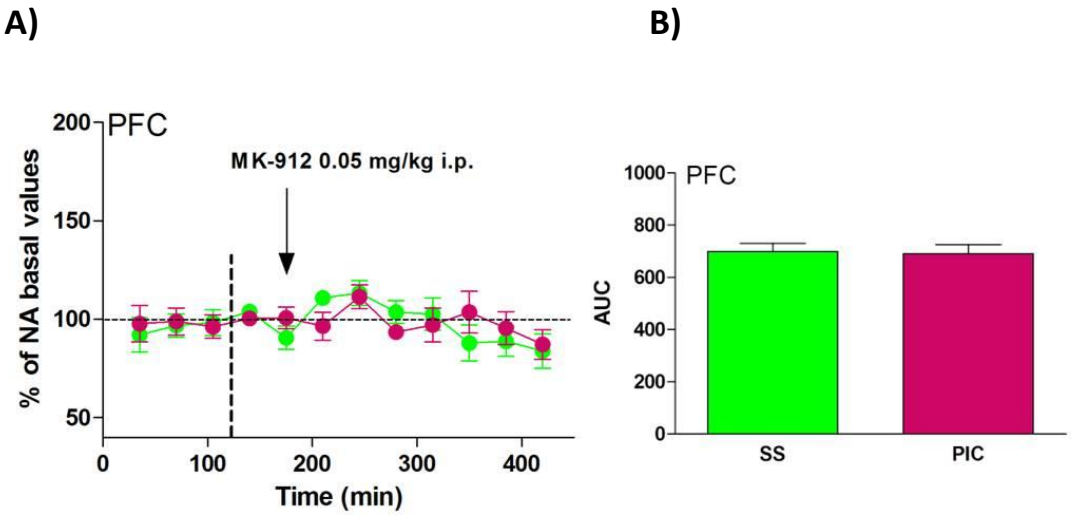

Figure 4.53: **A)** Graphic representation of the effect of systemic administration of MK-912 (0.05 mg/kg i.p.) on NA extracellular concentration in PFC of saline (•) (n=12) and Poly (I:C) (•) mice (n=9). The vertical arrow indicates the time of administration of MK-912. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **B)** Graphic representation of the AUC of the effect of systemic administration of MK-912 (0.05 mg/kg i.p.) on extracellular concentration of NA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.2.2.2.2. Effect of the systemic administration of MK-912 on DA concentrations in PFC

Systemic administration of the α_{2C}AR antagonist MK-912 (0.05 mg/kg i.p.) produced an increase in DA concentrations in PFC of saline mice (Emax=126.28±11%, *p=ns* vs. basal conditions) with the maximum effect occurring 70 min after the injection. The analysis by the one-way ANOVA revealed the drug modified significantly DA concentrations of saline mice during the experiment (*F[8,88]=2.486, p=0.0176, n=12*) (**Figure 4.54-A**). As for the Poly (I:C) mice, the MK-912 did not exert a significant effect modifying DA concentrations in PFC (*F[8,56]=0.787, p=0.616, n=8*), and the maximum effect was 120.68±16% (*p=ns* vs. basal conditions), reached 70 min after the injection. The two-way ANOVA did not show differences between groups (F_t*[8,144]=2.068, p=0.042;* F_{tr}*[1,144]=0.182, p=0.674;* F_i*[8,144]=0.809, p=0.595; n=20)*.

Equally, the analysis of the AUC of the curves of the two group of mice did not reveal differences (*t=0.424, p=0.677*) (**Figure 4.54-B**).

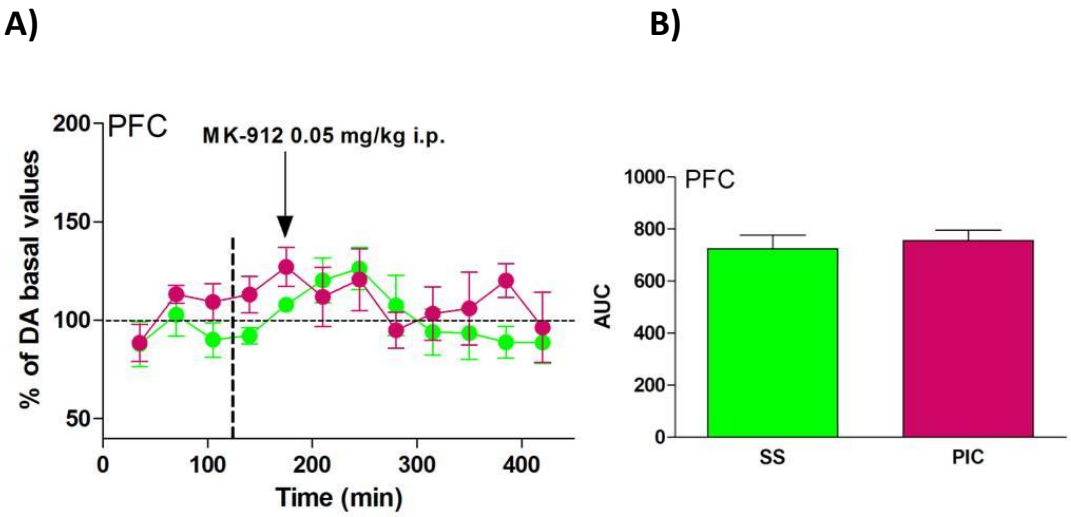

Figure 4.54: **A)** Graphic representation of the effect of systemic administration of MK-912 (0.05 mg/kg i.p.) on DA extracellular concentration in PFC of saline (•) (n=12) and Poly (I:C) (•) mice (n=8). The vertical arrow indicates the time of administration of MK-912. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values **B)** Graphic representation of the AUC of the effect of systemic administration of MK-912 (0.05 mg/kg i.p.) on extracellular concentration of DA in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values.

4.2.2.2.3. Effect of the systemic administration of MK-912 on 5-HT concentrations in PFC

When it comes to 5-HT in PFC, its concentrations were increased significantly by systemic MK-912 (0.05 mg/kg i.p.) in saline mice, with a maximum effect of 149.55±9% ($p<0.05$ *vs.* basal values) reached 70 min after the administration of the drug. One-way ANOVA revealed a statistically significant effect of the drug in these mice (*$F[8,88]=6.575$, $p<0.0001$, $n=12$*) (**Figure 4.55-A**). In the case of the Poly (I:C) mice, systemic administration of the MK-912 modified 5-HT concentrations in Poly (I:C) mice in a statistically significant manner (Emax=130.63±13%, *p=ns* vs. basal conditions) with the maximum effect occurring 35 min after the injection (*$F[8,72]=4.485$, $p=0.0002$, $n=10$*). The analysis by the two-way ANOVA showed statistical differences between saline and Poly (I:C) mice (*$F_t[8,160]=8.858$, $p<0.0001$; $F_{tr}[1,160]=10.34$, $p=0.0043$; $F_i[8,160]=2.012$, $p=0.0481$; $n=22$),* suggesting a reduced response of Poly (I:C) mice to the administration of the drug.

The analysis of the AUC of the curves showed a statistically significant reduction in the response of 5-HT concentrations to MK-912 in the Poly (I:C) mice compared to saline mice (*$t=3.460$, $p=0.002$*) (**Figure 4.55-B**).

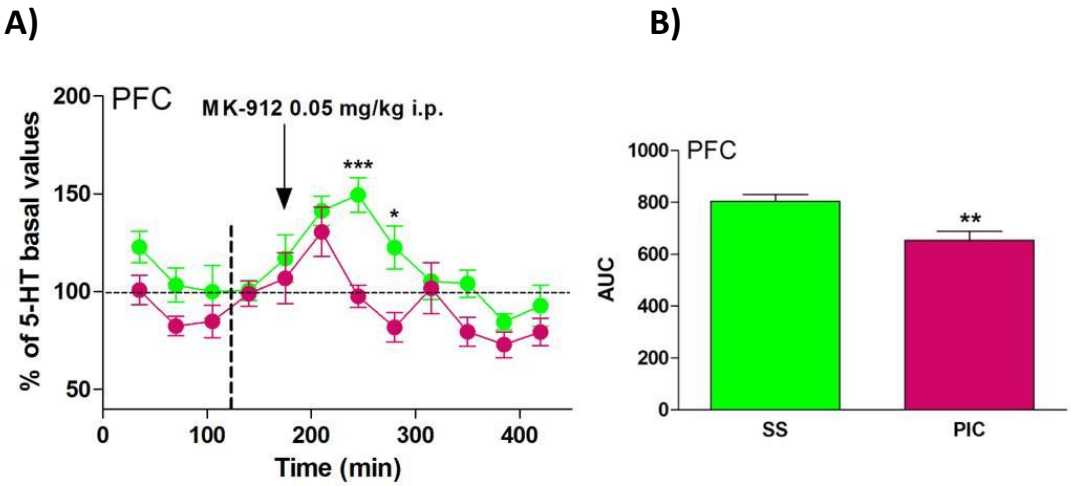

Figure 4.55: **A)** Graphic representation of the effect of systemic administration of MK-912 (0.05 mg/kg i.p.) on 5-HT extracellular concentration in PFC of saline (•) (n=12) and Poly (I:C) (•) mice (n=10). The vertical arrow indicates the time of administration of MK-912. The vertical discontinuous line marks the point from which the basal concentrations have been taken into consideration. Points are representations of the mean ± SEM values and are expressed as percentages of basal values. **p<0.05, ***p<0.001*, Two-way ANOVA followed by Bonferroni *post-hoc* test. **B)** Graphic representation of the AUC of the effect of systemic administration of MK-912 (0.05 mg/kg i.p.) on extracellular concentration of 5-HT in the PFC of saline and Poly (I:C) mice. Bars are a representation of the mean ± SEM values. ***p<0.01*, Student's unpaired *t*-test vs. saline group.

5. DISCUSSION

During the last decades, a great effort has been made in the search for innovative pharmacological targets and new therapeutic strategies for the effective treatment of the whole range of symptoms that a disease such as schizophrenia presents. Concretely, negative symptoms and cognitive deficiencies deserve special attention, since they are still resistant to any commercially available pharmacotherapy.

With regard to the research in the field of mental and psychiatric disorders, animal models have become a useful and promising tool in the way to a better understanding of the etiology and pathophysiology of schizophrenia, trying to smooth the way a little in the struggle of finding better and more effective pharmacotherapy. Despite the obstacles in creating animal models capable of simulating the clinical symptoms presented by schizophrenia patients, the new animal models focus especially in imitating the biological phenomena associated with clinical manifestations. In particular, the changes in the neurodevelopment of certain brain areas, the dysfunctions in the signaling pathways of the different neurotransmission systems, and the abnormalities related to specific behavior and their possible association with the interaction of environmental and genetic factors. It is precisely the PFC the area that is fundamentally implicated in all the so-called "higher functions", including attention, WM, decision making, sensorimotor gating, and cognitive plasticity; considered all of them impaired in schizophrenia. Those functions carried out by the PFC seem to be the result of a correct and exact equilibrium between the excitatory and inhibitory signaling, as much as of the negative feedback and the correct concentrations of all the neuromodulators and neurotransmitters implicated. Thus, abnormalities in the PFC functionality would come from the breakdown of this equilibrium and a disruption of the functionality of all these circuits and signaling pathways. These aberrations could have their origin in genetic factors, environmental factors or an interaction of both. Indeed, between environmental factors, pre/perinatal risk factors play an important role in the etiopathogenesis of the disorder. Actually, evidence coming from epidemiological studies suggest a strong association between maternal infection and neurodevelopmental disorders. It seems that disturbances directed at the maternal host during pregnancy can result in physiological changes in the fetal environment and negatively affect the normal course of the neurodevelopment in the offspring (Rees & Inder, 2005). Specifically, schizophrenia appears to be related to aberrations in the early developmental processes caused by a combination of environmental and genetic factors; therefore acting as a disease primer and predisposing the offspring to long-lasting psychopathology and neuropathology (Estes & McAllister, 2016b).

Concretely, through studies in animal models, it has been demonstrated that maternal infection during pregnancy increases the risk for several neuropsychiatric disorders in the offspring later in life, including schizophrenia (Brown & Susser, 2002; Fatemi et al., 2005). Indeed, structural and functional abnormalities implicated in the endophenotypes of this disorder have been detected following prenatal exposure to immunostimulatory agents, such as the Poly (I:C) (reviewed in Meyer et al., 2005; 2007). The Poly (I:C) model of MIA has been demonstrated to possess a high construct validity (Meyer et al., 2006a; 2008a; Estes & McAllister, 2016b). As for its face validity, although a partial validation of this model has been carried out (Bergdolt & Dunaevsky, 2019), much remains to be done at the molecular, neurochemical and behavioral level that would allow the full definition of its real face validity as an animal model of schizophrenia. Finally, its predictive validity is being challenged for

testing different therapies and drug treatments (Meyer et al., 2010; Hadar et al., 2015; Bikovsky et al., 2016; MacDowell et al., 2017; Prades et al., 2017; Casquero-Veiga et al., 2019).

In this context, this work aimed to perform a neurochemical and behavioral characterization of the Poly (I:C) model, empathizing especially its potential use as an animal model of CIAS. With that purpose, on the one hand, an *in vivo* neurochemical evaluation of the monoamine neurotransmission systems has been carried out in brain areas such as the PFC and striatum. On the other hand, *in vitro* studies have been done to determine the status of the different components of the noradrenergic and dopaminergic system in the mentioned brain areas. In addition, in the search for novel pharmacological approaches, different behavioral studies have been carried out to evaluate the cognitive status of the model and its response to potential procognitive drugs oriented to schizophrenia. Finally, using optogenetics, specific and selective targeting of the LC-PFC circuitry was performed, with the objective of being able to study in the model the possible existence of alterations that could be significant in the neurobiology of schizophrenia.

5.1. NEUROCHEMICAL CHARACTERIZATION OF THE POLY (I:C) MIA MODEL: ROLE ON THE PFC FUNCTION

5.1.1. The DA and NA systems in the PFC of Poly (I:C) MIA model

When extracellular DA concentrations in the PFC were measured, it was observed that Poly (I:C) mice presented a significant reduction in their DA basal concentrations (**Figure 4.27 A**), but unaltered *Ed* function (**Figure 4.27 B**) compared with the saline mice. DA tissue concentrations were not different between the two groups of mice (**Figure 4.3**). Moreover, to test whether the potential-dependent neurotransmitter release was altered, the effect of local hiperK$^+$ aCSF was also evaluated, since it would force the depolarization of the neuron membrane and the release of neurotransmitters. In this case, the response of DA concentrations to hiperK$^+$ aCSF in Poly (I:C) mice was not different compared with the saline mice **(Figure 4.29 A-B**). In addition, the potential-independent release of neurotransmitter was evaluated by the administration of systemic amphetamine. It was found that DA response in the Poly (I:C) mice was significantly lower compared with the saline mice (**Figure 4.32 A-B**). Regarding the effect of the systemic administration of the NMDA receptor antagonist MK-801, there was an increase in DA concentrations but no differences were reported between Poly (I:C) and saline mice (**Figure 4.35**). As for TH (**Figures 4.7-4.8**) and D_1- and D_2Rs (**Figures 4.15** and **4.16**) expression levels, no differences were detected between the two groups of mice. However, increased expression levels of DAT were found in the Poly (I:C) mice compared with saline mice (**Figure 4.5**).

Interestingly, in the present study, the results obtained for NA go in the same line as those obtained for DA, suggesting that these neurotransmitter systems behave in a very similar way in PFC. In fact, regarding NA in the PFC, there were no differences between saline and Poly (I:C) groups neither in the NA basal concentrations, the *Ed* value (**Figure 4.25 A**), nor in NA tissue concentrations (**Figure 4.2**). NET expression levels were also unaltered (**Figure 4.6**). Nevertheless, similar to what happened with DA, the Poly (I:C) mice presented a reduced response of NA concentrations to the effect of systemic administration of amphetamine and no differences were observed between groups after the local administration of hiperK$^+$ aCSF in the PFC (**Figure 4.31 A-B** and **Figure 4.28 A-B**). Regarding the effect of the systemic administration of the NMDA receptor antagonist MK-801, NA concentrations were increased, but no differences were observed between groups either (**Figure 4.34 A-B**). These data suggest that DA and NA systems respond similarly in the PFC of the Poly (I:C) model. Besides, these observations could also imply the existence of a possible cortical presynaptic catecholaminergic hypoactivity in the Poly (I:C) MIA model. **Table 5.1** summarizes the results obtained in the PFC for the Poly (I:C) model with respect to the saline group.

Table 5.1: Summary of the findings of the neurochemical characterization of the Poly (I:C) model in the PFC. The results expressed correspond to the Poly (I:C) group with respect to the saline group. Abbreviations: PIC: Poly (I:C); µD: microdialysis. ↑↑/↓↓: statistically significant difference. ↑/↓: tendency but not statistically significant difference. =: no differences.

PFC results	NA	DA	5-HT
Tissue monoamines	=	=	↓↓
Western Blot	TH =; NET=	TH =; DAT ↑↑ D_1R ↑; D_2R ↑	-
Non-net flow µD (basal levels and *Ed* fuction)	Basal levels = *Ed* =	Basal levels ↓↓ *Ed* =	-
Local K^+ CSF (50 mM)	=	=	↑↑
Systemic amphetamine (2.5 mg/kg i.p.)	↓↓	↓↓	↑
Systemic MK-801 (0.5 mg/kg i.p.)	=	=	=

The fact that the Poly (I:C) mice showed increased DAT expression levels (**Figure 4.5**) seem to be in agreement with decreased DA extracellular concentrations detected in the PFC (**Figure 4.27 A**). However, in the PFC of Poly (I:C) mice, the *Ed* value for DA was not different compared with the saline mice (**Figure 4.27 B**). The *Ed* value is indicative of the reuptake function that the neurotransmitter undergoes by its specific transporter (DAT, NET), but also by all the additional transport mechanisms (for instance, OCTs and PMATs (Bacq et al., 2012) and metabolic enzymes (COMT) that eliminate it from the synaptic space. In the case of DA in the Poly (I:C) mice, it was observed that despite having an increased amount of DAT, the *Ed* value is not augmented compared with saline mice. There are several studies that had reported observations in this regard. For instance, DAT appears not to be effective enough in the clearing process of DA in the PFC because of its sparse concentration in this area (Carboni et al., 1990; Di Chiara et al., 1992; Tanda et al., 1994). In fact, in this study, it was necessary to load twice the amount of protein to be able to detect and quantify it. Besides, the NA transporter, the NET, has more affinity for DA than for NA, and that the DAT itself for DA (Giros et al., 1994; Gu et al., 1994; Eshleman et al., 1999; Moron et al., 2002; Devoto & Flore, 2006), being able to transport NA as well as DA (Moron et al., 2002; Devoto & Flore, 2006). Therefore, due to the limited concentration and lack of efficiency of DAT in the PFC, DA reuptake in this area would depend primarily on the NET (Moron et al., 2002; Devoto & Flore, 2006; Slifstein et al., 2015). Hence, considering that the levels of NET in the cortex are not different between the

two groups of mice, the fact that there is not an increase in the *Ed* value of Poly (I:C) mice could be justified with the NET as the main responsible for DA reuptake the PFC, blurring the increased expression of DAT observed in the Poly (I:C) mice.

A reduction in the amphetamine-induced release of catecholamines in the PFC of Poly (I:C) mice compared to saline mice (**Figure 4.32 A-B**) could reflect alterations in any of the processes happening from the synthesis of the neurotransmitter to its release: vesicular storage, metabolism, and release/reuptake by specific transporters. Previous studies have evaluated the sensitivity to the locomotor-stimulating effects of amphetamine in different MIA models in mice (Ozawa et al., 2006; Meyer et al., 2008a) and rats (Zuckerman et al., 2003; Bronson et al., 2011; Vorhees et al., 2015); but there is no evidence addressing the effects of systemic amphetamine on monoamine concentrations in the PFC after prenatal Poly (I:C) administration. Related to this, one question that arises is whether the increased expression levels of DAT truly correspond to a higher function of the transporter. Since only DAT expression levels have been measured and not its functionality, an abnormal function of the DAT could not be ruled out. In any case, these data point to a possible presynaptic deficiency in the Poly (I:C) animal model. For further deepen in this issue it would be interesting to measure DAT functionality in the Poly (I:C) model in future studies.

Moreover, there are no studies describing DAT expression levels in the PFC in MIA models. In fact, all the studies where the expression of DAT is reported are focused on other brain regions, such as the striatum, nucleus accumbens core or shell regions. This is not surprising taking into account the very low density of DAT in the PFC region and the difficulty to measure it (Baharnoori et al., 2013; van Heesch et al., 2014). In the present work, however, we managed to measure its expression loading almost twice the amount of protein we did for other proteins, such as D_1-D_2Rs. Therefore, there is no reliable data about DAT to compare these results. Similarly, in human studies, the low DAT density in the PFC and its measurement difficulties have also led to a lack of reliable studies on this matter. Indeed, most studies focus on the striatum and midbrain areas. Among the studies that have tried, Artiges and cols. did not succeed (Artiges et al., 2017), and there was one study reporting decreased DAT levels in layer VI of area 9 in the cortex (Akil et al., 1999). In addition, one study described increased mRNA DAT levels in blood peripheral leucocytes (Liu et al., 2013). Consequently, there is no directly comparable data on DAT expression in PFC of MIA animal models, nor in schizophrenic patients.

However, regarding PFC functionality in human schizophrenic patients, there is abundant evidence reporting a widespread alteration of the DA system. Although the repeatedly observed and replicated excessive DA release in the striatum of schizophrenic patients after administration of DA-releasing stimulants (Guillin et al., 2007; Toda & Abi-Dargham, 2007; Miyake et al., 2011; Howes et al., 2012), one study using PET technology demonstrated a generalized blunting in DA release in dorsolateral PFC of schizophrenic patients after the administration of amphetamine. This effect was not limited to the dorsolateral PFC; it was also extended to other regions including the midbrain (Slifstein et al., 2015). Also, an association between the index of DA release capacity and WM-related activation in the dorsolateral PFC was detected, suggesting this lower release of DA may affect frontal cortical cognitive function. These findings seem to imply a differential regulation of striatal DA release compared with

regions such as the PFC (Weinberger et al., 1992a; Slifstein et al., 2015), thus supporting the present findings and the proposed cortical presynaptic deficiency in the Poly (I:C) model.

As it occurred with DA, the effect of systemic amphetamine on NA concentrations was lower in the Poly (I:C) mice (**Figure 4.31 A-B**). It has been described that amphetamine, apart from its effect upon the DAT, can also interact with the NET and therefore evoke NA release. In fact, amphetamine shows a higher affinity for the NET than for the DAT (Gu et al., 1994; Rothman & Baumann, 2003; Han & Gu, 2006). In this study there were no differences in the NET expression levels (**Figure 4.6**) and, besides, there are no studies measuring PFC NET expression levels in the Poly (I:C) MIA model. In humans, the majority of studies have evaluated the influences of the NET gene polymorphisms in schizophrenia and/or their influence in executive processes. Any of the studies found reported differences in NET expression between schizophrenia patients and healthy controls (Stober et al., 1996; Leszczynska-Rodziewicz et al., 2002; Szoke et al., 2006) or in the possible polymorphism effects on executive functions (Szoke et al., 2006). According to all this data, the possible existence of a presynaptic catecholaminergic hypoactivity in the PFC of the Poly (I:C) model seems to be reinforced.

In the present work, MK-801 was systemically administered to evaluate the effect on monoamine release in the PFC of a non-competitive NMDA receptor antagonist, observing a significant increase in DA concentrations in the PFC in both groups of mice (**Figure 4.35 A-B**). These results are supported by several studies describing the stimulatory effect on DA concentrations in the PFC by systemic NMDA receptor antagonist administration (Wedzony et al., 1993; Loscher et al., 1993; Kashiwa et al., 1995; Schmidt & Fadayel, 1996; Adams & Moghaddam, 1998; Vishnoi et al., 2015). The specific mechanism by which NMDA receptors can regulate cortical DA neurons and neurotransmitter release in the PFC is not yet fully described. On the one hand, it has been suggested that the effect of MK-801 on DA cortical neurotransmission is selective towards the DA system in the VTA that projects to the PFC (Deutch et al., 1987; Rao et al., 1990; Kashiwa et al., 1995). These observations are also supported by another study (French & Ceci, 1990) in which i.v. injection of MK-801 increased the firing rate of VTA DA cells that project to cortical areas. On the other hand, it has also been described that local infusion of NMDA antagonists in the PFC can enhance extracellular DA concentrations in the area (Hata et al., 1990; Nishijima et al., 1994). This view supports the hypothesis that suggests NMDA receptors located in GABAergic interneurons in the PFC are under a tonic activation of endogenous glutamate, hence controlling the activity of pyramidal neurons that project to the VTA (Nishijima et al., 1994). Therefore, it has been postulated that NMDA receptor antagonists might block this tonic excitatory drive on GABAergic interneurons, increasing the activity of PFC glutamatergic neurons and therefore, augmenting the activity of VTA DA neurons. Finally, this would result in an increase in DA release the PFC (PFC-VTA-PFC circuit)(Hata et al., 1990; Kashiwa et al., 1995; Nishijima et al., 1994; Adell & Artigas, 2004). Regarding the Poly (I:C) MIA model, an increase in DA concentrations in the PFC was observed after MK-801 systemic administration, with no differences compared with the saline mice. These data seem to suggest that the activity of NMDA receptors located in GABAergic interneurons in the PFC, and supposedly responsible for the effect of MK-801 on DA concentrations, is not altered in the model. Despite this, the possibility that other subcortical mechanisms are contributing to this effect should not be ruled out.

As for the effect of the NMDA receptor antagonist MK-801 on NA response, similar to what happened with DA, MK-801 was able to produce an increase in NA concentrations in both groups of mice (**Figure 4.34 A-B**). As it happens for DA, the role of NMDA receptors in relation with the noradrenergic system has not yet been fully elucidated, and some studies have reported dissenting results with respect to the effects of NMDA receptor agonists and antagonist on NA concentrations in the PFC. Thus, increased NA concentrations in the PFC are reported when MK-801 was systemically administered (Lena et al., 2007; Tose et al., 2009). Nevertheless, other studies have described that the systemic administration of MK-801 does not alter NA concentrations in the rat PFC (Loscher et al., 1993; Bowers & Morton, 1994), and one study reported a decrease in NA concentrations in the PFC of rats after MK-801 administration (Dai et al., 1995). These results cannot be directly compared with those of the present work since different doses (0.3 mg/kg i.p. *vs.* 0.5 mg/kg i.p.) (Loscher et al., 1993; Dai et al., 1995) and animal species (rat *vs.* mice) were used (Bowers & Morton, 1994). Apart from that, when applying MK-801 locally in the PFC, there was no effect on NA concentrations (Tose et al., 2009). Contrary to what is supposed to happen for DA, this observation might suggest that there is not a tonic inhibitory control of NMDA receptors over pyramidal neurons projecting to the LC and controlling NA release in the PFC. Then, another mechanism via NMDA receptors involving disinhibition of the LC-neurons projecting to the PFC should be implicated, and currently, this exact mechanism is not known (Tose et al., 2009). However, against to these data, it has recently been described that both systemic and local administration in the PFC of NMDA receptor antagonists such as ketamine increases the NA in the PFC (Lopez-Gil et al., 2019). This way, NMDA receptor-mediated disinhibition of glutamatergic neurons projecting to the LC is speculated, thus increasing NA concentration in the area (loop PFC-LC-PFC) (Lopez-Gil et al., 2019). All this evidence considered, and given the similarity of the responses that have been reported in this work by both the DA and NA systems to the NMDA receptor antagonist, the hypothesis of NMDA receptor-mediated disinhibition of pyramidal neurons projecting to the LC and controlling cortical NA concentrations would seem the most plausible explanation. In fact, there were no differences in the Poly (I:C) model compared to saline mice after MK-801 administration, therefore suggesting the responsible mechanisms for this effect would not be altered in the model. It is presumable that, as it was hypothesized for DA, the effect of systemic MK-801 in NA concentrations in the PFC would be mediated by a mechanism via disinhibition of the LC neurons projecting to the PFC.

Concerning the expression levels of the enzyme responsible for the catecholamine synthesis, TH, there were no differences between the Poly (I:C) mice and saline mice (**Figures 4.7-4.8**). In this regard, there is a lack of studies quantifying the expression of the TH in the PFC of MIA models to compare the present results with. For instance, Meyer and cols. (Meyer et al., 2008b) could not quantify the TH because of its low levels of immunoreactivity in the PFC. This seems surprising since in this work it is reported measurements of the TH expression with two different antibodies in the PFC. Another study by Deslauriers and cols. reported an increased expression of the TH in the PFC of the Poly (I:C) model (Deslauriers et al., 2013). However, it should be noted that they used a much higher dose (20 mg/kg i.p. *vs*. 7.5 mg/kg i.p.) and that they administered it later in the pregnancy course (GD12 *vs.* GD9). In any case, this data seems to indicate that the catecholamine synthesis process is not altered in the model. On the

contrary, human *postmortem* studies have reported a decrease in the TH immunolabeling in the PFC in schizophrenic patients, especially in layer VI (Akil et al., 2000). Also, in the rostral entorhinal cortex, which has been described to receive a dense DA innervation and to be implicated as a site of dysfunction in schizophrenia (Arnold et al., 1977; Lewis & Akil, 1997; Harrison, 1999), a decrease of the TH in layers III and VI but not in layer I, and reduced density of the synaptic boutons has been reported (Akil & Lewis, 1995; Akil et al., 2000). Comparisons to developmental studies in nonhuman primates suggest that the regional and laminar differences in the cortical layers exhibiting reduced TH axon immunoreactivity in schizophrenia might reflect an early developmental insult (Akil et al., 2000). These regional and laminar differences may imply that TH immunoreactive axons may be differentially sensitive to adverse events at different stages of maturation (Rosenberg & Lewis, 1995). This study reported no differences in TH expression levels in the PFC of the Poly (I:C) animal model, which could be precisely due to these changes in the sensitivity of these neurons throughout the neurodevelopment process. In addition, the samples used in this work comprise the entire PFC area, without discriminating between different layers. Therefore, abnormalities in the DA-NA innervation in the PFC and the rostral entorhinal cortex in schizophrenic patients may be consistent with the hypotheses of an early developmental insult in the pathogenesis of this disorder.

In the case of the D_1R and D_2R expression, the same absence of difference was noticed, although a slight trend to an increase was observed for both receptors in the Poly (I:C) mice (**Figures 4.15-4.16**). In relation to this, previous studies have shown contradictory results. Although with a different dose of Poly (I:C) (20 mg/kg i.p. *vs.* 7.5 mg/kg i.p) and prenatal timing (GD12 *vs.* GD9), findings in the present work are consistent with results from one study measuring D_2R mRNA and protein expression in the PFC, as they reported increased levels of both mRNA and protein expression in the Poly (I:C) model (Deslauriers et al., 2013). Other studies also using a different dose and route of administration of Poly (I:C) (5 mg/kg i.v. *vs.* 7.5 mg/kg i.p.) observed a decreased expression of D_1R and D_2Rs in the PFC (Meyer et al., 2008b). In addition, one study in rats injected with LPS on GD15 and GD16 also reported decreased levels of D_2R but no changes in D_1R expression levels (Baharnoori et al., 2013). As previously mentioned, human and animals studies have described that D_1R and D_2Rs are expressed in deep layers of the PFC, on both principal pyramidal neurons as well as different classes of interneurons (Vincent et al., 1993; Muly et al., 1998), with a more prominent expression of D_1R than D_2R in the PFC (Gaspar et al., 1995; Lidow et al., 1997; Santana et al., 2009). D_1R is mainly postsynaptic (Muly et al., 1998; Baharnoori et al., 2013) while D_2R has been reported to be localized both pre-and post-synaptically, and in extra- and peri-synaptic structures of the PFC (Bentivoglio & Morelli, 2005; Negyessy & Goldman-Rakic, 2005). These data, and the fact that in this work a slight trend to an increase in the expression of both DA receptors was detected, led us to emphasize about the possibility that the hypothesized cortical hypodopaminergic state may be presynaptic, and therefore it could be resulting in an up-regulation of these postsynaptic receptors in the area. In human schizophrenic patients, PET and binding studies have evaluated the availability and density of D_1Rs and there seem to be certain discrepancies between them. Binding and imaging studies have reported clear increases in D_1R availability in dorsolateral PFC of drug-naïve patients with schizophrenia (Abi-Dargham et al., 2002; 2012), supporting the trend observed in the results of the present work with the Poly (I:C) mice. Also,

a nonsignificant increase in D_1R autoradiography was also reported (Knable et al., 1996). However, another study reported decreased D_1R binding potential in the PFC of schizophrenic patients who had never been treated with neuroleptics (Okubo et al., 1997). Additionally, two other studies reported no differences in D_1R binding (Knable et al., 1996), and unchanged D_1R mRNA levels in the PFC (Meador-Woodruff et al., 1997). As for the D_2R, results obtained in this work are consistent with previous studies measuring PFC D_2R mRNA and protein levels, which have reported no differences between both AP-free and AP-treated schizophrenic patients and controls (Meador-Woodruf et al., 1997; Urigüen et al., 2009). Similarly, several binding and PET studies which have described unchanged D_2R levels in the PFC of human schizophrenic patients (Ruiz et al., 1992; Meador-Woodruff et al., 1997; Yasuno et al., 2005; Glenthoj et al., 2006; Buchsbaum et al., 2006; Slifstein et al., 2015). However, another study showed decreased binding properties of the D_2R in the PFC of drug-naïve schizophrenic patients (Lehrer et al., 2010) and another one reported an elevation of D_2R mRNA levels in the PFC of schizophrenic patients compared with controls (Tallerico et al., 2001). Besides, mRNA levels of D_2R were evaluated in peripheral blood leucocytes of schizophrenic patients and healthy controls and there were no significant differences, but those levels correlated with positive symptoms in acute schizophrenia patients (Liu et al., 2013). This evident lack of consensus may result from the very low density and limited anatomical distribution of these receptors in the PFC (Kessler et al., 1993; Slifstein et al., 2015) and the consequent difficulties that their measurement entails.

When DA tissue concentrations were measured in the PFC, no differences were detected between the two groups of mice (**Figure 4.3**). This observation is concordant with previous studies (Ozawa et al., 2006; Hadar et al., 2015) where no differences were described either when measuring DA tissue concentrations in the PFC between Poly (I:C) and control mice. However, opposite results have also been reported. Thus, higher DA tissue concentrations in the PFC of Poly (I:C) mice (Winter et al., 2009) and decreased DA concentrations have been found (Bitanihirwe et al., 2010). It should be noted that the dose of Poly (I:C) (5 mg/kg *vs.* 7.5 mg/kg) and the route of administration (i.v. *vs*. i.p.) were different from the one used in the present work, hence these results cannot be directly compared. As commented previously, among other factors, the dose of Poly (I:C) used will determine the intensity of the immune response. Therefore, the final pregnancy outcome and the long-term consequences affecting the offspring would vary substantially (*see section 1.6.4.1.4.3.1.*). Nevertheless, a possible explanation for the absence of differences in DA tissue concentrations in the model is the fact that DA can be located in both dopaminergic and noradrenergic terminals. Indeed, there is evidence reporting extracellular DA in the PFC may come from dopaminergic but also noradrenergic terminals, where it acts as a precursor and probably as cotransmitter of NA (Devoto et al., 2001; Moron et al., 2002; Devoto & Flore, 2006). Considering the wide noradrenergic innervation of the PFC, the expected deficit of DA tissue concentrations in the area could be masked by the DA content belonging to noradrenergic terminals. This view is supported by the fact that extracellular DA in different cortices was found to be modified by drugs acting on NA transmission but not, or only slightly, by drugs altering DA activity (Gresch et al., 1995; Devoto et al., 2004; Swanson et al., 2006; Masana et al., 2011). Indeed, α_2AR agonists and antagonists have been shown to produce concomitant changes in extracellular DA and NA in the PFC (Gresch et al., 1995; Devoto et al., 2001; Kawahara et al., 2001; Devoto et

al., 2005; Devoto & Flore, 2006; Masana et al., 2011; 2012b). Similarly, atypical antipsychotics such as clozapine have been demonstrated to augment extracellular DA and NA concentrations not only in the PFC but also in the occipital cortex, although it is predominantly a noradrenergic area (Devoto et al., 2003). In addition, NET inhibitors increase extracellular NA and DA in the PFC, but not in the nucleus accumbens or dorsal striatum (Carboni et al., 1990; Di Chiara et al., 1992; Tanda et al., 1994; Mazei et al., 2002; Masana et al., 2011). This is not surprising since it has been described DA reuptake in the PFC depends primarily on NET, given the scarce presence of DAT in the area (Giros et al., 1994; Gu et al., 1994; Eshleman et al., 1999; Moron et al., 2002). Hence, it seems that even with a scarce dopaminergic innervation in the PFC, the NA system and DA originating from noradrenergic terminals would be fundamental to preserve adequately cognitive functions that depend on DA in the PFC.

Likewise, NA tissue concentrations in the PFC were unaltered between Poly (I:C) and saline mice (**Figure 4.2**), in agreement to previous studies (Bitanihirwe et al., 2010). It was also observed that DA tissue concentrations in PFC were considerably higher than NA concentrations. It is known that noradrenergic innervation coming from the LC is diffuse and widespread throughout all the cerebral cortex being especially abundant in the PFC, while dopaminergic innervations are mainly focalized to discrete areas of the cortex, such as medial PFC, anterior cingulate, rhinal and entorhinal cortices (Descarries et al., 1987; Seguela et al., 1990; Devoto et al., 2001; 2005; Devoto & Flore, 2006). Considering tissue concentration measurement cannot determine the exact location of the neurotransmitter (cytosol, vesicles…), it is plausible that higher DA over NA tissue concentrations could be representing a pool of DA waiting to be converted through the DBH enzyme to NA, which eventually would be released from the noradrenergic terminal to the extracellular space. Additionally, in agreement with this hypothesis, it has been described that the conversion-rate of DA into NA is slower than the conversion-rate of tyrosine to DA by the TH enzyme (Devoto & Flore, 2006). Actually, Anh and Klinman showed that the rate of the DBH-catalyzed conversion of DA to NA appeared to be approximately 2-fold lower than the maximal rate of the previous DA transport inside the vesicles (Ahn & Klinman, 1987). Accordingly, there are studies describing that the NA synthesis rate is 2.5-fold slower than the conversion-rate of tyrosine to DA (Levine, 1986) and that the DA uptake-rate into vesicles exceeds the rate of NA formation by 2.8-fold (Levine et al., 1985).

As a result, all these data seem to go in accordance with the hypothesis that the Poly (I:C) model is characterized by a presynaptic catecholaminergic deficit in the PFC.

5.1.2. The 5-HT system in the PFC of the Poly (I:C) MIA model

In addition to catecholamines, 5-HT was also evaluated in the PFC. One of the most marked findings was that Poly (I:C) mice presented significantly decreased 5-HT tissue concentrations compared with saline mice (**Figure 4.4**) although extracellular basal levels of 5-HT were not different between groups. Apart from that, the response to the local hiperK^+ aCSF was significantly higher in Poly (I:C) mice compared with saline mice (**Figure 4.30**), opposingly to what was observed in the case of DA and NA. Moreover, also contrary to what we reported for the two catecholamines, the response of 5-HT to systemic amphetamine in Poly (I:C) mice showed a tendency to be higher than in saline mice (**Figure 4.33**). Finally, the response of 5-HT

to the NMDA receptor antagonist MK-801 was not different between Poly (I:C) and saline animals (**Figure 4.36**).

The significantly increased response of 5-HT to the local hiperK$^+$ aCSF and to the systemic amphetamine administration in the Poly (I:C) mice might be considered relevant given that 5-HT tissue levels in the PFC were considerably lower compared with saline mice (**Figures 4.30-4.33**). This latter finding goes in agreement with one study reporting that intracerebroventricular injection of INF-α (one of the cytokines released in response to immunostimulants such as Poly (I:C) (Meyer et al., 2006b; Cunningham et al., 2007) decreased 5-HT tissue concentrations in the PFC (Kamata et al., 2000). However, there are also studies reporting unaltered 5-HT tissue concentrations in the PFC in MIA animal models (Winter et al., 2009; Hadar et al., 2015). As in the present study, neither tryptophan hydroxylase, SERT expression levels, nor any other element from the 5-HT system was measured to shed light on this issue, it cannot be ruled out the possibility that the responses to the local hiperK$^+$ aCSF and to systemic amphetamine may be part of adaptive mechanisms put in place to compensate for this apparent deficiency in 5-HT availability.

It is worth emphasizing that the 5-HT system activity is apparently opposite to that of the two catecholamines. This may suggest that the modulation of cortical 5-HT is controlled differently from the regulation of cortical catecholamines. In fact, it is known that monoamine systems interact differently with each other (Aston-Jones et al., 1991; Kaehler et al., 1999). Concretely, the different roles of the DA and 5-HT systems and their interaction with each other related to disorders such as schizophrenia have been repeatedly reported (Kapur & Remington, 1996; Daw et al., 2002; Guiard et al., 2008; Hashemi et al., 2012; Devroye et al., 2017). On the one hand, the excitatory effect of the VTA neurons projecting to the dorsal raphe has been described (Guiard et al., 2008). Lesions of VTA DA neurons by intracerebroventricular administration of 6-hydroxydopamine produced a profound and selective reduction in the discharge rate of dorsal raphe 5-HT neurons, indicating that DA input exerts a tonic excitatory effect on 5-HT neurons in the intact brain. Also, previous in-vivo electrophysiological and neurochemical studies have shown that the systemic administration of the non-selective DA receptor agonist apomorphine, increases the firing rate of 5-HT neurons (Martin-Ruiz et al., 2001), enhancing 5-HT outflow in the rat dorsal raphe (Ferre & Artigas, 1993; Ferre et al., 1994). On the other hand, there is also abundant evidence demonstrating the opposed inhibitory role of the 5-HT system over the DA system (Deakin & Graeff, 1991; Kapur & Remington, 1996; Daw et al., 2002). Serotonergic projections from the dorsal raphe project via the medial forebrain to the ventral striatum (nucleus accumbens shell) and the PFC, among other regions (Jacobs & Azmitia, 1992) and similarly, other projections go directly to the substantia nigra (Fibiger & Miller, 1977; Olpe & Koella, 1977). Also, the medial raphe nucleus innervates the core of the nucleus accumbens and VTA that innervate, in turn, the PFC (Lechin et al., 2006). Raphe nuclei stimulation produces an inhibition of the firing of the DA cells in the VTA and blocks DA-mediated behaviors (Fibiger & Miller, 1977; Olpe & Koella, 1977; Dray et al., 1978; Kelland et al., 1990). These effects seem to be mediated by the 5-HT$_{2A}$R, which presence has been described by previous studies in subcortical GABA neurons controlling DA neuronal activity (Hensler et al., 2013). Consistent with this inhibitory influence of 5-HT over the DA system, lesioning serotonergic projections disinhibits the DA system and causes an increase in DA concentrations (Fibiger & Miller, 1977; Dray et al., 1978), probably by a

diminished action upon subcortical 5-HT_{2A}Rs located in GABA neurons controlling VTA DA cells. Although presumably this might be the mechanism explaining the effect of the 5-HT system over the DA system, it should be noted that 5-HT_{2A}Rs have also been described in a large proportion of pyramidal neurons and GABAergic interneurons in the PFC (Amargos-Bosch et al., 2004; Santana et al., 2004). Thus, other possible mechanisms involved should not be ruled out. It has also been reported that 5-HT_{2A}R antagonists block this serotonergic inhibitory action and result in augmented DA concentrations in cortical areas (Ashby et al., 1990; Hamon et al., 1990). Therefore, this evidence suggests that serotonergic projections inhibit the function of DA neurons innervating terminal areas by preventing the synaptic release of DA. Considering that in this study it is hypothesized the CIAS is based on a hypodopaminergic function in the PFC, increasing DA function in this area would probably be beneficial to counteract this symptomatology. In this context, manipulations that inhibit the 5-HT system (5-HT_{1A}R agonists and/or 5-HT_{2A}R antagonists) could disinhibit the DA system and offer an indirect strategy to alleviate the cortical DA deficiency associated to cognitive symptoms in schizophrenia. This idea is supported by studies suggesting that the beneficial effects of the antipsychotic clozapine on cognitive symptoms may result from its 5-HT_{2A}R/D_2R antagonism properties (Kapur & Remington, 1996; Kuroki et al., 1999; Meltzer & McGurk, 1999). Actually, a prominent role of 5-HT_{2A}Rs in antipsychotic action is to stabilize dopaminergic tone in the mesocortical and mesolimbic pathways (Masana et al., 2012b). In relation to the PFC of the Poly (I:C) mice, there is an apparently over-response of the 5-HT system coexisting with a deficiency in the DA system. At the moment it cannot be clarified whether this overactivity of the 5-HT system is a cause or a consequence of a DA deficiency. Nevertheless, considering results shown in this work, it is also plausible that a primary overactivation of the serotonergic system may result in an inhibition of the DA system and thus, result in a cortical DA deficiency. In any case, either increasing cortical DA output or blocking the excess of 5-HT transmission would seem reasonable strategies to overcome this symptomatology.

On the other hand, the response of increased 5-HT concentrations after administration of MK-801 was not different between the two groups of mice (**Figure 4.36**), similar to the response of DA and NA. Several studies have reported NMDA receptor antagonists such as MK-801 (Gorter et al., 1992; Loscher et al., 1993; Lejeune et al., 1994; Lopez-Gil et al., 2007; 2009), PCP and ketamine (Etou et al., 1998; Adams & Moghaddam, 2001; Amargos-Bosch et al., 2006) produced a generalized increase of the 5-HT activity in rat brain. Also in the rat, the MK-801-induced increase in 5-HT output is accompanied by a concurrent enhancement of the 5-HT neuronal activity, which suggests a generalized activation of the 5-HT system by MK-801 (Lejeune et al., 1994). As previously mentioned, the neural mechanisms responsible for the effects of MK-801 on cortical monoamines are still poorly understood. It is known that systemic MK-801 administration produces an overall increase in PFC activity (Suzuki et al., 2002; Jackson et al., 2004; Kargieman et al., 2007). Indeed, pyramidal neurons of the PFC project to the midbrain raphe nuclei and control the activity of 5-HT neurons (Carr & Sesack, 2000; Celada et al., 2001; Bortolozzi et al., 2005). Thus, augmented activity in the PFC produced by MK-801 may evoke an increase in 5-HT activity via descending excitatory pathways, which would subsequently result in an enhanced release in the PFC (Castane et al., 2008). In fact, it has been recently reported that local PFC administration of the NMDA receptor antagonist ketamine increases 5-HT concentrations in the area (Lopez-Gil et al.,

2019). This observation suggests a possible PFC-DR–PFC circuit, where NMDA receptor antagonists would disinhibit cortical pyramidal neurons projecting to the dorsal raphe and eventually result in an increased 5-HT release in the PFC. Although this may seem the responsible mechanism for the reported effect of MK-801 in this work, other mechanisms cannot be ruled out. Indeed, absence of effect on 5-HT concentrations in the PFC has also been described in previous studies after local application of NMDA receptor antagonists (Amargos-Bosch et al., 2006; Lopez-Gil et al., 2007; Castane et al., 2008). According to this, it has been proposed that the blockade of NMDA receptors might occur in extracortical GABAergic neurons instead, which would produce disinhibition of excitatory inputs to the PFC, evoking the increase of neurotransmitter release (Olney & Farber, 1995; Moghaddam et al., 1997; Lopez-Gil et al., 2007; Castane et al., 2008). In addition to this, a direct effect of MK-801 on 5-HT neurons of the dorsal raphe projecting to the PFC could not be discarded either, as it has also been suggested (Callado et al., 2000; Tao & Auerbach, 2000). In any case, the effect mediated by NMDA receptors on 5-HT concentrations in the PFC does not appear to be altered in the Poly (I:C) MIA model, since, similar to what happened for catecholamines, there is no difference in the 5-HT response to MK-801 compared with the saline mice.

In conclusion, the Poly (I:C) model is characterized by a catecholaminergic presynaptic deficit along with feasible serotonergic hyperactivity in the PFC.

5.2. NEUROCHEMICAL CHARACTERIZATION OF THE POLY (I:C) MIA MODEL: ROLE ON THE STRIATUM FUNCTION

As mentioned before, an imbalance between a PFC hypodopaminergic state underlying negative symptoms and a subcortical hyperdopaminergic state related to positive symptoms is the classical concept proposed to account for the coexistence of both excess and deficiency of DA transmission in schizophrenia (Weinberger, 1987; Davis et al., 1991; O'Donnell & Grace, 1998; Akil et al., 1999; Abi-Dargham & Moore, 2003; Winterer & Weinberger, 2004). Hence, for a more complete characterization of the animal model, it was considered convenient to compare the results obtained in the PFC with the striatum, an area of great interest in schizophrenia.

5.2.1. The DA and NA systems in the striatum of Poly (I:C) MIA model

When DA extracellular concentrations were measured in the striatum, Poly (I:C) mice presented decreased DA extracellular levels and increased *Ed* function compared with saline mice (**Figure 4.38**). In addition, in the striatum of the Poly (I:C) mice, DAT and TH protein expression levels were reduced (**Figures 4.11**, **4.13** and **4.14**) while the expression levels of the D_1R and D_2Rs were not different (**Figures 4.15-4.16**). Striatal DA tissue concentrations were not altered in the model either (**Figure 4.3**). As for the response to systemic amphetamine, no differences in the DA response were detected between Poly (I:C) and saline mice (**Figure 4.40**).

Regarding the NA system, there were no differences in striatal NA tissue concentrations (**Figure 4.2**), nor in the NET expression levels (**Figure 4.12**). NA extracellular basal concentrations in the striatum were not different between the two groups either. However, the response of NA to systemic amphetamine was significantly higher in the Poly (I:C) compared with saline mice (**Figure 4.39**).

Table 5.2 summarizes the results obtained in the striatum for the Poly (I:C) model with respect to the saline group.

Table 5.2: Summary of the findings of the neurochemical characterization of the Poly (I:C) model in the striatum. The results expressed correspond to the Poly (I:C) group with respect to the saline group. Abbreviations: PIC: Poly (I:C); µD: microdialysis. ↑↑/↓↓: statistically significant difference. =: no differences.

Striatum results	NA	DA	5-HT
Tissue monoamines	=	=	=
Western Blot	TH↓↓; NET =	TH↓↓;DAT ↓↓ D_1R = ; D_2R =	-
Non-net flow µD (basal levels and *Ed* fuction)	-	Basal levels ↓↓ *Ed* ↑↑	-
Local K^+ CSF (50 mM)	-	-	-
Systemic amphetamine (2.5 mg/kg i.p.)	↑↑	=	=
Systemic MK-801 (0.5 mg/kg i.p.)	-	-	-

As for DA, these data seem to suggest there is an apparent deficiency in the dopaminergic system in the striatum of the Poly (I:C) mice. Lower levels of TH expression (**Figure 4.13-4.14**) together with decreased extracellular DA basal concentrations (**Figure 4.38 A**) may allude to a shortage in the DA release to the extracellular space. As for extracellular basal levels of DA, the only study that reported extracellular DA basal concentration in the Poly (I:C) model was in the shell of the nucleus accumbens, and they were found increased (Luchicchi et al., 2016). This study is not completely comparable with the present work since the measurement was made in another species (rats *vs.* mice), with a different dose and route for administration of Poly (I:C) (4 mg/kg i.v. *vs.* 7.5 mg/kg i.p.) and in a more restricted area (shell of nucleus accumbens *vs.* dorso/ventral striatum). Many imaging studies describe alterations in the dopaminergic system in the striatum of schizophrenic patients (Meyer-Lindenberg et al., 2002; Goto & Grace, 2007; Jarskog et al., 2007; Brisch et al., 2014; Kahn et al., 2015; Bolkan et al., 2016; Forray & Buller, 2017). Specifically, it is reported that in the striatum there is greater synthesis and release of DA of AP-free patients compared with controls (Laruelle et al., 1996; Breier et al., 1997; Abi-Dargham et al., 1998), which was correlated with the worsening of psychotic symptoms at disease onset and during exacerbations. Also, augmented baseline synaptic DA levels were detected in the striatum in schizophrenic patients and correlated with the amphetamine-induced DA release in AP-free patients (Abi-Dargham et al., 2009). It should be

noted that in the majority of studies they do not specify the exact brain area measured (dorsal striatum *vs*. ventral striatum), therefore there are results not directly comparable with each other. Regarding the TH expression levels, *postmortem* human studies have reported contradictory evidence describing increases, decreases, or no differences in TH protein and mRNA levels (Ichinose et al., 1994; Perez-Costas et al., 2012; Howes et al., 2013; McCollum & Roberts, 2015; Purves-Tyson et al., 2017). It is probable that these discrepancies are due to the heterogeneity of the cohorts of patients, differences in the methods of analysis and/or the processed brain sections, since measurements in most of the studies were not limited to a specific area, but larger areas such as the substantia nigra/VTA complex. As for MIA animal models, there are studies reporting several contradictory results about TH expression. The present work supports findings from Vuillermont and cols., who reported reduced TH expression levels in the dorsal and ventral striatum of mice at PND35 (peripubertal stage), but increased expression in nucleus accumbens shell and core at PND70 (adulthood) (Vuillermot et al., 2010). Also, another study described reduced TH expression in the offspring of rats injected with LPS at GD10.5 (Rose et al., 2014). Using a higher dose (20 mg/kg *vs.* 7.5 mg/kg), prenatal timing (GD12 *vs*. GD9.5) and different strain of mice (C57 *vs*. CD1), no differences in TH mRNA or protein levels were detected in the striatum (Deslauriers et al., 2013). On the contrary, increased TH expression levels were found in the dorsal striatum and nucleus accumbens shell, and unaltered levels in the nucleus accumbens core (Meyer et al., 2008), but using a different dose (5 mg/kg *vs.* 7.5 mg/kg), administration route (i.v. *vs*. i.p.) and mice strain (C57 *vs*. CD1). Apart from the influence of the methodological differences in both animal and human studies, all these inconsistencies could also be justified considering the distinct roles of the dorsal striatum and the ventral striatum in the regulation and modulation of a variety of cognitive, attentional, emotional, and reward-related behaviors (Spanagel & Weiss, 1999; Nieoullon, 2002; Weiner, 2003). It is important to take into account that although dorsal and ventral striatum coexist in a single anatomically delimited area, they are part of different pathways, such as the mesolimbic and mesocortical pathways (Fallon et al., 1978; Brinley-Reed & McDonald, 1999). Indeed, further studies are clearly needed to identify the critical mechanisms underlying the different effects on the distinct areas that constitute the striatum.

Since decreased TH expression levels were detected in the model, it could be speculated the existence of a possible alteration of the overall functionality of the dopaminergic terminals in the area, or even an insufficiency of DA terminals in the striatum of the MIA model. In this regard, one study in a MIA model with rats reported a reduction in both the firing rate and population activity of DA cells in the VTA projecting to the nucleus accumbens (Luchicchi et al., 2016). Although the dose (4 mg/kg *vs*. 7.5 mg/kg), the route of administration (i.v. *vs*. i.p.), prenatal timing (GD15 *vs*. GD9.5) and the species (rats *vs*. mice) are different from the ones in this work, it is indeed evidence to consider and lead to the need of more concrete studies in MIA models to evaluate the functionality of dopaminergic neurons in these areas. There are hardly studies reporting DAT expression levels after prenatal Poly (I:C) exposure in striatal areas. In dorsal and ventral (both shell and core of nucleus accumbens) regions of the striatum of peripubertal mice (PND35) decreased DAT expression levels were detected, although they were unaltered in all striatal regions in adult mice (PND70) (Vuillermot et al., 2010). Despite not being totally comparable, other study using LPS on GD15/16 in rats reported decreased DAT binding in the ventral striatum (both core and shell of nucleus accumbens) at PND35

(Baharnoori et al., 2013). Also using LPS on GD10.5, striatal DAT expression was found unaltered (Rose et al., 2014). Therefore, it is difficult to compare any of these results with the ones presented in this work, since the disparities between immunogenic agents, prenatal timing, doses, and regions of the brain, are completely diverse. As for human schizophrenic subjects, DAT levels have been reported normal in most studies. Indeed, PET and SPECT studies described no changes or unaltered DAT expression levels between schizophrenic patients and healthy controls (Laruelle et al., 2000; Howes et al., 2012; Chen et al., 2013; Fusar-Poli & Meyer-Lindenberg, 2013; Purves-Tyson et al., 2017; Tseng et al., 2017). However, discrepancies have also been reported. Similarly to what is supposed to happen it the Poly (I:C) animal model, *postmortem* studies have described reduced DAT binding levels in schizophrenia (Joyce et al., 1988; Dean & Hussain, 2001). On the contrary, a SPECT study detected increased DAT binding sites in basal ganglia of schizophrenic patients treated with neuroleptics (Sjoholm et al., 2004) suggesting a biological adaptation to counteract postsynaptic D_2R blockade. Also, a high-resolution PET study reported increased DAT availability in striatum and midbrain of schizophrenic patients compared with healthy controls (Artiges et al., 2017). Although the precise anatomical origin of DA disfunction in the striatum remains unclear, it has been suggested that this disfunction is greater in the dorsal striatum compared to limbic subdivisions for patients with schizophrenia (McCutcheon et al., 2018). Dissimilarities found in the dorsal *vs*. ventral striatum and the differences in their regulation could justify, at least in part, why there are so many inconsistencies in the results of studies in both animals and humans.

The present work reported no differences in DA tissue concentrations between the Poly (I:C) and saline mice (**Figure 4.3**). Actually, our results support findings from another study where unaltered DA tissue levels were also found in the striatum of Poly (I:C) mice (Ozawa et al., 2006), even though the Poly (I:C) administration protocol differed from the one followed in this work. There are no other studies measuring DA tissue levels in MIA animals models.

In this study, expression levels of D_1Rs and D_2Rs were not altered (**Figures 4.15-4.16**). Other findings regarding D_1Rs and D_2Rs reflect a high heterogeneity between studies. Immunoreactivity studies have reported unaltered expression of D_1Rs and D_2Rs both in the dorsal and ventral striatum between Poly (I:C) and saline mice (Meyer et al., 2008). However, other study found increased immunoreactivity of D_1Rs in both dorsal striatum and nucleus accumbens shell and increased D_2R levels in the nucleus accumbens shell (Vuillermot et al., 2010). Binding studies reported unaltered binding for the D_1Rs but decreased binding for the D_2Rs in the striatum (Ozawa et al., 2006). Another study measured mRNA levels and protein content for the D_2R and described increases of both parameters in the striatum (Deslauriers et al., 2013). Finally, unaltered binding of the D_1Rs was described in the ventral striatum using LPS in rats (Baharnoori et al., 2013; Zavitsanou et al., 2013), although increased (Zavitsanou et al., 2013) and unaltered (Baharnoori et al., 2013) binding for the D_2R was reported. Regarding D_1Rs, this work supports results obtain in human studies. In fact, striatal D_1Rs have been reported unaltered in schizophrenic patients (Pimoule et al., 1985; Seeman, 1987; Okubo et al., 1997; Karlsson et al., 2002; Abi-Dargham et al., 2002; 2012). However, great variability has been reported for striatal D_2R availability between schizophrenic patients. Actually, it is generally assumed that there are no differences in D_2R binding between AP-free patients compared with controls (Howes et al., 2012). On the contrary, when previously medicated

patients were compared with controls, increased binding was reported (Abi-Dargham et al., 2000; 2009), although another study also found increased binding levels for both AP-free patients and controls (Kegeles et al., 2010). This led to speculate about the possible effect of the treatment on D_2R availability in these patients. Furthermore, excess of stimulated and baseline DA release have been shown to predict treatment response, since is higher in patients who respond to APs (Abi-Dargham et al., 2009; Demjaha et al., 2012).

Regarding the response to systemic amphetamine, DA response was not different in the Poly (I:C) compared with saline mice (**Figure4.40 A-B**). There are no studies describing the effect of systemic amphetamine on monoamine concentrations in the striatum of Poly (I:C) MIA mice. Nevertheless, in animal models of schizophrenia, it has been reported that the hyperlocomotion caused by psychostimulants such as amphetamine is related to DA increases in striatal regions (Kelly et al., 1975; Pijnenburg et al., 1976; Kesby et al., 2018), concretely in the ventral striatum, and that it can be blocked by drugs that prevent dopaminergic transmission in that area (Sharp et al., 1987; Kelly et al., 1975; Pijnenburg et al., 1975; 1976; David et al., 2004). In fact, animal studies have shown that the dorsal and ventral striatum may respond differently to amphetamine-like drugs and thus modulate different types of behaviors through a distinct DA response, such as locomotion and stereotypy (Staton & Solomon, 1984; Pijnenburg et al., 1975). In human schizophrenic patients, according to the "revised dopaminergic hypothesis of schizophrenia" which defends the existence of a DA hyperactivity in mesolimbic pathways coexisting with DA hypoactivity in the PFC (Brisch et al., 2014), striatal increased amphetamine-induced DA release has been found (Laruelle et al., 1996; Abi-Dargham et al., 1998; 2009; Pogarell et al., 2012), which correlated with the emergence or worsening of positive psychotic symptoms. In general, these studies do not discriminate if this alteration corresponds specifically to the dorsal or the ventral striatum, but when they do, they mostly refer to the mesolimbic pathway (ventral striatum) (Karam et al., 2010; Kesby et al., 2018). Taking this into account, and when trying to interpret the results presented in this work regarding the response to amphetamine in the MIA model, there is another important methodological connotation to consider. For microdialysis experiments, the probe was placed across dorsal and ventral striatum, following coordinates that reached very close to the shell of the nucleus accumbens (**Figure 3.19**). Thus, the dialysate comes from both the dorsal and ventral striatum together. As a consequence, the probe would be collecting DA mainly from the dorsal striatum but also from the ventral striatum (probably the shell region), resulting in a summation of DA from both regions. This may be the reason why in this study the supposed dopaminergic hyperactivity in the mesolimbic pathway (VTA-ventral striatum) is not completely detectable through these experiments.

In addition, the probe's location could justify the higher NA response to amphetamine observed in the Poly (I:C) model with respect with the saline (**Figure 4.39 A-B**), since it would be dialyzing NA from the dorsal striatum (a preferentially dopaminergic area (Macdonald & Monchi, 2011; Ferrucci et al., 2013)) but also from the ventral striatum (a noradrenergic and dopaminergic area (Berridge et al., 1997; Schroeter et al., 2000)). In fact, noradrenergic innervations from the LC have been described in the ventral striatum (Berridge et al., 1997; Schroeter et al., 2000; Ferrucci et al., 2013) as well as measurements of NA extracellular concentrations (Cenci et al., 1992; Li et al., 1996; Reith et al., 1997; Li et al., 1998; Dawson et al., 2000; Hajnal & Norgren, 2004; McKittrick & Abercrombie, 2007; Karkhanis et al., 2014). In

the present study, the probe is recollecting NA from the D/V striatum, showing an augmented NA response to amphetamine in the Poly (I:C) mice. Thus, this data could imply a possible NA hyperactivity in the ventral striatum of the Poly (I:C) model. Nevertheless, with the objective of clarifying these differences between NA and DA in the two distinct striatal regions in the Poly (I:C) model, it would be interesting to repeat these experiments by placing the microdialysis probe precisely and separately in each of the different striatal areas.

Finally, the fact that the *Ed* function is augmented (**Figure 4.38 B**) but DAT expression levels are decreased (**Figure 4.11**) could also seem incongruent. But, as it has been discussed previously, the *Ed* function includes, apart from the reuptake by the specific transporter, all the possible additional mechanisms that contribute to the clearance of the neurotransmitter from the synaptic space. Therefore, is possible that other metabolic enzymes (COMT) and/or transport mechanism as OCTs, PMATs or even extraneuronal transporters (Bacq et al., 2012; Blakely & Bauman, 2000) could be up-regulated as a compensatory mechanism for the DAT deficiency, ending in an increased *Ed* function.

5.2.2. The 5-HT system in the striatum of Poly (I:C) MIA model

There were no differences in the 5-HT striatal tissue concentrations between Poly (I:C) and saline mice (**Figure 4.4**). The same way, 5-HT extracellular basal concentrations in the area were unaltered, and the response to systemic amphetamine was not different between Poly (I:C) and saline mice (**Figure 4.41**). This data seems to suggest that the 5-HT system is not altered in the striatal areas of the Poly (I:C) model. Despite this, it should be taken into consideration that reported 5-HT extracellular concentrations are also a global outcome from both dorsal and ventral striatum.

Unlike what it is observed in the PFC, the results of the interconnections between the 5-HT system and the DA system do not seem to be as straightforward in striatal areas. As previously commented, several studies have described projections from the raphe nuclei to the two main DA nuclei, the VTA (Jacobs & Azmitia, 1992; Lechin et al., 2006) and the substantia nigra (Dray et al., 1978; Gervais & Rouillard, 2000). Despite the repeatedly described prominent inhibitory role of the raphe nuclei over the VTA neurons (Fibiger & Miller, 1977; Olpe & Koella, 1977) and the possible results of this modulation observed in the PFC of the model, discussed previously, the role of the serotonergic nuclei over the substantia nigra is still ambiguous. Although the majority of studies have reported a predominantly inhibitory role of the dorsal raphe over the substantia nigra neurons (Dray et al., 1978; Gervais & Rouillard, 2000; Delaville et al., 2012), other studies have reported mixed inhibitory and excitatory effects (Collingridge & Davies, 1981; Dray, 1981; Gongora-Alfaro et al., 1997). Therefore, it seems that the raphe modulates differently the VTA and the substantia nigra (Gervais & Rouillard, 2000).

Apart from that, results observed in this work with regard to the monoamine regulation in the PFC may be a reflection of a complex network of interconnections between the PFC, VTA, raphe nuclei, and probably other areas that could be contributing to those effects. Since this interconnections are probably different in relation to the substantia nigra, it should not be odd to identify in the striatum a dissimilar situation in relation to the 5-HT-DA relationship as it has been observed in the PFC of the Poly (I:C) model. In fact, even if projections coming from the

PFC to the VTA are well characterized (Sesack & Carr, 2002), there is still some debate about the direct regulation from the PFC to the substantia nigra. In fact, although in rodents this pathway has been described (Bunney & Aghajanian, 1976; Sesack et al., 1989; Hurley et al., 1991), it is not that evident in the case of primates (Leichnetz & Astruc, 1976; Kunzle, 1978; Frankle et al., 2006; Haber & Behrens, 2014). Hence, it is likely that the modulation of 5-HT concentrations in the striatum is mediated by multiple brain areas and distinctively to the PFC, and therefore observed results between the PFC and the striatum are not comparable.

Consequently, these data suggest there are some differences in the striatum of the Poly (I:C) mice that could imply possible alterations in the mesolimbic and/or nigrostriatal pathways. Nevertheless, the catecholaminergic hypoactivity hypothesized for the PFC seems to be characteristic of that area, and not of the striatum of the Poly (I:C) MIA model.

5.3. COGNITIVE STATUS OF THE POLY (I:C) MIA MODEL

5.3.1. Performance in the NORT

Saline and Poly (I:C) mice performed the NORT test with the objective to assess their spatial/visual learning and memory evocation capacity. Neither the time devoted to the exploration of the familiar object nor the time devoted to the novel object were different between the Poly (I:C) and saline mice (**Figure 4.17-A** and **Figure 4.17-B**). Consequently, the total time devoted to the exploration of both objects was not different either (**Figure 4.17-C**), even if a trend to a lower exploration time was detected in the Poly (I:C) group. However, when evaluating the discrimination index of the two group of mice, Poly (I:C) mice showed a significant reduction in this parameter (**Figure 4.18**), indicative of potential cognitive impairment in the MIA animal model.

There are several studies that have used the NORT as a reliable test to measure cognitive impairment in different MIA animal models. Actually, the Poly (I:C) model has been reported to show disrupted recognition memory and lower discrimination index in other studies using mice (Shi et al., 2003; Li et al., 2014; Prades et al., 2017; Osborne et al., 2017) and rats (Ozawa et al., 2006; Wolff et al., 2011; Luchicchi et al., 2016; Gray et al., 2019) after both short (10 min – 2 h) and long (24 h) delays. It should be noted that even if in each study the followed Poly (I:C) protocol is slightly different (prenatal timing, dose, via of administration, species), cognitive deficits are maintained as a relevant feature of this MIA model. In addition, other studies using different type of MIA models (LPS, Influenza) have also revealed disruption of cognitive abilities such as deficits in PPI and LI (Borrell et al., 2002; Zuckerman et al., 2003; Zuckerman & Weiner, 2005; Romero et al., 2007; Patterson, 2009). In addition to MIA models, other models of schizophrenia have also been tested in the NORT. Models based on the administration of NMDA receptor antagonists, also considered as putative models of schizophrenia, have been described to induce cognitive impairment in the NORT. For instance, acute and subchronic administration of PCP in rats (Grayson et al., 2007; Redrobe et al., 2010) and mice (Hashimoto et al., 2005; Nagai et al., 2009) has been described to produce significant disruption of recognition memory. Also, acute ketamine administration in rats consistently impaired performance in the NORT (Pitsikas et al., 2008). Acute MK-801 administration has been described to produce disrupted performance in the NORT in rats (King et al., 2004; Pichat et al., 2007; Karasawa et al., 2008); however in mice, depending on the stage of the task when it was administered, MK-801 could increase exploration of the novel object (Nilsson et al., 2007). Impairments in the NORT have also been described for the isolation-rearing model of schizophrenia in rats (Bianchi et al., 2006; McLean et al., 2011).

In human schizophrenic patients, visual learning and memory deficits have been observed (Heinrichs & Zakzanis, 1998; Wood et al., 2002; Nuechterlein et al., 2004), as well as in people at ultra-high risk for psychosis (Brewer et al., 2005). Following MATRICS recommendations, several studies have used the Brief Visual Memory Test to describe deficits in visual learning and memory in schizophrenia patients (Woodward et al., 2004; Schretlen et al., 2007; Unschuld et al., 2014; Reed et al., 2016; Mohn & Torgalsboen, 2018). In fact, schizophrenia patients showed deficits in the immediate recall, delayed recall, and the recognition components of the task (Schretlen et al., 2007). In addition, apart from Brief Visual Memory

Test, other tasks similar to the NORT in rodents have been used in schizophrenic patients, showing deficits in 2-D object recognition (Clare et al., 1993; Aleman et al., 1999; Heckers et al., 2000; Tek et al., 2002). Although no imaging studies have been carried out in patients performing the Brief Visual Memory Test, it has been shown a different pattern of thalamic and prefrontal brain activation between patients and controls when performing object recognition tasks (Heckers et al., 2000).

Thus, the Poly (I:C) MIA model presents visual learning and memory deficits measured by the NORT, which reinforces its validity as a model of CIAS.

5.3.2. Performance in the 5-CSRTT

In the present work, a variation of a touchscreen-based cognitive testing method (Bussey et al., 2001; 2012) of a 5-CSRTT for mice previously described (Humby et al., 1999; Robbins, 2002; Mar et al., 2013) was used for measuring different components of attention (sustained, divided attention) and cognitive speed processing, as well as behavioral responses related to impulsivity and motivation in the Poly (I:C) and saline mice. This test consists of different stages the animals have to go through until they "learn" to perform the test ("pre-training" and "training to baseline"). Once they have learned (reach the "baseline"), their cognitive abilities can be assessed through test manipulations ("testing/task manipulations").

5.3.2.1. Pre-training phase: weight registration, preference-test, must touch stimuli and must initiate training

The protocol of 5-CSRTT followed in the present study involved water deprivation in order to motivate the animals for a liquid reward during the performance of the task. In this study, it was decided to use a liquid reward as it is believed to be a better option for mice (Mar et al., 2013). Actually, liquid rewards reduce the risk of satiation and associated reduced motivation and besides, mice are quick to consume the reward and are ready to attend to the test as soon as exiting the reward tray. Since this deprivation started before the training itself, monitoring the weight of the animals during the whole experiment (see **Figure 3.12** for chronogram) was essential. **Figure 4.19** shows weight evolution through the experiment. Although the analysis by the two-way ANOVA revealed differences between Poly (I:C) and saline mice, this difference corresponds to the distinct grow slope of each of the group of mice. Indeed, saline and Poly (I:C) mice have a differentiated growth rate over time, causing the differences detected by the statistical analysis. In any case, by the time the training of the task started, weights were comparable between both groups of mice.

In order to motivate the animals to the liquid reward (10 % CM in water), a preference-test CM *vs.* water was carried out until both groups of animals showed a preference for the CM. Apart from motivating animals to the reward, the aim of this test was to detect any possible difference that could influence their behavior based on the reward. Regarding the total consumed volume (**Figure 4.20-A**), there were increases across time until both groups consume the same volume on day 12^{th}. Even if there were slight differences between Poly (I:C) and saline during the days the preference-test lasted, the last day of the test (day 12^{th}) both groups of mice consumed the same total volume of liquid. As for the preference for the CM *vs.*

water (**Figure 4.20-B**), it was increased over time in both groups of mice without any differences. On day 12th, both Poly (I:C) and saline mice showed equal preference for the CM over water. This data indicates that there were no alterations in weight, palatability (preference for CM) and/or food needs between saline and Poly (I:C) mice, and also that these mice were ready to start the training phase based on reward behavior.

Before finishing the pre-training stage, mice went through the Must Touch Stimuli and Must Initiate pre-training phases. The main objective of these phases was to get the animals ready, both by familiarizing them to the operant chamber and teaching them to nose-poke the touch-screen and collect the reward. Both groups of mice were capable of reaching the criterion required in each of both phases (complete 30 trials in 60 min) to pass to the following phase, and there were no differences in the number of days Poly (I:C) and saline mice spent in each of the phases (**Figure 4.21A–B**). These data suggest that both groups of animals would be able to perform the task in the next phases since none of the groups suffers any motor, visual or any other type of sensorial impairment that could affect them negatively in the performance.

5.3.2.2. Training to baseline

After the pre-training and when animals were ready, the training to baseline started. During this phase consisting of a total of eight sessions with increased difficulty and a specific SD, mice had to reach an established criterion to pass to the following session (≥80 % of accuracy and ≤20 % of omissions during two consecutive days). As for accuracy percentage, both groups of mice were able to manage to reach and exceed the 80 % of accuracy and maintain it during the whole training, with no differences between the two groups (**Figure 4.22-A**). Regarding the percentage of omissions, the percentage increases during the first sessions as the difficulty of the task augments (**Figure 4.22-B**). Despite this, at a certain point during the training and although the SD decreases, the percentage of omissions stabilizes and remains unchanged until the task ends, showing no differences between Poly (I:C) and saline mice. Finally, as for the days each group of mice spent in each of the sessions for a specific SD, there were no differences between Poly (I:C) and saline mice (**Figure 4.22-C**).

Therefore, all this data suggest that the Poly (I:C) mice do not need more time to perform equally well to saline mice in the training phase. Additionally, it could also be said that the capacity to process spatial information and the behavior based on reward is not affected in this MIA model.

5.3.2.3. Testing: task manipulations

In order to further asses the attentional function of these mice, especially the cognitive speed processing and cognitive flexibility, the basic 5-CSRTT was modified to get two different variations of the task based on modifications of the standard parameters. “Variation 2” was made adding a distractor noise during the delay time, with the objective to distract the animals from their task. “Variation 1” was made altering the duration of the delay time and making it shorter, variable in duration and offered randomly, in order to increase attentional load required to complete the task. First “Variation 2” was introduced and consecutively, “Variation 1”.

Regarding the accuracy, both Poly (I:C) and saline mice were able to reach accuracy requirement in both "Variation 2" (distractor noise) and "Variation 1" (alteration of the delay) (**Figure 4.23-A**), suggesting both groups were able to perform equally well. As for the omissions, "Variation 2" did not affect the performance of saline or Poly (I:C) mice (**Figure 4.23-B**). However, the introduction of "Variation 1" showed differences in the performance of both saline and Poly (I:C) mice. On the one hand, saline mice reduced their omission percentage with respect to the baseline and more importantly, with respect to "Variation 2", performed previously. This is indicative of the ability of saline mice to learn how to perform the task accurately as time goes by even if the complexity increases. However, on the other hand, Poly (I:C) mice maintained their omissions in both "Variation 2" and "Variation 1" with respect to baseline. In addition, Poly (I:C) percentage of omissions in "Variation 1" was higher compared with saline mice (**Figure 4.23-B**). This data suggest that Poly (I:C) mice, contrary to what happens with saline mice, are not able to adapt correctly to perform a more complex task when requirements are more strict. Thus, it seems that the findings are indicative of lack of cognitive flexibility.

When measuring premature responses in these mice, there were no differences between Poly (I:C) and saline mice. As for saline mice, "Variation 2" did not modify their premature responses, but when performing "Variation 1" they reduced their premature responses significantly compared with both baseline and "Variation 2" (**Figure 4.23-C**). Therefore, as it happened with omissions, saline mice are able to learn during the process and the complexity of the task does not prevent them from decreasing their premature responses thus improving their performance. Regarding Poly (I:C) mice, a similar pattern can be observed, since "Variation 2" did not modify their behavior, but in "Variation 1" they also reduced their premature responses with respect to both baseline and "Variation 2" (**Figure 4.23-C**). Hence, both groups of mice are capable of improving their performance and reduce their premature responses across time. This is indicating that Poly (I:C) exposure does not affect their inhibitory control and does not result in increased impulsivity in these mice.

Finally, the time needed by each group of mice to make a correct response was also measured (latency to correct response). In this case, saline mice were not affected by any of the two variations of the task (**Figure 4.23-D**), demonstrating that as a result of the learning process and despite the complexity of the task increases, they do not need more time to respond correctly. Poly (I:C) mice, however, performed differently. Although in "Variation 2" they did not increase their latency to correct response, in "Variation 1" their latency was significantly higher compared to baseline and to "Variation 2". Moreover, in "Variation 2" the time Poly (I:C) mice needed to respond correctly was significantly higher compared to saline mice (**Figure 4.23-D**). Therefore, similar to what it was observed with the number of omissions, these data suggest that Poly (I:C) mice need more time to respond correctly when the requirements of the task are more complex.

As a conclusion, this data demonstrates that Poly (I:C) mice do not show metabolic, locomotor or reward behavior alterations. Actually, these mice are able to learn at the same speed as the saline mice and they are also capable to perform the task as accurately as saline animals do. However, when the complexity and/or the requirements of the task change or increase, Poly (I:C) animals show attentional impairment and signs of deficits in adaptation. That is, although

they manage to complete the task correctly as saline mice, they need more time to do so, thus lacking cognitive flexibility and speed of processing, as is characteristic of schizophrenia patients. Therefore, the Poly (I:C) MIA model could be considered a good model of CIAS.

Although several studies have been conducted in rodents and especially in rats performing the 5-CSRTT, regarding MIA models, currently there are no studies evaluating the cognitive status of the Poly (I:C) model by the 5-CRSTT, being this study the first one to do so. However, in a MIA model of intrauterine injection of LPS followed by acute LPS administration during adulthood ("second hit"), performance in the 5-CSRTT of the offspring animals was impaired compared to when they were only affected by the first LPS administration (Makinson et al., 2019). In relation to publications specifically addressing perturbations in potential models of schizophrenia, there are limited and usually mixed results about them. The neonatal ventral hippocampal lesion model of schizophrenia developed by Lipska and Weinberger (Lipska & Weinberger, 1995) has been shown to induce some attentional deficits in the 5-CSRTT, but posterior studies with the same lesion performed in adulthood also exhibited deficits, indicating non-specificity of this effect to neurodevelopmental origins (Le Pen et al., 2003). The isolation-rearing model of schizophrenia has also been explored with the 5-CSRTT, although results on attentional processes were not encouraging. The performance was not impaired in the baseline task, and when task manipulations were introduced, no attentional deficits were observed, although they showed increased perseverative responses (Dalley et al., 2002). Models based on the administration of NMDA receptor antagonist as PCP or MK-801 have also been tested with the 5-CSRTT. PCP systemically administered in rats showed a wide range of impairments in the 5-CSRTT and in other variations of it (Jin et al., 1997; Le Pen et al., 2003; Amitai et al., 2007), such as reductions in accuracy and correct responses and increases in latency to correct response, premature and perseverative responses. MK-801 administration has also been shown to produce an impaired performance in rats, with reductions in accuracy and increments in omissions and premature responses (Paine et al., 2007; Paine & Carlezon, 2009). As for genetically modified models of schizophrenia, the α_7 nicotinic acetylcholine receptor KO mice exhibited impaired attentional performance in the 5-CSRTT, with increased omissions and premature responses and reduced accuracy (Young et al., 2004; 2007; Hoyle et al., 2006).

What has been described for animal models go in overall accordance with results of performance in cognitive tests in human schizophrenic patients. As commented before, in human studies the tests more frequently used to measure attention and cognitive performance are the Continuous Performance Test and the Wisconsin Card Sorting Test, being widely accepted that these patients suffer from cognitive impairment as a core symptom of the disorder (Young et al., 2009). There are several studies reporting impaired cognitive performance in schizophrenia patients measured by those tests (Nelson et al., 1998; Avila et al., 2006; Sanz et al., 2012; Ertekin et al., 2017; Wang et al., 2018) and in different versions of variations of them (Elvevag et al., 2000; Salgado-Pineda et al., 2003; Rapisarda et al., 2014; Ang et al., 2017). In general, it is described these patients present reduced accuracy and impaired sustained and focused attention. In addition, they show augmented reaction times, higher number of omissions and difficulties in "rapidly encoding information" (Elvevag et al., 2000). In fact, reduced focused and sustained attention has been reported to correlate with negative

symptoms (Sanz et al., 2012). Therefore, cognitive deficits in schizophrenia still remain an unmet clinical need.

5.4. EFFECTS OF α_2-ADRENOCEPTOR COMPOUNDS ON THE NEUROCHEMICAL AND BEHAVIORAL DEFICITS OF THE POLY (I:C) MIA MODEL

5.4.1. Effects of the α_{2A}-adrenoceptor agonist guanfacine on the performance in the NORT and on monoamine release in the PFC of the Poly (I:C) mice

For evaluating the effect of the α_{2A}AR agonist guanfacine in the NORT, a previous dose-response pilot study was conducted in saline animals in order to find the most appropriate dose of guanfacine that lacked one of its most common side effects, sedation (Hunter et al., 1997). As previously commented, antihypertensive and bradycardic effects are some of the most characteristic effects provoked by α_2AR agonists and specifically, sedation and hypotension seem to be mediated mostly by the α_{2A}AR subtype (Arnsten et al., 1988; MacMillan et al., 1996; Hunter et al., 1997). The related literature has reported a wide dose range of guanfacine eliciting behavioral changes (from 0.001 to 3 mg/kg) and the majority of rodent studies where WM was improved have reported doses between 0.05-0.3 mg/kg (Arnsten & Contant, 1992; Franowicz & Arnsten, 2002; Marrs et al., 2005; Hains et al., 2015). Hence, it was decided to test guanfacine doses of 0.1 mg/kg i.p. and 0.3 mg/kg i.p. *vs.* vehicle (saline) i.p. (**Figure 4.45**). The dose of 0.1 mg/kg i.p. did not affect the exploration time of the animals, contrary to what happened with the dose of 0.3 mg/kg, which decreased significantly their exploration time. Thus, it was decided that 0.1 mg/kg i.p. was the most appropriate dose to use in the subsequent behavioral assays.

When guanfacine was administered to saline and Poly (I:C) animals, it was observed that the exploration time devoted to the familiar object was lower in the Poly (I:C) compared with saline mice when receiving guanfacine and also between Poly(I:C) mice receiving guanfacine *vs*. vehicle (**Figure 4.46-A**). These results suggest that guanfacine affected more intensely the Poly (I:C) mice reducing their exploration time. Regarding the novel object exploration time (**Figure 4.46-B**), there were no differences between Poly (I:C) and saline mice. When considering the total exploration time of both objects, guanfacine decreased the exploration time of Poly (I:C) mice compared to saline mice (**Figure 4.46-C**). This decrease in the total exploration time could be due to possible sedation caused by guanfacine on the Poly (I:C) mice. In fact, as previously commented, sedation is a classical effect of α_2AR agonism, and seem to be mediated mostly by the α_{2A}AR subtype (Arnsten et al., 1988; MacMillan et al., 1996; Hunter et al., 1997). Therefore, Poly (I:C) mice show greater sensitivity to the effects of guanfacine than saline animals. As it has been previously discussed *(section 5.3.1.*), Poly (I:C) animals in basal conditions showed a significant reduction in the discrimination index parameter compared to saline animals, indicative of cognitive impairment (**Figure 4.18**). This observation is corroborated when evaluating the discrimination index of these animals receiving vehicle (**Figure 4.47**) since again Poly (I:C) mice showed lower discrimination index when administered vehicle compared to saline animals. However, when Poly (I:C) animals received guanfacine, their lower discrimination index was reversed (**Figure 4.47**). Therefore, the α_{2A}AR agonist guanfacine was able to neutralize the cognitive deficits showed by Poly (I:C)

mice in the NORT, without producing any significant effect on the performance of saline mice. Besides, even if the total exploration time of the Poly (I:C) mice was lower than saline mice when receiving guanfacine, these mice were able to discriminate better the novel object *vs*. the familiar object. Therefore, although causing certain degree of sedation, guanfacine was capable of producing beneficial effects in the Poly (I:C) MIA model.

Currently, the present study is the first one assessing the effects of guanfacine on the cognitive performance of the Poly (I:C) mice. In fact, there are not studies evaluating guanfacine's beneficial effects on cognition in any other MIA model. However, there is abundant evidence reporting the beneficial effects on spatial memory and WM performance of α_{2A}AR agonists. Studies in both healthy (Franowicz & Arnsten, 1998), aged monkeys (Arnsten, 1997; Arnsten & Goldman-Rakic, 1998; Mao et al., 1999), and aged rats (Tanila et al., 1996; Ramos et al., 2006) have shown improved performance on spatial WM tasks with α_{2A}AR agonists. Besides, imaging studies in monkeys prior to performing a memory task showed that α_{2A}AR agonist administration increased blood flow in the dorsolateral PFC (Avery et al., 2000) and in the principal sulcus of the PFC, an essential region for this cognitive function (Goldman & Rosvold, 1970). Actually, the effects of these compounds seemed to be more potent in cases of catecholamine depletion, suggesting actions at postsynaptic α_{2A}ARs (Arnsten & Goldman-Rakic, 1985; Cai et al., 1993). Guanfacine, as a selective α_{2A}AR agonist, has been reported to be the most effective compound improving WM without marked side effects in aged monkeys (Arnsten et al., 1988; Rama et al., 1996). Guanfacine presents the highest affinity for the α_{2A}AR (dissociation constant (K_d)= 17 nM), compared to the α_{2C}AR (K_d= 835 nM) and the α_{2B}AR (K_d= 2000 nM) (Uhlen & Wikberg, 1991; Uhlen et al., 1994; 1995). Clonidine however, is considered a non-selective α_2AR agonist which despite having higher affinity for the α_{2A}AR subtype (Kd= 20 nM), it also binds to the α_{2C}ARs (Kd= 155 nM) and α_{2B}ARs (Kd= 147 nM) (Uhlen & Wikberg, 1991). Actually, guanfacine presents a more selective profile compared to clonidine since guanfacine is about 10 times weaker in inhibiting the firing of the NA cell bodies in the LC or in decreasing NA release (Engberg & Eriksson, 1991), but improving WM in aged monkeys is 10–100 times more potent than clonidine (Arnsten et al., 1988). Besides, it was observed that guanfacine's beneficial effects on performance could be dissociated from the sedative and hypotensive effects if the dose is adequate (Arnsten et al., 1988) since higher doses could also bind to presynaptic α_{2A}ARs on PFC terminals and impair WM (Hassani et al., 2017). In addition, guanfacine has been described to preferentially bind to postsynaptic α_{2A}ARs in the PFC (Uhlen et al., 1995; Kawaura et al., 2014). This hypothesis is supported by observations where guanfacine's beneficial effects in an attentional test were not affected by treatment with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), a selective noradrenergic neuron neurotoxin (Kawaura et al., 2014). Indeed, DSP-4 has been reported to destroy NA terminals in the PFC (Heal et al., 1993), to reduce the number of presynaptic α_2ARs (Heal et al., 1993), and to attenuate α_2AR-mediated NA release in the PFC (Prieto & Giralt, 2001). Apart from that, it has been demonstrated that in rats guanfacine dose-dependently prevented deficits on spatial WM induced by the administration of NMDA receptor antagonists such as PCP (Marrs et al., 2005). Supporting that beneficial effects of α_{2A}AR agonists like guanfacine are mediated through postsynaptic α_{2A}ARs (Arnsten & Cai, 1993), there are studies showing that these favorable effects of α_{2A}AR agonism become more evident in animals with destroyed or

depleted presynaptic elements by 6-hydroxydopamine (Arnsten & Goldman-Rakic, 1985) or reserpine (Cai et al., 1993).

In the case of human studies, guanfacine has been widely used for the treatment of a variety of PFC cognitive dysfunctions and there is clinical evidence supporting its beneficial effects on cognition. Between its positive therapeutic effects, guanfacine has been proven to increase regional blood flow in the frontal lobe of healthy adults when measured by PET imaging (Swartz et al., 2000). In the case of ADHD patients, guanfacine showed to help these patients to control their own behavior and improve PFC tasks such as vigilance and WM (Biederman et al., 2008; Sallee et al., 2009). Also, it has been proven to be effective in different open-label trials (Chappell et al., 1995; Hunt et al., 1995; Boon-yasidhi et al., 2005) and in placebo-controlled trials (Scahill et al., 2001; Taylor & Russo, 2001), where it improved patients' tics and characteristic symptoms. Apart from that, guanfacine has been demonstrated to be useful in treating PFC dysfunctions in other disorders too. It enhanced WM in patients with a schizotypal disorder with cognitive deficits (McClure et al., 2007) and improved PFC function and metabolism in patients with epilepsy (Swartz et al., 2000). It is being tested in patients with autism to treat behaviors related to social deficits (Scahill et al., 2006; McCracken et al., 2010), and it has been proven useful in schizophrenia patients too (Friedman et al., 2001). Indeed, in subjects with schizotypal disorder guanfacine showed efficacy normalizing cognitive performance (Friedman et al., 1999; McClure et al., 2007) and it has been proven to be more effective than placebo when combined with cognitive psychotherapy and social skills training (McClure et al., 2019).

It might worth wondering if beneficial effects of α_{2A}AR agonists as guanfacine are exclusively due to action on postsynaptic α_{2A}ARs or if they would also be a result of the increase in cerebral vascular flow provoked by α_{2A}AR-mediated vasodilatation (MacMillan et al., 1996), resulting in improved cognitive performance. In fact, there is evidence indicating the emerging role of endothelial NO in linking cognition with cerebrovascular function, given its ability to affect the functional status of microglia and thus modulate cognitive function (Katusic & Austin, 2014).

In order to confirm the mechanism responsible for the beneficial results of guanfacine on cognitive performance, its effects on the PFC monoamine concentrations were also evaluated in both groups of mice. It was observed that there were no remarkable effects of guanfacine on any of the monoamine concentrations, neither in the Poly (I:C) mice nor in the saline mice (**Figures 4.50 A-B; 4.51 A-B; 4.52 A-B**). Concretely in the cases of NA and DA concentrations and especially in saline mice, a transient increase was observed just after the systemic administration of the drug, effect that could be attributed precisely to the stress generated to the animal during the processes of handling and injection of the drug (**Figures 4.51-A** and **4.52-A**).

As previously introduced, the α_{2A}AR is the most prevalent α_2AR in the rodent PFC, and has been found both presynaptically on NA terminals acting as an autoreceptor, but also postsynaptically on dendritic spines and cellular soma of pyramidal neurons of the PFC (Aoki et al., 1994; Ramos & Arnsten, 2007; Wang et al., 2011b). In addition, α_{2A}ARs can also be located in the LC, presynaptically and/or in postsynaptic/somatodendritic areas, acting as

autoreceptors and controlling the release of NA in the LC and terminal areas (Aoki et al., 1994; Wang et al., 2007; Okada et al., 2019). In human PFC, the α_{2A}AR subtype is the most abundant subtype and it seems to be located mainly postsynaptically (Erdozain et al., 2018a). The α_{2A}AR agonist guanfacine, as it has been commented before, shows the highest selectivity for the α_{2A}AR subtype (Kd-ratio α_{2A}/α_{2C}= 0.021; α_{2A}/α_{2B}= 0.0095) among α_{2A}AR agonists (Uhlen & Wikberg, 1991). Taking this data into consideration, the effects exerted by guanfacine could be mediated by action either on presynaptic α_{2A} autoreceptors located in PFC NA terminals or the ones located in the LC or on cortical postsynaptic α_{2A}ARs. Considering the results of this study, if guanfacine would be acting preferentially on presynaptic/somatodendritic α_{2A}ARs located in the LC, or α_{2A}ARs of the axon terminals of NA cells projecting to the PFC, a decrease in NA concentrations would have been expected in the PFC. In fact, guanfacine has been previously described to reduce NA concentrations as opposed to the increases provoked by α_2AR antagonist administration (Fresquet et al., 2007; Okada et al., 2019). Also, other microdialysis studies have shown that administration of α_2AR agonists/antagonists contributes to control NA release acting through α_2ARs located in presynaptic/somatodendritic areas and NA axon terminals (Dennis et al., 1987; Mateo et al., 1998; Ortega et al., 2010). In this study, however, no decreases in NA concentrations were detected when guanfacine was administered, and more importantly, this drug was able to reverse the cognitive deficits showed by the Poly (I:C) mice in the NORT, as discussed previously. Therefore, it is likely that guanfacine would be acting preferentially on the postsynaptic α_{2A}ARs located on pyramidal neurons of the PFC, where its major mechanism of action would be the modulation of cortical neurotransmission (Ramos & Arnsten, 2007). When activating postsynaptic α_{2A}ARs, probably by a reduction of the AC/cAMP signaling pathway, inhibition of HCN channels would occur. Closure of these channels would strengthen PFC synaptic connections, increasing the efficacy of network inputs and facilitating the PFC to regulate more effectively attention, behavior, and emotions (Wang et al., 2007; 2011a). Based on recordings from behaving primates, it is postulated that through postsynaptic α_{2A}ARs guanfacine would strengthen the signal-to-noise ratio during the performance of an attentional task either by increasing task-related activity (Li et al., 1999) or decreasing background activity (Sawaguchi et al., 1990). Since no differences in cortical NA concentrations were observed, and cognitive improvement of the Poly (I:C) mice was achieved in the NORT, it seems feasible that the dose of guanfacine used in this study was the most appropriate to ensure guanfacine's action at postsynaptic α_{2A}ARs in pyramidal neurons of the PFC, without acting on presynaptic/inhibitory autoreceptors in the LC or PFC NA axon terminals.

Regarding DA and 5-HT, the presence of presynaptic α_{2A}ARs has also been demonstrated in the VTA and DA terminal projections to the PFC (Castelli et al., 2016) as well as in the raphe nuclei and 5-HT projection terminals (Unnerstall et al., 1984; Scheibner et al., 2001b). However, the absence of effects exerted by guanfacine on DA and 5-HT concentrations in the PFC supports the previous hypothesis that guanfacine is not acting either upon presynaptic α_{2A}ARs located in the VTA or raphe nuclei nor in the α_{2A}ARs of the correspondent DA or 5-HT terminals in the PFC, since no decreases in the two monoamine cortical concentrations were detected in this study.

Therefore, the α_{2A}AR agonist guanfacine at a dose of 0.1 mg/kg i.p. is able to improve cognitive impairment in the Poly (I:C) MIA model probably acting through postsynaptic α_{2A}ARs in cortical

pyramidal neurons, reinforcing synaptic connections and allowing the PFC to regulate more efficiently cognitive functions. At the same time, the selected dose in the present study prevents guanfacine from acting on presynaptic α_{2A}ARs and the consequent monoamine decrease in the PFC.

5.4.2. Effects of the α_{2C}-adrenoceptor antagonist MK-912 on the performance in the NORT and on monoamine release in the PFC of the Poly (I:C) mice

As it was conducted for guanfacine, the effect of the α_{2C}AR antagonist MK-912 on the NORT performance of the Poly (I:C) and saline mice was evaluated. It has been described that MK-912 is a potent α_{2C}AR antagonist and several studies have demonstrated preferential binding at α_{2C}ARs compared to α_{2A}ARs. Indeed, MK-912 has shown to present lower K_d values for the α_{2C}AR compared to the α_{2A}AR; 0.086 nM *vs.* 1.25 nM respectively. Therefore, it has been reported its selectivity for the α_{2C}AR is 14-fold *vs.* the α_{2A}AR (Uhlen et al., 1992; Marjamaki et al., 1993; Uhlen et al., 1994; 1995; 1998). In the NORT test, it was decided to use 0.05 mg/kg i.p. based on previous literature using MK-912 for cognitive improvement (Franowicz & Arnsten, 2002).

When MK-912 was administered to both groups of animals, there was not a significant effect of the drug neither on the time mice spent exploring the familiar object (**Figure 4.48-A**), nor in the time spent exploring the novel object (**Figure 4.48-B**) in any of the two groups of mice. Despite this, the Poly (I:C) mice that received vehicle showed reduced exploration time of the novel object, data that support once again their reduced object discrimination abilities. As for the total exploration time of both objects, MK-912 did not produce any effect on any of the groups of mice (**Figure 4.48-C**). Finally, when evaluating the discrimination index (**Figure 4.49**), two main significant effects were observed. On the one hand, the Poly (I:C) mice receiving vehicle showed reduced discrimination index compared with saline mice, supporting what has been repeatedly described in the present study regarding their deficiency in cognitive functions. On the other hand, MK-912 administration produced a significant reduction in the discrimination index of saline mice compared to vehicle, suggesting MK-912 may produce impairment in the NORT performance of saline animals. More importantly and in the same way that happened with guanfacine, MK-912 reversed the reduced discrimination index showed by Poly (I:C) mice, although without reaching statistical significance (**Figure 4.49**). Hence, the α_{2C}AR antagonist MK-912 is able to reverse cognitive deficits in the Poly (I:C) mice without affecting the total exploration time of any of the groups of animals. However, MK-912 also showed disruptive effects in the NORT performance in the saline mice, suggesting a selective beneficial effect of the drug exclusively in the group of mice that showed basal cognitive impairment.

Rodent studies have corroborated the important role of the α_{2C}ARs in managing disorders accompanied by cognitive dysfunction. The present work is the first study assessing the effects of the α_{2C}AR antagonist MK-912 in the Poly (I:C) MIA model. Although there are not studies evaluating the effects of α_{2C}AR antagonists on the cognitive performance of MIA models, reports with other potential models of schizophrenia have described the favorable effects of

the α_{2C}AR antagonism. One study showed that the selective α_{2C}AR antagonist ORM12741 was able to attenuate the NMDA receptor antagonist MK-801-induced disruption in the Morris Water Maze and to improve PCP-induced performance disruption in the 8-arm radial maze in rodents (Sallinen et al., 2013b). Also in PCP/MK-801-administered mice, α_{2C}AR antagonists JP1302 and ORM10921 showed antipsychotic-like properties as they reversed PPI dysfunction (Sallinen et al., 2007; 2013a). Besides, the hypoglutamatergic states provoked in these animals were alleviated, supporting the important role of the α_{2C}AR antagonism in these effects. Similarly, in the social isolation rearing model of schizophrenia in rats, the α_{2C}AR antagonist ORM10921 showed improvement in the NORT performance and reversion of the PPI deficits in these animals, both alone and in combination with antipsychotics like clozapine or haloperidol (Uys et al., 2016). In fact, the combination of α_{2C}AR and D_2R antagonism has been described to potentiate the antipsychotic-like effects in rats, reducing the dose of antipsychotic and improving cognitive parameters (Marcus et al., 2005; Wadenberg et al., 2007). Moreover, observations that activation of α_{2C}ARs produced disruptive spatial search and memory function in mice (Bjorklund et al., 2001) confirm that the beneficial effects described above come from action through these receptors. Nevertheless, there are studies reporting that non-selective α_2AR antagonism can produce detrimental effects on cognition. Actually, Arnsten and Goldman Rakic (Arnsten & Goldman-Rakic, 1985) described PFC function impairment produced by α_2AR antagonism in monkeys when developing cognitive tasks. Following this line, it has been demonstrated that direct infusions of the α_2AR antagonist yohimbine in the monkey PFC impaired spatial WM in a delayed-response task (Li & Mei, 1994). This observation was confirmed by another study describing that yohimbine directly infused in the PFC produced disruptive effects on the inhibitory control of animals performing an attentional task (Ma et al., 2003). Therefore, these observations suggest that selective α_{2C}AR antagonism seems to be necessary for achieving improvements and beneficial effects on cognitive functions whereas the presence of α_{2A}AR antagonism would contribute to undesirable effects on cognitive activity.

Findings described in human studies seem to go in accordance with the reported results from preclinical studies. As previously commented, almost all atypical antipsychotics, apart from the ability to block the D_2R, show moderate-potent levels of α_2AR antagonism, which has been suggested to contribute to the atypical profile of these compounds (Kalkman and Loetscher, 2003). In fact, in schizophrenic patients a combination of mirtazapine (α_2AR and 5-$HT_{2/3}$R antagonist) with the atypical antipsychotic clozapine reduced negative symptoms such as apathy and sociability deficits (Zoccali et al., 2004). Indeed, a meta-analysis evaluating the effects of the addition of α_2AR antagonists to D_2R antagonists showed that in schizophrenic patients combined treatment improved the efficacy of the D_2R antagonists in reducing negative symptoms (Hecht & Landy, 2012). Moreover, it has been suggested that a specifically higher α_{2C}AR/D_2R selectivity ratio is responsible for mediating the improved antipsychotic effect on cognitive function (Shalid et al., 2009). These observations could be explained taking into account that antipsychotics have been described to increase levels of DA but also of NA in the PFC (Li et al., 1998; Westerlink et al., 1998). Considering that NA shows a higher affinity for the α_{2C}AR (Hein et al., 1999; Bunemmann et al., 2001), it would preferentially bind to these receptors. Therefore, it could be speculated that beneficial effects resulting from combination of antipsychotics and α_2AR antagonists could be due to a blockade of the α_{2C}ARs. Supporting

this idea, in another study with patients suffering from AD, it was observed that the α_{2C}AR antagonist OR12741 showed potential efficacy in improving cognitive domains in these patients (Rouru et al., 2013). Furthermore, associations between genetic polymorphism of the α_{2C}ARs and certain aspects of psychotic disorders have also been described (Rivero et al., 2016).

In addition to the NORT performance, in both saline and Poly (I:C) mice the effects on monoamine concentrations in the PFC of the systemic administration of the α_{2C}AR antagonist MK-912 were evaluated. In the case of catecholamines, no significant differences were detected in NA and DA concentrations in either saline or Poly (I:C) mice (**Figures 4.53 A-B** and **4.54 A-B**), suggesting MK-912 at this dose does not alter catecholamine concentrations in the PFC.

It has been previously introduced that the α_{2C}AR distribution in the brain is quite reduced compared to that of the α_{2A}AR. Techniques such as in situ hybridization, binding, and immunohistochemistry have revealed α_{2C}AR can be found in the cerebral cortex, but it is most abundantly expressed in the striatum, modulating presynaptic DA release and DA-mediated behaviors. Other areas include the LC acting as an autoreceptor, the raphe nuclei, the substantia nigra and the VTA, as well as the axon terminals of their projections acting as presynaptic heteroreceptor (MacDonald et al., 1997; Rosin et al., 1996; Scheibner et al., 2001b). In human PFC, the α_{2C}AR subtype has been reported to be distributed more homogeneously in both pre- and postsynaptic locations (60% *vs*. 40%) contrary to the α_{2A}AR, mainly postsynaptic (Erdozain et al., 2018a). Prior studies have described that administration of α_2AR antagonists such as RX821002, idazoxan or RS79948, induced the release of NA (Mateo et al., 1998; Devoto et al., 2004; Fresquet et al., 2007) and DA (Matsumoto et al., 1998; Hertel et al., 1999; Devoto et al., 2004) in the PFC, effects supposedly mediated by presynaptic α_2ARs located on the PFC axon terminals of these catecholaminergic projections. Additionally, the selective α_{2C}AR antagonist ORM10921 has been reported to increase DA concentrations in the PFC, in line with the previous studies (Sallinen et al., 2013a). Thus, this data suggests the existence of a tonic inhibitory effect on cortical catecholamine concentrations mediated by the α_2ARs located in projection axon terminals in the PFC. As for the effect of the α_{2C}AR antagonist MK-912 at the dose tested, if acting through these presynaptic α_2ARs, increases in catecholamine concentrations would have been expected after receptor blockade. In this study, however, NA and DA concentrations were not altered after the administration of MK-912 and additionally, the cognitive impairment shown by the Poly (I:C) mice was also reversed. Then, it could be speculated that the effects of MK-912 on cognitive performance and the absence of effect on cortical catecholamine concentration could be mediated by different α_{2C}ARs. Cognitive improvement could be induced by α_{2C}ARs located in other neurons controlling the activity of cortical pyramidal neurons. Indeed, in the PFC, pyramidal and interneuronal interactions are a key neuronal substrate for the correct PFC functions (Wilson et al., 1994). The activity of PFC is the result of mutual excitation between glutamatergic pyramidal neurons, and it is regulated by inhibitory GABA interneurons. Thus, when performing a specific task, the activity of the PFC is modulated by GABA from intracortical interneurons and also by glutamate from the cortex and the thalamus (Kritzer & Goldman-Rakic, 1995). Besides, the presence of α_2ARs in cortical GABAergic interneurons has been reported (Wang et al., 2011b). However, there is no clear evidence about which α_2AR subtypes

are included in these subpopulations since the classification of α_2AR subtypes in GABA interneurons is still unclear (Kawaguchi & Shindou, 1998). Despite this, NA has been described to inhibit GABA release in the PFC when acting upon α_2ARs located in GABAergic terminals (Stevens et al., 2004) probably acting at somatodendritic domains of these interneurons (Hirono & Obata, 2006). Moreover, it has been reported that the α_2AR agonist clonidine reduces GABA-evoked inhibitory response in the majority of PFC pyramidal neurons, suggesting that stimulation of α_2ARs in GABAergic terminals leads to a disinhibition of cortical pyramidal neurons (Hirono & Obata, 2006; Wang et al., 2011b). Numerous evidence has suggested that WM impairments in schizophrenia could be a consequence of a reduction in GABA-mediated inhibitory modulation over pyramidal neurons (Schmidt & Mirnics, 2015). Actually, dysfunctional GABA interneuron activity can disrupt the excitatory/inhibitory balance in the PFC, which is considered a pathophysiological mechanism underlying cognitive dysfunction in schizophrenia (Uhlhaas, 2011; Xu & Wong, 2018). Indeed, as a result of a decline in GABA synthesis in certain interneuron population in the cortex (Akbarian et al., 1995; Benes & Berretta, 2001; Lewis et al., 2001), a lack of inhibition in the PFC default mode network has been described to affect patients when executing WM and attentional tasks (Sakurai et al., 2015). Also, the severity of positive symptoms seems to be correlated with over-increased functional connectivity of the medial PFC in schizophrenia (Whitfield-Gabrieli et al., 2009). In addition, deficiency in GABA signaling over pyramidal neurons is also consistent with the glutamatergic hypothesis of schizophrenia (Goff & Coyle, 2001), which postulates deficits in the NMDA receptor-mediated signaling on GABA interneurons leading to a lack of cortical synchronization and loss of efficacy in cortical neural connections.

Accordingly, if the α_{2C}AR antagonist MK-912 would be acting on α_2ARs located in GABAergic interneurons, it would provoke an increase of GABA release and enhance GABA-mediated inhibitory output over pyramidal neurons, thus suggesting a tonic inhibitory control of GABA release over pyramidal neurons, as it has been previously suggested (Kawaguchi and Shindou, 1998). This way, it would restore pyramidal neuronal inhibition, normalizing cortical aberrant neuronal oscillations and therefore improving performance on WM and attentional tasks. Since in this study MK-912 reversed cognitive impairment in the Poly (I:C) mice and did not alter cortical catecholamine concentrations, MK-912 acting through α_2ARs in GABAergic terminals could be a feasible mechanism for the observed effects. Following this hypothesis, the reported negative effects of MK-912 in the performance of saline mice in the NORT might also go in accordance with the proposed mechanism. Unlike the Poly (I:C) MIA animal model, in saline animals there should not be a hypofunction of the GABA-mediated inhibitory signaling activity, thus there would not be an inefficacious pyramidal neuron synchronization leading to a potential cognitive deficiency (*as demonstrated in section 4.1.3.*). Therefore, the action of MK-912 on α_2ARs on GABA interneurons would increase GABA output over pyramidal neurons, reducing their normal basal synchronized activity. This effect could result in a disruption of the correct performance of these animals in cognitive tasks as the NORT. Therefore, the reported effects of MK-912 and produced by α_2AR antagonism are presumably mediated by α_{2C}ARs located in GABAergic neurons. However, it cannot be confirmed with certainty that observed effects would exclusively be α_{2C}AR-mediated since all the evidence describing the presence of α_2ARs in GABAergic neurons do not specify which α_2AR subtypes conform these subpopulations (Pittaluga & Raiteri, 1988; Moroni et al., 1983; Beani et al., 1988; Hirono &

Obata, 2006). Hence, it would be interesting to measure the expression levels of these α_2AR subtypes in GABAergic neurons in order to clarify this issue.

Unlike catecholamines, 5-HT concentrations were increased by systemic administration of MK-912, showing a higher response of 5-HT concentrations in the PFC of saline mice compared to Poly (I:C) mice (**Figure 4.55 A-B**). Previous studies have described that cortical 5-HT concentrations are subjected to a tonic negative noradrenergic control by α_2ARs located on 5-HT nerve terminals in the PFC as well as on noradrenergic terminals located in the raphe nuclei (Svensson et al., 1975; Gothert et al., 1981; Freedman & Aghajanian, 1984; Starke et al., 1989; Garratt et al., 1991; Maura et al., 1992). In fact, it has been reported that cortical administration of the α_2AR antagonist idazoxan increases cortical 5-HT concentrations in a dose-dependent manner (Doxey et al., 1983; Garratt et al., 1991). Other study described the α_2AR agonist medetomidine inhibited the release of 5-HT, an effect that was antagonized by α_2AR antagonist rauwolscine (Scheibner et al., 2001a). Furthermore, other reports supported these findings describing that activation of α_2ARs in the cortical serotonergic terminals decreased 5-HT release at the same time that α_2AR antagonists blocked this effect (Uys et al., 2017a). As for α_2ARs located in NA terminals in the raphe nuclei, systemic administration of the α_2AR antagonist idazoxan increased the firing rate of the raphe nuclei and 5-HT release in the PFC (Hertel et al., 1997). Additionally, it has been described that activation of these receptors by the α_2AR agonists clonidine and S3341 has an inhibitory effect on the dorsal raphe's activity (Dresse & Scuvee-Moreau, 1986), probably by decreasing the tonic noradrenergic excitatory influence of α_1ARs located on 5-HT cell bodies, which modulate the activity of ascending 5-HT projections (Baraban & Aghajanian, 1980; Verbanac et al., 1996; Adell & Artigas, 1999). Regarding to this, a basal excitatory tonic activation of α_1ARs of the raphe nuclei has been described, which seems to be already maximal at resting conditions (Bortolozzi & Artigas, 2003; Pudovkina et al., 2003) since the selective α_1AR antagonist prazosin was capable of dose-dependently inhibit 5-HT cell firing in the raphe (Gartside et al., 1997) and the 5-HT release in the PFC (Pudovkina et al., 2003). However, there are other studies reporting that further stimulation of α_1ARs in the 5-HT neurons in the raphe nuclei is possible, which enhances the firing rate of 5-HT cells (Svensson et al., 1975) and increases 5-HT concentrations in terminal areas (Rouquier et al., 1994; Hjorth et al., 1995; Gartside et al., 1997). In any case, it seems that the α_1ARs located in the raphe nuclei could be involved in the α_2AR-mediated effect on 5-HT cortical concentrations.

Therefore, considering all the aforementioned evidence and the results of this work, it could be speculated that MK-912 could act on α_{2C}ARs by two different mechanisms. On the one hand, if acting upon α_2ARs in 5-HT terminals in the PFC, blockade of these receptors would increase 5-HT concentrations in the area. Indeed, there is evidence describing the presence of both α_{2A}ARs and α_{2C}ARs controlling 5-HT release located on 5-HT axon terminals in the PFC (Scheibner et al., 2001b), although it is not known which α_2AR subtype is the main responsible of this effect. On the other hand, if acting upon presynaptic α_2ARs located on NA terminals in the raphe nuclei, α_2AR blockade would increase NA concentrations, stimulate 5-HT cell activity in the raphe probably via α_1ARs, and eventually increase 5-HT release in the PFC. In this case there is also no clear evidence of which α_2AR subtype would be the principal responsible for this effect since the presence of both α_{2A}AR and α_{2C}AR subtypes has been described in the area (Rosin et al., 1996; Scheinin et al., 1994; Hopwood and Stamford, 2001). In any case, both

possibilities seem plausible for explaining the reported effects of MK-912. As for the lower response of 5-HT concentrations observed in the Poly (I:C) mice compared to saline when MK-912 was administered, this data seems to suggest that the tonic inhibitory control of α_2ARs over 5-HT concentrations is somehow attenuated in the Poly (I:C) MIA model. Since in the present study only the systemic effect of MK-912 on cortical monoamine concentration was evaluated, it would be interesting to perform local administration of α_2AR agonist/antagonists in the raphe nuclei and PFC to clarify the possible mechanisms involved in the observed effects in the MIA animal model.

In conclusion, despite through different mechanisms and acting upon α_2ARs on distinct regional and subcellular localization, both the selective α_{2A}AR agonist guanfacine and the α_{2C}AR antagonist MK-912 are able to improve CIAS in the Poly (I:C) MIA model. Thus, both the selective α_{2A}AR agonism on cortical pyramidal neurons and α_{2C}AR antagonism on GABAergic neurons are suggested as promising targets for treating cognition in schizophrenia. Besides, the Poly (I:C) MIA model confirms and reinforces its predictive validity as a useful and translational MIA animal model of CIAS.

5.5. CELL-TYPE SELECTIVE TARGETING OF THE LC-PFC PROJECTION IN MICE

It has been previously commented that regulation of attention, memory and behavioral flexibility are important functions attributed to the networks of the PFC (Devito & Eichenbaum, 2011; Janitzky et al., 2015). There is abundant evidence supporting that NA innervation of the PFC is critical for functions like attention and WM (Devauges & Sara, 1990; Lapiz & Morilak, 2006; Chamberlain & Robbins, 2013). NA projections to the PFC seem to be essential for the ability to rapidly switch attention between stimuli leading to cognitive flexibility, therefore supporting the special role of the NA system in the PFC-dependent functions (Lapiz et al., 2007; Tait et al., 2007; McGaughy et al., 2008). Considering that small changes of catecholaminergic activity in the PFC profoundly affect cognitive functions (Janitzky et al., 2015), it is interesting to study how and to which extent specific changes originated in the LC could affect PFC-dependent cognitive functions.

Optogenetics refers to the integration of optics and genetics to achieve gain or loss of function of well-defined events within specific cells of living tissue (Deisseroth, 2011; Yizhar et al., 2011). It is a method for delivering millisecond precision control (for activation or inhibition) to targeted cells using light within freely behaving mammals (Deisseroth, 2014). As most commonly practiced today, the components of this method involve lasers and fiber optics for light delivery into the CNS (Deisseroth, 2014) and genes called microbial opsins (Gunaydin et al., 2014), which encode light-activated proteins that regulate transmembrane ion conductance. Microbial opsins display diverse color sensitivity and encode ion flow modulators which control the excitability of a neuron by direct manipulation of its membrane potential: either bringing it nearer to or above the threshold of an action potential generation or hyperpolarizing the cell and therefore inhibiting its spiking (Yizhar et al., 2011). These opsins can be targeted using genetic techniques, thereby specifying light-induced current flow in cells defined by function and anatomy. During the last decade, optogenetic methods have started to allow a reversible manipulation of neuronal activity with high genetic and temporal precision (Deisseroth, 2015). Their ability to regulate cellular functions, such as excitability, mobility, proliferation and secretion has transformed many areas of research, such as neuroscience. These methods are opening new perspectives in synthetic biology and promise to make a significant impact on human precision medicine (Bamberg et al., 2018). **Figure 5.1** summarizes the components and steps of optogenetic research.

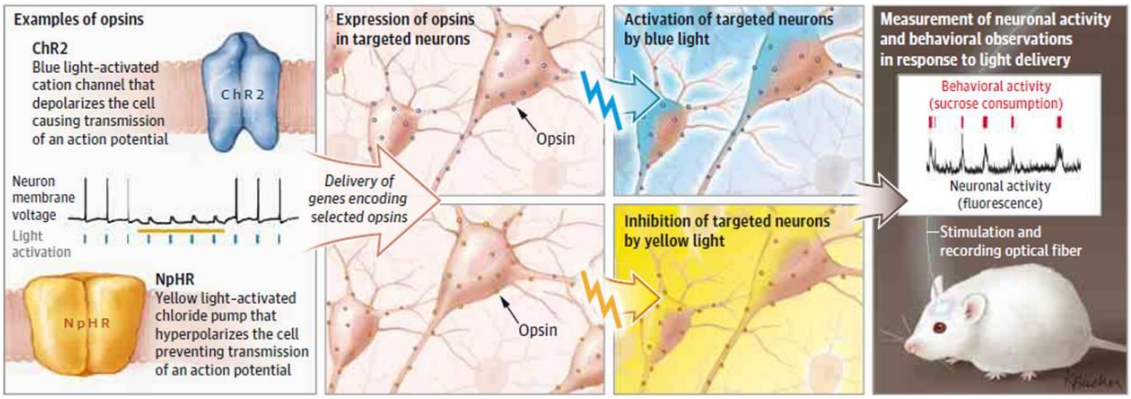

Figure 5.1: Representation of the components and steps in optogenetic research. Adapted from *Deisseroth et al., 2015.*

Furthermore, optogenetic methods are considered well-suited to selectively study the role of small nuclei such as the LC on complex behavior (Carter et al., 2010; de Lecea et al., 2012). While the anatomical projections from the LC and their cell types have been studied for several years, the precise mechanism by which fibers from the LC can directly influence PFC and its role in cognition are still not well described. Bearing this in mind, it was considered intriguing to study more deeply the involvement of the NA system on the PFC-dependent cognitive functions, given that optogenetic techniques would allow a selective and precise study of the neural circuits underlying cognitive behavior. With this objective, a temporary research stay was made under the supervision of Jordan McCall, Ph.D., MPH in the Department of Anesthesiology at Washington University in St. Louis (MO, USA). This research group is interested in the modulatory role of the LC-NA system as a tractable target for clinical intervention and they have extensive experience with optogenetic techniques, which are applied routinely to the study of neurological diseases. **Figure 5.2** schematically summarizes the different key steps on which optogenetic techniques are based.

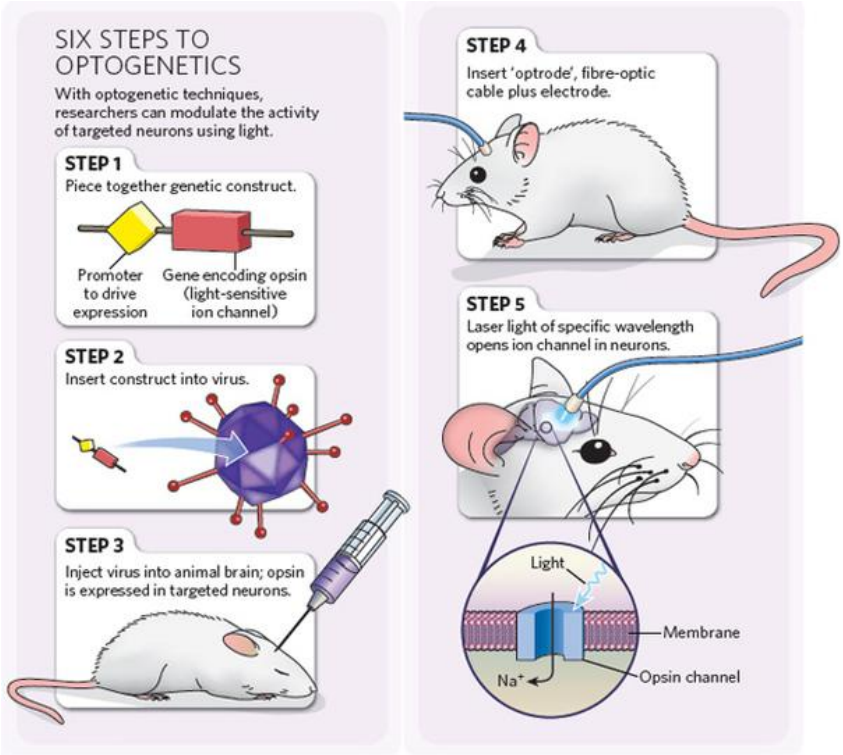

Figure 5.2: Schematic representation of the key fundamental steps on which optogenetic techniques are based. Adapted from *https://optogenetics.weebly.com/why--how.html.*

The principal aim of this stay was to learn the basic fundamentals of the optogenetic techniques and their potential application to study the LC-PFC projection circuitry and its role in cognition. In order to achieve this goal, it was decided to try to optogenetically target the LC-NA input to the PFC selectively and precisely by viral expression systems. Indeed, targeting with viruses has numerous advantages for optogenetics, including rapidity and flexibility of experimental implementation, potency linked to high gene copy number, and capacity for multiplexing genetic and anatomical specificity (Yizhar et al., 2011). Currently, they represent the most popular means of delivering optogenetic tools to intact systems (Deisseroth, 2015; Tervo et al., 2016). Since the previous experience of McCall's group was with strains of mice different from CD1, it was necessary to identify the most suitable strategy that would work out best for this strain. Three different approaches were attempted to identify and isolate the projection by the utilization of viruses; two different retrograde tracing approaches and one anterograde tracing approach.

The first retrograde tracing approach consisted of two viral injections: one in the PFC, a retrograde Adeno Associated Virus (AAV) expressing Cre recombinase and a cytoplasmic red fluorescent protein, mCherry, under the Ef1α promoter of a ubiquitous elongation factor protein in charge of the enzymatic delivery of aminoacyl transfer RNAs to the ribosome for protein synthesis (AAVrg-Ef1α-mCherry-IRES-Cre). In the LC, a Cre-dependent reporter (AAV5-DIO-Ef1α-eYFP), that would allow Cre-dependent expression of an enhanced fluorescent protein (eYFP) (**Figure 4.42**). With this first approach, it was expected that the retrograde virus injected in the PFC would be able to travel backward to the LC through the LC fibers projecting to the PFC. That way, with the Cre-dependent reporter injected in the LC, the fluorescent protein eYPF would only be expressed in the presence of Cre (indicating that the retrograde

virus had been able to travel from the PFC to the LC and infect the cells in the LC). However, although there was expression of the retrograde virus correctly injected in the PFC (in red) (**Figure 4.42-C**), there was no expression in the LC (absence of green and red fibers) (**Figure 4.42-B**). These results indicate that the retrograde virus injected the PFC was not able to travel backward to the LC and infect the cells there. Thus, this approach was discarded.

The second approach consisted of a double viral injection in the LC. The first injection included an anterograde virus expressing Cre under a fragment of the promoter for TH enzyme (AAV9.rTH.PI.Cre.SV40) to ensure that Cre would express selectively in NA neurons of the LC, while the second injection consisted of a Cre-dependent reporter (AAV5-DIO-Ef1α-eYFP), which would only express in the presence of Cre (**Figure 4.43**). With this attempt, it was expected that the anterograde virus would travel forward from the LC via NA projections to terminal areas including the PFC, and thus targeting exclusively the circuitry of interest. In **Figure 4.43-B** it can be observed that there is viral expression in the LC-NA cells (in red and green, a result of the immunohistochemistry staining for the TH positive cells and the expression of eYFP), but not exclusively in this area; there is also expression in non-TH positive cells in areas surrounding the LC (green). Consequently, the expression that can be observed in the PFC (green) (**Figure 4.43-C**) could come from the LC but not in an exclusive manner. That is, it could not be assured that the final outcome in the PFC after stimulation in the LC area would not be affected by the effects exerted by the other infected cells in the surrounding areas of the LC. Therefore, this approach also failed to target selectively the LC-PFC circuit. However, in the case that instead of stimulating the LC, the stimulation would happen in terminal areas of the PFC, it may be a useful approach since we would be stimulating NA terminals coming selectively from NA cells in the LC.

Finally, the third approach was similar to the first one, consisting of two viral injections: in the PFC, a different retrograde virus expressing Cre under the promoter of the PGK, an enzyme necessary in every cell for glycolysis (AAVrg-PGK-Cre), and the Cre-dependent reporter (AAV5-DIO-Ef1α-eYFP) in the LC (**Figure 4.44**). Similar to the first approach, the retrograde virus was expected to travel retrogradely to the LC via NA projections. There, the presence of the injected Cre-dependent reporter would enable expression only in the NA cells projecting to the PFC. Indeed, **Figure 4.44-A** shows viral expression specifically in the NA neurons of the LC (green and red) and **Figure 4.44-B** depicts expression on the PFC projections that come exclusively from the LC (green). This third approach was eventually selected since it best enabled to target selectively the LC-PFC projection in CD1 mice.

Some other authors have also targeted the LC-PFC circuitry using different approaches in order to study LC-NA cell role in analgesia and stress-like adverse effects provoked by pain. For example, Hirschberg and colleagues (Hirschberg et al., 2017) selectively targeted LC neurons from their projections to the PFC in wild-type male rats using axon terminal uptake and retrograde transport of canine adenovirus type 2 (CAV-2) vectors. This vector expresses a chemogenetic actuator (Pharmaco-Selective Actuator Module, PSAM) specifically in NA neurons since it expresses under the NA-cell specific DBH promoter PRSx8. Through this approach, they described that activation of LC neurons is analgesic and that this effect is mediated by NA acting of α_2AR in the spinal cord since the effect was completely blocked by the intrathecal administration of the α_2AR antagonist yohimbine. In addition, Uematsu and

cols. (Uematsu et al., 2017) aimed to study the role in flexible reversal learning, extinction learning and fear of different populations of NA neurons in the LC projecting to the PFC. With that purpose, in the PFC of TH-Cre-recombinase rats, they injected adeno-associated viral vectors (AAV) carrying a construct that encodes a light-activated inhibitory opsin archaerhodopsin (ArchT) fused with enhanced GFP fusion protein under the control of Cre-recombinase (RVΔG-ArchT3.0-EGFP). This virus is taken up by synaptic terminals and transported retrogradely to cell bodies that project to the injected region. By this approach, the PFC-projecting LC-NA neuron activity was optogenetically inhibited.

5.5.1. Future directions

After successfully achieved the selective targeting of the LC-PFC circuit, there is a wide range of options to be explored. For instance, one of the most immediate intentions is to implement this selective targeting into the Poly (I:C) MIA model, and assess whether is possible to analyze the cognitive performance of both saline and Poly (I:C) animals while stimulation of the LC neurons is happening. In this case, it would be possible to inject selective viruses allowing the expression of specific opsins (for example channelrodopsin-2, a blue light-activated cation channel that depolarizes the cell and causes transmission of action potentials) that would allow the stimulation of selective brain areas using blue light (Deisseroth, 2015). This strategy would enable evaluating if the optical stimulation of the LC neurons projecting to the PFC has any effect on their performance on memory and attentional tasks. In the same way, stimulation in the PFC could also be performed, and evaluate the effects on performance while the selective excitation of NA terminals in the PFC is occurring.

Additionally, it would also be possible to test if the effects of electrical stimulation of LC-PFC circuitry could be enhanced or diminished by potential procognitive drugs such as α_2AR agonists/antagonists. Another alternative to evaluate the LC-NA system's role in cognition would be the specific LC silencing during brief periods of the task performance, in order to investigate these effects on memory acquisition and learning processes. Optogenetic silencing can be restricted to a specific phase of the test and does not interfere with LC functions during the rest of the task time (Janitzky et al., 2015). For these cases, viruses allowing the expression of another opsin, halorhodopsin, could be used. This opsin is a yellow light-activated chloride pump that hyperpolarizes the cell, preventing the transmission of action potentials (Deisseroth et al., 2015). In addition, it would also be intriguing to assess the possibility to combine both optogenetic and microdialysis experiments, in order to have a more complete neurochemical and behavioral profile of the effects resulting from the selective manipulation of the LC-PFC circuitry. Eventually, different optogenetic strategies could also be applied for the study of other areas of interest for the neurobiology of schizophrenia, such as the selective targeting of the VTA-PFC circuitry and the influence of the particular manipulation of the DA system on cognitive functions.

6. CONCLUSIONS

From the results obtained in the present work, it can be concluded that:

1. NA and DA tissue concentrations are not altered in the Poly (I:C) model neither in the PFC nor in the striatum. However, reduced 5-HT tissue concentrations are observed in the PFC of the animal model, finding that it is not replicated in the striatum.

2. The Poly (I:C) model shows increased protein expression levels of DAT in the PFC, while expression levels of the TH, D_1R, D_2R, and NET are not altered. In the striatum, DAT and TH protein expression levels are reduced in the animal model, while D_1R, D_2R, and NET are unaltered.

3. In the PFC, the Poly (I:C) model displays reduced DA extracellular basal concentrations but unaltered *Ed* function. As for NA, neither NA extracellular basal concentrations nor *Ed* function are altered in the PFC of the animal model. However, in the striatum, lower DA extracellular basal concentrations and increased *Ed* function are present in the animal model.

4. Potential-dependent DA and NA release is not altered in the PFC of the Poly (I:C) model after local cortical hiperK^+ aCSF administration, while potential-dependent release of 5-HT is augmented.

5. Potential-independent DA and NA release in the PFC is diminished in the Poly (I:C) model after systemic amphetamine administration, and conversely, 5-HT release shows a tendency to be higher. As for the striatum, potential-independent NA release is increased whilst it is unaffected for DA and 5-HT.

6. The NMDA receptor antagonist MK-801 increases monoamine concentrations in the PFC with similar effects on control and Poly (I:C) animals.

7. The Poly (I:C) model presents a lower discrimination index in the NORT, thus demonstrating visual and learning memory deficiencies, indicative of cognitive impairment.

8. In the 5-CSRTT, the Poly (I:C) model shows attentional impairment and signs of deficits in cognitive adaptation. These findings indicate that the animal model lacks cognitive flexibility and speed of processing. Nevertheless, it does not present alterations in locomotor or reward behaviors.

9. These findings suggest the Poly (I:C) MIA model is characterized by a catecholaminergic presynaptic hypoactivity along with probable serotonergic hyperactivity, a condition that seems to be distinctive of the PFC. Additionally, the Poly (I:C) MIA model exhibits cognitive impairment along with reduced cognitive flexibility and speed of processing.

10. The selective α_{2A}AR agonist guanfacine reverses the cognitive impairment showed by the Poly (I:C) model in the NORT, whilst it does not modify monoamine concentrations in the PFC. These findings seem to discard that guanfacine is acting at presynaptic inhibitory α_{2A}ARs, and instead suggest activation of postsynaptic α_{2A}ARs in cortical pyramidal neurons, leading to more efficient control of the PFC cognitive functions.

11. The selective α_{2C}AR antagonist MK-912 partially reverses cognitive deficits shown by the Poly (I:C) model in the NORT and deteriorates the performance of control animals, while it

does not alter PFC catecholamine concentrations. It seems feasible that MK-912 would be acting on cortical α_{2C}ARs located on GABAergic interneurons responsible for controlling the activity of cortical pyramidal neurons and thus, affecting the tonic inhibitory control of GABA over the cortical pyramidal function.

12. In contrast to what happens for catecholamines, the selective α_{2C}AR antagonist MK-912 increases 5-HT concentrations in the PFC of both groups of mice. This effect on 5-HT concentrations could be mediated by disruption of the noradrenergic tonic inhibitory control exerted either by α_{2C}ARs located on 5-HT terminals in the PFC or by α_{2C}ARs located on NA terminals in the raphe nuclei. Besides, since the Poly (I:C) model shows a reduced cortical increase of 5-HT concentrations induced by MK-912, the tonic inhibitory control of the α_{2C}ARs might be attenuated in the model.

13. The findings presented in this work propose the selective α_{2A}AR agonism on cortical pyramidal neurons and the selective α_{2C}AR antagonism on GABAergic neurons as potential targets for treating cognitive impairment in schizophrenia. In addition, together with the previous results, the Poly I:C) MIA model confirms and reinforces its high face, construct and predictive validity as a promising and translational MIA animal model of CIAS.

7. REFERENCES

Aantaa, R., Marjamaki, A., & Scheinin, M. (1995). Molecular pharmacology of alpha 2-adrenoceptor subtypes. *Ann.Med., 27*(4), 439-449.

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R. M., Seibyl, J. P., et al. (1998). Increased striatal dopamine transmission in schizophrenia: Confirmation in a second cohort. *Am.J.Psychiatry, 155*(6), 761-767.

Abi-Dargham, A., Mawlawi, O., Lombardo, I., Gil, R., Martinez, D., et al. (2002). Prefrontal dopamine D1 receptors and working memory in schizophrenia. *J.Neurosci., 22*(9), 3708-3719.

Abi-Dargham, A., & Moore, H. (2003). Prefrontal DA transmission at D1 receptors and the pathology of schizophrenia. *Neuroscientist, 9*(5), 404-416.

Abi-Dargham, A., Rodenhiser, J., Printz, D., Zea-Ponce, Y., Gil, R., et al. (2000). Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc.Natl.Acad.Sci.U.S.A., 97*(14), 8104-8109.

Abi-Dargham, A., van de Giessen, E., Slifstein, M., Kegeles, L. S., & Laruelle, M. (2009). Baseline and amphetamine-stimulated dopamine activity are related in drug-naive schizophrenic subjects. *Biol.Psychiatry, 65*(12), 1091-1093.

Abi-Dargham, A., Xu, X., Thompson, J. L., Gil, R., Kegeles, L. S., et al. (2012). Increased prefrontal cortical D(1) receptors in drug naive patients with schizophrenia: A PET study with [(1)(1)C]NNC112. *J.Psychopharmacol., 26*(6), 794-805.

Adams, B., & Moghaddam, B. (1998). Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J.Neurosci., 18*(14), 5545-5554.

Adams, B., & Moghaddam, B. (2001). Effect of clozapine, haloperidol, or M100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. *Biol.Psychiatry, 50*(10), 750-757.

Adams, D. H., Kinon, B. J., Baygani, S., Millen, B. A., Velona, I., et al. (2013). A long-term, phase 2, multicenter, randomized, open-label, comparative safety study of pomaglumetad methionil (LY2140023 monohydrate) versus atypical antipsychotic standard of care in patients with schizophrenia. *BMC Psychiatry, 13*, 14-143.

Adams, D. H., Zhang, L., Millen, B. A., Kinon, B. J., & Gomez, J. C. (2014). Pomaglumetad methionil (LY2140023 monohydrate) and aripiprazole in patients with schizophrenia: A phase 3, multicenter, double-blind comparison. *Schizophr.Res.Treatment, 2014*, 758212.

Adell, A., & Artigas, F. (1999). Regulation of the release of 5-hydroxytryptamine in the median raphe nucleus of the rat by catecholaminergic afferents. *Eur.J.Neurosci., 11*(7), 2305-2311.

Adell, A., & Artigas, F. (2004). The somatodendritic release of dopamine in the ventral tegmental area and its regulation by afferent transmitter systems. *Neurosci.Biobehav.Rev., 28*(4), 415-431.

Aghajanian, G. K., & Marek, G. J. (1999). Serotonin and hallucinogens. *Neuropsychopharmacology, 21*(2 Suppl), 16-23S.

Ahn, N. G., & Klinman, J. P. (1987). Activation of dopamine beta-monooxygenase by external and internal electron donors in resealed chromaffin granule ghosts. *J.Biol.Chem., 262*(4), 1485-1492.

Akbarian, S. (2014). Epigenetic mechanisms in schizophrenia. *Dialogues Clin.Neurosci., 16*(3), 405-417.

Akbarian, S., Bunney, W. E., Jr, Potkin, S. G., Wigal, S. B., Hagman, J. O., et al. (1993a). Altered distribution of nicotinamide-adenine dinucleotide phosphate-diaphorase cells in frontal lobe of schizophrenics implies disturbances of cortical development. *Arch.Gen.Psychiatry, 50*(3), 169-177.

Akbarian, S., Kim, J. J., Potkin, S. G., Hagman, J. O., Tafazzoli, A., et al. (1995). Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Arch.Gen.Psychiatry, 52*(4), 258-266.

Akbarian, S., Vinuela, A., Kim, J. J., Potkin, S. G., Bunney, W. E.,Jr, & Jones, E. G. (1993b). Distorted distribution of nicotinamide-adenine dinucleotide phosphate-diaphorase neurons in temporal lobe of schizophrenics implies anomalous cortical development. *Arch.Gen.Psychiatry, 50*(3), 178-187.

Akil, M., Edgar, C. L., Pierri, J. N., Casali, S., & Lewis, D. A. (2000). Decreased density of tyrosine hydroxylase-immunoreactive axons in the entorhinal cortex of schizophrenic subjects. *Biol.Psychiatry, 47*(5), 361-370.

Akil, M., Kolachana, B. S., Rothmond, D. A., Hyde, T. M., Weinberger, D. R., & Kleinman, J. E. (2003). Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. *J.Neurosci., 23*(6), 2008-2013.

Akil, M., & Lewis, D. A. (1995). The catecholaminergic innervation of the human entorhinal cortex: Comparisons of schizophrenics and controls.*15*(1-2), 3-235.

Akil, M., Pierri, J. N., Whitehead, R. E., Edgar, C. L., Mohila, C., et al. (1999). Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. *Am.J.Psychiatry, 156*(10), 1580-1589.

Alberati, D., Moreau, J. L., Lengyel, J., Hauser, N., Mory, R., et al. (2012). Glycine reuptake inhibitor RG1678: A pharmacologic characterization of an investigational agent for the treatment of schizophrenia. *Neuropharmacology*, *62*(2), 1152-1161.

Aleman, A., Hijman, R., de Haan, E. H., & Kahn, R. S. (1999). Memory impairment in schizophrenia: A meta-analysis. *Am.J.Psychiatry, 156*(9), 1358-1366.

Aleman, A., Kahn, R. S., & Selten, J. P. (2003). Sex differences in the risk of schizophrenia: Evidence from meta-analysis. *Arch.Gen.Psychiatry, 60*(6), 565-571.

Alexander, M. P., Stuss, D. T., Shallice, T., Picton, T. W., & Gillingham, S. (2005). Impaired concentration due to frontal lobe damage from two distinct lesion sites. *Neurology*, *65*(4), 572-579.

Altman, J. D., Trendelenburg, A. U., MacMillan, L., Bernstein, D., Limbird, L., et al. (1999). Abnormal regulation of the sympathetic nervous system in alpha2A-adrenergic receptor knockout mice. *Mol.Pharmacol., 56*(1), 154-161.

Amara, S. G., & Kuhar, M. J. (1993). Neurotransmitter transporters: Recent progress. *Annu.Rev.Neurosci., 16*, 73-93.

Amaral, D. G., & Sinnamon, H. M. (1977). The locus coeruleus: Neurobiology of a central noradrenergic nucleus. *Prog.Neurobiol., 9*(3), 147-196.

Amargos-Bosch, M., Bortolozzi, A., Puig, M. V., Serrats, J., Adell, A., et al. (2004). Co-expression and in vivo interaction of serotonin1A and serotonin2A receptors in pyramidal neurons of prefrontal cortex. *Cereb.Cortex, 14*(3), 281-299.

Amargos-Bosch, M., Lopez-Gil, X., Artigas, F., & Adell, A. (2006). Clozapine and olanzapine, but not haloperidol, suppress serotonin efflux in the medial prefrontal cortex elicited by phencyclidine and ketamine. *Int.J.Neuropsychopharmacol., 9*(5), 565-573.

Amitai, N., Semenova, S., & Markou, A. (2007). Cognitive-disruptive effects of the psychotomimetic phencyclidine and attenuation by atypical antipsychotic medications in rats. *Psychopharmacology (Berl), 193*(4), 521-537.

Anden, N. E., Carlsson, A., Dahlstroem, A., Fuxe, K., Hillarp, N. A., & Larsson, K. (1964). Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life.Sci.(1962), 3*, 523-530.

Anderson, S. W., Bechara, A., Damasio, H., Tranel, D., & Damasio, A. R. (1999). Impairment of social and moral behavior related to early damage in human prefrontal cortex. *Nat.Neurosci., 2*(11), 1032-1037.

Andreeva, S. G., Dikkes, P., Epstein, P. M., & Rosenberg, P. A. (2001). Expression of cGMP-specific phosphodiesterase 9A mRNA in the rat brain. *J.Neurosci., 21*(22), 9068-9076.

Ang, M. S., Abdul Rashid, N. A., Lam, M., Rapisarda, A., Kraus, M., et al. (2017). The impact of medication anticholinergic burden on cognitive performance in people with schizophrenia. *J.Clin.Psychopharmacol., 37*(6), 651-656.

Angrist, B. (1987). Neurochemistry and neuropharmacology of schizophrenia. In Henn F.A., DeLisi L. E. (Ed.), *Pharmacological models of schizophrenia* (pp. 391-424) Elservier.

Angst, J. (2002). Historical aspects of the dichotomy between manic-depressive disorders and schizophrenia. *Schizophr.Res., 57*(1), 5-13.

Anticevic, A., Repovs, G., & Barch, D. M. (2013). Working memory encoding and maintenance deficits in schizophrenia: Neural evidence for activation and deactivation abnormalities. *Schizophr.Bull., 39*(1), 168-178.

Antonova, E., Sharma, T., Morris, R., & Kumari, V. (2004). The relationship between brain structure and neurocognition in schizophrenia: A selective review. *Schizophr.Res., 70*(2-3), 117-145.

Antunes, M., & Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cogn.Process., 13*(2), 93-110.

Aoki, C., Go, C. G., Venkatesan, C., & Kurose, H. (1994). Perikaryal and synaptic localization of alpha 2A-adrenergic receptor-like immunoreactivity. *Brain Res., 650*(2), 181-204.

Aoki, C., Venkatesan, C., Go, C. G., Forman, R., & Kurose, H. (1998). Cellular and subcellular sites for noradrenergic action in the monkey dorsolateral prefrontal cortex as revealed by the immunocytochemical localization of noradrenergic receptors and axons. *Cereb.Cortex, 8*(3), 269-277.

Arguello, P. A., & Gogos, J. A. (2006). Modeling madness in mice: One piece at a time. *Neuron*, *52*(1), 179-196.

Arif, M., Chikuma, T., Ahmed, M. M., Yoshida, S., & Kato, T. (2007). Suppressive effect of clozapine but not haloperidol on the increases of neuropeptide-degrading enzymes and glial cells in MK-801-treated rat brain regions. *Neurosci.Res., 57*(2), 248-258.

Arnold, E. B., Molinoff, P. B., & Rutledge, C. O. (1977). The release of endogenous norepinephrine and dopamine from cerebral cortex by amphetamine. *J.Pharmacol.Exp.Ther., 202*(3), 544-557.

Arnsten, A. F. (1997). Catecholamine regulation of the prefrontal cortex. *J.Psychopharmacol., 11*(2), 151-162.

Arnsten, A. F. (2000). Stress impairs prefrontal cortical function in rats and monkeys: Role of dopamine D1 and norepinephrine α-1 receptor mechanisms. *Prog. Brain Res*. 126, 183-92.

Arnsten, A. F. (2004). Adrenergic targets for the treatment of cognitive deficits in schizophrenia. *Psychopharmacology (Berl), 174*(1), 25-31.

Arnsten, A. F. (2009). Stress signaling pathways that impair prefrontal cortex structure and function. *Nat.Rev.Neurosci., 10*(6), 410-422.

Arnsten, A. F. (2011). Catecholamine influences on dorsolateral prefrontal cortical networks. *Biol.Psychiatry, 69*(12), 89.

Arnsten, A. F. (2013). The neurobiology of thought: The groundbreaking discoveries of Patricia Goldman-Rakic 1937-2003. *Cereb.Cortex, 23*(10), 2269-2281.

Arnsten, A. F., Cai, J. X., & Goldman-Rakic, P. S. (1988). The alpha-2 adrenergic agonist guanfacine improves memory in aged monkeys without sedative or hypotensive side effects: Evidence for alpha-2 receptor subtypes. *J.Neurosci., 8*(11), 4287-4298.

Arnsten, A. F., & Contant, T. A. (1992). Alpha-2 adrenergic agonists decrease distractibility in aged monkeys performing the delayed response task. *Psychopharmacology (Berl), 108*(1-2), 159-169.

Arnsten, A. F., & Goldman-Rakic, P. S. (1985). Alpha 2-adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. *Science, 230*(4731), 1273-1276.

Arnsten, A. F., & Cai, J. X. (1993). Postsynaptic alpha-2 receptor stimulation improves memory in aged monkeys: Indirect effects of yohimbine versus direct effects of clonidine. *Neurobiol.Aging, 14*(6), 597-603.

Arnsten, A. F., Cai, J. X., Murphy, B. L., & Goldman-Rakic, P. S. (1994). Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology (Berl), 116*(2), 143-151.

Arnsten, A. F., & Goldman-Rakic, P. S. (1998). Noise stress impairs prefrontal cortical cognitive function in monkeys: Evidence for a hyperdopaminergic mechanism. *Arch.Gen.Psychiatry, 55*(4), 362-368.

Arnsten, A. F., & Jin, L. E. (2012). Guanfacine for the treatment of cognitive disorders: A century of discoveries at yale. *Yale J.Biol.Med., 85*(1), 45-58.

Arnsten, A. F., & Leslie, F. M. (1991). Behavioral and receptor binding analysis of the alpha 2-adrenergic agonist, 5-bromo-6 [2-imidazoline-2-yl amino] quinoxaline (UK-14304): Evidence for cognitive enhancement at an alpha 2-adrenoceptor subtype. *Neuropharmacology*, *30*(12A), 1279-1289.

Arnsten, A. F., & Li, B. M. (2005). Neurobiology of executive functions: Catecholamine influences on prefrontal cortical functions. *Biol.Psychiatry, 57*(11), 1377-1384.

Arnsten, A. F., Mathew, R., Ubriani, R., Taylor, J. R., & Li, B. M. (1999). Alpha-1 noradrenergic receptor stimulation impairs prefrontal cortical cognitive function. *Biol.Psychiatry, 45*(1), 26-31.

Arnsten, A. F., Paspalas, C. D., Gamo, N. J., Yang, Y., & Wang, M. (2010). Dynamic network connectivity: A new form of neuroplasticity. *Trends Cogn.Sci., 14*(8), 365-375.

Arnsten, A. F., & Pliszka, S. R. (2011). Catecholamine influences on prefrontal cortical function: Relevance to treatment of attention-deficit/hyperactivity disorder and related disorders. *Pharmacol.Biochem.Behav., 99*(2), 211-216.

Arnsten, A. F., Scahill, L., & Findling, R. L. (2007). Alpha2-adrenergic receptor agonists for the treatment of attention-deficit/hyperactivity disorder: Emerging concepts from new data. *J.Child Adolesc.Psychopharmacol., 17*(4), 393-406.

Arnsten, A. F., Steere, J. C., & Hunt, R. D. (1996). The contribution of alpha 2-noradrenergic mechanisms of prefrontal cortical cognitive function. potential significance for attention-deficit hyperactivity disorder. *Arch.Gen.Psychiatry, 53*(5), 448-455.

Arnsten, A. F., Wang, M. J., & Paspalas, C. D. (2012). Neuromodulation of thought: Flexibilities and vulnerabilities in prefrontal cortical network synapses. *Neuron*, *76*(1), 223-239.

Arnt, J. (1998). Pharmacological differentiation of classical and novel antipsychotics. *Int.Clin.Psychopharmacol., 13 Suppl 3*, 7.

Arnt, J., & Skarsfeldt, T. (1998). Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology, 18*(2), 63-101.

Arrowsmith, C. H., Bountra, C., Fish, P. V., Lee, K., & Schapira, M. (2012). Epigenetic protein families: A new frontier for drug discovery. *Nat.Rev.Drug Discov., 11*(5), 384-400.

Artiges, E., Leroy, C., Dubol, M., Prat, M., Pepin, A., et al. (2017). Striatal and extrastriatal dopamine transporter availability in schizophrenia and its clinical correlates: A voxel-based and high-resolution PET study. *Schizophr.Bull., 43*(5), 1134-1142.

Ashby, C. R.,Jr, Jiang, L. H., Kasser, R. J., & Wang, R. Y. (1990). Electrophysiological characterization of 5-hydroxytryptamine2 receptors in the rat medial prefrontal cortex. *J.Pharmacol.Exp.Ther., 252*(1), 171-178.

Aston-Jones, G., & Bloom, F. E. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J.Neurosci., 1*(8), 876-886.

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: Adaptive gain and optimal performance. *Annu.Rev.Neurosci., 28*, 403-450.

Aston-Jones, G., Rajkowski, J., & Cohen, J. (1999). Role of locus coeruleus in attention and behavioral flexibility. *Biol.Psychiatry, 46*(9), 1309-1320.

Aston-Jones, G., Shipley, M. T., Chouvet, G., Ennis, M., van Bockstaele, E., et al. (1991). Afferent regulation of locus coeruleus neurons: Anatomy, physiology and pharmacology. *Prog.Brain Res., 88*, 47-75.

Avery, R. A., Franowicz, J. S., Studholme, C., van Dyck, C. H., & Arnsten, A. F. (2000). The alpha-2A-adrenoceptor agonist, guanfacine, increases regional cerebral blood flow in dorsolateral prefrontal cortex of monkeys performing a spatial working memory task. *Neuropsychopharmacology, 23*(3), 240-249.

Avila, M. T., Robles, O., Hong, L. E., Blaxton, T. A., Myers, C. S., et al. (2006). Deficits on the continuous performance test within the schizophrenia spectrum and the mediating effects of family history of schizophrenia. *J.Abnorm.Psychol., 115*(4), 771-778.

Awad, A. G., & Voruganti, L. N. (2008). The burden of schizophrenia on caregivers: A review. *Pharmacoeconomics, 26*(2), 149-162.

Ayhan, Y., Sawa, A., Ross, C.,A., & Pletnikov, M.,V. (2009). Animal models of gene-environment interactions in schizophrenia. *Behav. Brain Res*. *204*(2), 274-81.

Azzaro, A. J., King, J., Kotzuk, J., Schoepp, D. D., Frost, J., & Schochet, S. (1985). Guinea pig striatum as a model of human dopamine deamination: The role of monoamine oxidase isozyme ratio, localization, and affinity for substrate in synaptic dopamine metabolism. *J.Neurochem., 45*(3), 949-956.

Babri, S., Doosti, M. H., & Salari, A. A. (2014). Strain-dependent effects of prenatal maternal immune activation on anxiety- and depression-like behaviors in offspring. *Brain Behav.Immun., 37*, 164-176.

Bach, M. E., Simpson, E. H., Kahn, L., Marshall, J. J., Kandel, E. R., & Kellendonk, C. (2008). Transient and selective overexpression of D2 receptors in the striatum causes persistent deficits in conditional associative learning. *Proc.Natl.Acad.Sci.U.S.A., 105*(41), 16027-16032.

Bacq, A., Balasse, L., Biala, G., Guiard, B., Gardier, A. M., et al. (2012). Organic cation transporter 2 controls brain norepinephrine and serotonin clearance and antidepressant response. *Mol.Psychiatry, 17*(9), 926-939.

Baharnoori, M., Bhardwaj, S. K., & Srivastava, L. K. (2013). Effect of maternal lipopolysaccharide administration on the development of dopaminergic receptors and transporter in the rat offspring. *PLoS One, 8*(1), e54439.

Baker, S. C., Rogers, R. D., Owen, A. M., Frith, C. D., Dolan, R. J., et al. (1996). Neural systems engaged by planning: A PET study of the Tower of London task. *Neuropsychologia*, *34*(6), 515-526.

Baldessarini, R. J., Huston-Lyons, D., Campbell, A., Marsh, E., & Cohen, B. M. (1992). Do central antiadrenergic actions contribute to the atypical properties of clozapine? *Br.J.Psychiatry Suppl., (17)*(17), 12-16.

Bamberg, E., Gartner, W., & Trauner, D. (2018). Introduction: Optogenetics and photopharmacology. *Chem.Rev., 118*(21), 10627-10628.

Baraban, J. M., & Aghajanian, G. K. (1980). Suppression of serotonergic neuronal firing by alpha-adrenoceptor antagonists: Evidence against GABA mediation. *Eur.J.Pharmacol., 66*(4), 287-294.

Barbelivien, A., Ruotsalainen, S., & Sirvio, J. (2001). Metabolic alterations in the prefrontal and cingulate cortices are related to behavioral deficits in a rodent model of attention-deficit hyperactivity disorder. *Cereb.Cortex, 11*(11), 1056-1063.

Barbier, A. J., Hilhorst, M., Van Vliet, A., Snyder, P., Palfreyman, M. G., et al. (2015). Pharmacodynamics, pharmacokinetics, safety, and tolerability of encenicline, a selective alpha7 nicotinic receptor partial agonist, in single ascending-dose and bioavailability studies. *Clin.Ther., 37*(2), 311-324.

Bard, J. A., Zgombick, J., Adham, N., Vaysse, P., Branchek, T. A., & Weinshank, R. L. (1993). Cloning of a novel human serotonin receptor (5-HT7) positively linked to adenylate cyclase. *J.Biol.Chem., 268*(31), 23422-23426.

Bardgett, M. E., Jackson, J. L., Taylor, G. T., & Csernansky, J. G. (1995). Kainic acid decreases hippocampal neuronal number and increases dopamine receptor binding in the nucleus accumbens: An animal model of schizophrenia. *Behav.Brain Res., 70*(2), 153-164.

Bardgett, M. E., Jacobs, P. S., Jackson, J. L., & Csernansky, J. G. (1997). Kainic acid lesions enhance locomotor responses to novelty, saline, amphetamine, and MK-801. *Behav.Brain Res., 84*(1-2), 47-55.

Bari, A., Dalley, J. W., & Robbins, T. W. (2008). The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nat.Protoc., 3*(5), 759-767.

Barik, J., & Wonnacott, S. (2009). Molecular and cellular mechanisms of action of nicotine in the CNS. *Handb.Exp.Pharmacol., (192):*173-207.

Barker, G. R., Bird, F., Alexander, V., & Warburton, E. C. (2007). Recognition memory for objects, place, and temporal order: A disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *J.Neurosci., 27*(11), 2948-2957.

Barnett, J. H., Robbins, T. W., Leeson, V. C., Sahakian, B. J., Joyce, E. M., & Blackwell, A. D. (2010). Assessing cognitive function in clinical trials of schizophrenia. *Neurosci.Biobehav.Rev., 34*(8), 1161-1177.

Barrett, R. M., Malvaez, M., Kramar, E., Matheos, D. P., Arrizon, A., et al. (2011). Hippocampal focal knockout of CBP affects specific histone modifications, long-term potentiation, and long-term memory. *Neuropsychopharmacology, 36*(8), 1545-1556.

Barth, A. M., Vizi, E. S., Zelles, T., & Lendvai, B. (2008). Alpha2-adrenergic receptors modify dendritic spike generation via HCN channels in the prefrontal cortex. *J.Neurophysiol., 99*(1), 394-401.

Bassett, D. S., Bullmore, E., Verchinski, B. A., Mattay, V. S., Weinberger, D. R., & Meyer-Lindenberg, A. (2008). Hierarchical organization of human cortical networks in health and schizophrenia. *J.Neurosci., 28*(37), 9239-9248.

Beani, L., Bianchi, C., Tanganelli, S., Antonelli, T., Simonato, M., & Rando, S. (1988). Inversion of the alpha-2 and alpha-1 noradrenergic control of the cortical release of acetylcholine and gamma-aminobutyric acid in morphine-tolerant guinea pigs. *J.Pharmacol.Exp.Ther., 247*(1), 294-301.

Beaulieu, J. M., & Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol.Rev., 63*(1), 182-217.

Beaulieu, J. M., Sotnikova, T. D., Marion, S., Lefkowitz, R. J., Gainetdinov, R. R., & Caron, M. G. (2005). An akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell*, *122*(2), 261-273.

Beck, L. H., Bransome, E. D.,Jr, Mirsky, A. F., Rosvold, H. E., & Saraon, I. (1956). A continuous performance test of brain damage. *J.Consult.Psychol., 20*(5), 343-350.

Beckstead, M. J., Grandy, D. K., Wickman, K., & Williams, J. T. (2004). Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. *Neuron*, *42*(6), 939-946.

Bel, N., & Artigas, F. (1996). In vivo effects of the simultaneous blockade of serotonin and norepinephrine transporters on serotonergic function. microdialysis studies. *J.Pharmacol.Exp.Ther., 278*(3), 1064-1072.

Ben Abdallah, N. M., Fuss, J., Trusel, M., Galsworthy, M. J., Bobsin, K., et al. (2011). The puzzle box as a simple and efficient behavioral test for exploring impairments of general cognition and executive functions in mouse models of schizophrenia. *Exp.Neurol., 227*(1), 42-52.

Benarroch, E. E. (2013). HCN channels: Function and clinical implications. *Neurology, 80*(3), 304-310.

Benedict, R. H., Amato, M. P., Boringa, J., Brochet, B., Foley, F., et al. (2012). Brief international cognitive assessment for MS (BICAMS): International standards for validation. *BMC Neurol., 12*, 5-55.

Benedict, R. H. B., & Groninger, L. (1995). Preliminary standardization of a new visuospatial memory test with six alternate forms. *Clin.Neuropsychol., 9*(1), 11-16.

Benes, F. M., & Berretta, S. (2001). GABAergic interneurons: Implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology, 25*(1), 1-27.

Bengel, D., Murphy, D. L., Andrews, A. M., Wichems, C. H., Feltner, D., et al. (1998). Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4-methylenedioxymethamphetamine ("ecstasy") in serotonin transporter-deficient mice. *Mol.Pharmacol., 53*(4), 649-655.

Bentivoglio, & Morelli, M. (2005). Chapter I the organization and circuits of mesencephalic dopaminergic neurons and the distribution of dopamine receptors in the brain. *Handbook of Chemical Neuroanatomy*, *21*,1-107.

Bergdolt, L., & Dunaevsky, A. (2019). Brain changes in a maternal immune activation model of neurodevelopmental brain disorders. *Prog.Neurobiol., 175*, 1-19.

Berger, P. A. (1981). Biochemistry and the schizophrenia. old concepts and new hypothesis. *J.Nerv.Ment.Dis., 169*(2), 90-99.

Berger, T., Larkum, M. E., & Luscher, H. R. (2001). High I(h) channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. *J.Neurophysiol., 85*(2), 855-868.

Bergink, V., Gibney, S. M., & Drexhage, H. A. (2014). Autoimmunity, inflammation, and psychosis: A search for peripheral markers. *Biol.Psychiatry, 75*(4), 324-331.

Bergson, C., Mrzljak, L., Smiley, J. F., Pappy, M., Levenson, R., & Goldman-Rakic, P. S. (1995). Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J.Neurosci., 15*(12), 7821-7836.

Berman, K. F., Weinberger, D. R., Shelton, R. C., & Zec, R. F. (1987). A relationship between anatomical and physiological brain pathology in schizophrenia: Lateral cerebral ventricular size predicts cortical blood flow. *Am.J.Psychiatry, 144*(10), 1277-1282.

Berridge, C. W., & Spencer, R. C. (2016). Differential cognitive actions of norepinephrine a2 and a1 receptor signaling in the prefrontal cortex. *Brain Res., 1641*(Pt B), 189-196.

Berridge, C. W., Stratford, T. L., Foote, S. L., & Kelley, A. E. (1997). Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens. *Synapse, 27*(3), 230-241.

Berridge, C. W., & Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. *Brain Res.Brain Res.Rev., 42*(1), 33-84.

Bianchi, M., Fone, K. F., Azmi, N., Heidbreder, C. A., Hagan, J. J., & Marsden, C. A. (2006). Isolation rearing induces recognition memory deficits accompanied by cytoskeletal alterations in rat hippocampus. *Eur.J.Neurosci., 24*(10), 2894-2902.

Biederman, J., Melmed, R. D., Patel, A., McBurnett, K., Konow, J., et al. (2008). A randomized, double-blind, placebo-controlled study of guanfacine extended release in children and adolescents with attention-deficit/hyperactivity disorder. *Pediatrics*, *121*(1), 73-84.

Bikovsky, L., Hadar, R., Soto-Montenegro, M. L., Klein, J., Weiner, I., et al. (2016). Deep brain stimulation improves behavior and modulates neural circuits in a rodent model of schizophrenia. *Exp.Neurol., 283*(Pt A), 142-150.

Bilder, R. M., Loo, S. K., McGough, J. J., Whelan, F., Hellemann, G., et al. (2016). Cognitive effects of stimulant, guanfacine, and combined treatment in child and adolescent attention-deficit/hyperactivity disorder. *J.Am.Acad.Child Adolesc.Psychiatry, 55*(8), 667-673.

Birnbaum, S. G., Yuan, P. X., Wang, M., Vijayraghavan, S., Bloom, A. K., et al. (2004). Protein kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science, 306*(5697), 882-884.

Birnbaum, S., Gobeske, K. T., Auerbach, J., Taylor, J. R., & Arnsten, A. F. (1999). A role for norepinephrine in stress-induced cognitive deficits: Alpha-1-adrenoceptor mediation in the prefrontal cortex. *Biol.Psychiatry, 46*(9), 1266-1274.

Bissiere, S., Humeau, Y., & Luthi, A. (2003). Dopamine gates LTP induction in lateral amygdala by suppressing feedforward inhibition. *Nat.Neurosci., 6*(6), 587-592.

Bitanihirwe, B. K., Peleg-Raibstein, D., Mouttet, F., Feldon, J., & Meyer, U. (2010). Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia. *Neuropsychopharmacology, 35*(12), 2462-2478.

Bjorklund, M., Sirvio, J., Sallinen, J., Scheinin, M., Kobilka, B. K., & Riekkinen, P.,Jr. (1999). Alpha2C-adrenoceptor overexpression disrupts execution of spatial and non-spatial search patterns. *Neuroscience*, *88*(4), 1187-1198.

Bjorklund, M., Siverina, I., Heikkinen, T., Tanila, H., Sallinen, J., et al. (2001). Spatial working memory improvement by an alpha2-adrenoceptor agonist dexmedetomidine is not mediated through alpha2C-adrenoceptor. *Prog.Neuropsychopharmacol.Biol.Psychiatry, 25*(8), 1539-1554.

Black, J. E., Kodish, I. M., Grossman, A. W., Klintsova, A. Y., Orlovskaya, D., et al. (2004). Pathology of layer V pyramidal neurons in the prefrontal cortex of patients with schizophrenia. *Am.J.Psychiatry, 161*(4), 742-744.

Blakely, R. D., & Bauman, A. L. (2000). Biogenic amine transporters: Regulation in flux. *Curr.Opin.Neurobiol., 10*(3), 328-336.

Blandizzi, C., Natale, G., Colucci, R., Carignani, D., Lazzeri, G., & Del Tacca, M. (1995). Characterization of alpha 2-adrenoceptor subtypes involved in the modulation of gastric acid secretion. *Eur.J.Pharmacol., 278*(2), 179-182.

Blaxall, H. S., Hass, N. A., & Bylund, D. B. (1994). Expression of alpha 2-adrenergic receptor genes in rat tissues. *Receptor*, *4*(3), 191-199.

Blaxall, H. S., Murphy, T. J., Baker, J. C., Ray, C., & Bylund, D. B. (1991). Characterization of the alpha-2C adrenergic receptor subtype in the opossum kidney and in the OK cell line. *J.Pharmacol Exp.Ther*., (259), 32-329.

Bleich, A., Brown, S. L., Kahn, R., & van Praag, H. M. (1988). The role of serotonin in schizophrenia. *Schizophr.Bull., 14*(2), 297-315.

Bloom, F. E. (1993). Advancing a neurodevelopmental origin for schizophrenia. *Arch.Gen.Psychiatry, 50*(3), 224-227.

Bobetsis, Y. A., Barros, S. P., & Offenbacher, S. (2006). Exploring the relationship between periodontal disease and pregnancy complications. *J.Am.Dent.Assoc., 137 Suppl*, 7-13S.

Bobker, D. H., & Williams, J. T. (1989). Serotonin augments the cationic current ih in central neurons. *Neuron*, *2*(6), 1535-1540.

Bocchio Chiavetto, L., Boin, F., Zanardini, R., Popoli, M., Michelato, A., et al. (2002). Association between promoter polymorphic haplotypes of interleukin-10 gene and schizophrenia. *Biol.Psychiatry, 51*(6), 480-484.

Boehm, S., Huck, S., & Freissmuth, M. (1996). Involvement of a phorbol ester-insensitive protein kinase C in the alpha2-adrenergic inhibition of voltage-gated calcium current in chick sympathetic neurons. *J.Neurosci., 16*(15), 4596-4603.

Boess, F. G., & Martin, I. L. (1994). Molecular biology of 5-HT receptors. *Neuropharmacology*, *33*(3-4), 275-317.

Boin, F., Zanardini, R., Pioli, R., Altamura, C. A., Maes, M., & Gennarelli, M. (2001). Association between -G308A tumor necrosis factor alpha gene polymorphism and schizophrenia. *Mol.Psychiatry, 6*(1), 79-82.

Boksa, P. (2008). Maternal infection during pregnancy and schizophrenia. *J.Psychiatry Neurosci., 33*(3), 183-185.

Boland, K., Moschetti, V., Dansirikul, C., Pichereau, S., Gheyle, L., et al. (2017). A phase I, randomized, proof-of-clinical-mechanism study assessing the pharmacokinetics and pharmacodynamics of the oral PDE9A inhibitor BI 409306 in healthy male volunteers. *Hum.Psychopharmacol., 32*(1), 10.1002/hup.2569.

Bolkan, S. S., Carvalho Poyraz, F., & Kellendonk, C. (2016). Using human brain imaging studies as a guide toward animal models of schizophrenia. *Neuroscience*, *321*, 77-98.

Bolme, P., Forsyth, R. P., Ishizaki, T., & Melmon, K. L. (1975). Hemodynimic effects of systemic and central administration of clonidine in the monkey. *Am.J.Physiol., 228*(4), 1276-1279.

Bondy, B., Ackenheil, M., Birzle, W., Elbers, R., & Frohler, M. (1984). Catecholamines and their receptors in blood: Evidence for alterations in schizophrenia. *Biol.Psychiatry, 19*(10), 1377-1393.

Bonhomme, N., De Deurwaerdere, P., Le Moal, M., & Spampinato, U. (1995). Evidence for 5-HT4 receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: A microdialysis study in the halothane-anesthetized rat. *Neuropharmacology*, *34*(3), 269-279.

Boon-yasidhi, V., Kim, Y. S., & Scahill, L. (2005). An open-label, prospective study of guanfacine in children with ADHD and tic disorders. *J.Med.Assoc.Thai., 88 (8)*, 156.

Borrell, J., Vela, J. M., Arevalo-Martin, A., Molina-Holgado, E., & Guaza, C. (2002). Prenatal immune challenge disrupts sensorimotor gating in adult rats. implications for the etiopathogenesis of schizophrenia. *Neuropsychopharmacology, 26*(2), 204-215.

Borrelli, E., Nestler, E. J., Allis, C. D., & Sassone-Corsi, P. (2008). Decoding the epigenetic language of neuronal plasticity. *Neuron*, *60*(6), 961-974.

Bortolozzi, A., & Artigas, F. (2003). Control of 5-hydroxytryptamine release in the dorsal raphe nucleus by the noradrenergic system in rat brain. Role of alpha-adrenoceptors. *Neuropsychopharmacology, 28*(3), 421-434.

Bortolozzi, A., Diaz-Mataix, L., Scorza, M. C., Celada, P., & Artigas, F. (2005). The activation of 5-HT receptors in prefrontal cortex enhances dopaminergic activity. *J.Neurochem., 95*(6), 1597-1607.

Bouret, S., & Sara, S. J. (2005). Network reset: A simplified overarching theory of locus coeruleus noradrenaline function. *Trends Neurosci., 28*(11), 574-582.

Bowers, M. B., Jr, & Morton, J. B. (1994). Regional brain catecholamines and metabolites following THC, PCP and MK-801. *Prog.Neuropsychopharmacol.Biol.Psychiatry, 18*(5), 961-964.

Bowie, C. R., & Harvey, P. D. (2005). Cognition in schizophrenia: Impairments, determinants, and functional importance. *Psychiatr.Clin.North Am., 28*(3), 61-33, 626.

Bowie, C. R., & Harvey, P. D. (2006). Cognitive deficits and functional outcome in schizophrenia. *Neuropsychiatr.Dis.Treat., 2*(4), 531-536.

Boydell, J., van Os, J., McKenzie, K., Allardyce, J., Goel, R., et al. (2001). Incidence of schizophrenia in ethnic minorities in London: Ecological study into interactions with environment. *BMJ, 323*(7325), 1336-1338.

Bradberry, C. W., & Rubino, S. R. (2004). Phasic alterations in dopamine and serotonin release in striatum and prefrontal cortex in response to cocaine predictive cues in behaving rhesus macaques. *Neuropsychopharmacology, 29*(4), 676-685.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal.Biochem., 72*, 248-254.

Braff, D. L., & Geyer, M. A. (1990). Sensorimotor gating and schizophrenia. human and animal model studies. *Arch.Gen.Psychiatry, 47*(2), 181-188.

Brake, W. G., Boksa, P., & Gratton, A. (1997). Effects of perinatal anoxia on the acute locomotor response to repeated amphetamine administration in adult rats. *Psychopharmacology (Berl), 133*(4), 389-395.

Braun, A. R., Jaskiw, G. E., Vladar, K., Sexton, R. H., Kolachana, B. S., & Weinberger, D. R. (1993). Effects of ibotenic acid lesion of the medial prefrontal cortex on dopamine agonist-related behaviors in the rat. *Pharmacol.Biochem.Behav., 46*(1), 51-60.

Breier, A., Adler, C. M., Weisenfeld, N., Su, T. P., Elman, I., et al. (1998). Effects of NMDA antagonism on striatal dopamine release in healthy subjects: Application of a novel PET approach. *Synapse, 29*(2), 142-147.

Breier, A., Su, T. P., Saunders, R., Carson, R. E., Kolachana, B. S., et al. (1997). Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: Evidence from a novel positron emission tomography method. *Proc.Natl.Acad.Sci.U.S.A., 94*(6), 2569-2574.

Brenner, B., Harney, J. T., Ahmed, B. A., Jeffus, B. C., Unal, R., et al. (2007). Plasma serotonin levels and the platelet serotonin transporter. *J.Neurochem., 102*(1), 206-215.

Brewer, W. J., Francey, S. M., Wood, S. J., Jackson, H. J., Pantelis, C., et al. (2005). Memory impairments identified in people at ultra-high risk for psychosis who later develop first-episode psychosis. *Am.J.Psychiatry, 162*(1), 71-78.

Briand, L. A., Gritton, H., Howe, W. M., Young, D. A., & Sarter, M. (2007). Modulators in concert for cognition: Modulator interactions in the prefrontal cortex. *Prog.Neurobiol., 83*(2), 69-91.

Brinley-Reed, M., & McDonald, A. J. (1999). Evidence that dopaminergic axons provide a dense innervation of specific neuronal subpopulations in the rat basolateral amygdala. *Brain Res., 850*(1-2), 127-135.

Brisch, R., Saniotis, A., Wolf, R., Bielau, H., Bernstein, H. G., et al. (2014). The role of dopamine in schizophrenia from a neurobiological and evolutionary perspective: Old fashioned, but still in vogue. *Front.Psychiatry., 5*, 47. eCollection 2014.

Broadbent, N. J., Gaskin, S., Squire, L. R., & Clark, R. E. (2009). Object recognition memory and the rodent hippocampus. *Learn.Mem., 17*(1), 5-11.

Bronson, S. L., Ahlbrand, R., Horn, P. S., Kern, J. R., & Richtand, N. M. (2011). Individual differences in maternal response to immune challenge predict offspring behavior: Contribution of environmental factors. *Behav.Brain Res., 220*(1), 55-64.

Brown, A. S. (2006). Prenatal infection as a risk factor for schizophrenia. *Schizophr.Bull., 32*(2), 200-202.

Brown, A. S., Begg, M. D., Gravenstein, S., Schaefer, C. A., Wyatt, R. J., et al. (2004). Serologic evidence of prenatal influenza in the etiology of schizophrenia. *Arch.Gen.Psychiatry, 61*(8), 774-780.

Brown, A. S., & Susser, E. S. (2002). In utero infection and adult schizophrenia. *Ment.Retard.Dev.Disabil.Res.Rev., 8*(1), 51-57.

Brown, D., Nakagome, K., Cordes, J., Brenner, R., Grunder, G., et al. (2019). Evaluation of the efficacy, safety, and tolerability of BI 409306, a novel phosphodiesterase 9 inhibitor, in cognitive impairment in schizophrenia: A randomized, double-blind, placebo-controlled, phase II trial. *Schizophr.Bull., 45*(2), 350-359.

Brown, R. G., & Marsden, C. D. (1990). Cognitive function in Parkinson's disease: From description to theory. *Trends Neurosci., 13*(1), 21-29.

Brown, V. J., & Bowman, E. M. (2002). Rodent models of prefrontal cortical function. *Trends Neurosci., 25*(7), 340-343.

Brozoski, T. J., Brown, R. M., Rosvold, H. E., & Goldman, P. S. (1979). Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science, 205*(4409), 929-932.

Brum, P. C., Hurt, C. M., Shcherbakova, O. G., Kobilka, B., & Angelotti, T. (2006). Differential targeting and function of alpha2A and alpha2C adrenergic receptor subtypes in cultured sympathetic neurons. *Neuropharmacology*, *51*(3), 397-413.

Buchanan, R. W., Keefe, R. S., Umbricht, D., Green, M. F., Laughren, T., & Marder, S. R. (2011). The FDA-NIMH-MATRICS guidelines for clinical trial design of cognitive-enhancing drugs: What do we know 5 years later? *Schizophr.Bull., 37*(6), 1209-1217.

Bucheler, M. M., Hadamek, K., & Hein, L. (2002). Two alpha(2)-adrenergic receptor subtypes, alpha(2A) and alpha(2C), inhibit transmitter release in the brain of gene-targeted mice. *Neuroscience*, *109*(4), 819-826.

Buchsbaum, M. S., Christian, B. T., Lehrer, D. S., Narayanan, T. K., Shi, B., et al. (2006). D2/D3 dopamine receptor binding with [F-18]fallypride in thalamus and cortex of patients with schizophrenia. *Schizophr.Res., 85*(1-3), 232-244.

Buckley, P. F. (2005). Neuroimaging of schizophrenia: Structural abnormalities and pathophysiological implications. *Neuropsychiatr.Dis.Treat., 1*(3), 193-204.

Buckner, R. L., & Wheeler, M. E. (2001). The cognitive neuroscience of remembering. *Nat.Rev.Neurosci., 2*(9), 624-634.

Bugarski-Kirola, D., Iwata, N., Sameljak, S., Reid, C., Blaettler, T., et al. (2016). Efficacy and safety of adjunctive bitopertin versus placebo in patients with suboptimally controlled symptoms of schizophrenia treated with antipsychotics: Results from three phase 3, randomized, double-blind, parallel-group, placebo-controlled, multicentre studies in the SearchLyte clinical trial program. *Lancet Psychiatry., 3*(12), 1115-1128.

Buhot, M. C., Martin, S., & Segu, L. (2000). Role of serotonin in memory impairment. *Ann.Med., 32*(3), 210-221.

Bunemann, M., Bucheler, M. M., Philipp, M., Lohse, M. J., & Hein, L. (2001). Activation and deactivation kinetics of alpha 2A- and alpha 2C-adrenergic receptor-activated G protein-activated inwardly rectifying K+ channel currents. *J.Biol.Chem., 276*(50), 47512-47517.

Bunney, B. S., & Aghajanian, G. K. (1976). The precise localization of nigral afferents in the rat as determined by a retrograde tracing technique. *Brain Res., 117*(3), 423-435.

Bussey, T. J., Holmes, A., Lyon, L., Mar, A. C., McAllister, K. A., et al. (2012). New translational assays for preclinical modeling of cognition in schizophrenia: The touchscreen testing method for mice and rats. *Neuropharmacology*, *62*(3), 1191-1203.

Bussey, T. J., Saksida, L. M., & Rothblat, L. A. (2001). Discrimination of computer-graphic stimuli by mice: A method for the behavioral characterization of transgenic and gene-knockout models. *Behav.Neurosci., 115*(4), 957-960.

Buzsaki, G., Kennedy, B., Solt, V. B., & Ziegler, M. (1991). Noradrenergic control of thalamic oscillation: The role of alpha-2 receptors. *Eur.J.Neurosci., 3*(3), 222-229.

Byerly, M. J., Weber, M. T., Brooks, D. L., Snow, L. R., Worley, M. A., & Lescouflair, E. (2001). Antipsychotic medications and the elderly: Effects on cognition and implications for use. *Drugs Aging, 18*(1), 45-61.

Bylund, D. B. (1988). Subtypes of alpha 2-adrenoceptors: Pharmacological and molecular biological evidence converge. *Trends Pharmacol.Sci., 9*(10), 356-361.

Bylund, D. B., Eikenberg, D. C., Hieble, J. P., Langer, S. Z., Lefkowitz, R. J., et al. (1994). International union of pharmacology nomenclature of adrenoceptors. *Pharmacol.Rev., 46*(2), 121-136.

Bylund, D. B., Ray-Prenger, C., & Murphy, T. J. (1988). Alpha-2A and alpha-2B adrenergic receptor subtypes: Antagonist binding in tissues and cell lines containing only one subtype. *J.Pharmacol.Exp.Ther., 245*(2), 600-607.

Bylund, D. B., (1985). Heterogeneity of alpha-2 adrenergic receptors. *Pharmacol. Biochem. Behav. 5*, 835-43.

Bymaster, F. P., Rasmussen, K., Calligaro, D. O., Nelson, D. L., DeLapp, N. W., et al. (1997). In vitro and in vivo biochemistry of olanzapine: A novel, atypical antipsychotic drug. *J.Clin.Psychiatry, 58 Suppl 10*, 28-36.

Byrne, M., Agerbo, E., Bennedsen, B., Eaton, W. W., & Mortensen, P. B. (2007). Obstetric conditions and risk of first admission with schizophrenia: A Danish national register-based study. *Schizophr.Res., 97*(1-3), 51-59.

Cahill, L., & McGaugh, J. L. (1996). Modulation of memory storage. *Curr.Opin.Neurobiol., 6*(2), 237-242.

Cai, J. X., Ma, Y. Y., Xu, L., & Hu, X. T. (1993). Reserpine impairs spatial working memory performance in monkeys: Reversal by the alpha 2-adrenergic agonist clonidine. *Brain Res., 614*(1-2), 191-196.

Callado, L. F., Hopwood, S. E., Hancock, P. J., & Stamford, J. A. (2000). Effects of dizocilpine (MK 801) on noradrenaline, serotonin and dopamine release and uptake. *Neuroreport*, *11*(1), 173-176.

Callado, L. F., & Stamford, J. A. (1999). Alpha2A- but not alpha2B/C-adrenoceptors modulate noradrenaline release in rat locus coeruleus: Voltammetric data. *Eur.J.Pharmacol., 366*(1), 35-39.

Callicott, J. H., Egan, M. F., Mattay, V. S., Bertolino, A., Bone, A. D., et al. (2003). Abnormal fMRI response of the dorsolateral prefrontal cortex in cognitively intact siblings of patients with schizophrenia. *Am.J.Psychiatry, 160*(4), 709-719.

Cannon, M., Jones, P. B., & Murray, R. M. (2002). Obstetric complications and schizophrenia: Historical and meta-analytic review. *Am.J.Psychiatry, 159*(7), 1080-1092.

Cannon, T. D., & Keller, M. C. (2006). Endophenotypes in the genetic analyses of mental disorders. *Annu.Rev.Clin.Psychol., 2*, 267-290.

Cantor-Graae, E., & Selten, J. P. (2005). Schizophrenia and migration: A meta-analysis and review. *Am.J.Psychiatry, 162*(1), 12-24.

Carbon, M., & Marie, R. M. (2003). Functional imaging of cognition in Parkinson's disease. *Curr.Opin.Neurol., 16*(4), 475-480.

Carboni, E., Tanda, G. L., Frau, R., & Di Chiara, G. (1990). Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: Evidence that dopamine is taken up in vivo by noradrenergic terminals. *J.Neurochem., 55*(3), 1067-1070.

Careaga, M., Taylor, S. L., Chang, C., Chiang, A., Ku, K. M., et al. (2018). Variability in PolyIC induced immune response: Implications for preclinical maternal immune activation models. *J.Neuroimmunol., 323*, 87-93.

Carhart-Harris, R. L., Erritzoe, D., Williams, T., Stone, J. M., Reed, L. J., et al. (2012). Neural correlates of the psychedelic state as determined by fMRI studies with psilocybin. *Proc.Natl.Acad.Sci.U.S.A., 109*(6), 2138-2143.

Carli, M., Calcagno, E., Mainini, E., Arnt, J., & Invernizzi, R. W. (2011a). Sertindole restores attentional performance and suppresses glutamate release induced by the NMDA receptor antagonist CPP. *Psychopharmacology (Berl), 214*(3), 625-637.

Carli, M., Calcagno, E., Mainolfi, P., Mainini, E., & Invernizzi, R. W. (2011b). Effects of aripiprazole, olanzapine, and haloperidol in a model of cognitive deficit of schizophrenia in rats: Relationship with glutamate release in the medial prefrontal cortex. *Psychopharmacology (Berl), 214*(3), 639-652.

Carli, M., Robbins, T. W., Evenden, J. L., & Everitt, B. J. (1983). Effects of lesions to ascending noradrenergic neurons on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behav.Brain Res., 9*(3), 361-380.

Carlson, S., Tanila, H., Rama, P., Mecke, E., & Pertovaara, A. (1992). Effects of medetomidine, an alpha-2 adrenoceptor agonist, and atipamezole, an alpha-2 antagonist, on spatial memory performance in adult and aged rats. *Behav.Neural Biol., 58*(2), 113-119.

Carlsson, A. (1959). Detection and assay of dopamine. *Pharmacol.Rev., 11*(2, Part 2), 300-304.

Carlsson, A. (1974). Antipsychotic drugs and catecholamine synapses. *J.Psychiatr.Res., 11*, 57-64.

Carlsson, A., Lindqvist, M., Magnusson, T., & Waldeck, B. (1958). On the presence of 3-hydroxytyramine in the brain. *Science, 127*(3296), 471.

Carr, D. B., Andrews, G. D., Glen, W. B., & Lavin, A. (2007). Alpha2-noradrenergic receptors activation enhances excitability and synaptic integration in rat prefrontal cortex pyramidal neurons via inhibition of HCN currents. *J.Physiol., 584*(Pt 2), 437-450.

Carr, D. B., & Sesack, S. R. (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J.Neurosci., 20*(10), 3864-3873.

Carter, M. E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., et al. (2010). Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat.Neurosci., 13*(12), 1526-1533.

Casquero-Veiga, M., Garcia-Garcia, D., MacDowell, K. S., Perez-Caballero, L., Torres-Sanchez, S., et al. (2019). Risperidone administered during adolescence induced metabolic, anatomical and inflammatory/oxidative changes in adult brain: A PET and MRI study in the maternal immune stimulation animal model. *Eur.Neuropsychopharmacol., 29*(7), 880-896.

Castane, A., Artigas, F., & Bortolozzi, A. (2008). The absence of 5-HT(1A) receptors has minor effects on dopamine but not serotonin release evoked by MK-801 in mice prefrontal cortex. *Psychopharmacology (Berl), 200*(2), 281-290.

Castelli, M. P., Spiga, S., Perra, A., Madeddu, C., Mulas, G., et al. (2016). Alpha2A adrenergic receptors highly expressed in mesoprefrontal dopamine neurons. *Neuroscience*, *332*, 130-139.

Castle, D. J., & Murray, R. M. (1991). The neurodevelopmental basis of sex differences in schizophrenia. *Psychol.Med., 21*(3), 565-575.

Castle, D., Sham, P., & Murray, R. (1998). Differences in distribution of ages of onset in males and females with schizophrenia. *Schizophr.Res., 33*(3), 179-183.

Cathala, L., & Paupardin-Tritsch, D. (1997). Neurotensin inhibition of the hyperpolarization-activated cation current (ih) in the rat substantia nigra pars compacta implicates the protein kinase C pathway. *J.Physiol., 503 (Pt 1)*, 87-97.

Cedarbaum, J. M., & Aghajanian, G. K. (1978). Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. *J.Comp.Neurol., 178*(1), 1-16.

Celada, P., Bortolozzi, A., & Artigas, F. (2013). Serotonin 5-HT1A receptors as targets for agents to treat psychiatric disorders: Rationale and current status of research. *CNS Drugs, 27*(9), 703-716.

Celada, P., Puig, M. V., Casanovas, J. M., Guillazo, G., & Artigas, F. (2001). Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: Involvement of serotonin-1A, GABA(A), and glutamate receptors. *J.Neurosci., 21*(24), 9917-9929.

Cenci, M. A., Kalen, P., Mandel, R. J., & Bjorklund, A. (1992). Regional differences in the regulation of dopamine and noradrenaline release in medial frontal cortex, nucleus accumbens and caudate-putamen: A microdialysis study in the rat. *Brain Res., 581*(2), 217-228.

Chabre, O., Conklin, B. R., Brandon, S., Bourne, H. R., & Limbird, L. E. (1994). Coupling of the alpha 2A-adrenergic receptor to multiple G-proteins. A simple approach for estimating receptor-G-protein coupling efficiency in a transient expression system. *J.Biol.Chem., 269*(8), 5730-5734.

Chamberlain, S. R., & Robbins, T. W. (2013). Noradrenergic modulation of cognition: Therapeutic implications. *J.Psychopharmacol., 27*(8), 694-718.

Chappell, P. B., Riddle, M. A., Scahill, L., Lynch, K. A., Schultz, R., et al. (1995). Guanfacine treatment of comorbid attention-deficit hyperactivity disorder and Tourette's syndrome: Preliminary clinical experience. *J.Am.Acad.Child Adolesc.Psychiatry, 34*(9), 1140-1146.

Charney, D. S. (2003). Neuroanatomical circuits modulating fear and anxiety behaviors. *Acta Psychiatr.Scand.Suppl., (417)*(417), 38-50.

Chatterjee, P., Chiasson, V. L., Kopriva, S. E., Young, K. J., Chatterjee, V., et al. (2011). Interleukin 10 deficiency exacerbates toll-like receptor 3-induced preeclampsia-like symptoms in mice. *Hypertension, 58*(3), 489-496.

Chaudhry, F. A., Boulland, J. L., Jenstad, M., Bredahl, M. K., & Edwards, R. H. (2008). Pharmacology of neurotransmitter transport into secretory vesicles. *Handb.Exp.Pharmacol., (184):77-106.*

Chen, K. C., Yang, Y. K., Howes, O., Lee, I. H., Landau, S., et al. (2013). Striatal dopamine transporter availability in drug-naive patients with schizophrenia: A case-control SPECT study with [(99m)tc]-TRODAT-1 and a meta-analysis. *Schizophr.Bull., 39*(2), 378-386.

Chen, L., & Yang, C. R. (2002). Interaction of dopamine D1 and NMDA receptors mediates acute clozapine potentiation of glutamate EPSPs in rat prefrontal cortex. *J.Neurophysiol., 87*(5), 2324-2336.

Chen, L., Zhai, H., Lu, L., Chen, S., Ning, Y., & Wang, W. (2011). Effects of polyinosinic-polycytidylic acid (poly I:C) on naloxone-precipitated withdrawal in morphine-dependent mice. *Neurosci.Lett., 487*(3), 341-344.

Chen, P. L., Avramopoulos, D., Lasseter, V. K., McGrath, J. A., Fallin, M. D., et al. (2009). Fine mapping on chromosome 10q22-q23 implicates neuregulin 3 in schizophrenia. *Am.J.Hum.Genet., 84*(1), 21-34.

Cho, S. C., Kim, J. W., Kim, B. N., Hwang, J. W., Park, M., et al. (2008a). Possible association of the alpha-2A-adrenergic receptor gene with response time variability in attention deficit hyperactivity disorder. *Am.J.Med.Genet.B.Neuropsychiatr.Genet., 147B*(6), 957-963.

Cho, S. C., Kim, J. W., Kim, B. N., Hwang, J. W., Shin, M. S., et al. (2008b). Association between the alpha-2C-adrenergic receptor gene and attention deficit hyperactivity disorder in a Korean sample. *Neurosci.Lett., 446*(2-3), 108-111.

Choi, D. S., Ward, S. J., Messaddeq, N., Launay, J. M., & Maroteaux, L. (1997). 5-HT2B receptor-mediated serotonin morphogenetic functions in mouse cranial neural crest and myocardiac cells. *Development, 124*(9), 1745-1755.

Choi, G. B., Yim, Y. S., Wong, H., Kim, S., Kim, H., et al. (2016). The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science, 351*(6276), 933-939.

Chotani, M. A., Flavahan, S., Mitra, S., Daunt, D., & Flavahan, N. A. (2000). Silent alpha(2C)-adrenergic receptors enable cold-induced vasoconstriction in cutaneous arteries. *Am.J.Physiol.Heart Circ.Physiol., 278*(4), 1075.

Chou-Green, J. M., Holscher, T. D., Dallman, M. F., & Akana, S. F. (2003). Compulsive behavior in the 5-HT2C receptor knockout mouse. *Physiol.Behav., 78*(4-5), 641-649.

Chu, X., & Agmo, A. (2016). The adrenergic alpha2-receptor, sexual incentive motivation and copulatory behavior in the male rat. *Pharmacol.Biochem.Behav., 144*, 33-44.

Chudasama, Y. (2011). Animal models of prefrontal-executive function. *Behav.Neurosci., 125*(3), 327-343.

Chudasama, Y., Passetti, F., Rhodes, S. E., Lopian, D., Desai, A., & Robbins, T. W. (2003). Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: Differential effects on selectivity, impulsivity and compulsivity. *Behav.Brain Res., 146*(1-2), 105-119.

Chudasama, Y., & Robbins, T. W. (2004). Psychopharmacological approaches to modulating attention in the five-choice serial reaction time task: Implications for schizophrenia. *Psychopharmacology (Berl), 174*(1), 86-98.

Chudasama, Y., & Robbins, T. W. (2006). Functions of frontostriatal systems in cognition: Comparative neuropsychopharmacological studies in rats, monkeys and humans. *Biol.Psychol., 73*(1), 19-38.

Civantos Calzada, B., & Aleixandre de Artinano, A. (2001). Alpha-adrenoceptor subtypes. *Pharmacol.Res., 44*(3), 195-208.

Civelli, O., Bunzow, J. R., & Grandy, D. K. (1993). Molecular diversity of the dopamine receptors. *Annu.Rev.Pharmacol.Toxicol., 33*, 281-307.

Clare, L., McKenna, P. J., Mortimer, A. M., & Baddeley, A. D. (1993). Memory in schizophrenia: What is impaired and what is preserved?*31*(11), 1225-1241.

Clark, L., Cools, R., & Robbins, T. W. (2004). The neuropsychology of ventral prefrontal cortex: Decision-making and reversal learning. *Brain Cogn., 55*(1), 41-53.

Clark, R. E., Zola, S. M., & Squire, L. R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *J.Neurosci., 20*(23), 8853-8860.

Clerkin, S. M., Schulz, K. P., Halperin, J. M., Newcorn, J. H., Ivanov, I., et al. (2009). Guanfacine potentiates the activation of prefrontal cortex evoked by warning signals. *Biol.Psychiatry, 66*(4), 307-312.

Clozapine in drug-induced psychosis in Parkinson's disease. The French clozapine Parkinson study group.(1999). *Lancet, 353*(9169), 2041-2042.

Cochet, M., Donneger, R., Cassier, E., Gaven, F., Lichtenthaler, S. F., et al. (2013). 5-HT4 receptors constitutively promote the non-amyloidogenic pathway of APP cleavage and interact with ADAM10. *ACS Chem.Neurosci., 4*(1), 130-140.

Cohen, S. J., & Stackman, R. W., Jr. (2015). Assessing rodent hippocampal involvement in the novel object recognition task. A review. *Behav.Brain Res., 285*, 105-117.

Cole, M. W., Anticevic, A., Repovs, G., & Barch, D. (2011). Variable global dysconnectivity and individual differences in schizophrenia. *Biol.Psychiatry, 70*(1), 43-50.

Collin, G., de Reus, M. A., Cahn, W., Hulshoff Pol, H. E., Kahn, R. S., & van den Heuvel, M. P. (2013). Disturbed grey matter coupling in schizophrenia. *Eur.Neuropsychopharmacol., 23*(1), 46-54.

Collingridge, G. L., & Davies, J. (1981). The influence of striatal stimulation and putative neurotransmitters on identified neurones in the rat substantia nigra. *Brain Res., 212*(2), 345-359.

Connell, P. H. (Philip Henry) (1961). Amphetamine psychosis. *Oxford University Press*, London; New York.

Connor, D. F., Findling, R. L., Kollins, S. H., Sallee, F., Lopez, F. A., et al. (2010). Effects of guanfacine extended-release on oppositional symptoms in children aged 6-12 years with attention-deficit hyperactivity disorder and oppositional symptoms: A randomized, double-blind, placebo-controlled trial. *CNS Drugs, 24*(9), 755-768.

Corcoran, C., Walker, E., Huot, R., Mittal, V., Tessner, K., et al. (2003). The stress cascade and schizophrenia: Etiology and onset. *Schizophr.Bull., 29*(4), 671-692.

Cornblatt, B. A., & Keilp, J. G. (1994). Impaired attention, genetics, and the pathophysiology of schizophrenia. *Schizophr.Bull., 20*(1), 31-46.

Cornblatt, B., Obuchowski, M., Schnur, D., & O'Brien, J. D. (1998). Hillside study of risk and early detection in schizophrenia. *Br.J.Psychiatry Suppl., 172*(33), 26-32.

Correll, C. U. (2010). From receptor pharmacology to improved outcomes: Individualising the selection, dosing, and switching of antipsychotics. *Eur.Psychiatry, 25 Suppl 2*, 12.

Cortes, R., Soriano, E., Pazos, A., Probst, A., & Palacios, J. M. (1988). Autoradiography of antidepressant binding sites in the human brain: Localization using [3H]imipramine and [3H]paroxetine. *Neuroscience*, *27*(2), 473-496.

Costall B., N. R. (1995). Animal neuropharmacology and its prediction of clinical response. In Hirsch S.R., Weinberger D. R. (Ed.), *Schizophrenia* (pp. 275-307) Oxford.

Cotecchia, S., Kobilka, B. K., Daniel, K. W., Nolan, R. D., Lapetina, E. Y., et al. (1990). Multiple second messenger pathways of alpha-adrenergic receptor subtypes expressed in eukaryotic cells. *J.Biol.Chem., 265*(1), 63-69.

Cottingham, C., & Wang, Q. (2012). α2 adrenergic receptor dysregulation in depressive disorders: Implications for the neurobiology of depression and antidepressant therapy. *Neurosci.Biobehav.Rev., 36*(10), 2214-2225.

Coull, J. T. (1994). Pharmacological manipulations of the alpha 2-noradrenergic system. effects on cognition. *Drugs Aging, 5*(2), 116-126.

Coull, J. T., Buchel, C., Friston, K. J., & Frith, C. D. (1999). Noradrenergically mediated plasticity in a human attentional neuronal network. *Neuroimage, 10*(6), 705-715.

Coull, J. T., Frith, C. D., Dolan, R. J., Frackowiak, R. S., & Grasby, P. M. (1997). The neural correlates of the noradrenergic modulation of human attention, arousal and learning. *Eur.J.Neurosci., 9*(3), 589-598.

Coull, J. T., Sahakian, B. J., & Hodges, J. R. (1996). The alpha(2) antagonist idazoxan remediates certain attentional and executive dysfunction in patients with dementia of frontal type. *Psychopharmacology (Berl), 123*(3), 239-249.

Cowen, P., & Sherwood, A. C. (2013). The role of serotonin in cognitive function: Evidence from recent studies and implications for understanding depression. *J.Psychopharmacol., 27*(7), 575-583.

Coyle, J. T., & Tsai, G. (2004). NMDA receptor function, neuroplasticity, and the pathophysiology of schizophrenia. *Int.Rev.Neurobiol., 59*, 491-515.

Coyle, J. T., Tsai, G., & Goff, D. (2003). Converging evidence of NMDA receptor hypofunction in the pathophysiology of schizophrenia. *Ann.N.Y.Acad.Sci., 1003*, 318-327.

Crabtree, G. W., Park, A. J., Gordon, J. A., & Gogos, J. A. (2016). Cytosolic accumulation of L-proline disrupts GABA-ergic transmission through GAD blockade. *Cell.Rep., 17*(2), 570-582.

Craine, J. E., Daniels, G. H., & Kaufman, S. (1973). Dopamine-beta-hydroxylase. the subunit structure and anion activation of the bovine adrenal enzyme. *J.Biol.Chem., 248*(22), 7838-7844.

Crassous, P. A., Cardinaletti, C., Carrieri, A., Bruni, B., Di Vaira, M., et al. (2007). Alpha2-adrenoreceptors profile modulation. 3.1 (R)-(+)-m-nitrobiphenyline, a new efficient and alpha2C-subtype-selective agonist. *J.Med.Chem., 50*(16), 3964-3968.

Creese, I., Burt, D. R., & Snyder, S. H. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science, 192*(4238), 481-483.

Crow, T. J., Baker, H. F., Cross, A. J., Joseph, M. H., Lofthouse, R., et al. (1979). Monoamine mechanisms in chronic schizophrenia: Post-mortem neurochemical findings. *Br.J.Psychiatry, 134*, 249-256.

Csernansky, J. G., Csernansky, C. A., Kogelman, L., Montgomery, E. M., & Bardgett, M. E. (1998). Progressive neurodegeneration after intracerebroventricular kainic acid administration in rats: Implications for schizophrenia? *Biol.Psychiatry, 44*(11), 1143-1150.

Cunningham, C., Campion, S., Teeling, J., Felton, L., & Perry, V. H. (2007). The sickness behavior and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). *Brain Behav.Immun., 21*(4), 490-502.

Curley, A. A., & Lewis, D. A. (2012). Cortical basket cell dysfunction in schizophrenia. *J.Physiol., 590*(4), 715-724.

Dahlstrom, A., & Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol.Scand.Suppl.,* SUPPL 232:-55.

Dai, H., Gebhardt, K., & Carey, R. J. (1995). Time course effects of MK-801: The relationship between brain neurochemistry and behavior. *Brain Res.Bull., 36*(2), 175-180.

Dajas, F., Barbeito, L., Martinez-Pesquera, G., Lista, A., Puppo, D., & Puppo-Touriz, H. (1983). Plasma noradrenaline and clinical psychopathology in schizophrenia. A correlation analysis. *Neuropsychobiology*, *10*(2-3), 70-74.

Dalley, J. W., & Stanford, S. C. (1995). Contrasting effects of the imidazole(in)e alpha 2-adrenoceptor agonists, medetomidine, clonidine and UK 14,304 on extraneuronal levels of noradrenaline in the rat frontal cortex: Evaluation using in vivo microdialysis and synaptosomal uptake studies. *Br.J.Pharmacol., 114*(8), 1717-1723.

Dalley, J. W., Theobald, D. E., Pereira, E. A., Li, P. M., & Robbins, T. W. (2002). Specific abnormalities in serotonin release in the prefrontal cortex of isolation-reared rats measured during behavioral performance of a task assessing visuospatial attention and impulsivity. *Psychopharmacology (Berl), 164*(3), 329-340.

Dalman, C., Allebeck, P., Cullberg, J., Grunewald, C., & Koster, M. (1999). Obstetric complications and the risk of schizophrenia: A longitudinal study of a national birth cohort. *Arch.Gen.Psychiatry, 56*(3), 234-240.

Damsbo, A. G., Kraglund, K. L., Buttenschon, H. N., Johnsen, S. P., Andersen, G., & Mortensen, J. K. (2019). Serotonergic regulation and cognition after stroke: The role of antidepressant treatment and genetic variation. *Cerebrovasc.Dis., 47*(1-2), 72-79.

Dao-Castellana, M. H., Paillere-Martinot, M. L., Hantraye, P., Attar-Levy, D., Remy, P., et al. (1997). Presynaptic dopaminergic function in the striatum of schizophrenic patients. *Schizophr.Res., 23*(2), 167-174.

Daunt, D. A., Hurt, C., Hein, L., Kallio, J., Feng, F., & Kobilka, B. K. (1997). Subtype-specific intracellular trafficking of alpha2-adrenergic receptors. *Mol.Pharmacol., 51*(5), 711-720.

David, H. N., Sissaoui, K., & Abraini, J. H. (2004). Modulation of the locomotor responses induced by D1-like and D2-like dopamine receptor agonists and D-amphetamine by NMDA and non-NMDA glutamate receptor agonists and antagonists in the core of the rat nucleus accumbens. *Neuropharmacology*, *46*(2), 179-191.

Davidson, M., Galderisi, S., Weiser, M., Werbeloff, N., Fleischhacker, W. W., et al. (2009). Cognitive effects of antipsychotic drugs in first-episode schizophrenia and schizophreniform disorder: A randomized, open-label clinical trial (EUFEST). *Am.J.Psychiatry, 166*(6), 675-682.

Davies, W., Humby, T., Kong, W., Otter, T., Burgoyne, P. S., & Wilkinson, L. S. (2009). Converging pharmacological and genetic evidence indicates a role for steroid sulfatase in attention. *Biol.Psychiatry, 66*(4), 360-367.

Davis, K. L., Kahn, R. S., Ko, G., & Davidson, M. (1991). Dopamine in schizophrenia: A review and reconceptualization. *Am.J.Psychiatry, 148*(11), 1474-1486.

Davis, R., Whittington, R., & Bryson, H. M. (1997). Nefazodone. A review of its pharmacology and clinical efficacy in the management of major depression. *Drugs*, *53*(4), 608-636.

Daw, N. D., Kakade, S., & Dayan, P. (2002). Opponent interactions between serotonin and dopamine. *Neural Netw., 15*(4-6), 603-616.

Dawson, L. A., Langmead, C. J., Dada, A., Watson, J. M., Wu, Z., et al. (2010). In vitro and in vivo comparison of two non-peptide tachykinin NK3 receptor antagonists: Improvements in efficacy achieved through enhanced brain penetration or altered pharmacological characteristics. *Eur.J.Pharmacol., 627*(1-3), 106-114.

Dawson, L. A., Nguyen, H. Q., & Li, P. (2000). In vivo effects of the 5-HT(6) antagonist SB-271046 on striatal and frontal cortex extracellular concentrations of noradrenaline, dopamine, 5-HT, glutamate and aspartate. *Br.J.Pharmacol., 130*(1), 23-26.

Day, M., Balci, F., Wan, H. I., Fox, G. B., Rutkowski, J. L., & Feuerstein, G. (2008). Cognitive endpoints as disease biomarkers: Optimizing the congruency of preclinical models to the clinic. *Curr.Opin.Investig Drugs, 9*(7), 696-706.

Day, M., Carr, D. B., Ulrich, S., Ilijic, E., Tkatch, T., & Surmeier, D. J. (2005). Dendritic excitability of mouse frontal cortex pyramidal neurons is shaped by the interaction among HCN, Kir2, and kleak channels. *J.Neurosci., 25*(38), 8776-8787.

de Almeida, J., & Mengod, G. (2008). Serotonin 1A receptors in human and monkey prefrontal cortex are mainly expressed in pyramidal neurons and in a GABAergic interneuron subpopulation: Implications for schizophrenia and its treatment. *J.Neurochem., 107*(2), 488-496.

De Hert, M., Detraux, J., van Winkel, R., Yu, W., & Correll, C. U. (2011). Metabolic and cardiovascular adverse effects associated with antipsychotic drugs. *Nat.Rev.Endocrinol., 8*(2), 114-126.

De Keyser, J., Ebinger, G., & Vauquelin, G. (1989). Evidence for a widespread dopaminergic innervation of the human cerebral neocortex. *Neurosci.Lett., 104*(3), 281-285.

de la Flor, R., & Dawson, L. A. (2009). Augmentation of antipsychotic-induced neurochemical changes by the NK3 receptor antagonist talnetant (SB-223412). *Neuropharmacology*, *56*(2), 342-349.

de Lange, E. C., Danhof, M., de Boer, A. G., & Breimer, D. D. (1997). Methodological considerations of intracerebral microdialysis in pharmacokinetic studies on drug transport across the blood-brain barrier. *Brain Res.Brain Res.Rev., 25*(1), 27-49.

de Lecea, L., Carter, M. E., & Adamantidis, A. (2012). Shining light on wakefulness and arousal. *Biol.Psychiatry, 71*(12), 1046-1052.

De Ponti, F., & Tonini, M. (2001). Irritable bowel syndrome: New agents targeting serotonin receptor subtypes. *Drugs*, *61*(3), 317-332.

De Vos, H., Vauquelin, G., De Keyser, J., De Backer, J. P., & Van Liefde, I. (1992). Regional distribution of alpha 2A- and alpha 2B-adrenoceptor subtypes in postmortem human brain. *J.Neurochem., 58*(4), 1555-1560.

Deakin, J. F., & Graeff, F. G. (1991). 5-HT and mechanisms of defence. *J.Psychopharmacol., 5*(4), 305-315.

Dean, B., & Hussain, T. (2001). Studies on dopaminergic and GABAergic markers in striatum reveals a decrease in the dopamine transporter in schizophrenia. *Schizophr.Res., 52*(1-2), 107-114.

Deisseroth, K. (2011). Optogenetics. *Nat.Methods, 8*(1), 26-29.

Deisseroth, K. (2014). Circuit dynamics of adaptive and maladaptive behavior. *Nature*, *505*(7483), 309-317.

Deisseroth, K. (2015). Optogenetics: 10 years of microbial opsins in neuroscience. *Nat.Neurosci., 18*(9), 1213-1225.

Deisseroth, K., Etkin, A., & Malenka, R. C. (2015). Optogenetics and the circuit dynamics of psychiatric disease. *Jama, 313*(20), 2019-2020.

Del Pino, I., Garcia-Frigola, C., Dehorter, N., Brotons-Mas, J. R., Alvarez-Salvado, E., et al. (2013). Erbb4 deletion from fast-spiking interneurons causes schizophrenia-like phenotypes. *Neuron*, *79*(6), 1152-1168.

Delaville, C., Navailles, S., & Benazzouz, A. (2012). Effects of noradrenaline and serotonin depletions on the neuronal activity of globus pallidus and substantia nigra pars reticulata in experimental parkinsonism. *Neuroscience, 202*, 424-433.

Deleidi, M., Hallett, P. J., Koprich, J. B., Chung, C. Y., & Isacson, O. (2010). The toll-like receptor-3 agonist polyinosinic:Polycytidylic acid triggers nigrostriatal dopaminergic degeneration. *J.Neurosci., 30*(48), 16091-16101.

Demjaha, A., Murray, R. M., McGuire, P. K., Kapur, S., & Howes, O. D. (2012). Dopamine synthesis capacity in patients with treatment-resistant schizophrenia. *Am.J.Psychiatry, 169*(11), 1203-1210.

Dennis, T., L'Heureux, R., Carter, C., & Scatton, B. (1987). Presynaptic alpha-2 adrenoceptors play a major role in the effects of idazoxan on cortical noradrenaline release (as measured by in vivo dialysis) in the rat. *J.Pharmacol.Exp.Ther., 241*(2), 642-649.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neurosci.Biobehav.Rev., 31*(5), 673-704.

Dere, E., Kart-Teke, E., Huston, J. P., & De Souza Silva, M. A. (2006). The case for episodic memory in animals. *Neurosci.Biobehav.Rev., 30*(8), 1206-1224.

Descarries, L., Lemay, B., Doucet, G., & Berger, B. (1987). Regional and laminar density of the dopamine innervation in adult rat cerebral cortex. *Neuroscience*, *21*(3), 807-824.

Deslauriers, J., Larouche, A., Sarret, P., & Grignon, S. (2013). Combination of prenatal immune challenge and restraint stress affects prepulse inhibition and dopaminergic/GABAergic markers. *Prog.Neuropsychopharmacol.Biol.Psychiatry, 45*, 156-164.

Deutch, A. Y., & Roth, R. H. (1990). The determinants of stress-induced activation of the prefrontal cortical dopamine system. *Prog.Brain Res., 85*, 36-3.

Deutch, A. Y., Tam, S. Y., Freeman, A. S., Bowers, M. B.,Jr, & Roth, R. H. (1987). Mesolimbic and mesocortical dopamine activation induced by phencyclidine: Contrasting pattern to striatal response. *Eur.J.Pharmacol., 134*(3), 257-264.

Devauges, V., & Sara, S. J. (1990). Activation of the noradrenergic system facilitates an attentional shift in the rat. *Behav.Brain Res., 39*(1), 19-28.

Devito, L. M., & Eichenbaum, H. (2011). Memory for the order of events in specific sequences: Contributions of the hippocampus and medial prefrontal cortex. *J.Neurosci., 31*(9), 3169-3175.

Devoto, P., & Flore, G. (2006). On the origin of cortical dopamine: Is it a co-transmitter in noradrenergic neurons? *Curr.Neuropharmacol., 4*(2), 115-125.

Devoto, P., Flore, G., Pani, L., & Gessa, G. L. (2001). Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. *Mol.Psychiatry, 6*(6), 657-664.

Devoto, P., Flore, G., Pira, L., Longu, G., & Gessa, G. L. (2004). Alpha2-adrenoceptor mediated co-release of dopamine and noradrenaline from noradrenergic neurons in the cerebral cortex. *J.Neurochem., 88*(4), 1003-1009.

Devoto, P., Flore, G., Saba, P., Fa, M., & Gessa, G. L. (2005). Stimulation of the locus coeruleus elicits noradrenaline and dopamine release in the medial prefrontal and parietal cortex. *J.Neurochem., 92*(2), 368-374.

Devoto, P., Flore, G., Vacca, G., Pira, L., Arca, A., et al. (2003). Co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex induced by clozapine, the prototype atypical antipsychotic. *Psychopharmacology (Berl), 167*(1), 79-84.

Devroye, C., Haddjeri, N., Cathala, A., Rovera, R., Drago, F., et al. (2017). Opposite control of mesocortical and mesoaccumbal dopamine pathways by serotonin2B receptor blockade: Involvement of medial prefrontal cortex serotonin1A receptors. *Neuropharmacology*, *119*, 91-99.

Di Chiara, G., Tanda, G. L., Frau, R., & Carboni, E. (1992). Heterologous monoamine reuptake: Lack of transmitter specificity of neuron-specific carriers. *Neurochem.Int., 20 Suppl*, 231-235S.

Di Forti, M., Marconi, A., Carra, E., Fraietta, S., Trotta, A., et al. (2015). Proportion of patients in south london with first-episode psychosis attributable to use of high potency cannabis: A case-control study. *Lancet Psychiatry., 2*(3), 233-238.

Di Forti, M., Quattrone, D., Freeman, T. P., Tripoli, G., Gayer-Anderson, C., et al. (2019). The contribution of cannabis use to variation in the incidence of psychotic disorder across europe (EU-GEI): A multicentre case-control study. *Lancet Psychiatry., 6*(5), 427-436.

DiCicco-Bloom, E., Lord, C., Zwaigenbaum, L., Courchesne, E., Dager, S. R., et al. (2006). The developmental neurobiology of autism spectrum disorder. *J.Neurosci., 26*(26), 6897-6906.

Dickey, C. C., McCarley, R. W., Voglmaier, M. M., Niznikiewicz, M. A., Seidman, L. J., et al. (2003). A MRI study of fusiform gyrus in schizotypal personality disorder. *Schizophr.Res., 64*(1), 35-39.

Dickinson, D., Iannone, V. N., Wilk, C. M., & Gold, J. M. (2004). General and specific cognitive deficits in schizophrenia. *Biol.Psychiatry, 55*(8), 826-833.

Didriksen, M. (1995). Effects of antipsychotics on cognitive behaviour in rats using the delayed non-match to position paradigm. *Eur.J.Pharmacol., 281*(3), 241-250.

Dillon, C., Serrano, C. M., Castro, D., Leguizamon, P. P., Heisecke, S. L., & Taragano, F. E. (2013). Behavioral symptoms related to cognitive impairment. *Neuropsychiatr.Dis.Treat., 9*, 1443-1455.

Docherty, J. R. (1998). Subtypes of functional alpha1- and alpha2-adrenoceptors. *Eur.J.Pharmacol., 361*(1), 1-15.

Dominguez Mde, G., Viechtbauer, W., Simons, C. J., van Os, J., & Krabbendam, L. (2009). Are psychotic psychopathology and neurocognition orthogonal? A systematic review of their associations. *Psychol.Bull., 135*(1), 157-171.

Doxey, J. C., Roach, A. G., & Smith, C. F. (1983). Studies on RX 781094: A selective, potent and specific antagonist of alpha 2-adrenoceptors. *Br.J.Pharmacol., 78*(3), 489-505.

Dray, A. (1981). Serotonin in the basal ganglia: Functions and interactions with other neuronal pathways. *J.Physiol.(Paris), 77*(2-3), 393-403.

Dray, A., Davies, J., Oakley, N. R., Tongroach, P., & Vellucci, S. (1978). The dorsal and medial raphe projections to the substantia nigra in the rat: Electrophysiological, biochemical and behavioural observations. *Brain Res., 151*(3), 431-442.

Dresse, A., & Scuvee-Moreau, J. (1986). Influence of the alpha-2 agonist oxaminozoline (S3341) on firing rate of central noradrenergic and serotonergic neurons in the rat. comparison with clonidine. *Arch.Int.Physiol.Biochim., 94*(2), 99-106.

Drew, M. R., Simpson, E. H., Kellendonk, C., Herzberg, W. G., Lipatova, O., et al. (2007). Transient overexpression of striatal D2 receptors impairs operant motivation and interval timing. *J.Neurosci., 27*(29), 7731-7739.

Driesen, N. R., Leung, H. C., Calhoun, V. D., Constable, R. T., Gueorguieva, R., et al. (2008). Impairment of working memory maintenance and response in schizophrenia: Functional magnetic resonance imaging evidence. *Biol.Psychiatry, 64*(12), 1026-1034.

Drouin, C., Darracq, L., Trovero, F., Blanc, G., Glowinski, J., et al. (2002). Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. *J.Neurosci., 22*(7), 2873-2884.

Drzyzga, L., Obuchowicz, E., Marcinowska, A., & Herman, Z. S. (2006). Cytokines in schizophrenia and the effects of antipsychotic drugs. *Brain Behav.Immun., 20*(6), 532-545.

Duman, R. S. (2002). Synaptic plasticity and mood disorders. *Mol.Psychiatry, 7 Suppl 1*, 29.

Duncan, G., Miyamoto, S., Gu, H., Lieberman, J., Koller, B., & Snouwaert, J. (2002). Alterations in regional brain metabolism in genetic and pharmacological models of reduced NMDA receptor function. *Brain Res., 951*(2), 166-176.

Durstewitz, D., Seamans, J. K., & Sejnowski, T. J. (2000). Neurocomputational models of working memory. *Nat.Neurosci., 3 Suppl*, 1184-1191.

Duxon, M. S., Kennett, G. A., Lightowler, S., Blackburn, T. P., & Fone, K. C. (1997). Activation of 5-HT2B receptors in the medial amygdala causes anxiolysis in the social interaction test in the rat. *Neuropharmacology*, *36*(4-5), 601-608.

Eason, M. G., Jacinto, M. T., & Liggett, S. B. (1994). Contribution of ligand structure to activation of alpha 2-adrenergic receptor subtype coupling to gs. *Mol.Pharmacol., 45*(4), 696-702.

Eason, M. G., Kurose, H., Holt, B. D., Raymond, J. R., & Liggett, S. B. (1992). Simultaneous coupling of alpha 2-adrenergic receptors to two G-proteins with opposing effects. subtype-selective coupling of alpha 2C10, alpha 2C4, and alpha 2C2 adrenergic receptors to gi and gs. *J.Biol.Chem., 267*(22), 15795-15801.

Eggers, A. E. (2013). A serotonin hypothesis of schizophrenia. *Med.Hypotheses, 80*(6), 791-794.

Eisenhofer, G. (2001). The role of neuronal and extraneuronal plasma membrane transporters in the inactivation of peripheral catecholamines. *Pharmacol.Ther., 91*(1), 35-62.

Elston, G. N. (2003). Cortex, cognition and the cell: New insights into the pyramidal neuron and prefrontal function. *Cereb.Cortex, 13*(11), 1124-1138.

Elvevag, B., Weinberger, D. R., Suter, J. C., & Goldberg, T. E. (2000). Continuous performance test and schizophrenia: A test of stimulus-response compatibility, working memory, response readiness, or none of the above? *Am.J.Psychiatry, 157*(5), 772-780.

Emamian, E. S., Hall, D., Birnbaum, M. J., Karayiorgou, M., & Gogos, J. A. (2004). Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat.Genet., 36*(2), 131-137.

Engberg, G., & Eriksson, E. (1991). Effects of alpha 2-adrenoceptor agonists on locus coeruleus firing rate and brain noradrenaline turnover in N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)-treated rats. *Naunyn Schmiedebergs Arch.Pharmacol., 343*(5), 472-477.

Engel, K., Zhou, M., & Wang, J. (2004). Identification and characterization of a novel monoamine transporter in the human brain. *J.Biol.Chem., 279*(48), 50042-50049.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav.Brain Res., 31*(1), 47-59.

Erdozain, A. M., Brocos-Mosquera, I., Gabilondo, A. M., Meana, J. J., & Callado, L. F. (2018a). Differential alpha2A- and alpha2C-adrenoceptor protein expression in presynaptic and postsynaptic density fractions of postmortem human prefrontal cortex. *J.Psychopharmacol., 33*,(2),244-249.

Erdozain, A. M., De Gois, S., Bernard, V., Gorgievski, V., Pietrancosta, N., et al. (2018b). Structural and functional characterization of the interaction of snapin with the dopamine transporter: Differential modulation of psychostimulant actions. *Neuropsychopharmacology, 43*(5), 1041-1051.

Erlenmeyer-Kimling, L., Rock, D., Roberts, S. A., Janal, M., Kestenbaum, C., et al. (2000). Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: The new york high-risk project. *Am.J.Psychiatry, 157*(9), 1416-1422.

Erspamer, V., & Asero, B. (1952). Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature*, *169*(4306), 800-801.

Ertekin, E., Ucok, A., Keskin-Ergen, Y., & Devrim-Ucok, M. (2017). Deficits in go and NoGo P3 potentials in patients with schizophrenia. *Psychiatry Res., 254*, 126-132.

Eschenko, O., & Sara, S. J. (2008). Learning-dependent, transient increase of activity in noradrenergic neurons of locus coeruleus during slow wave sleep in the rat: Brain stem-cortex interplay for memory consolidation? *Cereb.Cortex, 18*(11), 2596-2603.

Eshleman, A. J., Carmolli, M., Cumbay, M., Martens, C. R., Neve, K. A., & Janowsky, A. (1999). Characteristics of drug interactions with recombinant biogenic amine transporters expressed in the same cell type. *J.Pharmacol.Exp.Ther., 289*(2), 877-885.

Estes, M. L., & McAllister, A. K. (2015). Immune mediators in the brain and peripheral tissues in autism spectrum disorder. *Nat.Rev.Neurosci., 16*(8), 469-486.

Estes, M. L., & McAllister, A. K. (2016a). Immunology. Maternal TH17 cells take a toll on baby's brain. *Science, 351*(6276), 919-920.

Estes, M. L., & McAllister, A. K. (2016b). Maternal immune activation: Implications for neuropsychiatric disorders. *Science, 353*(6301), 772-777.

Etou, K., Kuroki, T., Kawahara, T., Yonezawa, Y., Tashiro, N., & Uchimura, H. (1998). Ceruletide inhibits phencyclidine-induced dopamine and serotonin release in rat prefrontal cortex. *Pharmacol.Biochem.Behav., 61*(4), 427-434.

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. *Nat.Neurosci., 8*(11), 1481-1489.

Fagerholm, V., Rokka, J., Nyman, L., Sallinen, J., Tiihonen, J., et al. (2008). Autoradiographic characterization of alpha(2C)-adrenoceptors in the human striatum. *Synapse, 62*(7), 508-515.

Fallon, J. H., Koziell, D. A., & Moore, R. Y. (1978a). Catecholamine innervation of the basal forebrain. II. amygdala, suprarhinal cortex and entorhinal cortex. *J.Comp.Neurol., 180*(3), 509-532.

Fallon, J. H., & Moore, R. Y. (1978b). Catecholamine innervation of the basal forebrain. III. olfactory bulb, anterior olfactory nuclei, olfactory tubercle and piriform cortex. *J.Comp.Neurol., 180*(3), 533-544.

Farde, L., Wiesel, F. A., Hall, H., Halldin, C., Stone-Elander, S., & Sedvall, G. (1987). No D2 receptor increase in PET study of schizophrenia. *Arch.Gen.Psychiatry, 44*(7), 671-672.

Farley, I. J., Price, K. S., McCullough, E., Deck, J. H., Hordynski, W., & Hornykiewicz, O. (1978). Norepinephrine in chronic paranoid schizophrenia: Above-normal levels in limbic forebrain. *Science, 200*(4340), 456-458.

Farovik, A., Dupont, L. M., Arce, M., & Eichenbaum, H. (2008). Medial prefrontal cortex supports recollection, but not familiarity, in the rat. *J.Neurosci., 28*(50), 13428-13434.

Fatemi, S. H., Cuadra, A. E., El-Fakahany, E. E., Sidwell, R. W., & Thuras, P. (2000). Prenatal viral infection causes alterations in nNOS expression in developing mouse brains. *Neuroreport*, *11*(7), 1493-1496.

Fatemi, S. H., Emamian, E. S., Sidwell, R. W., Kist, D. A., Stary, J. M., et al. (2002). Human influenza viral infection in utero alters glial fibrillary acidic protein immunoreactivity in the developing brains of neonatal mice. *Mol.Psychiatry, 7*(6), 633-640.

Fatemi, S. H., Pearce, D. A., Brooks, A. I., & Sidwell, R. W. (2005). Prenatal viral infection in mouse causes differential expression of genes in brains of mouse progeny: A potential animal model for schizophrenia and autism. *Synapse, 57*(2), 91-99.

Fatemi, S. H., Reutiman, T. J., Folsom, T. D., Huang, H., Oishi, K., et al. (2008). Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: Implications for genesis of neurodevelopmental disorders. *Schizophr.Res., 99*(1-3), 56-70.

Fazzari, P., Paternain, A. V., Valiente, M., Pla, R., Lujan, R., et al. (2010). Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. *Nature*, *464*(7293), 1376-1380.

Feinberg, I. (1982). Schizophrenia: Caused by a fault in programmed synaptic elimination during adolescence? *J.Psychiatr.Res., 17*(4), 319-334.

Ferezou, I., Cauli, B., Hill, E. L., Rossier, J., Hamel, E., & Lambolez, B. (2002). 5-HT3 receptors mediate serotonergic fast synaptic excitation of neocortical vasoactive intestinal peptide/cholecystokinin interneurons. *J.Neurosci., 22*(17), 7389-7397.

Fernández-Atucha, A., Echevarría, A., Larrinaga, G., Gil, J., Martínez-Cengotitabengoa, M., et al. (2015). Plasma peptidases as prognostic biomarkers in patients with fist-episode psychosis. *Psychiatry Res*., *228*(2), 197-202.

Fernández-Pastor, B. (2003). Caracterización y regulación farmacológica in vivo de la neurotransmisión noradrenérgica y su modulación por fármacos serotonérgicos. Un estudio en el locus coeruleus de rata. University of the Basque Country (UPV-EHU) (Spain).

Fernández-Pastor, B., & Meana, J. J. (2002). In vivo tonic modulation of the noradrenaline release in the rat cortex by locus coeruleus somatodendritic alpha(2)-adrenoceptors. *Eur.J.Pharmacol., 442*(3), 225-229.

Ferre, S., & Artigas, F. (1993). Dopamine D2 receptor-mediated regulation of serotonin extracellular concentration in the dorsal raphe nucleus of freely moving rats. *J.Neurochem., 61*(2), 772-775.

Ferre, S., Cortes, R., & Artigas, F. (1994). Dopaminergic regulation of the serotonergic raphe-striatal pathway: Microdialysis studies in freely moving rats. *J.Neurosci., 14*(8), 4839-4846.

Ferrucci, M., Giorgi, F. S., Bartalucci, A., Busceti, C. L., & Fornai, F. (2013). The effects of locus coeruleus and norepinephrine in methamphetamine toxicity. *Curr.Neuropharmacol., 11*(1), 80-94.

Fibiger, & Miller, J. J. (1977). An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat. *Neuroscience, 2(*6), 975-987.

Fidalgo, S., Ivanov, D. K., & Wood, S. H. (2013). Serotonin: From top to bottom. *Biogerontology*, *14*(1), 21-45.

Fields, R. B., Van Kammen, D. P., Peters, J. L., Rosen, J., Van Kammen, W. B., et al. (1988). Clonidine improves memory function in schizophrenia independently from change in psychosis. preliminary findings. *Schizophr.Res., 1*(6), 417-423.

Finlay, J. M., Zigmond, M. J., & Abercrombie, E. D. (1995). Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: Effects of diazepam. *Neuroscience*, *64*(3), 619-628.

Finnema, S. J., Hughes, Z. A., Haaparanta-Solin, M., Stepanov, V., Nakao, R., et al. (2014). Amphetamine decreases alpha2C-adrenoceptor binding of [11C]ORM-13070: A PET study in the primate brain. *Int.J.Neuropsychopharmacol., 18*(3), 10.1093/ijnp/pyu081.

Fitzgerald, P. J. (2014). Is elevated norepinephrine an etiological factor in some cases of schizophrenia? *Psychiatry Res., 215*(3), 497-504.

Flashman, L. A., & Green, M. F. (2004). Review of cognition and brain structure in schizophrenia: Profiles, longitudinal course, and effects of treatment. *Psychiatr.Clin.North Am., 27*(1), 1-18, vii.

Fleming, K., Goldberg, T. E., Gold, J. M., & Weinberger, D. R. (1995). Verbal working memory dysfunction in schizophrenia: Use of a Brown-Peterson paradigm. *Psychiatry Res., 56*(2), 155-161.

Floresco, S. B., Geyer, M. A., Gold, L. H., & Grace, A. A. (2005). Developing predictive animal models and establishing a preclinical trials network for assessing treatment effects on cognition in schizophrenia. *Schizophr.Bull., 31*(4), 888-894.

Floresco, S. B., Magyar, O., Ghods-Sharifi, S., Vexelman, C., & Tse, M. T. (2006). Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. *Neuropsychopharmacology, 31*(2), 297-309.

Floresco, S. B., Todd, C. L., & Grace, A. A. (2001). Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. *J.Neurosci., 21*(13), 4915-4922.

Floresco, S. B., West, A. R., Ash, B., Moore, H., & Grace, A. A. (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat.Neurosci., 6*(9), 968-973.

Florin-Lechner, S. M., Druhan, J. P., Aston-Jones, G., & Valentino, R. J. (1996). Enhanced norepinephrine release in prefrontal cortex with burst stimulation of the locus coeruleus. *Brain Res., 742*(1-2), 89-97.

Foley, P., Gerlach, M., Youdim, M. B., & Riederer, P. (2000). MAO-B inhibitors: Multiple roles in the therapy of neurodegenerative disorders? *Parkinsonism Relat.Disord., 6*(1), 25-47.

Foote, S. L., Aston-Jones, G., & Bloom, F. E. (1980). Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. *Proc.Natl.Acad.Sci.U.S.A., 77*(5), 3033-3037.

Foote, S. L., & Morrison, J. H. (1987). Extrathalamic modulation of cortical function. *Annu.Rev.Neurosci., 10*, 67-95.

Ford, J. M., Roach, B. J., Faustman, W. O., & Mathalon, D. H. (2008). Out-of-synch and out-of-sorts: Dysfunction of motor-sensory communication in schizophrenia. *Biol.Psychiatry, 63*(8), 736-743.

Fornito, A., Harrison, B. J., Goodby, E., Dean, A., Ooi, C., et al. (2013). Functional dysconnectivity of corticostriatal circuitry as a risk phenotype for psychosis. *JAMA Psychiatry., 70*(11), 1143-1151.

Forray, C., & Buller, R. (2017). Challenges and opportunities for the development of new antipsychotic drugs. *Biochem.Pharmacol., 143*, 10-24.

Fortier, M. E., Kent, S., Ashdown, H., Poole, S., Boksa, P., & Luheshi, G. N. (2004). The viral mimic, polyinosinic:Polycytidylic acid, induces fever in rats via an interleukin-1-dependent mechanism. *Am.J.Physiol.Regul.Integr.Comp.Physiol., 287*(4), R759-66.

Fortier, M. E., Luheshi, G. N., & Boksa, P. (2007). Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy. *Behav.Brain Res., 181*(2), 270-277.

Foster, B. L., Kaveh, A., Dastjerdi, M., Miller, K. J., & Parvizi, J. (2013). Human retrosplenial cortex displays transient theta phase locking with medial temporal cortex prior to activation during autobiographical memory retrieval. *J.Neurosci., 33*(25), 10439-10446.

Fowler, J. S., Logan, J., Shumay, E., Alia-Klein, N., Wang, G. J., & Volkow, N. D. (2015). Monoamine oxidase: Radiotracer chemistry and human studies. *J.Labelled Comp.Radiopharm., 58*(3), 51-64.

Fox, H., Sofuoglu, M., & Sinha, R. (2015). Guanfacine enhances inhibitory control and attentional shifting in early abstinent cocaine-dependent individuals. *J.Psychopharmacol., 29*(3), 312-323.

Frankhuyzen, A. L., & Mulder, A. H. (1980). Noradrenaline inhibits depolarization-induced 3H-serotonin release from slices of rat hippocampus. *Eur.J.Pharmacol., 63*(2-3), 179-182.

Frankle, W. G., Laruelle, M., & Haber, S. N. (2006). Prefrontal cortical projections to the midbrain in primates: Evidence for a sparse connection. *Neuropsychopharmacology, 31*(8), 1627-1636.

Franowicz, J. S., & Arnsten, A. F. (2002). Actions of alpha-2 noradrenergic agonists on spatial working memory and blood pressure in rhesus monkeys appear to be mediated by the same receptor subtype. *Psychopharmacology (Berl), 162*(3), 304-312.

Franowicz, J. S., Kessler, L. E., Borja, C. M., Kobilka, B. K., Limbird, L. E., & Arnsten, A. F. (2002). Mutation of the alpha2A-adrenoceptor impairs working memory performance and annuls cognitive enhancement by guanfacine. *J.Neurosci., 22*(19), 8771-8777.

Franowicz, J. S., & Arnsten, A. F. (1998). The alpha-2a noradrenergic agonist, guanfacine, improves delayed response performance in young adult rhesus monkeys. *Psychopharmacology (Berl), 136*(1), 8-14.

Franowicz, J. S., & Arnsten, A. F. (1999). Treatment with the noradrenergic alpha-2 agonist clonidine, but not diazepam, improves spatial working memory in normal young rhesus monkeys. *Neuropsychopharmacology, 21*(5), 611-621.

Fraser, C. M., Arakawa, S., McCombie, W. R., & Venter, J. C. (1989). Cloning, sequence analysis, and permanent expression of a human alpha 2-adrenergic receptor in Chinese hamster ovary cells. evidence for independent pathways of receptor coupling to adenylate cyclase attenuation and activation. *J.Biol.Chem., 264*(20), 11754-11761.

Freedman, J. E., & Aghajanian, G. K. (1984). Idazoxan (RX 781094) selectively antagonizes alpha 2-adrenoceptors on rat central neurons. *Eur.J.Pharmacol., 105*(3-4), 265-272.

Freeman, T. P., van der Pol, P., Kuijpers, W., Wisselink, J., Das, R. K., et al. (2018). Changes in cannabis potency and first-time admissions to drug treatment: A 16-year study in the netherlands. *Psychol.Med., 48*(14), 2346-2352.

French, E. D., & Ceci, A. (1990). Non-competitive N-methyl-D-aspartate antagonists are potent activators of ventral tegmental A10 dopamine neurons. *Neurosci.Lett., 119*(2), 159-162.

French, E. D., & Vantini, G. (1984). Phencyclidine-induced locomotor activity in the rat is blocked by 6-hydroxydopamine lesion of the nucleus accumbens: Comparisons to other psychomotor stimulants. *Psychopharmacology (Berl), 82*(1-2), 83-88.

Fresquet, N., Angst, M. J., Schleef, C., Gobaille, S., & Sandner, G. (2007). Adrenergic drugs modify the level of noradrenaline in the insular cortex and alter extinction of conditioned taste aversion in rats. *Behav.Brain Res., 178*(1), 39-46.

Fribourg, M., Moreno, J. L., Holloway, T., Provasi, D., Baki, L., et al. (2011). Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. *Cell*, *147*(5), 1011-1023.

Friedman, J. I. (2004). Cholinergic targets for cognitive enhancement in schizophrenia: Focus on cholinesterase inhibitors and muscarinic agonists. *Psychopharmacology (Berl), 174*(1), 45-53.

Friedman, J. I., Adler, D. N., & Davis, K. L. (1999). The role of norepinephrine in the pathophysiology of cognitive disorders: Potential applications to the treatment of cognitive dysfunction in schizophrenia and Alzheimer's disease. *Biol.Psychiatry, 46*(9), 1243-1252.

Friedman, J. I., Adler, D. N., Temporini, H. D., Kemether, E., Harvey, P. D., et al. (2001). Guanfacine treatment of cognitive impairment in schizophrenia. *Neuropsychopharmacology, 25*(3), 402-409.

Funahashi, S. (2001). Neuronal mechanisms of executive control by the prefrontal cortex. *Neurosci.Res., 39*(2), 147-165.

Funahashi, S., Chafee, M. V., & Goldman-Rakic, P. S. (1993). Prefrontal neuronal activity in rhesus monkeys performing a delayed anti-saccade task. *Nature*, *365*(6448), 753-756.

Fusar-Poli, P., & Meyer-Lindenberg, A. (2013). Striatal presynaptic dopamine in schizophrenia, part I: Meta-analysis of dopamine active transporter (DAT) density. *Schizophr.Bull., 39*(1), 22-32.

Fuster, J. M. (1997). *The prefrontal cortex: Anatomy, physiology, and neuropsychology of the frontal lobe*. New York: Lippincott-Raven Publishers.

Gage, S. H., Hickman, M., & Zammit, S. (2016). Association between cannabis and psychosis: Epidemiologic evidence. *Biol.Psychiatry, 79*(7), 549-556.

Gamo, N. J., Lur, G., Higley, M. J., Wang, M., Paspalas, C. D., et al. (2015). Stress impairs prefrontal cortical function via D1 dopamine receptor interactions with hyperpolarization-activated cyclic nucleotide-gated channels. *Biol.Psychiatry, 78*(12), 860-870.

Garcia-Alloza, M., Hirst, W. D., Chen, C. P., Lasheras, B., Francis, P. T., & Ramirez, M. J. (2004). Differential involvement of 5-HT(1B/1D) and 5-HT6 receptors in cognitive and non-cognitive symptoms in Alzheimer's disease. *Neuropsychopharmacology, 29*(2), 410-416.

Garey, L. J., Ong, W. Y., Patel, T. S., Kanani, M., Davis, A., et al. (1998). Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *J.Neurol.Neurosurg.Psychiatry., 65*(4), 446-453.

Garratt, J. C., Crespi, F., Mason, R., & Marsden, C. A. (1991). Effects of idazoxan on dorsal raphe 5-hydroxytryptamine neuronal function. *Eur.J.Pharmacol., 193*(1), 87-93.

Gartside, S. E., Umbers, V., & Sharp, T. (1997). Inhibition of 5-HT cell firing in the DRN by non-selective 5-HT reuptake inhibitors: Studies on the role of 5-HT1A autoreceptors and noradrenergic mechanisms. *Psychopharmacology (Berl), 130*(3), 261-268.

Gaspar, P., Bloch, B., & Le Moine, C. (1995). D1 and D2 receptor gene expression in the rat frontal cortex: Cellular localization in different classes of efferent neurons. *Eur.J.Neurosci., 7*(5), 1050-1063.

Gavin, K. T., Colgan, M. P., Moore, D., Shanik, G., & Docherty, J. R. (1997). Alpha 2C-adrenoceptors mediate contractile responses to noradrenaline in the human saphenous vein. *Naunyn Schmiedebergs Arch.Pharmacol., 355*(3), 406-411.

Gervais, J., & Rouillard, C. (2000). Dorsal raphe stimulation differentially modulates dopaminergic neurons in the ventral tegmental area and substantia nigra. *Synapse, 35*(4), 281-291.

Geyer, M. A. (1998). Behavioral studies of hallucinogenic drugs in animals: Implications for schizophrenia research. *Pharmacopsychiatry*, *31 Suppl 2*, 73-79.

Geyer, M. A., & Braff, D. L. (1987). Startle habituation and sensorimotor gating in schizophrenia and related animal models. *Schizophr.Bull., 13*(4), 643-668.

Geyer, M. A., Olivier, B., Joels, M., & Kahn, R. S. (2012). From antipsychotic to anti-schizophrenia drugs: Role of animal models. *Trends Pharmacol.Sci., 33*(10), 515-521.

Geyer, M. A., Segal, D. S., & Greenberg, B. D. (1984). Increased startle responding in rats treated with phencyclidine. *Neurobehav.Toxicol.Teratol., 6*(2), 161-164.

Geyer, M. A., & Vollenweider, F. X. (2008). Serotonin research: Contributions to understanding psychoses. *Trends Pharmacol.Sci., 29*(9), 445-453.

Gilmore, J. H., & Jarskog, L. F. (1997). Exposure to infection and brain development: Cytokines in the pathogenesis of schizophrenia. *Schizophr.Res., 24*(3), 365-367.

Gilmore, J. H., Jarskog, L. F., & Vadlamudi, S. (2005). Maternal poly I:C exposure during pregnancy regulates TNF alpha, BDNF, and NGF expression in neonatal brain and the maternal-fetal unit of the rat. *J.Neuroimmunol., 159*(1-2), 106-112.

Gilsbach, R., & Hein, L. (2012). Are the pharmacology and physiology of alpha(2) adrenoceptors determined by alpha(2)-heteroreceptors and autoreceptors respectively? *Br.J.Pharmacol., 165*(1), 90-102.

Gilsbach, R., Kouta, M., Bonisch, H., & Bruss, M. (2006). Comparison of in vitro and in vivo reference genes for internal standardization of real-time PCR data. *Biotechniques*, *40*(2), 173-177.

Giros, B., Wang, Y. M., Suter, S., McLeskey, S. B., Pifl, C., & Caron, M. G. (1994). Delineation of discrete domains for substrate, cocaine, and tricyclic antidepressant interactions using chimeric dopamine-norepinephrine transporters. *J.Biol.Chem., 269*(23), 15985-15988.

Glahn, D. C., Therman, S., Manninen, M., Huttunen, M., Kaprio, J., et al. (2003). Spatial working memory as an endophenotype for schizophrenia. *Biol.Psychiatry, 53*(7), 624-626.

Glantz, L. A., & Lewis, D. A. (2000). Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch.Gen.Psychiatry, 57*(1), 65-73.

Glausier, J. R., Fish, K. N., & Lewis, D. A. (2014). Altered parvalbumin basket cell inputs in the dorsolateral prefrontal cortex of schizophrenia subjects. *Mol.Psychiatry, 19*(1), 30-36.

Glausier, J. R., & Lewis, D. A. (2013). Dendritic spine pathology in schizophrenia. *Neuroscience*, *251*, 90-107.

Gleason, M. M., & Hieble, J. P. (1992). The alpha 2-adrenoceptors of the human retinoblastoma cell line (Y79) may represent an additional example of the alpha 2C-adrenoceptor. *Br.J.Pharmacol., 107*(1), 222-225.

Glennon, R. A., Titeler, M., & McKenney, J. D. (1984). Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents. *Life Sci., 35*(25), 2505-2511.

Glenthoj, B. Y., Mackeprang, T., Svarer, C., Rasmussen, H., Pinborg, L. H., et al. (2006). Frontal dopamine D(2/3) receptor binding in drug-naive first-episode schizophrenic patients correlates with positive psychotic symptoms and gender. *Biol.Psychiatry, 60*(6), 621-629.

Goff, D. C., & Coyle, J. T. (2001). The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am.J.Psychiatry, 158*(9), 1367-1377.

Gold, J. M. (2004). Cognitive deficits as treatment targets in schizophrenia. *Schizophr.Res., 72*(1), 21-28.

Gold, J. M., Fuller, R. L., Robinson, B. M., Braun, E. L., & Luck, S. J. (2007). Impaired top-down control of visual search in schizophrenia. *Schizophr.Res., 94*(1-3), 148-155.

Goldberg, T. E., Greenberg, R. D., Griffin, S. J., Gold, J. M., Kleinman, J. E., et al. (1993). The effect of clozapine on cognition and psychiatric symptoms in patients with schizophrenia. *Br.J.Psychiatry, 162*, 43-48.

Goldman, P. S., & Rosvold, H. E. (1970). Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. *Exp.Neurol., 27*(2), 291-304.

Goldman-Rakic, P. S. (1987). Circuitry of the frontal association cortex and its relevance to dementia. *Arch.Gerontol.Geriatr., 6*(3), 299-309.

Goldman-Rakic, P. S. (1994). Working memory dysfunction in schizophrenia. *J.Neuropsychiatry Clin.Neurosci., 6*(4), 348-357.

Goldman-Rakic, P. S. (1995). Cellular basis of working memory. *Neuron*, *14*(3), 477-485.

Goldman-Rakic, P. S., Castner, S. A., Svensson, T. H., Siever, L. J., & Williams, G. V. (2004). Targeting the dopamine D1 receptor in schizophrenia: Insights for cognitive dysfunction. *Psychopharmacology (Berl), 174*(1), 3-16.

Goldman-Rakic, P. S., & Selemon, L. D. (1997). Functional and anatomical aspects of prefrontal pathology in schizophrenia. *Schizophr.Bull., 23*(3), 437-458.

Gomes, U. C., Shanley, B. C., Potgieter, L., & Roux, J. T. (1980). Noradrenergic overactivity in chronic schizophrenia: Evidence based on cerebrospinal fluid noradrenaline and cyclic nucleotide concentrations. *Br.J.Psychiatry, 137*, 346-351.

Gongora-Alfaro, J. L., Hernandez-Lopez, S., Flores-Hernandez, J., & Galarraga, E. (1997). Firing frequency modulation of substantia nigra reticulata neurons by 5-hydroxytryptamine. *Neurosci.Res., 29*(3), 225-231.

Gonzalez-Burgos, G., Hashimoto, T., & Lewis, D. A. (2010). Alterations of cortical GABA neurons and network oscillations in schizophrenia. *Curr.Psychiatry Rep., 12*(4), 335-344.

Gonzalez-Maeso, J., Ang, R. L., Yuen, T., Chan, P., Weisstaub, N. V., et al. (2008). Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature*, *452*(7183), 93-97.

Gonzalez-Maeso, J., & Sealfon, S. C. (2009). Psychedelics and schizophrenia. *Trends Neurosci., 32*(4), 225-232.

Gonzalez-Maeso, J., Weisstaub, N. V., Zhou, M., Chan, P., Ivic, L., et al. (2007). Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron*, *53*(3), 439-452.

Goodman, R. L., Maltby, M. J., Millar, R. P., Hileman, S. M., Nestor, C. C., et al. (2012). Evidence that dopamine acts via kisspeptin to hold GnRH pulse frequency in check in anestrous ewes. *Endocrinology*, *153*(12), 5918-5927.

Gorenstein, E. E., Mammato, C. A., & Sandy, J. M. (1989). Performance of inattentive-overactive children on selected measures of prefrontal-type function. *J.Clin.Psychol., 45*(4), 619-632.

Gorter, J. A., Botterblom, M. H., Feenstra, M. G., & Boer, G. J. (1992). Chronic neonatal NMDA receptor blockade with MK-801 alters monoamine metabolism in the adult rat. *Neurosci.Lett., 137*(1), 97-100.

Gothert, M., Huth, H., & Schlicker, E. (1981). Characterization of the receptor subtype involved in alpha-adrenoceptor-mediated modulation of serotonin release from rat brain cortex slices. *Naunyn Schmiedebergs Arch.Pharmacol., 317*(3), 199-203.

Goto, Y., & Grace, A. A. (2005). Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nat.Neurosci., 8*(6), 805-812.

Goto, Y., & Grace, A. A. (2007). The dopamine system and the pathophysiology of schizophrenia: A basic science perspective. *Int.Rev.Neurobiol., 78*, 41-68.

Gouzoulis-Mayfrank, E., Heekeren, K., Neukirch, A., Stoll, M., Stock, C., et al. (2005). Psychological effects of (S)-ketamine and N,N-dimethyltryptamine (DMT): A double-blind, cross-over study in healthy volunteers. *Pharmacopsychiatry*, *38*(6), 301-311.

Grace, A. A., & Bunney, B. S. (1984). The control of firing pattern in nigral dopamine neurons: Burst firing. *J.Neurosci., 4*(11), 2877-2890.

Grace, A. A., & Rosenkranz, J. A. (2002). Regulation of conditioned responses of basolateral amygdala neurons. *Physiol.Behav., 77*(4-5), 489-493.

Grailhe, R., Waeber, C., Dulawa, S. C., Hornung, J. P., Zhuang, X., et al. (1999). Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. *Neuron*, *22*(3), 581-591.

Granon, S., Passetti, F., Thomas, K. L., Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2000). Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *J.Neurosci., 20*(3), 1208-1215.

Gray, A., Tattoli, R., Dunn, A., Hodgson, D. M., Michie, P. T., & Harms, L. (2019). Maternal immune activation in mid-late gestation alters amphetamine sensitivity and object recognition, but not other schizophrenia-related behaviours in adult rats. *Behav.Brain Res., 356*, 358-364.

Graybiel, A. M. (1997). The basal ganglia and cognitive pattern generators. *Schizophr.Bull., 23*(3), 459-469.

Graybiel, A. M. (1998). The basal ganglia and chunking of action repertoires. *Neurobiol.Learn.Mem., 70*(1-2), 119-136.

Grayson, B., Idris, N. F., & Neill, J. C. (2007). Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. *Behav.Brain Res., 184*(1), 31-38.

Green, M. F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *Am.J.Psychiatry, 153*(3), 321-330.

Green, M. F. (2006). Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. *J.Clin.Psychiatry, 67*(9), 3-8.

Green, M. F., Nuechterlein, K. H., Gold, J. M., Barch, D. M., Cohen, J., et al. (2004). Approaching a consensus cognitive battery for clinical trials in schizophrenia: The NIMH-MATRICS conference to select cognitive domains and test criteria. *Biol.Psychiatry, 56*(5), 301-307.

Greene, C. M., Bellgrove, M. A., Gill, M., & Robertson, I. H. (2009). Noradrenergic genotype predicts lapses in sustained attention. *Neuropsychologia*, *47*(2), 591-594.

Greengard, P., Allen, P. B., & Nairn, A. C. (1999). Beyond the dopamine receptor: The DARPP-32/protein phosphatase-1 cascade. *Neuron*, *23*(3), 435-447.

Greer, S., Honeywell, R., Geletu, M., Arulanandam, R., & Raptis, L. (2010). Housekeeping genes; expression levels may change with density of cultured cells. *J.Immunol.Methods, 355*(1-2), 76-79.

Gresch, P. J., Sved, A. F., Zigmond, M. J., & Finlay, J. M. (1995). Local influence of endogenous norepinephrine on extracellular dopamine in rat medial prefrontal cortex. *J.Neurochem., 65*(1), 111-116.

Griebel, G., & Holsboer, F. (2012). Neuropeptide receptor ligands as drugs for psychiatric diseases: The end of the beginning? *Nat.Rev.Drug Discov., 11*(6), 462-478.

Grijalba B., Callado, L. F., Meana, J. J., García-Sevilla, J. A., & Pazos, A. (1996). α2-adrenoceptor subtypes in the human brain: A pharmacological delineation of [3H]RX-821002 binding to membranes and tissue sections*. Eur. J. Pharcol., 310*(1), 83-93.

Groenewegen, H. J., & Uylings, H. B. (2000). The prefrontal cortex and the integration of sensory, limbic and autonomic information. *Prog.Brain Res., 126*, 3-28.

Groenewegen, H. J., Vermeulen-Van der Zee, E., te Kortschot, A., & Witter, M. P. (1987). Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of phaseolus vulgaris leucoagglutinin. *Neuroscience*, *23*(1), 103-120.

Groenewegen, H. J., Wright, C. I., & Beijer, A. V. (1996). The nucleus accumbens: Gateway for limbic structures to reach the motor system? *Prog.Brain Res., 107*, 485-511.

Grueninger, W. E., & Pribram, K. H. (1969). Effects of spatial and nonspatial distractors on performance latency of monkeys with frontal lesions. *J.Comp.Physiol.Psychol., 68*(2), 203-209.

Grunder, G., Hippius, H., & Carlsson, A. (2009). The 'atypicality' of antipsychotics: A concept re-examined and re-defined. *Nat.Rev.Drug Discov., 8*(3), 197-202.

Gu, H., Wall, S. C., & Rudnick, G. (1994). Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. *J.Biol.Chem., 269*(10), 7124-7130.

Guiard, B. P., El Mansari, M., Merali, Z., & Blier, P. (2008). Functional interactions between dopamine, serotonin and norepinephrine neurons: An in-vivo electrophysiological study in rats with monoaminergic lesions. *Int.J.Neuropsychopharmacol., 11*(5), 625-639.

Guillin, O., Abi-Dargham, A., & Laruelle, M. (2007). Neurobiology of dopamine in schizophrenia. *Int.Rev.Neurobiol., 78*, 1-39.

Gunaydin, L. A., Grosenick, L., Finkelstein, J. C., Kauvar, I. V., Fenno, L. E., et al. (2014). Natural neural projection dynamics underlying social behavior. *Cell*, *157*(7), 1535-1551.

Gurden, H., Tassin, J. P., & Jay, T. M. (1999). Integrity of the mesocortical dopaminergic system is necessary for complete expression of in vivo hippocampal-prefrontal cortex long-term potentiation. *Neuroscience*, *94*(4), 1019-1027.

Gustafson, E. L., Durkin, M. M., Bard, J. A., Zgombick, J., & Branchek, T. A. (1996). A receptor autoradiographic and in situ hybridization analysis of the distribution of the 5-ht7 receptor in rat brain. *Br.J.Pharmacol., 117*(4), 657-666.

Gyires, K., Zadori, Z. S., Shujaa, N., Minorics, R., Falkay, G., & Matyus, P. (2007). Analysis of the role of central and peripheral alpha2-adrenoceptor subtypes in gastric mucosal defense in the rat. *Neurochem.Int., 51*(5), 289-296.

Gyires, K., Zadori, Z. S., Torok, T., & Matyus, P. (2009). Alpha(2)-adrenoceptor subtypes-mediated physiological, pharmacological actions. *Neurochem.Int., 55*(7), 447-453.

Haber, S. N., & Behrens, T. E. (2014). The neural network underlying incentive-based learning: Implications for interpreting circuit disruptions in psychiatric disorders. *Neuron*, *83*(5), 1019-1039.

Hadar, R., Soto-Montenegro, M. L., Gotz, T., Wieske, F., Sohr, R., et al. (2015). Using a maternal immune stimulation model of schizophrenia to study behavioral and neurobiological alterations over the developmental course. *Schizophr.Res., 166*(1-3), 238-247.

Haenschel, C., Bittner, R. A., Waltz, J., Haertling, F., Wibral, M., et al. (2009). Cortical oscillatory activity is critical for working memory as revealed by deficits in early-onset schizophrenia. *J.Neurosci., 29*(30), 9481-9489.

Haettig, J., Stefanko, D. P., Multani, M. L., Figueroa, D. X., McQuown, S. C., & Wood, M. A. (2011). HDAC inhibition modulates hippocampus-dependent long-term memory for object location in a CBP-dependent manner. *Learn.Mem., 18*(2), 71-79.

Haig, G. M., Wang, D., Zhao, J., Othman, A. A., & Bain, E. E. (2018). Efficacy and safety of the alpha7-nicotinic acetylcholine receptor agonist ABT-126 in the treatment of cognitive impairment associated with schizophrenia: Results from a phase 2b randomized controlled study in smokers. *J.Clin.Psychiatry, 79*(3), 10.4088/JCP.16m11162.

Haig, G., Wang, D., Othman, A. A., & Zhao, J. (2016). The alpha7 nicotinic agonist ABT-126 in the treatment of cognitive impairment associated with schizophrenia in nonsmokers: Results from a randomized controlled phase 2b study. *Neuropsychopharmacology, 41*(12), 2893-2902.

Hains, A. B., Yabe, Y., & Arnsten, A. F. (2015). Chronic stimulation of alpha-2A-adrenoceptors with guanfacine protects rodent prefrontal cortex dendritic spines and cognition from the effects of chronic stress. *Neurobiol.Stress, 2*, 1-9.

Hajnal, A., & Norgren, R. (2004). Sucrose sham feeding decreases accumbens norepinephrine in the rat. *Physiol.Behav., 82*(1), 43-47.

Hammond, R. S., Tull, L. E., & Stackman, R. W. (2004). On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiol.Learn.Mem., 82*(1), 26-34.

Hamon, M., Gozlan, H., el Mestikawy, S., Emerit, M. B., Bolanos, F., & Schechter, L. (1990). The central 5-HT1A receptors: Pharmacological, biochemical, functional, and regulatory properties. *Ann.N.Y.Acad.Sci., 600*, 11-31.

Han, D. D., & Gu, H. H. (2006). Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacol., 6*, 6.

Hannesson, D. K., Vacca, G., Howland, J. G., & Phillips, A. G. (2004). Medial prefrontal cortex is involved in spatial temporal order memory but not spatial recognition memory in tests relying on spontaneous exploration in rats. *Behav.Brain Res., 153*(1), 273-285.

Harley, C. W. (1987). A role for norepinephrine in arousal, emotion and learning?: Limbic modulation by norepinephrine and the Kety hypothesis. *Prog.Neuropsychopharmacol.Biol.Psychiatry, 11*(4), 419-458.

Harley, C. W. (2007). Norepinephrine and the dentate gyrus. *Prog.Brain Res., 163*, 299-318.

Harrison, P. J. (1999). The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain, 122 (Pt 4)*, 593-624.

Harrison, P. J., & Burnet, P. W. (1997). The 5-HT2A (serotonin2A) receptor gene in the aetiology, pathophysiology and pharmacotherapy of schizophrenia. *J.Psychopharmacol., 11*(1), 18-20.

Harvey, L., & Boksa, P. (2012). Prenatal and postnatal animal models of immune activation: Relevance to a range of neurodevelopmental disorders. *Dev.Neurobiol., 72*(10), 1335-1348.

Harvey, P. D. (2014). Assessing disability in schizophrenia: Tools and contributors. *J.Clin.Psychiatry, 75*(10), e27.

Hasan, A., Mitchell, A., Schneider, A., Halene, T., & Akbarian, S. (2013). Epigenetic dysregulation in schizophrenia: Molecular and clinical aspects of histone deacetylase inhibitors. *Eur.Arch.Psychiatry Clin.Neurosci., 263*(4), 273-284.

Hashemi, P., Dankoski, E. C., Lama, R., Wood, K. M., Takmakov, P., & Wightman, R. M. (2012). Brain dopamine and serotonin differ in regulation and its consequences. *Proc.Natl.Acad.Sci.U.S.A., 109*(29), 11510-11515.

Hashimoto, K., Fujita, Y., Shimizu, E., & Iyo, M. (2005). Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of clozapine, but not haloperidol. *Eur.J.Pharmacol., 519*(1-2), 114-117.

Hassani, S. A., Oemisch, M., Balcarras, M., Westendorff, S., Ardid, S., et al. (2017). A computational psychiatry approach identifies how alpha-2A noradrenergic agonist guanfacine affects feature-based reinforcement learning in the macaque. *Sci.Rep., 7*, 40606.

Hasselbalch, S. G., Madsen, K., Svarer, C., Pinborg, L. H., Holm, S., et al. (2008). Reduced 5-HT2A receptor binding in patients with mild cognitive impairment. *Neurobiol.Aging, 29*(12), 1830-1838.

Hata, N., Nishikawa, T., Umino, A., & Takahashi, K. (1990). Evidence for involvement of N-methyl-D-aspartate receptor in tonic inhibitory control of dopaminergic transmission in rat medial frontal cortex. *Neurosci.Lett., 120*(1), 101-104.

Hawco, C., Berlim, M. T., & Lepage, M. (2013). The dorsolateral prefrontal cortex plays a role in self-initiated elaborative cognitive processing during episodic memory encoding: rTMS evidence. *PLoS One, 8*(9), e73789.

Hazlett, E. A., Dawson, M. E., Buchsbaum, M. S., & Nuechterlein, K. H. (1993). Reduced regional brain glucose metabolism assessed by positron emission tomography in electrodermal nonresponder schizophrenics: A pilot study. *J.Abnorm.Psychol., 102*(1), 39-46.

Heal, D. J., Butler, S. A., Prow, M. R., & Buckett, W. R. (1993). Quantification of presynaptic alpha 2-adrenoceptors in rat brain after short-term DSP-4 lesioning. *Eur.J.Pharmacol., 249*(1), 37-41

Hecht, E. M., & Landy, D. C. (2012). Alpha-2 receptor antagonist add-on therapy in the treatment of schizophrenia; a meta-analysis. *Schizophr.Res., 134*(2-3), 202-206.

Heckers, S., Curran, T., Goff, D., Rauch, S. L., Fischman, A. J., et al. (2000). Abnormalities in the thalamus and prefrontal cortex during episodic object recognition in schizophrenia. *Biol.Psychiatry, 48*(7), 651-657.

Heidbreder, C. A., & Groenewegen, H. J. (2003). The medial prefrontal cortex in the rat: Evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. *Neurosci.Biobehav.Rev., 27*(6), 555-579.

Hein, L., Altman, J. D., & Kobilka, B. K. (1999). Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. *Nature*, *402*(6758), 181-184.

Heinrichs, R. W., & Zakzanis, K. K. (1998). Neurocognitive deficit in schizophrenia: A quantitative review of the evidence. *Neuropsychology*, *12*(3), 426-445.

Hensler, J. G. (2006). Serotonergic modulation of the limbic system. *Neurosci.Biobehav.Rev., 30*(2), 203-214.

Hensler, J. G., Artigas, F., Bortolozzi, A., Daws, L. C., De Deurwaerdere, P., et al. (2013). Catecholamine/serotonin interactions: Systems thinking for brain function and disease. *Adv.Pharmacol., 68*, 167-197.

Herry, C., & Garcia, R. (2002). Prefrontal cortex long-term potentiation, but not long-term depression, is associated with the maintenance of extinction of learned fear in mice. *J.Neurosci., 22*(2), 577-583.

Hertel, P., Fagerquist, M. V., & Svensson, T. H. (1999). Enhanced cortical dopamine output and antipsychotic-like effects of raclopride by alpha2 adrenoceptor blockade. *Science, 286*(5437), 105-107.

Hertel, P., Nomikos, G. G., Schilstrom, B., Arborelius, L., & Svensson, T. H. (1997). Risperidone dose-dependently increases extracellular concentrations of serotonin in the rat frontal cortex: Role of alpha 2-adrenoceptor antagonism. *Neuropsychopharmacology, 17*(1), 44-55.

Hess, C., Reif, A., Strobel, A., Boreatti-Hummer, A., Heine, M., et al. (2009). A functional dopamine-beta-hydroxylase gene promoter polymorphism is associated with impulsive personality styles, but not with affective disorders. *J.Neural Transm.(Vienna), 116*(2), 121-130.

Hieble, J. P., Bondinell, W. E., & Ruffolo, R. R., Jr. (1995). Alpha- and beta-adrenoceptors: From the gene to the clinic. 1. molecular biology and adrenoceptor subclassification. *J.Med.Chem., 38*(18), 3415-3444.

Hietala, J., Syvalahti, E., Vilkman, H., Vuorio, K., Rakkolainen, V., et al. (1999). Depressive symptoms and presynaptic dopamine function in neuroleptic-naive schizophrenia. *Schizophr.Res., 35*(1), 41-50.

Hietala, J., Syvalahti, E., Vuorio, K., Rakkolainen, V., Bergman, J., et al. (1995). Presynaptic dopamine function in striatum of neuroleptic-naive schizophrenic patients. *Lancet, 346*(8983), 1130-1131.

Hill, S. K., Bishop, J. R., Palumbo, D., & Sweeney, J. A. (2010). Effect of second-generation antipsychotics on cognition: Current issues and future challenges. *Expert Rev.Neurother, 10*(1), 43-57.

Hirono, M., & Obata, K. (2006). Alpha-adrenoceptive dual modulation of inhibitory GABAergic inputs to purkinje cells in the mouse cerebellum. *J.Neurophysiol., 95*(2), 700-708.

Hirschberg, S., Li, Y., Randall, A., Kremer, E. J., & Pickering, A. E. (2017). Functional dichotomy in spinal- vs prefrontal-projecting locus coeruleus modules splits descending noradrenergic analgesia from ascending aversion and anxiety in rats. *Elife, 6*, 10.7554/eLife.29808.

Hjorth, S., Bengtsson, H. J., Milano, S., Lundberg, J. F., & Sharp, T. (1995). Studies on the role of 5-HT1A autoreceptors and alpha 1-adrenoceptors in the inhibition of 5-HT release--I. BMY7378 and prazosin. *Neuropharmacology*, *34*(6), 615-620.

Hobson, J. A., McCarley, R. W., & Wyzinski, P. W. (1975). Sleep cycle oscillation: Reciprocal discharge by two brainstem neuronal groups. *Science, 189*(4196), 55-58.

Hoek, H., W., Brown, A., S., & Susser, E. (1998). The Dutch famine and schizophrenia spectrum disorders. *Soc.Psychiatry.Psychiatr.Epidemiol*., *33*(8), 373-379.

Hoek, H. W., Susser, E., Buck, K. A., Lumey, L. H., Lin, S. P., & Gorman, J. M. (1996). Schizoid personality disorder after prenatal exposure to famine. *Am.J.Psychiatry, 153*(12), 1637-1639.

Hoffman, R. E., Fernandez, T., Pittman, B., & Hampson, M. (2011). Elevated functional connectivity along a corticostriatal loop and the mechanism of auditory/verbal hallucinations in patients with schizophrenia. *Biol.Psychiatry, 69*(5), 407-414.

Hofling, C., Kulesskaya, N., Jaako, K., Peltonen, I., Mannisto, P. T., et al. (2016). Deficiency of prolyl oligopeptidase in mice disturbs synaptic plasticity and reduces anxiety-like behaviour, body weight, and brain volume. *Eur.Neuropsychopharmacol., 26*(6), 1048-1061.

Hofmann, C., Pizzagalli, F., Boetsch, C., Alberati, D., Ereshefsky, L., et al. (2016). Effects of the glycine reuptake inhibitors bitopertin and RG7118 on glycine in cerebrospinal fluid: Results of two proofs of mechanism studies in healthy volunteers. *Psychopharmacology (Berl), 233*(13), 2429-2439.

Holmberg, M., Fagerholm, V., & Scheinin, M. (2003). Regional distribution of alpha(2C)-adrenoceptors in brain and spinal cord of control mice and transgenic mice overexpressing the alpha(2C)-subtype: An autoradiographic study with [(3)H]RX821002 and [(3)H]rauwolscine. *Neuroscience*, *117*(4), 875-898.

Holmberg, M., Scheinin, M., Kurose, H., & Miettinen, R. (1999). Adrenergic alpha2C-receptors reside in rat striatal GABAergic projection neurons: Comparison of radioligand binding and immunohistochemistry. *Neuroscience*, *93*(4), 1323-1333.

Holthausen, E. A., Wiersma, D., Cahn, W., Kahn, R. S., Dingemans, P. M., et al. (2007). Predictive value of cognition for different domains of outcome in recent-onset schizophrenia. *Psychiatry Res., 149*(1-3), 71-80.

Honea, R., Crow, T. J., Passingham, D., & Mackay, C. E. (2005). Regional deficits in brain volume in schizophrenia: A meta-analysis of voxel-based morphometry studies. *Am.J.Psychiatry, 162*(12), 2233-2245.

Hornykiewicz, O. (1971). Pharmacology and pathophysiology of dopaminergic neurons. *Adv.Cytopharmacol., 1*, 369-377.

Hoskins, B., Peeler, D. F., Lawson, K., Barnes, A. M., & Ho, I. K. (1991). Effects of haloperidol on motor and cognitive functioning in aged mice. *Brain Res.Bull., 27*(2), 279-281.

Hotte, M., Naudon, L., & Jay, T. M. (2005). Modulation of recognition and temporal order memory retrieval by dopamine D1 receptor in rats. *Neurobiol.Learn.Mem., 84*(2), 85-92.

Howes, O. D., Bose, S. K., Turkheimer, F., Valli, I., Egerton, A., et al. (2011). Dopamine synthesis capacity before onset of psychosis: A prospective [18F]-DOPA PET imaging study. *Am.J.Psychiatry, 168*(12), 1311-1317.

Howes, O. D., Kambeitz, J., Kim, E., Stahl, D., Slifstein, M., et al. (2012). The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch.Gen.Psychiatry, 69*(8), 776-786.

Howes, O. D., Montgomery, A. J., Asselin, M. C., Murray, R. M., Grasby, P. M., & McGuire, P. K. (2007). Molecular imaging studies of the striatal dopaminergic system in psychosis and predictions for the prodromal phase of psychosis. *Br.J.Psychiatry Suppl., 51*, 13.

Howes, O. D., Montgomery, A. J., Asselin, M. C., Murray, R. M., Valli, I., et al. (2009). Elevated striatal dopamine function linked to prodromal signs of schizophrenia. *Arch.Gen.Psychiatry, 66*(1), 13-20.

Howes, O. D., Williams, M., Ibrahim, K., Leung, G., Egerton, A., et al. (2013). Midbrain dopamine function in schizophrenia and depression: A post-mortem and positron emission tomographic imaging study. *Brain, 136*(Pt 11), 3242-3251.

Hoyer, D., Clarke, D. E., Fozard, J. R., Hartig, P. R., Martin, G. R., et al. (1994). International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol.Rev., 46*(2), 157-203.

Hoyer, D., Hannon, J. P., & Martin, G. R. (2002). Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol.Biochem.Behav., 71*(4), 533-554.

Hoyle, E., Genn, R. F., Fernandes, C., & Stolerman, I. P. (2006). Impaired performance of alpha7 nicotinic receptor knockout mice in the five-choice serial reaction time task. *Psychopharmacology (Berl), 189*(2), 211-223.

Huang, M., Felix, A. R., Flood, D. G., Bhuvaneswaran, C., Hilt, D., et al. (2014). The novel alpha7 nicotinic acetylcholine receptor agonist EVP-6124 enhances dopamine, acetylcholine, and glutamate efflux in rat cortex and nucleus accumbens. *Psychopharmacology (Berl), 231*(23), 4541-4551.

Huang, M., Ichiwaka, J., Li, Z., Dai, J., & Meltzer, H. Y. (2006a). Augmentation by citalopram of risperidone-induced monoamine release in rat prefrontal cortex. *Psychopharmacology (Berl), 185*(3), 274-281.

Huang, T., McDonough, C., B., & Abel, T. (2006b). Compartmentalized PKA signaling events are required for synaptic tagging and capture during hippocampal late-phase long-term potentiation. *Eur. J. Cell. Biol*., *85*(7), 635-642.

Huether, G. (1996). The central adaptation syndrome: Psychosocial stress as a trigger for adaptive modifications of brain structure and brain function. *Prog.Neurobiol., 48*(6), 569-612.

Hulshoff Pol, H. E., Hoek, H. W., Susser, E., Brown, A. S., Dingemans, A., et al. (2000). Prenatal exposure to famine and brain morphology in schizophrenia. *Am.J.Psychiatry, 157*(7), 1170-1172.

Humby, T., Laird, F. M., Davies, W., & Wilkinson, L. S. (1999). Visuospatial attentional functioning in mice: Interactions between cholinergic manipulations and genotype. *Eur.J.Neurosci., 11*(8), 2813-2823.

Humby, T., Wilkinson, L., & Dawson, G. (2005). Assaying aspects of attention and impulse control in mice using the 5-choice serial reaction time task. *Curr.Protoc.Neurosci., Chapter 8*, Unit 8.5H.

Hunt, R. D., Arnsten, A. F., & Asbell, M. D. (1995). An open trial of guanfacine in the treatment of attention-deficit hyperactivity disorder. *J.Am.Acad.Child Adolesc.Psychiatry, 34*(1), 50-54.

Hunter, J. C., Fontana, D. J., Hedley, L. R., Jasper, J. R., Lewis, R., et al. (1997). Assessment of the role of alpha2-adrenoceptor subtypes in the antinociceptive, sedative and hypothermic action of dexmedetomidine in transgenic mice. *Br.J.Pharmacol., 122*(7), 1339-1344.

Hurley, K. M., Herbert, H., Moga, M. M., & Saper, C. B. (1991). Efferent projections of the infralimbic cortex of the rat. *J.Comp.Neurol., 308*(2), 249-276.

Hutchinson, G., Takei, N., Fahy, T. A., Bhugra, D., Gilvarry, C., et al. (1996). Morbid risk of schizophrenia in first-degree relatives of white and African-Caribbean patients with psychosis. *Br.J.Psychiatry, 169*(6), 776-780.

Hvoslef-Eide, M., Nilsson, S. R., Saksida, L. M., & Bussey, T. J. (2016). Cognitive translation using the rodent touchscreen testing approach. *Curr.Top.Behav.Neurosci., 28*, 423-447.

Hyde, K. J., & Schust, D. J. (2016). Immunologic challenges of human reproduction: An evolving story. *Fertil.Steril., 106*(3), 499-510.

Hyde, T. M., & Crook, J. M. (2001). Cholinergic systems and schizophrenia: Primary pathology or epiphenomena? *J.Chem.Neuroanat., 22*(1-2), 53-63.

Hyman, S. E., & Fenton, W. S. (2003). Medicine. what are the right targets for psychopharmacology? *Science, 299*(5605), 350-351.

Ibi, D., Nagai, T., Koike, H., Kitahara, Y., Mizoguchi, H., et al. (2010). Combined effect of neonatal immune activation and mutant DISC1 on phenotypic changes in adulthood. *Behav.Brain Res., 206*(1), 32-37.

Ibrahim, H. M., & Tamminga, C. A. (2011). Schizophrenia: Treatment targets beyond monoamine systems. *Annu.Rev.Pharmacol.Toxicol., 51*, 189-209.

Ichinose, H., Ohye, T., Fujita, K., Pantucek, F., Lange, K., et al. (1994). Quantification of mRNA of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in the substantia nigra in Parkinson's disease and schizophrenia. *J.Neural Transm.Park.Dis.Dement.Sect., 8*(1-2), 149-158.

Idris, N., Neill, J., Grayson, B., Bang-Andersen, B., Witten, L. M., et al. (2010). Sertindole improves sub-chronic PCP-induced reversal learning and episodic memory deficits in rodents: Involvement of 5-HT(6) and 5-HT (2A) receptor mechanisms. *Psychopharmacology (Berl), 208*(1), 23-36.

Imperato, A., & Di Chiara, G. (1984). Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: A new method for the study of the in vivo release of endogenous dopamine and metabolites. *J.Neurosci., 4*(4), 966-977.

Innis, R. B., & Aghajanian, G. K. (1987). Pertussis toxin blocks 5-HT1A and GABAB receptor-mediated inhibition of serotonergic neurons. *Eur.J.Pharmacol., 143*(2), 195-204.

Insel, T. R., Voon, V., Nye, J. S., Brown, V. J., Altevogt, B. M., et al. (2013). Innovative solutions to novel drug development in mental health. *Neurosci.Biobehav.Rev., 37*(10 Pt 1), 2438-2444.

Insel, T. R., & Wang, P. S. (2010). Rethinking mental illness. *Jama, 303*(19), 1970-1971.

Inyushin, M. U., Arencibia-Albite, F., Vazquez-Torres, R., Velez-Hernandez, M. E., & Jimenez-Rivera, C. A. (2010). Alpha-2 noradrenergic receptor activation inhibits the hyperpolarization-activated cation current (ih) in neurons of the ventral tegmental area. *Neuroscience*, *167*(2), 287-297.

Ishibashi, T., Horisawa, T., Tokuda, K., Ishiyama, T., Ogasa, M., et al. (2010). Pharmacological profile of lurasidone, a novel antipsychotic agent with potent 5-hydroxytryptamine 7 (5-HT7) and 5-HT1A receptor activity. *J.Pharmacol.Exp.Ther., 334*(1), 171-181.

Jackson, M. E., Frost, A. S., & Moghaddam, B. (2001). Stimulation of prefrontal cortex at physiologically relevant frequencies inhibits dopamine release in the nucleus accumbens. *J.Neurochem., 78*(4), 920-923.

Jackson, M. E., Homayoun, H., & Moghaddam, B. (2004). NMDA receptor hypofunction produces concomitant firing rate potentiation and burst activity reduction in the prefrontal cortex. *Proc.Natl.Acad.Sci.U.S.A., 101*(22), 8467-8472.

Jackson, W. J., & Buccafusco, J. J. (1991). Clonidine enhances delayed matching-to-sample performance by young and aged monkeys. *Pharmacol.Biochem.Behav., 39*(1), 79-84.

Jacobs, B. L., & Azmitia, E. C. (1992). Structure and function of the brain serotonin system. *Physiol.Rev., 72*(1), 165-229.

Jacobsen, C. F. (1936). Studies of cerebral function in primates. I. the functions of the frontal association areas in monkeys. *Comparative Psychology Monographs*, *13, 3*, 1-60.

Jaiswal, S., Hockenbury, N., Pan, H., Knutsen, A., Dardzinski, B. J., & Byrnes, K. R. (2018). Alteration of FDG uptake by performing novel object recognition task in a rat model of traumatic brain injury. *Neuroimage, 188*, 419-426.

Jakala, P., Riekkinen, M., Sirvio, J., Koivisto, E., Kejonen, K., et al. (1999). Guanfacine, but not clonidine, improves planning and working memory performance in humans. *Neuropsychopharmacology, 20*(5), 460-470.

Jakala, P., Sirvio, J., Riekkinen, P., Jr, Haapalanna, A., & Riekkinen, P. (1992). Effects of atipamezole, an alpha 2-adrenoceptor antagonist, on the performance of rats in a five-choice serial reaction time task. *Pharmacol.Biochem.Behav., 42*(4), 903-907.

Jalkanen, A. J., Leikas, J. V., & Forsberg, M. M. (2014). Prolyl oligopeptidase inhibition decreases extracellular acetylcholine levels in rat hippocampus and prefrontal cortex. *Neurosci.Lett., 579*, 110-113.

Janitzky, K., Lippert, M. T., Engelhorn, A., Tegtmeier, J., Goldschmidt, J., et al. (2015). Optogenetic silencing of locus coeruleus activity in mice impairs cognitive flexibility in an attentional set-shifting task. *Front.Behav.Neurosci., 9*, 286.

Japha, K., & Koch, M. (1999). Picrotoxin in the medial prefrontal cortex impairs sensorimotor gating in rats: Reversal by haloperidol. *Psychopharmacology (Berl), 144*(4), 347-354.

Jarskog, L. F., Miyamoto, S., & Lieberman, J. A. (2007). Schizophrenia: New pathological insights and therapies. *Annu.Rev.Med., 58*, 49-61.

Jaskiw, G. E., Karoum, F., Freed, W. J., Phillips, I., Kleinman, J. E., & Weinberger, D. R. (1990). Effect of ibotenic acid lesions of the medial prefrontal cortex on amphetamine-induced locomotion and regional brain catecholamine concentrations in the rat. *Brain Res., 534*(1-2), 263-272.

Jaskiw, G. E., & Weinberger, D. R. (1992). Ibotenic acid lesions of medial prefrontal cortex augment swim-stress-induced locomotion. *Pharmacol.Biochem.Behav., 41*(3), 607-609.

Jasper, J. R., Lesnick, J. D., Chang, L. K., Yamanishi, S. S., Chang, T. K., et al. (1998). Ligand efficacy and potency at recombinant alpha2 adrenergic receptors: Agonist-mediated [35S]GTPgammaS binding. *Biochem.Pharmacol., 55*(7), 1035-1043.

Javitt, D. C., & Zukin, S. R. (1991). Recent advances in the phencyclidine model of schizophrenia. *Am.J.Psychiatry, 148*(10), 1301-1308.

Javoy-Agid, F., & Agid, Y. (1980). Is the mesocortical dopaminergic system involved in Parkinson disease? *Neurology*, *30*(12), 1326-1330.

Jay, T. M., Glowinski, J., & Thierry, A. M. (1989). Selectivity of the hippocampal projection to the prelimbic area of the prefrontal cortex in the rat. *Brain Res., 505*(2), 337-340.

Jenkins, T. A., Nguyen, J. C., Polglaze, K. E., & Bertrand, P. P. (2016). Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients*, *8*(1), pii: E56.

Jentsch, J. D., Redmond, D. E.,Jr, Elsworth, J. D., Taylor, J. R., Youngren, K. D., & Roth, R. H. (1997). Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. *Science, 277*(5328), 953-955.

Jentsch, J. D., Taylor, J. R., Redmond, D. E.,Jr, Elsworth, J. D., Youngren, K. D., & Roth, R. H. (1999). Dopamine D4 receptor antagonist reversal of subchronic phencyclidine-induced object retrieval/detour deficits in monkeys. *Psychopharmacology (Berl), 142*(1), 78-84.

Jiang, Z. G., Pessia, M., & North, R. A. (1993). Dopamine and baclofen inhibit the hyperpolarization-activated cation current in rat ventral tegmental neurones. *J.Physiol., 462*, 753-764.

Jiang, Z., Cowell, R. M., & Nakazawa, K. (2013). Convergence of genetic and environmental factors on parvalbumin-positive interneurons in schizophrenia. *Front.Behav.Neurosci., 7*, 116.

Jin, J., Yamamoto, T., & Watanabe, S. (1997). The involvement of sigma receptors in the choice reaction performance deficits induced by phencyclidine. *Eur.J.Pharmacol., 319*(2-3), 147-152.

Jog, M. S., Kubota, Y., Connolly, C. I., Hillegaart, V., & Graybiel, A. M. (1999). Building neural representations of habits. *Science, 286*(5445), 1745-1749.

Jonakait, G. M. (2007). The effects of maternal inflammation on neuronal development: Possible mechanisms. *Int.J.Dev.Neurosci., 25*(7), 415-425.

Jones, B. E., Halaris, A. E., McIlhany, M., & Moore, R. Y. (1977). Ascending projections of the locus coeruleus in the rat. I. axonal transport in central noradrenaline neurons. *Brain Res., 127*(1), 1-21.

Jones, S. B., Halenda, S. P., & Bylund, D. B. (1991). Alpha 2-adrenergic receptor stimulation of phospholipase A2 and of adenylate cyclase in transfected Chinese hamster ovary cells is mediated by different mechanisms. *Mol.Pharmacol., 39*(2), 239-245.

Jorgensen, H., Knigge, U., Kjaer, A., & Warberg, J. (1999). Adrenocorticotropic hormone secretion in rats induced by stimulation with serotonergic compounds. hsj@mfi.ku.dk. *J.Neuroendocrinol., 11*(4), 283-290.

Joyce, J. N., Lexow, N., Bird, E., & Winokur, A. (1988). Organization of dopamine D1 and D2 receptors in human striatum: Receptor autoradiographic studies in Huntington's disease and schizophrenia. *Synapse, 2*(5), 546-557.

Kable, J. W., Murrin, L. C., & Bylund, D. B. (2000). In vivo gene modification elucidates subtype-specific functions of alpha(2)-adrenergic receptors. *J.Pharmacol.Exp.Ther., 293*(1), 1-7.

Kaehler, S. T., Singewald, N., & Philippu, A. (1999). Dependence of serotonin release in the locus coeruleus on dorsal raphe neuronal activity. *Naunyn Schmiedebergs Arch.Pharmacol., 359*(5), 386-393.

Kahn, R. S., & Keefe, R. S. (2013). Schizophrenia is a cognitive illness: Time for a change in focus. *JAMA Psychiatry., 70*(10), 1107-1112.

Kahn, R. S., Sommer, I. E., Murray, R. M., Meyer-Lindenberg, A., Weinberger, D. R., et al. (2015). Schizophrenia. *Nat.Rev.Dis.Primers, 1*, 15067.

Kalkman, H. O., & Loetscher, E. (2003). alpha2C-adrenoceptor blockade by clozapine and other antipsychotic drugs. *Eur.J.Pharmacol., 462*(1-3), 33-40.

Kamata, M., Higuchi, H., Yoshimoto, M., Yoshida, K., & Shimizu, T. (2000). Effect of single intracerebroventricular injection of alpha-interferon on monoamine concentrations in the rat brain. *Eur.Neuropsychopharmacol., 10*(2), 129-132.

Kamei, H., Nagai, T., Nakano, H., Togan, Y., Takayanagi, M., et al. (2006). Repeated methamphetamine treatment impairs recognition memory through a failure of novelty-induced ERK1/2 activation in the prefrontal cortex of mice. *Biol.Psychiatry, 59*(1), 75-84.

Kamiya, A., Kubo, K., Tomoda, T., Takaki, M., Youn, R., et al. (2005). A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nat.Cell Biol., 7*(12), 1167-1178.

Kanaan, R. A., Kim, J. S., Kaufmann, W. E., Pearlson, G. D., Barker, G. J., & McGuire, P. K. (2005). Diffusion tensor imaging in schizophrenia. *Biol.Psychiatry, 58*(12), 921-929.

Kane, J., Honigfeld, G., Singer, J., & Meltzer, H. (1988). Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch.Gen.Psychiatry, 45*(9), 789-796.

Kane, J. M., & Correll, C. U. (2010). Past and present progress in the pharmacologic treatment of schizophrenia. *J.Clin.Psychiatry, 71*(9), 1115-1124.

Kane, J. M., Woerner, M. G., Pollack, S., Safferman, A. Z., & Lieberman, J. A. (1993). Does clozapine cause tardive dyskinesia? *J.Clin.Psychiatry, 54*(9), 327-330.

Kaneko, M., Honda, K., Kanno, T., Horikoshi, R., Manome, T., et al. (1992). Plasma free 3-methoxy-4-hydroxyphenylglycol in acute schizophrenics before and after treatment. *Neuropsychobiology*, *25*(3), 126-129.

Kannan, S., Saadani-Makki, F., Muzik, O., Chakraborty, P., Mangner, T. J., et al. (2007). Microglial activation in perinatal rabbit brain induced by intrauterine inflammation: Detection with 11C-(R)-PK11195 and small-animal PET. *J.Nucl.Med., 48*(6), 946-954.

Kao, W. T., Wang, Y., Kleinman, J. E., Lipska, B. K., Hyde, T. M., et al. (2010). Common genetic variation in neuregulin 3 (NRG3) influences risk for schizophrenia and impacts NRG3 expression in human brain. *Proc.Natl.Acad.Sci.U.S.A., 107*(35), 15619-15624.

Kapur, S., & Remington, G. (1996). Serotonin-dopamine interaction and its relevance to schizophrenia. *Am.J.Psychiatry, 153*(4), 466-476.

Kapur, S., Zipursky, R. B., & Remington, G. (1999). Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am.J.Psychiatry, 156*(2), 286-293.

Kapur, S., Zipursky, R., Jones, C., Remington, G., & Houle, S. (2000). Relationship between dopamine D(2) occupancy, clinical response, and side effects: A double-blind PET study of first-episode schizophrenia. *Am.J.Psychiatry, 157*(4), 514-520.

Karam, C. S., Ballon, J. S., Bivens, N. M., Freyberg, Z., Girgis, R. R., et al. (2010). Signaling pathways in schizophrenia: Emerging targets and therapeutic strategies. *Trends Pharmacol.Sci., 31*(8), 381-390.

Karasawa, J., Hashimoto, K., & Chaki, S. (2008). D-serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. *Behav.Brain Res., 186*(1), 78-83.

Kargieman, L., Santana, N., Mengod, G., Celada, P., & Artigas, F. (2007). Antipsychotic drugs reverse the disruption in prefrontal cortex function produced by NMDA receptor blockade with phencyclidine. *Proc.Natl.Acad.Sci.U.S.A., 104*(37), 14843-14848.

Karhunen, T., Tilgmann, C., Ulmanen, I., & Panula, P. (1995). Catechol-O-methyltransferase (COMT) in rat brain: Immunoelectron microscopic study with an antiserum against rat recombinant COMT protein. *Neurosci.Lett., 187*(1), 57-60.

Karkhanis, A. N., Locke, J. L., McCool, B. A., Weiner, J. L., & Jones, S. R. (2014). Social isolation rearing increases nucleus accumbens dopamine and norepinephrine responses to acute ethanol in adulthood. *Alcohol.Clin.Exp.Res., 38*(11), 2770-2779.

Karlsson, P., Farde, L., Halldin, C., & Sedvall, G. (2002). PET study of D(1) dopamine receptor binding in neuroleptic-naive patients with schizophrenia. *Am.J.Psychiatry, 159*(5), 761-767.

Kashiwa, A., Nishikawa, T., Nishijima, K., Umino, A., & Takahashi, K. (1995). Dizocilpine (MK-801) elicits a tetrodotoxin-sensitive increase in extracellular release of dopamine in rat medial frontal cortex. *Neurochem.Int., 26*(3), 269-279.

Katsel, P. L., Davis, K. L., & Haroutunian, V. (2005). Large-scale microarray studies of gene expression in multiple regions of the brain in schizophrenia and Alzheimer's disease. *Int.Rev.Neurobiol., 63*, 41-82.

Katusic, Z. S., & Austin, S. A. (2014). Endothelial nitric oxide: Protector of a healthy mind. *Eur.Heart J., 35*(14), 888-894.

Kawaguchi, Y., & Shindou, T. (1998). Noradrenergic excitation and inhibition of GABAergic cell types in rat frontal cortex. *J.Neurosci., 18*(17), 6963-6976.

Kawahara, H., Kawahara, Y., & Westerink, B. H. (2001). The noradrenaline-dopamine interaction in the rat medial prefrontal cortex studied by multi-probe microdialysis. *Eur.J.Pharmacol., 418*(3), 177-186.

Kawaura, K., Karasawa, J., Chaki, S., & Hikichi, H. (2014). Stimulation of postsynapse adrenergic alpha2A receptor improves attention/cognition performance in an animal model of attention deficit hyperactivity disorder. *Behav.Brain Res., 270*, 349-356.

Keefe, R. S., Bilder, R. M., Davis, S. M., Harvey, P. D., Palmer, B. W., et al. (2007). Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE trial. *Arch.Gen.Psychiatry, 64*(6), 633-647.

Keefe, R. S., Meltzer, H. A., Dgetluck, N., Gawryl, M., Koenig, G., et al. (2015). Randomized, double-blind, placebo-controlled study of encenicline, an alpha7 nicotinic acetylcholine receptor agonist, as a treatment for cognitive impairment in schizophrenia. *Neuropsychopharmacology, 40*(13), 3053-3060.

Keefe, R. S., Silva, S. G., Perkins, D. O., & Lieberman, J. A. (1999). The effects of atypical antipsychotic drugs on neurocognitive impairment in schizophrenia: A review and meta-analysis. *Schizophr.Bull., 25*(2), 201-222.

Keefe, R. S. E. (2008). Should cognitive impairment be included in the diagnostic criteria for schizophrenia? *World Psychiatry*, *7*(1), 22-28.

Keeler, B. E., Lallemand, P., Patel, M. M., de Castro Brás, L. E., & Clemens, S. (2016). Opposing aging-related shift of excitatory dopamine D1 and inhibitory D3 receptor protein expression in striatum and spinal cord. *J.Neurophysiol., 115*(1), 363-369.

Kegeles, L. S., Abi-Dargham, A., Frankle, W. G., Gil, R., Cooper, T. B., et al. (2010). Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. *Arch.Gen.Psychiatry, 67*(3), 231-239.

Kelland, M. D., Freeman, A. S., & Chiodo, L. A. (1990). Serotonergic afferent regulation of the basic physiology and pharmacological responsiveness of nigrostriatal dopamine neurons. *J.Pharmacol.Exp.Ther., 253*(2), 803-811.

Kellendonk, C. (2009). Modeling excess striatal D2 receptors in mice. *Prog.Brain Res., 179*, 59-65.

Kellendonk, C., Simpson, E. H., Polan, H. J., Malleret, G., Vronskaya, S., et al. (2006). Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. *Neuron*, *49*(4), 603-615.

Kelley, A. E., & Domesick, V. B. (1982). The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: An anterograde- and retrograde-horseradish peroxidase study. *Neuroscience, 7*(10), 2321-2335.

Kelly, P. H., Seviour, P. W., & Iversen, S. D. (1975). Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res., 94*(3), 507-522.

Kemali, D., Maj, M., Galderisi, S., Grazia Ariano, M., & Starace, F. (1990). Factors associated with increased noradrenaline levels in schizophrenic patients. *Prog.Neuropsychopharmacol.Biol.Psychiatry, 14*(1), 49-59.

Kepe, V., Barrio, J. R., Huang, S. C., Ercoli, L., Siddarth, P., et al. (2006). Serotonin 1A receptors in the living brain of Alzheimer's disease patients. *Proc.Natl.Acad.Sci.U.S.A., 103*(3), 702-707.

Kesby, J. P., Eyles, D. W., McGrath, J. J., & Scott, J. G. (2018). Dopamine, psychosis and schizophrenia: The widening gap between basic and clinical neuroscience. *Transl. Psychiatry*, *8*(1):30.

Kessler, R. M., Whetsell, W. O., Ansari, M. S., Votaw, J. R., de Paulis, T., et al. (1993). Identification of extrastriatal dopamine D2 receptors in post mortem human brain with [125I]epidepride. *Brain Res., 609*(1-2), 237-243.

Khan, D., Fernando, P., Cicvaric, A., Berger, A., Pollak, A., et al. (2014). Long-term effects of maternal immune activation on depression-like behavior in the mouse. *Transl.Psychiatry., 4*, e363.

Khan, Z. U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A., & De La Calle, A. (1998). Differential regional and cellular distribution of dopamine D2-like receptors: An immunocytochemical study of subtype-specific antibodies in rat and human brain. *J.Comp.Neurol., 402*(3), 353-371.

Kia, H. K., Brisorgueil, M. J., Hamon, M., Calas, A., & Verge, D. (1996). Ultrastructural localization of 5-hydroxytryptamine1A receptors in the rat brain. *J.Neurosci.Res., 46*(6), 697-708.

Kieling, C., Genro, J. P., Hutz, M. H., & Rohde, L. A. (2008). The -1021 C/T DBH polymorphism is associated with neuropsychological performance among children and adolescents with ADHD. *Am.J.Med.Genet.B.Neuropsychiatr.Genet., 147B*(4), 485-490.

Kimberg, D. Y., D'Esposito, M., & Farah, M. J. (1997). Effects of bromocriptine on human subjects depend on working memory capacity. *Neuroreport*, *8*(16), 3581-3585.

King, D. J. (1994). Psychomotor impairment and cognitive disturbances induced by neuroleptics. *Acta Psychiatr.Scand.Suppl., 380*, 53-58.

King, M. V., Sleight, A. J., Woolley, M. L., Topham, I. A., Marsden, C. A., & Fone, K. C. (2004). 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation--an effect sensitive to NMDA receptor antagonism. *Neuropharmacology*, *47*(2), 195-204.

Kinon, B. J., Millen, B. A., Zhang, L., & McKinzie, D. L. (2015). Exploratory analysis for a targeted patient population responsive to the metabotropic glutamate 2/3 receptor agonist pomaglumetad methionil in schizophrenia. *Biol.Psychiatry, 78*(11), 754-762.

Kish, S. J., Furukawa, Y., Chang, L. J., Tong, J., Ginovart, N., et al. (2005). Regional distribution of serotonin transporter protein in postmortem human brain: Is the cerebellum a SERT-free brain region? *Nucl.Med.Biol., 32*(2), 123-128.

Knable, M. B., Hyde, T. M., Murray, A. M., Herman, M. M., & Kleinman, J. E. (1996). A postmortem study of frontal cortical dopamine D1 receptors in schizophrenics, psychiatric controls, and normal controls. *Biol.Psychiatry, 40*(12), 1191-1199.

Knable, M. B., & Weinberger, D. R. (1997). Dopamine, the prefrontal cortex and schizophrenia. *J.Psychopharmacol., 11*(2), 123-131.

Knight, R. T., Grabowecky, M. F., & Scabini, D. (1995). Role of human prefrontal cortex in attention control. *Adv.Neurol., 66*, 2-6.

Knuesel, I., Chicha, L., Britschgi, M., Schobel, S.,A., Bodmer, M., et al. (2014). Maternal immune activation and abnormal brain development across CNS disorders. *Nat.Rev.Neurol.*, *10*(11), 643-660.

Kobilka, B. K., Matsui, H., Kobilka, T. S., Yang-Feng, T. L., Francke, U., et al. (1987). Cloning, sequencing, and expression of the gene coding for the human platelet alpha 2-adrenergic receptor. *Science, 238*(4827), 650-656.

Kohler, C., & Steinbusch, H. (1982). Identification of serotonin and non-serotonin-containing neurons of the mid-brain raphe projecting to the entorhinal area and the hippocampal formation. A combined immunohistochemical and fluorescent retrograde tracing study in the rat brain. *Neuroscience*, *7*(4), 951-975.

Kokkinidis, L., & Anisman, H. (1980). Amphetamine models of paranoid schizophrenia: An overview and elaboration of animal experimentation. *Psychol.Bull., 88*(3), 551-579.

Kolachana, B. S., Saunders, R. C., & Weinberger, D. R. (1995). Augmentation of prefrontal cortical monoaminergic activity inhibits dopamine release in the caudate nucleus: An in vivo neurochemical assessment in the rhesus monkey. *Neuroscience*, *69*(3), 859-868.

Kolb, B. (1984). Functions of the frontal cortex of the rat: A comparative review. *Brain Res., 320*(1), 65-98.

Koster, L. S., Carbon, M., & Correll, C. U. (2014). Emerging drugs for schizophrenia: An update. *Expert Opin.Emerg.Drugs, 19*(4), 511-531.

Krabbe, S., Duda, J., Schiemann, J., Poetschke, C., Schneider, G., et al. (2015). Increased dopamine D2 receptor activity in the striatum alters the firing pattern of dopamine neurons in the ventral tegmental area. *Proc.Natl.Acad.Sci.U.S.A., 112*(12), 1498.

Kritzer, M. F., & Goldman-Rakic, P. S. (1995). Intrinsic circuit organization of the major layers and sublayers of the dorsolateral prefrontal cortex in the rhesus monkey. *J.Comp.Neurol., 359*(1), 131-143.

Kroken, R. A., Loberg, E. M., Dronen, T., Gruner, R., Hugdahl, K., et al. (2014). A critical review of pro-cognitive drug targets in psychosis: Convergence on myelination and inflammation. *Front.Psychiatry., 5*, 11.

Krystal, J. H., Karper, L. P., Seibyl, J. P., Freeman, G. K., Delaney, R., et al. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch.Gen.Psychiatry, 51*(3), 199-214.

Kubicki, M., Westin, C. F., McCarley, R. W., & Shenton, M. E. (2005). The application of DTI to investigate white matter abnormalities in schizophrenia. *Ann.N.Y.Acad.Sci., 1064*, 134-148.

Kulig, K. (1990). Lsd. *Emerg.Med.Clin.North Am., 8*(3), 551-558.

Kunzle, H. (1978). An autoradiographic analysis of the efferent connections from premotor and adjacent prefrontal regions (areas 6 and 9) in macaca fascicularis. *Brain Behav.Evol., 15*(3), 185-234.

Kuperberg, G. R., Broome, M. R., McGuire, P. K., David, A. S., Eddy, M., et al. (2003). Regionally localized thinning of the cerebral cortex in schizophrenia. *Arch.Gen.Psychiatry, 60*(9), 878-888.

Kupfermann, I. (1979). Modulatory actions of neurotransmitters. *Annu.Rev.Neurosci., 2*, 447-465.

Kurita, M., Holloway, T., Garcia-Bea, A., Kozlenkov, A., Friedman, A. K., et al. (2012). HDAC2 regulates atypical antipsychotic responses through the modulation of mGlu2 promoter activity. *Nat.Neurosci., 15*(9), 1245-1254.

Kuroki, T., Meltzer, H. Y., & Ichikawa, J. (1999). Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *J.Pharmacol.Exp.Ther., 288*(2), 774-781.

Kyd, R. J., & Bilkey, D. K. (2003). Prefrontal cortex lesions modify the spatial properties of hippocampal place cells. *Cereb.Cortex, 13*(5), 444-451.

Lahdesmaki, J., Sallinen, J., MacDonald, E., Kobilka, B. K., Fagerholm, V., & Scheinin, M. (2002). Behavioral and neurochemical characterization of alpha(2A)-adrenergic receptor knockout mice. *Neuroscience*, *113*(2), 289-299.

Lahti, A. C., Holcomb, H. H., Medoff, D. R., & Tamminga, C. A. (1995a). Ketamine activates psychosis and alters limbic blood flow in schizophrenia. *Neuroreport*, *6*(6), 869-872.

Lahti, A. C., Koffel, B., LaPorte, D., & Tamminga, C. A. (1995b). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology, 13*(1), 9-19.

Lahti, A. C., Weiler, M. A., Tamara Michaelidis, B. A., Parwani, A., & Tamminga, C. A. (2001). Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology, 25*(4), 455-467.

Lai, M. K., Tsang, S. W., Alder, J. T., Keene, J., Hope, T., et al. (2005). Loss of serotonin 5-HT2A receptors in the postmortem temporal cortex correlates with rate of cognitive decline in Alzheimer's disease. *Psychopharmacology (Berl), 179*(3), 673-677.

Lai, M. K., Tsang, S. W., Francis, P. T., Esiri, M. M., Hope, T., et al. (2003). 3H]GR113808 binding to serotonin 5-HT(4) receptors in the postmortem neocortex of Alzheimer's disease: A clinicopathological study. *J.Neural Transm.(Vienna), 110*(7), 779-788.

Lake, C. R., Sternberg, D. E., van Kammen, D. P., Ballenger, J. C., Ziegler, M. G., et al. (1980). Schizophrenia: Elevated cerebrospinal fluid norepinephrine. *Science, 207*(4428), 331-333.

Lakhlani, P. P., MacMillan, L. B., Guo, T. Z., McCool, B. A., Lovinger, D. M., et al. (1997). Substitution of a mutant alpha2a-adrenergic receptor via "hit and run" gene targeting reveals the role of this subtype in sedative, analgesic, and anesthetic-sparing responses in vivo. *Proc.Natl.Acad.Sci.U.S.A., 94*(18), 9950-9955.

Lamouroux, A., Vigny, A., Faucon Biguet, N., Darmon, M. C., Franck, R., et al. (1987). The primary structure of human dopamine-beta-hydroxylase: Insights into the relationship between the soluble and the membrane-bound forms of the enzyme. *Embo J., 6*(13), 3931-3937.

Lanctot, K. L., Herrmann, N., & Mazzotta, P. (2001). Role of serotonin in the behavioral and psychological symptoms of dementia. *J.Neuropsychiatry Clin.Neurosci., 13*(1), 5-21.

Lapiz, M. D., Bondi, C. O., & Morilak, D. A. (2007). Chronic treatment with desipramine improves cognitive performance of rats in an attentional set-shifting test. *Neuropsychopharmacology, 32*(5), 1000-1010.

Lapiz, M. D., & Morilak, D. A. (2006). Noradrenergic modulation of cognitive function in rat medial prefrontal cortex as measured by attentional set shifting capability. *Neuroscience*, *137*(3), 1039-1049.

Laporte, A. M., Koscielniak, T., Ponchant, M., Verge, D., Hamon, M., & Gozlan, H. (1992). Quantitative autoradiographic mapping of 5-HT3 receptors in the rat CNS using [125I]iodo-zacopride and [3H]zacopride as radioligands. *Synapse, 10*(4), 271-281.

Laroche, S., Jay, T. M., & Thierry, A. M. (1990). Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. *Neurosci.Lett., 114*(2), 184-190.

Laruelle, M., Abi-Dargham, A., Gil, R., Kegeles, L., & Innis, R. (1999). Increased dopamine transmission in schizophrenia: Relationship to illness phases. *Biol.Psychiatry, 46*(1), 56-72.

Laruelle, M., Abi-Dargham, A., van Dyck, C. H., Gil, R., D'Souza, C. D., et al. (1996). Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc.Natl.Acad.Sci.U.S.A., 93*(17), 9235-9240.

Laruelle, M., Abi-Dargham, A., van Dyck, C., Gil, R., D'Souza, D. C., et al. (2000). Dopamine and serotonin transporters in patients with schizophrenia: An imaging study with [(123)I]beta-CIT. *Biol.Psychiatry, 47*(5), 371-379.

Laruelle, M., Vanisberg, M. A., & Maloteaux, J. M. (1988). Regional and subcellular localization in human brain of [3H]paroxetine binding, a marker of serotonin uptake sites. *Biol.Psychiatry, 24*(3), 299-309.

Laursen, T. M., Nordentoft, M., & Mortensen, P. B. (2014). Excess early mortality in schizophrenia. *Annu.Rev.Clin.Psychol., 10*, 425-448.

Le Pen, G., Grottick, A. J., Higgins, G. A., & Moreau, J. L. (2003). Phencyclidine exacerbates attentional deficits in a neurodevelopmental rat model of schizophrenia. *Neuropsychopharmacology, 28*(10), 1799-1809.

Lechin, F., van der Dijs, B., & Hernandez-Adrian, G. (2006). Dorsal raphe vs. median raphe serotonergic antagonism. anatomical, physiological, behavioral, neuroendocrinological, neuropharmacological and clinical evidences: Relevance for neuropharmacological therapy. *Prog.Neuropsychopharmacol.Biol.Psychiatry, 30*(4), 565-585.

Lee, A., Rosin, D. L., & Van Bockstaele, E. J. (1998). alpha2A-adrenergic receptors in the rat nucleus locus coeruleus: Subcellular localization in catecholaminergic dendrites, astrocytes, and presynaptic axon terminals. *Brain Res., 795*(1-2), 157-169.

Lee, D., Rushworth, M. F., Walton, M. E., Watanabe, M., & Sakagami, M. (2007). Functional specialization of the primate frontal cortex during decision making. *J.Neurosci., 27*(31), 8170-8173.

Lee, K. W., Woon, P. S., Teo, Y. Y., & Sim, K. (2012). Genome wide association studies (GWAS) and copy number variation (CNV) studies of the major psychoses: What have we learnt? *Neurosci.Biobehav.Rev., 36*(1), 556-571.

Lehrer, D. S., Christian, B. T., Kirbas, C., Chiang, M., Sidhu, S., et al. (2010). 18F-fallypride binding potential in patients with schizophrenia compared to healthy controls. *Schizophr.Res., 122*(1-3), 43-52.

Leichnetz, G. R., & Astruc, J. (1976). The efferent projections of the medial prefrontal cortex in the squirrel monkey (saimiri sciureus). *Brain Res., 109*(3), 455-472.

Lejeune, F., Gobert, A., Rivet, J. M., & Millan, M. J. (1994). Blockade of transmission at NMDA receptors facilitates the electrical and synthetic activity of ascending serotoninergic neurones. *Brain Res., 656*(2), 427-431.

Lelong, V., Lhonneur, L., Dauphin, F., & Boulouard, M. (2003). BIMU 1 and RS 67333, two 5-HT4 receptor agonists, modulate spontaneous alternation deficits induced by scopolamine in the mouse. *Naunyn Schmiedebergs Arch.Pharmacol., 367*(6), 621-628.

LeMoal, M., Stinus, L., & Galey, D. (1976). Radiofrequency lesion of the ventral mesencephalic tegmentum: Neurological and behavioral considerations. *Exp.Neurol., 50*(3), 521-535.

Lena, I., Chessel, A., Le Pen, G., Krebs, M. O., & Garcia, R. (2007). Alterations in prefrontal glutamatergic and noradrenergic systems following MK-801 administration in rats prenatally exposed to methylazoxymethanol at gestational day 17. *Psychopharmacology (Berl), 192*(3), 373-383.

Leszczynska-Rodziewicz, A., Czerski, P. M., Kapelski, P., Godlewski, S., Dmitrzak-Weglarz, M., et al. (2002). A polymorphism of the norepinephrine transporter gene in bipolar disorder and schizophrenia: Lack of association. *Neuropsychobiology*, *45*(4), 182-185.

Leucht, S., Pitschel-Walz, G., Abraham, D., & Kissling, W. (1999). Efficacy and extrapyramidal side-effects of the new antipsychotics olanzapine, quetiapine, risperidone, and sertindole compared to conventional antipsychotics and placebo. A meta-analysis of randomized controlled trials. *Schizophr.Res., 35*(1), 51-68.

Levaux, M. N., Potvin, S., Sepehry, A. A., Sablier, J., Mendrek, A., & Stip, E. (2007). Computerized assessment of cognition in schizophrenia: Promises and pitfalls of CANTAB. *Eur.Psychiatry, 22*(2), 104-115.

Levin, E. D., & Gunne, L. M. (1989). Chronic neuroleptic effects on spatial reversal learning in monkeys. *Psychopharmacology (Berl), 97*(4), 496-500.

Levine, M. (1986). Ascorbic acid specifically enhances dopamine beta-monooxygenase activity in resting and stimulated chromaffin cells. *J.Biol.Chem., 261*(16), 7347-7356.

Levine, M., Morita, K., Heldman, E., & Pollard, H. B. (1985). Ascorbic acid regulation of norepinephrine biosynthesis in isolated chromaffin granules from bovine adrenal medulla. *J.Biol.Chem., 260*(29), 15598-15603.

Levy, F. (2008). Pharmacological and therapeutic directions in ADHD: Specificity in the PFC. *Behav.Brain Funct., 4*, 1-12. eCollection 2008.

Lewis, D., A. (2011). The chandelier neuron in schizophrenia. *Dev.Neurobiol*. *71*(1), 118-127.

Lewis, D. A., & Akil, M. (1997). Cortical dopamine in schizophrenia: Strategies for postmortem studies. *J.Psychiatr.Res., 31*(2), 175-195.

Lewis, D. A., Cho, R. Y., Carter, C. S., Eklund, K., Forster, S., et al. (2008). Subunit-selective modulation of GABA type A receptor neurotransmission and cognition in schizophrenia. *Am.J.Psychiatry, 165*(12), 1585-1593.

Lewis, D. A., Cruz, D. A., Melchitzky, D. S., & Pierri, J. N. (2001). Lamina-specific deficits in parvalbumin-immunoreactive varicosities in the prefrontal cortex of subjects with schizophrenia: Evidence for fewer projections from the thalamus. *Am.J.Psychiatry, 158*(9), 1411-1422.

Lewis, D. A., Curley, A. A., Glausier, J. R., & Volk, D. W. (2012). Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. *Trends Neurosci., 35*(1), 57-67.

Lewis, D. A., & Gonzalez-Burgos, G. (2008). Neuroplasticity of neocortical circuits in schizophrenia. *Neuropsychopharmacology, 33*(1), 141-165.

Lewis, D. A., Hashimoto, T., & Volk, D. W. (2005). Cortical inhibitory neurons and schizophrenia. *Nat.Rev.Neurosci., 6*(4), 312-324.

Lewis, D. A., & Lieberman, J. A. (2000). Catching up on schizophrenia: Natural history and neurobiology. *Neuron*, *28*(2), 325-334

Lewis, D. A., Pierri, J. N., Volk, D. W., Melchitzky, D. S., & Woo, T. U. (1999). Altered GABA neurotransmission and prefrontal cortical dysfunction in schizophrenia. *Biol.Psychiatry, 46*(5), 616-626.

Li, B. M., Mao, Z. M., Wang, M., & Mei, Z. T. (1999). Alpha-2 adrenergic modulation of prefrontal cortical neuronal activity related to spatial working memory in monkeys. *Neuropsychopharmacology, 21*(5), 601-610.

Li, B. M., & Mei, Z. T. (1994). Delayed-response deficit induced by local injection of the alpha 2-adrenergic antagonist yohimbine into the dorsolateral prefrontal cortex in young adult monkeys. *Behav.Neural Biol., 62*(2), 134-139.

Li, C. J., Zhou, M., Li, H. G., Lv, Q., Xu, X. L., & Guo, L. J. (2013). Clonidine suppresses the induction of long-term potentiation by inhibiting HCN channels at the schaffer collateral-CA1 synapse in anesthetized adult rats. *Cell.Mol.Neurobiol., 33*(8), 1075-1086.

Li, M. Y., Yan, Q. S., Coffey, L. L., & Reith, M. E. (1996). Extracellular dopamine, norepinephrine, and serotonin in the nucleus accumbens of freely moving rats during intracerebral dialysis with cocaine and other monoamine uptake blockers. *J.Neurochem., 66*(2), 559-568.

Li, S., Cullen, W. K., Anwyl, R., & Rowan, M. J. (2003). Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. *Nat.Neurosci., 6*(5), 526-531.

Li, W. Y., Chang, Y. C., Lee, L. J., & Lee, L. J. (2014). Prenatal infection affects the neuronal architecture and cognitive function in adult mice. *Dev.Neurosci., 36*(5), 359-370.

Li, X. M., Perry, K. W., Wong, D. T., & Bymaster, F. P. (1998). Olanzapine increases in vivo dopamine and norepinephrine release in rat prefrontal cortex, nucleus accumbens and striatum. *Psychopharmacology (Berl), 136*(2), 153-161.

Li, Y. C., Kellendonk, C., Simpson, E. H., Kandel, E. R., & Gao, W. J. (2011). D2 receptor overexpression in the striatum leads to a deficit in inhibitory transmission and dopamine sensitivity in mouse prefrontal cortex. *Proc.Natl.Acad.Sci.U.S.A., 108*(29), 12107-12112.

Liang, M., Zhou, Y., Jiang, T., Liu, Z., Tian, L., et al. (2006). Widespread functional disconnectivity in schizophrenia with resting-state functional magnetic resonance imaging. *Neuroreport*, *17*(2), 209-213.

Lidow, M. S. (2003). Calcium signaling dysfunction in schizophrenia: A unifying approach. *Brain Res.Brain Res.Rev., 43*(1), 70-84.

Lidow, M. S., Elsworth, J. D., & Goldman-Rakic, P. S. (1997). Down-regulation of the D1 and D5 dopamine receptors in the primate prefrontal cortex by chronic treatment with antipsychotic drugs. *J.Pharmacol.Exp.Ther., 281*(1), 597-603.

Limberger, N., Bonanno, G., Späth, L., & Starke, K. (1986). Autoreceptors and α2-adrenoceptors at the serotonergic axons of rabbit brain cortex. *Naunyn Schmiedebergs Arch. Pharmacol*., *332*(4), 324-331.

Link, R. E., Desai, K., Hein, L., Stevens, M. E., Chruscinski, A., et al. (1996). Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c. *Science, 273*(5276), 803-805.

Link, R., Daunt, D., Barsh, G., Chruscinski, A., & Kobilka, B. (1992). Cloning of two mouse genes encoding alpha 2-adrenergic receptor subtypes and identification of a single amino acid in the mouse alpha 2-C10 homolog responsible for an interspecies variation in antagonist binding. *Mol.Pharmacol., 42*(1), 16-27.

Lins, B. R., Hurtubise, J. L., Roebuck, A. J., Marks, W. N., Zabder, N. K., et al. (2018). Prospective analysis of the effects of maternal immune activation on rat cytokines during pregnancy and behavior of the male offspring relevant to schizophrenia. *eNeuro*, *5*(4).pii: ENEURO.0249-18.2018.

Lipska, B. K. (2004). Using animal models to test a neurodevelopmental hypothesis of schizophrenia. *J.Psychiatry Neurosci., 29*(4), 282-286.

Lipska, B. K., Jaskiw, G. E., Braun, A. R., & Weinberger, D. R. (1995). Prefrontal cortical and hippocampal modulation of haloperidol-induced catalepsy and apomorphine-induced stereotypic behaviors in the rat. *Biol.Psychiatry, 38*(4), 255-262.

Lipska, B. K., & Weinberger, D. R. (1995). Genetic variation in vulnerability to the behavioral effects of neonatal hippocampal damage in rats. *Proc.Natl.Acad.Sci.U.S.A., 92*(19), 8906-8910.

Lipska, B. K., & Weinberger, D. R. (2000). To model a psychiatric disorder in animals: Schizophrenia as a reality test. *Neuropsychopharmacology, 23*(3), 223-239.

Lisman, J. E., & Grace, A. A. (2005). The hippocampal-VTA loop: Controlling the entry of information into long-term memory. *Neuron*, *46*(5), 703-713.

Litman, R. E., Smith, M. A., Desai, D. G., Simpson, T., Sweitzer, D., & Kanes, S. J. (2014). The selective neurokinin 3 antagonist AZD2624 does not improve symptoms or cognition in schizophrenia: A proof-of-principle study. *J.Clin.Psychopharmacol., 34*(2), 199-204.

Liu, L., Yuan, G., Cheng, Z., Zhang, G., Liu, X., & Zhang, H. (2013). Identification of the mRNA expression status of the dopamine D2 receptor and dopamine transporter in peripheral blood lymphocytes of schizophrenia patients. *PLoS One, 8*(9), e75259.

Liu, Y. P., Lin, Y. L., Chuang, C. H., Kao, Y. C., Chang, S. T., & Tung, C. S. (2009). Alpha adrenergic modulation on effects of norepinephrine transporter inhibitor reboxetine in five-choice serial reaction time task. *J.Biomed.Sci., 16*, 7-72.

Lloyd, K., & Hornykiewicz, O. (1970). Parkinson's disease: Activity of L-dopa decarboxylase in discrete brain regions. *Science*, (170), 1212–1213.

Lodge D., A. J. (1987). Excitatory amino acids and phencyclidine drugs. In HIcks T.P., Lodge D., McLennan H. (Ed.), *Excitatory amino acid transmission* (pp. 83-90)

Lomasney, J. W., Cotecchia, S., Lefkowitz, R. J., & Caron, M. G. (1991). Molecular biology of alpha-adrenergic receptors: Implications for receptor classification and for structure-function relationships. *Biochim.Biophys.Acta, 1095*(2), 127-139.

Lopez, A., Mendieta, L., Prades, R., Royo, S., Tarrago, T., & Giralt, E. (2013). Peptide POP inhibitors for the treatment of the cognitive symptoms of schizophrenia. *Future Med.Chem., 5*(13), 1509-1523.

Lopez-Gil, X., Artigas, F., & Adell, A. (2009). Role of different monoamine receptors controlling MK-801-induced release of serotonin and glutamate in the medial prefrontal cortex: Relevance for antipsychotic action. *Int.J.Neuropsychopharmacol., 12*(4), 487-499.

Lopez-Gil, X., Babot, Z., Amargos-Bosch, M., Sunol, C., Artigas, F., & Adell, A. (2007). Clozapine and haloperidol differently suppress the MK-801-increased glutamatergic and serotonergic transmission in the medial prefrontal cortex of the rat. *Neuropsychopharmacology, 32*(10), 2087-2097.

Lopez-Gil, X., Jimenez-Sanchez, L., Campa, L., Castro, E., Frago, C., & Adell, A. (2019). Role of serotonin and noradrenaline in the rapid antidepressant action of ketamine. *ACS Chem.Neurosci., 10*(7), 3318-3326.

Loscher, W., Annies, R., & Honack, D. (1993). Comparison of competitive and uncompetitive NMDA receptor antagonists with regard to monoaminergic neuronal activity and behavioural effects in rats. *Eur.J.Pharmacol., 242*(3), 263-274.

Lozeva-Thomas, V. (2004). Serotonin brain circuits with a focus on hepatic encephalopathy. *Metab.Brain Dis., 19*(3-4), 413-420.

Luchicchi, A., Lecca, S., Melis, M., De Felice, M., Cadeddu, F., et al. (2016). Maternal immune activation disrupts dopamine system in the offspring. *Int.J.Neuropsychopharmacol., 19*(7), pii: pyw007.

Lucki, I. (1998). The spectrum of behaviors influenced by serotonin. *Biol.Psychiatry, 44*(3), 151-162.

Luessen, D. J., Hinshaw, T. P., Sun, H., Howlett, A. C., Marrs, G., et al. (2016). RGS2 modulates the activity and internalization of dopamine D2 receptors in neuroblastoma N2A cells. *Neuropharmacology*, *110*(Pt A), 297-307.

Luhrs, L., Manlapaz, C., Kedzie, K., Rao, S., Cabrera-Ghayouri, S., et al. (2016). Function of brain alpha2B-adrenergic receptor characterized with subtype-selective alpha2B antagonist and KO mice. *Neuroscience*, *339*, 608-621.

Luscher, C., & Malenka, R. C. (2012). NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb Perspect.Biol., 4*(6), pii: a005710.

Lustig, C., Kozak, R., Sarter, M., Young, J. W., & Robbins, T. W. (2013). CNTRICS final animal model task selection: Control of attention. *Neurosci.Biobehav.Rev., 37*(9 Pt B), 2099-2110.

Luykx, J. J., Vinkers, C. H., Bakker, S. C., Visser, W. F., van Boxmeer, L., et al. (2012). A common variant in ERBB4 regulates GABA concentrations in human cerebrospinal fluid. *Neuropsychopharmacology, 37*(9), 2088-2092.

Lymer, G. K., Job, D. E., William, T., Moorhead, J., McIntosh, A. M., et al. (2006). Brain-behaviour relationships in people at high genetic risk of schizophrenia. *Neuroimage, 33*(1), 275-285.

Ma, C. L., Arnsten, A. F., & Li, B. M. (2005). Locomotor hyperactivity induced by blockade of prefrontal cortical alpha2-adrenoceptors in monkeys. *Biol.Psychiatry, 57*(2), 192-195.

Ma, C. L., Qi, X. L., Peng, J. Y., & Li, B. M. (2003). Selective deficit in no-go performance induced by blockade of prefrontal cortical alpha 2-adrenoceptors in monkeys. *Neuroreport*, *14*(7), 1013-1016.

MacDonald, E., Kobilka, B. K., & Scheinin, M. (1997). Gene targeting--homing in on alpha 2-adrenoceptor-subtype function. *Trends Pharmacol.Sci., 18*(6), 211-219.

MacDonald, E., & Scheinin, M. (1995). Distribution and pharmacology of alpha 2-adrenoceptors in the central nervous system. *J.Physiol.Pharmacol., 46*(3), 241-258.

Macdonald, P. A., & Monchi, O. (2011). Differential effects of dopaminergic therapies on dorsal and ventral striatum in Parkinson's disease: Implications for cognitive function. *Parkinsons Dis., 2011*, 572743.

MacDowell, K. S., Garcia-Bueno, B., Madrigal, J. L., Parellada, M., Arango, C., et al. (2013). Risperidone normalizes increased inflammatory parameters and restores anti-inflammatory pathways in a model of neuroinflammation. *Int.J.Neuropsychopharmacol., 16*(1), 121-135.

MacDowell, K. S., Munarriz-Cuezva, E., Caso, J. R., Madrigal, J. L., Zabala, A., et al. (2017). Paliperidone reverts toll-like receptor 3 signaling pathway activation and cognitive deficits in a maternal immune activation mouse model of schizophrenia. *Neuropharmacology*, *116*, 196-207.

Machado, C. J., Whitaker, A. M., Smith, S. E., Patterson, P. H., & Bauman, M. D. (2015). Maternal immune activation in nonhuman primates alters social attention in juvenile offspring. *Biol.Psychiatry, 77*(9), 823-832.

MacMillan, L. B., Hein, L., Smith, M. S., Piascik, M. T., & Limbird, L. E. (1996). Central hypotensive effects of the alpha2a-adrenergic receptor subtype. *Science, 273*(5276), 801-803.

Maddux, J. M., & Holland, P. C. (2011). Effects of dorsal or ventral medial prefrontal cortical lesions on five-choice serial reaction time performance in rats. *Behav.Brain Res., 221*(1), 63-74.

Madsen, K., Haahr, M. T., Marner, L., Keller, S. H., Baare, W. F., et al. (2011). Age and sex effects on 5-HT(4) receptors in the human brain: A [(11)C]SB207145 PET study. *J.Cereb.Blood Flow Metab., 31*(6), 1475-1481.

Maes, M., Goossens, F., Scharpe, S., Calabrese, J., Desnyder, R., & Meltzer, H. Y. (1995). Alterations in plasma prolyl endopeptidase activity in depression, mania, and schizophrenia: Effects of antidepressants, mood stabilizers, and antipsychotic drugs. *Psychiatry Res., 58*(3), 217-225.

Magee, J. C. (1998). Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. *J.Neurosci., 18*(19), 7613-7624.

Magee, J. C. (1999). Dendritic lh normalizes temporal summation in hippocampal CA1 neurons. *Nat.Neurosci., 2*(6), 508-514.

Mailman, R. B., & Murthy, V. (2010). Third generation antipsychotic drugs: Partial agonism or receptor functional selectivity? *Curr.Pharm.Des., 16*(5), 488-501.

Mair, R. D., Zhang, Y., Bailey, K. R., Toupin, M. M., & Mair, R. G. (2005). Effects of clonidine in the locus coeruleus on prefrontal- and hippocampal-dependent measures of attention and memory in the rat. *Psychopharmacology (Berl), 181*(2), 280-288.

Mair, R. G., & McEntee, W. J. (1986). Cognitive enhancement in Korsakoff's psychosis by clonidine: A comparison with L-dopa and ephedrine. *Psychopharmacology (Berl), 88*(3), 374-380.

Maixner, S. M., Mellow, A. M., & Tandon, R. (1999). The efficacy, safety, and tolerability of antipsychotics in the elderly. *J.Clin.Psychiatry, 60 Suppl 8*, 29-41.

Makaritsis, K. P., Handy, D. E., Johns, C., Kobilka, B., Gavras, I., & Gavras, H. (1999). Role of the alpha2B-adrenergic receptor in the development of salt-induced hypertension. *Hypertension, 33*(1), 14-17.

Makinson, R., Lloyd, K., Grissom, N., & Reyes, T. M. (2019). Exposure to in utero inflammation increases locomotor activity, alters cognitive performance and drives vulnerability to cognitive performance deficits after acute immune activation. *Brain Behav.Immun., 80*, 56-65.

Malaspina, D., Harlap, S., Fennig, S., Heiman, D., Nahon, D., et al. (2001). Advancing paternal age and the risk of schizophrenia. *Arch.Gen.Psychiatry, 58*(4), 361-367.

Malhotra, A. K., Pinals, D. A., Adler, C. M., Elman, I., Clifton, A., et al. (1997). Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology, 17*(3), 141-150.

Malhotra, A. K., Pinals, D. A., Weingartner, H., Sirocco, K., Missar, C. D., et al. (1996). NMDA receptor function and human cognition: The effects of ketamine in healthy volunteers. *Neuropsychopharmacology, 14*(5), 301-307.

Mancama, D., Arranz, M. J., Landau, S., & Kerwin, R. (2003). Reduced expression of the muscarinic 1 receptor cortical subtype in schizophrenia. *Am.J.Med.Genet.B.Neuropsychiatr.Genet., 119B*(1), 2-6.

Manji, H. K., & Lenox, R. H. (2000). Signaling: Cellular insights into the pathophysiology of bipolar disorder. *Biol.Psychiatry, 48*(6), 518-530.

Mannisto, P. T., & Kaakkola, S. (1999). Catechol-O-methyltransferase (COMT): Biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol.Rev., 51*(4), 593-628.

Mannisto, P. T., Venalainen, J., Jalkanen, A., & Garcia-Horsman, J. A. (2007). Prolyl oligopeptidase: A potential target for the treatment of cognitive disorders. *Drug News.Perspect., 20*(5), 293-305.

Manoach, D. S. (2003). Prefrontal cortex dysfunction during working memory performance in schizophrenia: Reconciling discrepant findings. *Schizophr.Res., 60*(2-3), 285-298.

Mao, Z. M., Arnsten, A. F., & Li, B. M. (1999). Local infusion of an alpha-1 adrenergic agonist into the prefrontal cortex impairs spatial working memory performance in monkeys. *Biol.Psychiatry, 46*(9), 1259-1265.

Mar, A. C., Horner, A. E., Nilsson, S. R., Alsio, J., Kent, B. A., et al. (2013). The touchscreen operant platform for assessing executive function in rats and mice. *Nat.Protoc., 8*(10), 1985-2005.

Marchetti-Gauthier, E., Roman, F. S., Dumuis, A., Bockaert, J., & Soumireu-Mourat, B. (1997). BIMU1 increases associative memory in rats by activating 5-HT4 receptors. *Neuropharmacology, 36*(4-5), 697-706.

Marconi, A., Di Forti, M., Lewis, C. M., Murray, R. M., & Vassos, E. (2016). Meta-analysis of the association between the level of cannabis use and risk of psychosis. *Schizophr.Bull., 42*(5), 1262-1269.

Marcotte, E. R., Pearson, D. M., & Srivastava, L. K. (2001). Animal models of schizophrenia: A critical review. *J.Psychiatry Neurosci., 26*(5), 395-410.

Marcus, M. M., Jardemark, K. E., Wadenberg, M. L., Langlois, X., Hertel, P., & Svensson, T. H. (2005). Combined alpha2 and D2/3 receptor blockade enhances cortical glutamatergic transmission and reverses cognitive impairment in the rat. *Int.J.Neuropsychopharmacol., 8*(3), 315-327.

Marcus, M. M., Jardemark, K., Malmerfelt, A., Bjorkholm, C., & Svensson, T. H. (2010a). Reboxetine enhances the olanzapine-induced antipsychotic-like effect, cortical dopamine outflow and NMDA receptor-mediated transmission. *Neuropsychopharmacology, 35*(9), 1952-1961.

Marcus, M. M., Wiker, C., Franberg, O., Konradsson-Geuken, A., Langlois, X., et al. (2010b). Adjunctive alpha2-adrenoceptor blockade enhances the antipsychotic-like effect of risperidone and facilitates cortical dopaminergic and glutamatergic, NMDA receptor-mediated transmission. *Int.J.Neuropsychopharmacol., 13*(7), 891-903.

Marder, S. R., & Fenton, W. (2004). Measurement and treatment research to improve cognition in schizophrenia: NIMH MATRICS initiative to support the development of agents for improving cognition in schizophrenia. *Schizophr.Res., 72*(1), 5-9.

Marder, S. R., Fenton, W., & Youens, K. (2004). Schizophrenia, IX: Cognition in schizophrenia--the MATRICS initiative. *Am.J.Psychiatry, 161*(1), 25.

Marek, G. J. (2010). Metabotropic glutamate2/3 (mGlu2/3) receptors, schizophrenia and cognition. *Eur.J.Pharmacol., 639*(1-3), 81-90.

Marek, G. J., & Aghajanian, G. K. (1999). 5-HT2A receptor or alpha1-adrenoceptor activation induces excitatory postsynaptic currents in layer V pyramidal cells of the medial prefrontal cortex. *Eur.J.Pharmacol., 367*(2-3), 197-206.

Marenco, S., Geramita, M., van der Veen, J. W., Barnett, A. S., Kolachana, B., et al. (2011). Genetic association of ErbB4 and human cortical GABA levels in vivo. *J.Neurosci., 31*(32), 11628-11632.

Marini, S., Bagnoli, S., Bessi, V., Tedde, A., Bracco, L., et al. (2011). Implication of serotonin-transporter (5-HTT) gene polymorphism in subjective memory complaints and mild cognitive impairment (MCI). *Arch.Gerontol.Geriatr., 52*(2), 71.

Marjamaki, A., Luomala, K., Ala-Uotila, S., & Scheinin, M. (1993). Use of recombinant human alpha 2-adrenoceptors to characterize subtype selectively of antagonist binding. *Eur.J.Pharmacol., 246*(3), 219-226.

Marrs, W., Kuperman, J., Avedian, T., Roth, R. H., & Jentsch, J. D. (2005). Alpha-2 adrenoceptor activation inhibits phencyclidine-induced deficits of spatial working memory in rats. *Neuropsychopharmacology, 30*(8), 1500-1510.

Martin, L. F., Kem, W. R., & Freedman, R. (2004). Alpha-7 nicotinic receptor agonists: Potential new candidates for the treatment of schizophrenia. *Psychopharmacology (Berl), 174*(1), 54-64.

Martinez, D., Hwang, D., Mawlawi, O., Slifstein, M., Kent, J., et al. (2001). Differential occupancy of somatodendritic and postsynaptic 5HT(1A) receptors by pindolol: A dose-occupancy study with [11C]WAY 100635 and positron emission tomography in humans. *Neuropsychopharmacology, 24*(3), 209-229.

Martin-Ruiz, R., Puig, M. V., Celada, P., Shapiro, D. A., Roth, B. L., et al. (2001). Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. *J.Neurosci., 21*(24), 9856-9866.

Masana, M., Bortolozzi, A., & Artigas, F. (2011). Selective enhancement of mesocortical dopaminergic transmission by noradrenergic drugs: Therapeutic opportunities in schizophrenia. *Int.J.Neuropsychopharmacol., 14*(1), 53-68.

Masana, M., Castane, A., Santana, N., Bortolozzi, A., & Artigas, F. (2012a). Noradrenergic antidepressants increase cortical dopamine: Potential use in augmentation strategies. *Neuropharmacology*, *63*(4), 675-684.

Masana, M., Santana, N., Artigas, F., & Bortolozzi, A. (2012b). Dopamine neurotransmission and atypical antipsychotics in prefrontal cortex: A critical review. *Curr.Top.Med.Chem., 12*(21), 2357-2374.

Mateo, Y., & Meana, J. J. (1999). Determination of the somatodendritic alpha2-adrenoceptor subtype located in rat locus coeruleus that modulates cortical noradrenaline release in vivo. *Eur.J.Pharmacol., 379*(1), 53-57.

Mateo, Y., Pineda, J., & Meana, J. J. (1998). Somatodendritic alpha2-adrenoceptors in the locus coeruleus are involved in the in vivo modulation of cortical noradrenaline release by the antidepressant desipramine. *J.Neurochem., 71*(2), 790-798.

Mathai, D. S., Holst, M., Rodgman, C., Haile, C. N., Keller, J., et al. (2018). Guanfacine attenuates adverse effects of dronabinol (THC) on working memory in adolescent-onset heavy cannabis users: A pilot study. *J.Neuropsychiatry Clin.Neurosci., 30*(1), 66-76.

Matsukawa, M., Ogawa, M., Nakadate, K., Maeshima, T., Ichitani, Y., et al. (1997). Serotonin and acetylcholine are crucial to maintain hippocampal synapses and memory acquisition in rats. *Neurosci.Lett., 230*(1), 13-16.

Matsumoto, M., & Hikosaka, O. (2009). Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature*, *459*(7248), 837-841.

Matsumoto, M., Yoshioka, M., Togashi, H., Mori, K., Ueno, K., & Saito, H. (1998). Effects of idazoxan on dopamine release in the prefrontal cortex of freely moving rats. *Eur.J.Pharmacol., 343*(2-3), 165-170.

Matsumoto, T., Maeno, Y., Kato, H., Seko-Nakamura, Y., Monma-Ohtaki, J., et al. (2014). 5-hydroxytryptamine- and dopamine-releasing effects of ring-substituted amphetamines on rat brain: A comparative study using in vivo microdialysis. *Eur.Neuropsychopharmacol., 24*(8), 1362-1370.

Matthies, H. J., Han, Q., Shields, A., Wright, J., Moore, J. L., et al. (2009). Subcellular localization of the antidepressant-sensitive norepinephrine transporter. *BMC Neurosci., 10*, 6-65.

Maura, G., Bonanno, G., & Raiteri, M. (1992). Presynaptic alpha 2-adrenoceptors mediating inhibition of noradrenaline and 5-hydroxytryptamine release in rat cerebral cortex: Further characterization as different alpha 2-adrenoceptor subtypes. *Naunyn Schmiedebergs Arch.Pharmacol., 345*(4), 410-416.

Maura, G., Gemignani, A., & Raiteri, M. (1982). Noradrenaline inhibits central serotonin release through alpha 2-adrenoceptors located on serotonergic nerve terminals. *Naunyn Schmiedebergs Arch.Pharmacol., 320*(3), 272-274.

Mazei, M. S., Pluto, C. P., Kirkbride, B., & Pehek, E. A. (2002). Effects of catecholamine uptake blockers in the caudate-putamen and subregions of the medial prefrontal cortex of the rat. *Brain Res., 936*(1-2), 58-67.

McAllister, T. W., Flashman, L. A., Sparling, M. B., & Saykin, A. J. (2004). Working memory deficits after traumatic brain injury: Catecholaminergic mechanisms and prospects for treatment -- a review. *Brain Inj., 18*(4), 331-350.

McCall, J. G., Al-Hasani, R., Siuda, E. R., Hong, D. Y., Norris, A. J., et al. (2015). CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. *Neuron*, *87*(3), 605-620.

McCall, J. G., Siuda, E. R., Bhatti, D. L., Lawson, L. A., McElligott, Z. A., et al. (2017). Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior. *Elife, 6*, 10.7554/eLife.18247.

McClure, M. M., Barch, D. M., Romero, M. J., Minzenberg, M. J., Triebwasser, J., et al. (2007). The effects of guanfacine on context processing abnormalities in schizotypal personality disorder. *Biol.Psychiatry, 61*(10), 1157-1160.

McClure, M. M., Graff, F., Triebwasser, J., Perez-Rodriguez, M., Rosell, D. R., et al. (2019). Guanfacine augmentation of a combined intervention of computerized cognitive remediation therapy and social skills training for schizotypal personality disorder. *Am.J.Psychiatry, 176*(4), 307-314.

McCollum, L. A., & Roberts, R. C. (2015). Uncovering the role of the nucleus accumbens in schizophrenia: A postmortem analysis of tyrosine hydroxylase and vesicular glutamate transporters. *Schizophr.Res., 169*(1-3), 369-373.

McCracken, J. T., Aman, M. G., McDougle, C. J., Tierney, E., Shiraga, S., et al. (2010). Possible influence of variant of the P-glycoprotein gene (MDR1/ABCB1) on clinical response to guanfacine in children with pervasive developmental disorders and hyperactivity. *J.Child Adolesc.Psychopharmacol., 20*(1), 1-5.

McCune, S. K., Voigt, M. M., & Hill, J. M. (1993). Expression of multiple alpha adrenergic receptor subtype messenger RNAs in the adult rat brain. *Neuroscience*, *57*(1), 143-151.

McCutcheon, R., Beck, K., Jauhar, S., & Howes, O. D. (2018). Defining the locus of dopaminergic dysfunction in schizophrenia: A meta-analysis and test of the mesolimbic hypothesis. *Schizophr.Bull., 44*(6), 1301-1311.

McGaugh, J. L., & Roozendaal, B. (2009). Drug enhancement of memory consolidation: Historical perspective and neurobiological implications. *Psychopharmacology (Berl), 202*(1-3), 3-14.

McGaughy, J., Ross, R. S., & Eichenbaum, H. (2008). Noradrenergic, but not cholinergic, deafferentation of prefrontal cortex impairs attentional set-shifting. *Neuroscience*, *153*(1), 63-71.

McGowan, S., Lawrence, A. D., Sales, T., Quested, D., & Grasby, P. (2004). Presynaptic dopaminergic dysfunction in schizophrenia: A positron emission tomographic [18F]fluorodopa study. *Arch.Gen.Psychiatry, 61*(2), 134-142.

McKittrick, C. R., & Abercrombie, E. D. (2007). Catecholamine mapping within nucleus accumbens: Differences in basal and amphetamine-stimulated efflux of norepinephrine and dopamine in shell and core. *J.Neurochem., 100*(5), 1247-1256.

McLean, S. L., Grayson, B., Idris, N. F., Lesage, A. S., Pemberton, D. J., et al. (2011). Activation of alpha7 nicotinic receptors improves phencyclidine-induced deficits in cognitive tasks in rats: Implications for therapy of cognitive dysfunction in schizophrenia. *Eur.Neuropsychopharmacol., 21*(4), 333-343.

McQuown, S. C., Barrett, R. M., Matheos, D. P., Post, R. J., Rogge, G. A., et al. (2011). HDAC3 is a critical negative regulator of long-term memory formation. *J.Neurosci., 31*(2), 764-774.

Meador-Woodruff, J. H., Haroutunian, V., Powchik, P., Davidson, M., Davis, K. L., & Watson, S. J. (1997). Dopamine receptor transcript expression in striatum and prefrontal and occipital cortex. focal abnormalities in orbitofrontal cortex in schizophrenia. *Arch.Gen.Psychiatry, 54*(12), 1089-1095.

Meana, J. J., Callado, L. F., Pazos, A., Grijalba, B., & Garcia-Sevilla, J. A. (1996). The subtype-selective alpha 2-adrenoceptor antagonists BRL 44408 and ARC 239 also recognize 5-HT1A receptors in the rat brain. *Eur.J.Pharmacol., 312*(3), 385-388.

Mednick, S. A., Machon, R. A., Huttunen, M. O., & Bonett, D. (1988). Adult schizophrenia following prenatal exposure to an influenza epidemic. *Arch.Gen.Psychiatry, 45*(2), 189-192.

Mehta, M. A., Swainson, R., Ogilvie, A. D., Sahakian, J., & Robbins, T. W. (2001). Improved short-term spatial memory but impaired reversal learning following the dopamine D(2) agonist bromocriptine in human volunteers. *Psychopharmacology (Berl), 159*(1), 10-20.

Meisenzahl, E. M., Schmitt, G. J., Scheuerecker, J., & Moller, H. J. (2007). The role of dopamine for the pathophysiology of schizophrenia. *Int.Rev.Psychiatry., 19*(4), 337-345.

Melchitzky, D. S., & Lewis, D. A. (2000). Tyrosine hydroxylase- and dopamine transporter-immunoreactive axons in the primate cerebellum. evidence for a lobular- and laminar-specific dopamine innervation. *Neuropsychopharmacology, 22*(5), 466-472.

Meltzer, C. C., Smith, G., DeKosky, S. T., Pollock, B. G., Mathis, C. A., et al. (1998). Serotonin in aging, late-life depression, and Alzheimer's disease: The emerging role of functional imaging. *Neuropsychopharmacology, 18*(6), 407-430.

Meltzer, H. Y. (1991). The mechanism of action of novel antipsychotic drugs. *Schizophr.Bull., 17*(2), 263-287.

Meltzer, H. Y., Arvanitis, L., Bauer, D., Rein, W., & Meta-Trial Study Group. (2004). Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. *Am.J.Psychiatry, 161*(6), 975-984.

Meltzer, H. Y., & McGurk, S. R. (1999). The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. *Schizophr.Bull., 25*(2), 233-255.

Meltzer, & Prus, A. (2006). NK3 receptor antagonists for the treatment of schizophrenia. *Drug Discovery Today Therapeutic Strategies*, *3*(4), 555-560.

Meneses, A. (1999). 5-HT system and cognition. *Neurosci.Biobehav.Rev., 23*(8), 1111-1125.

Meyer, U. (2014). Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems. *Biol.Psychiatry, 75*(4), 307-315.

Meyer, U., & Feldon, J. (2012). To poly(I:C) or not to poly(I:C): Advancing preclinical schizophrenia research through the use of prenatal immune activation models. *Neuropharmacology*, *62*(3), 1308-1321.

Meyer, U., Feldon, J., & Fatemi, S. H. (2009). In-vivo rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. *Neurosci.Biobehav.Rev., 33*(7), 1061-1079.

Meyer, U., Feldon, J., Schedlowski, M., & Yee, B. K. (2005). Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. *Neurosci.Biobehav.Rev., 29*(6), 913-947.

Meyer, U., Feldon, J., Schedlowski, M., & Yee, B. K. (2006a). Immunological stress at the maternal-foetal interface: A link between neurodevelopment and adult psychopathology. *Brain Behav.Immun., 20*(4), 378-388.

Meyer, U., Murray, P. J., Urwyler, A., Yee, B. K., Schedlowski, M., & Feldon, J. (2008b). Adult behavioral and pharmacological dysfunctions following disruption of the fetal brain balance between pro-inflammatory and IL-10-mediated anti-inflammatory signaling. *Mol.Psychiatry, 13*(2), 208-221.

Meyer, U., Nyffeler, M., Engler, A., Urwyler, A., Schedlowski, M., et al. (2006b). The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. *J.Neurosci., 26*(18), 4752-4762.

Meyer, U., Nyffeler, M., Schwendener, S., Knuesel, I., Yee, B. K., & Feldon, J. (2008b). Relative prenatal and postnatal maternal contributions to schizophrenia-related neurochemical dysfunction after in utero immune challenge. *Neuropsychopharmacology, 33*(2), 441-456.

Meyer, U., Schwendener, S., Feldon, J., & Yee, B. K. (2006c). Prenatal and postnatal maternal contributions in the infection model of schizophrenia. *Exp.Brain Res., 173*(2), 243-257.

Meyer, U., Spoerri, E., Yee, B. K., Schwarz, M. J., & Feldon, J. (2010). Evaluating early preventive antipsychotic and antidepressant drug treatment in an infection-based neurodevelopmental mouse model of schizophrenia. *Schizophr.Bull., 36*(3), 607-623.

Meyer, U., Yee, B. K., & Feldon, J. (2007). The neurodevelopmental impact of prenatal infections at different times of pregnancy: The earlier the worse? *Neuroscientist, 13*(3), 241-256.

Meyer-Lindenberg, A. S., Olsen, R. K., Kohn, P. D., Brown, T., Egan, M. F., et al. (2005). Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. *Arch.Gen.Psychiatry, 62*(4), 379-386.

Meyer-Lindenberg, A., Buckholtz, J. W., Kolachana, B., R Hariri, A., Pezawas, L., et al. (2006). Neural mechanisms of genetic risk for impulsivity and violence in humans. *Proc.Natl.Acad.Sci.U.S.A., 103*(16), 6269-6274.

Meyer-Lindenberg, A., Miletich, R. S., Kohn, P. D., Esposito, G., Carson, R. E., et al. (2002). Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia. *Nat.Neurosci., 5*(3), 267-271.

Millan, M. J., Andrieux, A., Bartzokis, G., Cadenhead, K., Dazzan, P., et al. (2016). Altering the course of schizophrenia: Progress and perspectives. *Nat.Rev.Drug Discov., 15*(7), 485-515.

Millan, M. J., & Bales, K. L. (2013). Towards improved animal models for evaluating social cognition and its disruption in schizophrenia: The CNTRICS initiative. *Neurosci.Biobehav.Rev., 37*(9 Pt B), 2166-2180.

Millan, M. J., Bervoets, K., Rivet, J. M., Widdowson, P., Renouard, A., et al. (1994). Multiple alpha-2 adrenergic receptor subtypes. II. evidence for a role of rat R alpha-2A adrenergic receptors in the control of nociception, motor behavior and hippocampal synthesis of noradrenaline. *J.Pharmacol.Exp.Ther., 270*(3), 958-972.

Millan, M. J., Dekeyne, A., & Gobert, A. (1998). Serotonin (5-HT)2C receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. *Neuropharmacology, 37*(7), 953-955.

Millan, M. J., Marin, P., Bockaert, J., & Mannoury la Cour, C. (2008). Signaling at G-protein-coupled serotonin receptors: Recent advances and future research directions. *Trends Pharmacol.Sci., 29*(9), 454-464.

Miller, R. J. (1998). Presynaptic receptors. *Annu.Rev.Pharmacol.Toxicol., 38*, 201-227.

Milner, B. (1963). Effects of different brain lesions on card sorting. *JAMA Neurology, 9*, 90-100.

Minneman, K. P., Dibner, M. D., Wolfe, B. B., & Molinoff, P. B. (1979). Beta1- and beta2-adrenergic receptors in rat cerebral cortex are independently regulated. *Science, 204*(4395), 866-868.

Minzenberg, M. J., Firl, A. J., Yoon, J. H., Gomes, G. C., Reinking, C., & Carter, C. S. (2010). Gamma oscillatory power is impaired during cognitive control independent of medication status in first-episode schizophrenia. *Neuropsychopharmacology, 35*(13), 2590-2599.

Minzenberg, M. J., Poole, J. H., Benton, C., & Vinogradov, S. (2004). Association of anticholinergic load with impairment of complex attention and memory in schizophrenia. *Am.J.Psychiatry, 161*(1), 116-124.

Minzenberg, M. J., & Yoon, J. H. (2011). An index of relative central alpha-adrenergic receptor antagonism by antipsychotic medications. *Exp.Clin.Psychopharmacol., 19*(1), 31-39.

Mirnics, K., Middleton, F. A., Stanwood, G. D., Lewis, D. A., & Levitt, P. (2001). Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia. *Mol.Psychiatry, 6*(3), 293-301.

Mitchell, E. S., & Neumaier, J. F. (2005). 5-HT6 receptors: A novel target for cognitive enhancement. *Pharmacol.Ther., 108*(3), 320-333.

Mittleman, G., LeDuc, P. A., & Whishaw, I. Q. (1993). The role of D1 and D2 receptors in the heightened locomotion induced by direct and indirect dopamine agonists in rats with hippocampal damage: An animal analogue of schizophrenia. *Behav.Brain Res., 55*(2), 253-267.

Miyakawa, T., Leiter, L. M., Gerber, D. J., Gainetdinov, R. R., Sotnikova, T. D., et al. (2003). Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. *Proc.Natl.Acad.Sci.U.S.A., 100*(15), 8987-8992.

Miyake, N., Thompson, J., Skinbjerg, M., & Abi-Dargham, A. (2011). Presynaptic dopamine in schizophrenia. *CNS Neurosci.Ther., 17*(2), 104-109.

Miyamoto, S., Miyake, N., Jarskog, L. F., Fleischhacker, W. W., & Lieberman, J. A. (2012). Pharmacological treatment of schizophrenia: A critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Mol.Psychiatry, 17*(12), 1206-1227.

Moffoot, A., O'Carroll, R. E., Murray, C., Dougall, N., Ebmeier, K., & Goodwin, G. M. (1994). Clonidine infusion increases uptake of 99mTc-exametazime in anterior cingulate cortex in Korsakoff's psychosis. *Psychol.Med., 24*(1), 53-61.

Mogenson, G. J., Jones, D. L., & Yim, C. Y. (1980). From motivation to action: Functional interface between the limbic system and the motor system. *Prog.Neurobiol., 14*(2-3), 69-97.

Moghaddam, B., Adams, B., Verma, A., & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J.Neurosci., 17*(8), 2921-2927.

Moghaddam, B., & Krystal, J. H. (2012). Capturing the angel in "angel dust": Twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. *Schizophr.Bull., 38*(5), 942-949.

Mohamed, S., Rosenheck, R., Swartz, M., Stroup, S., Lieberman, J. A., & Keefe, R. S. (2008). Relationship of cognition and psychopathology to functional impairment in schizophrenia. *Am.J.Psychiatry, 165*(8), 978-987.

Mohler, E. G., Franklin, S. R., Rueter, L. E., Fox, G. B., Decker, M. W., & Browman, K. E. (2010). ABT-594 improves performance in the 5-choice serial reaction time task under conditions of increased difficulty, sub-chronic dosing, and in poorly-performing subjects. *Pharmacol.Biochem.Behav., 95*(2), 146-157.

Mohn, C., & Torgalsboen, A. K. (2018). Details of attention and learning change in first-episode schizophrenia. *Psychiatry Res., 260*, 324-330.

Molina, V., Sanz, J., Reig, S., Martinez, R., Sarramea, F., et al. (2005). Hypofrontality in men with first-episode psychosis. *Br.J.Psychiatry, 186*, 203-208.

Moller, M., Swanepoel, T., & Harvey, B. H. (2015). Neurodevelopmental animal models reveal the convergent role of neurotransmitter systems, inflammation, and oxidative stress as biomarkers of schizophrenia: Implications for novel drug development. *ACS Chem.Neurosci., 6*(7), 987-1016.

Molliver, M. E. (1987). Serotonergic neuronal systems: What their anatomic organization tells us about function. *J.Clin.Psychopharmacol., 7*(6 Suppl), 3-23S.

Mongeau, R., Blier, P., & de Montigny, C. (1997). The serotonergic and noradrenergic systems of the hippocampus: Their interactions and the effects of antidepressant treatments. *Brain Res.Brain Res.Rev., 23*(3), 145-195.

Moore, H., Jentsch, J. D., Ghajarnia, M., Geyer, M. A., & Grace, A. A. (2006). A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: Implications for the neuropathology of schizophrenia. *Biol.Psychiatry, 60*(3), 253-264.

Moore, R. Y., Whone, A. L., McGowan, S., & Brooks, D. J. (2003). Monoamine neuron innervation of the normal human brain: An 18F-DOPA PET study. *Brain Res., 982*(2), 137-145.

Moreno, J. L., & Gonzalez-Maeso, J. (2013). Preclinical models of antipsychotic drug action. *Int.J.Neuropsychopharmacol., 16*(10), 2131-2144.

Moreno, J. L., Kurita, M., Holloway, T., Lopez, J., Cadagan, R., et al. (2011). Maternal influenza viral infection causes schizophrenia-like alterations of 5-HT(2)A and mGlu(2) receptors in the adult offspring. *J.Neurosci., 31*(5), 1863-1872.

Moreno, J. L., Sealfon, S. C., & Gonzalez-Maeso, J. (2009). Group II metabotropic glutamate receptors and schizophrenia. *Cell Mol.Life Sci., 66*(23), 3777-3785.

Morilak, D. A., Somogyi, P., Lujan-Miras, R., & Ciaranello, R. D. (1994). Neurons expressing 5-HT2 receptors in the rat brain: Neurochemical identification of cell types by immunocytochemistry. *Neuropsychopharmacology, 11*(3), 157-166.

Moron, J. A., Brockington, A., Wise, R. A., Rocha, B. A., & Hope, B. T. (2002). Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: Evidence from knock-out mouse lines. *J.Neurosci., 22*(2), 389-395.

Moroni, F., Tanganelli, S., Antonelli, T., Carla, V., Bianchi, C., & Beani, L. (1983). Modulation of cortical acetylcholine and gamma-aminobutyric acid release in freely moving guinea pigs: Effects of clonidine and other adrenergic drugs. *J.Pharmacol.Exp.Ther., 227*(2), 435-440.

Moschetti, V., Boland, K., Feifel, U., Hoch, A., Zimdahl-Gelling, H., & Sand, M. (2016). First-in-human study assessing safety, tolerability and pharmacokinetics of BI 409306, a selective phosphodiesterase 9A inhibitor, in healthy males. *Br.J.Clin.Pharmacol., 82*(5), 1315-1324.

Mrzljak, L., Bergson, C., Pappy, M., Huff, R., Levenson, R., & Goldman-Rakic, P. S. (1996). Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. *Nature*, *381*(6579), 245-248.

Mueller, F. S., Richetto, J., Hayes, L. N., Zambon, A., Pollak, D. D., et al. (2019). Influence of poly(I:C) variability on thermoregulation, immune responses and pregnancy outcomes in mouse models of maternal immune activation. *Brain Behav.Immun., 80*, 406-418.

Muguruza, C., Miranda-Azpiazu, P., Diez-Alarcia, R., Morentin, B., Gonzalez-Maeso, J., et al. (2014). Evaluation of 5-HT2A and mGlu2/3 receptors in postmortem prefrontal cortex of subjects with major depressive disorder: Effect of antidepressant treatment. *Neuropharmacology*, *86*, 311-318.

Muir, J. L., Bussey, T. J., Everitt, B. J., & Robbins, T. W. (1996a). Dissociable effects of AMPA-induced lesions of the vertical limb diagonal band of broca on performance of the 5-choice serial reaction time task and on acquisition of a conditional visual discrimination. *Behav.Brain Res., 82*(1), 31-44.

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1994). AMPA-induced excitotoxic lesions of the basal forebrain: A significant role for the cortical cholinergic system in attentional function. *J.Neurosci., 14*(4), 2313-2326.

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1996b). The cerebral cortex of the rat and visual attentional function: Dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. *Cereb.Cortex, 6*(3), 470-481.

Mullner, K., Gyires, K., & Furst, S. (2001). Involvement of the opioid system in the central antisecretory action of alpha-2 adrenoceptor agonists in rat. *J.Physiol.Paris, 95*(1-6), 209-214.

Mullner, K., Ronai, A. Z., Fulop, K., Furst, S., & Gyires, K. (2002). Involvement of central K(ATP) channels in the gastric antisecretory action of alpha2-adrenoceptor agonists and beta-endorphin in rats. *Eur.J.Pharmacol., 435*(2-3), 225-229.

Muly, E. C.,3rd, Szigeti, K., & Goldman-Rakic, P. S. (1998). D1 receptor in interneurons of macaque prefrontal cortex: Distribution and subcellular localization. *J.Neurosci., 18*(24), 10553-10565.

Munafo, M. R., Thiselton, D. L., Clark, T. G., & Flint, J. (2006). Association of the NRG1 gene and schizophrenia: A meta-analysis. *Mol.Psychiatry, 11*(6), 539-546.

Murchison, C. F., Zhang, X. Y., Zhang, W. P., Ouyang, M., Lee, A., & Thomas, S. A. (2004). A distinct role for norepinephrine in memory retrieval. *Cell*, *117*(1), 131-143.

Murphy, T. J., & Bylund, D. B. (1988). Characterization of alpha-2 adrenergic receptors in the OK cell, an opossum kidney cell line. *J.Pharmacol.Exp.Ther., 244*(2), 571-578.

Murray, R. M. (1994). Neurodevelopmental schizophrenia: The rediscovery of dementia praecox. *Br.J.Psychiatry Suppl., (25)*(25), 6-12.

Murray, R. M., Englund, A., Abi-Dargham, A., Lewis, D. A., Di Forti, M., et al. (2017). Cannabis-associated psychosis: Neural substrate and clinical impact. *Neuropharmacology*, *124*, 89-104.

Murray, R. M., Jones, P., & O'Callaghan, E. (1991). Fetal brain development and later schizophrenia. *Ciba Found.Symp., 156*, 15-70.

Murray, R. M., & Lewis, S. W. (1987). Is schizophrenia a neurodevelopmental disorder? *Br.Med.J.(Clin.Res.Ed), 295*(6600), 681-682.

Murray, R. M., O'Callaghan, E., Castle, D. J., & Lewis, S. W. (1992). A neurodevelopmental approach to the classification of schizophrenia. *Schizophr.Bull., 18*(2), 319-332.

Murray, R. M., Paparelli, A., Morrison, P. D., Marconi, A., & Di Forti, M. (2013). What can we learn about schizophrenia from studying the human model, drug-induced psychosis? *Am.J.Med.Genet.B.Neuropsychiatr.Genet., 162B*(7), 661-670.

Murray, R. M., Quigley, H., Quattrone, D., Englund, A., & Di Forti, M. (2016). Traditional marijuana, high-potency cannabis and synthetic cannabinoids: Increasing risk for psychosis. *World Psychiatry., 15*(3), 195-204.

Mustonen, A., Niemela, S., Nordstrom, T., Murray, G. K., Maki, P., et al. (2018). Adolescent cannabis use, baseline prodromal symptoms and the risk of psychosis. *Br.J.Psychiatry, 212*(4), 227-233.

Nader, K., & LeDoux, J. E. (1999). Inhibition of the mesoamygdala dopaminergic pathway impairs the retrieval of conditioned fear associations. *Behav.Neurosci., 113*(5), 891-901.

Nagai, T., Murai, R., Matsui, K., Kamei, H., Noda, Y., et al. (2009). Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D1 and serotonin 5-HT1A receptors. *Psychopharmacology (Berl), 202*(1-3), 315-328.

Nagai, T., Takuma, K., Kamei, H., Ito, Y., Nakamichi, N., et al. (2007). Dopamine D1 receptors regulate protein synthesis-dependent long-term recognition memory via extracellular signal-regulated kinase 1/2 in the prefrontal cortex. *Learn.Mem., 14*(3), 117-125.

Nazarian, A., Christianson, C. A., Hua, X. Y., & Yaksh, T. L. (2008). Dexmedetomidine and ST-91 analgesia in the formalin model is mediated by alpha2A-adrenoceptors: A mechanism of action distinct from morphine. *Br.J.Pharmacol., 155*(7), 1117-1126.

Neddens, J., Fish, K. N., Tricoire, L., Vullhorst, D., Shamir, A., et al. (2011). Conserved interneuron-specific ErbB4 expression in frontal cortex of rodents, monkeys, and humans: Implications for schizophrenia. *Biol.Psychiatry, 70*(7), 636-645.

Negyessy, L., & Goldman-Rakic, P. S. (2005). Subcellular localization of the dopamine D2 receptor and coexistence with the calcium-binding protein neuronal calcium sensor-1 in the primate prefrontal cortex. *J.Comp.Neurol., 488*(4), 464-475.

Neill, J. C., Barnes, S., Cook, S., Grayson, B., Idris, N. F., et al. (2010). Animal models of cognitive dysfunction and negative symptoms of schizophrenia: Focus on NMDA receptor antagonism. *Pharmacol.Ther., 128*(3), 419-432.

Nelson, D. L. (2004). 5-HT5 receptors. *Curr.Drug Targets CNS Neurol.Disord., 3*(1), 53-58.

Nelson, E. B., Sax, K. W., & Strakowski, S. M. (1998). Attentional performance in patients with psychotic and nonpsychotic major depression and schizophrenia. *Am.J.Psychiatry, 155*(1), 137-139.

Nestler, E. J., Hyman, S. E., & Malencka, R. C. (2001). Cathecolamines. *Molecular pharmacology. A foundation for clinical neuroscience* (pp. 167-190) McGraw HIll.

Neumeister, A., Drevets, W. C., Belfer, I., Luckenbaugh, D. A., Henry, S., et al. (2006). Effects of a alpha 2C-adrenoreceptor gene polymorphism on neural responses to facial expressions in depression. *Neuropsychopharmacology, 31*(8), 1750-1756.

Newcomer, J. W., Farber, N. B., Jevtovic-Todorovic, V., Selke, G., Melson, A. K., et al. (1999). Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. *Neuropsychopharmacology, 20*(2), 106-118.

Nicholas, A. P., Hokfelt, T., & Pieribone, V. A. (1996). The distribution and significance of CNS adrenoceptors examined with in situ hybridization. *Trends Pharmacol.Sci., 17*(7), 245-255.

Nicholas, A. P., Pieribone, V., & Hokfelt, T. (1993). Distributions of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: An in situ hybridization study. *J.Comp.Neurol., 328*(4), 575-594.

Nielsen, R. E., Levander, S., Kjaersdam Telleus, G., Jensen, S. O., Ostergaard Christensen, T., & Leucht, S. (2015). Second-generation antipsychotic effect on cognition in patients with schizophrenia--a meta-analysis of randomized clinical trials. *Acta Psychiatr.Scand., 131*(3), 185-196.

Nielsen, R. E., (2011). Cognition in schizophrenia – a systematic review. *Drug Discovery Today Therapeutic Strategies, 8*(1-2), 43-48.

Nieoullon, A. (2002). Dopamine and the regulation of cognition and attention. *Prog.Neurobiol., 67*(1), 53-83.

Nilsson, M., Hansson, S., Carlsson, A., & Carlsson, M. L. (2007). Differential effects of the N-methyl-d-aspartate receptor antagonist MK-801 on different stages of object recognition memory in mice. *Neuroscience*, *149*(1), 123-130.

Ninan, I., & Wang, R. Y. (2003). Modulation of the ability of clozapine to facilitate NMDA- and electrically evoked responses in pyramidal cells of the rat medial prefrontal cortex by dopamine: Pharmacological evidence. *Eur.J.Neurosci., 17*(6), 1306-1312.

Nirenberg, M. J., Vaughan, R. A., Uhl, G. R., Kuhar, M. J., & Pickel, V. M. (1996). The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. *J.Neurosci., 16*(2), 436-447.

Nishijima, K., Kashiwa, A., & Nishikawa, T. (1994). Preferential stimulation of extracellular release of dopamine in rat frontal cortex to striatum following competitive inhibition of the N-methyl-D-aspartate receptor. *J.Neurochem., 63*(1), 375-378.

Niwa, M., Jaaro-Peled, H., Tankou, S., Seshadri, S., Hikida, T., et al. (2013). Adolescent stress-induced epigenetic control of dopaminergic neurons via glucocorticoids. *Science, 339*(6117), 335-339.

Nolan, M. F., Malleret, G., Dudman, J. T., Buhl, D. L., Santoro, B., et al. (2004). A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. *Cell*, *119*(5), 719-732.

Nordstrom, A. L., Farde, L., Nyberg, S., Karlsson, P., Halldin, C., & Sedvall, G. (1995). D1, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: A PET study of schizophrenic patients. *Am.J.Psychiatry, 152*(10), 1444-1449.

Nuechterlein, K. H., Barch, D. M., Gold, J. M., Goldberg, T. E., Green, M. F., & Heaton, R. K. (2004). Identification of separable cognitive factors in schizophrenia. *Schizophr.Res., 72*(1), 29-39.

Numazawa, R., Yoshioka, M., Matsumoto, M., Togashi, H., Kemmotsu, O., & Saito, H. (1995). Pharmacological characterization of alpha 2-adrenoceptor regulated serotonin release in the rat hippocampus. *Neurosci.Lett., 192*(3), 161-164.

Nyffeler, M., Meyer, U., Yee, B. K., Feldon, J., & Knuesel, I. (2006). Maternal immune activation during pregnancy increases limbic GABAA receptor immunoreactivity in the adult offspring: Implications for schizophrenia. *Neuroscience*, *143*(1), 51-62.

Oberhauser, A. F., & Fernandez, J. M. (1996). A fusion pore phenotype in mast cells of the ruby-eye mouse. *Proc.Natl.Acad.Sci.U.S.A., 93*(25), 14349-14354.

Odagaki, Y., & Toyoshima, R. (2008). Pharmacological characterization of alpha2D-adrenergic receptor-mediated [35S]GTPgammaS binding in rat cerebral cortical membranes. *Pharmacol.Res., 57*(6), 435-444.

O'Donnell, P., & Grace, A. A. (1998). Dysfunctions in multiple interrelated systems as the neurobiological bases of schizophrenic symptom clusters. *Schizophr.Bull., 24*(2), 267-283.

Ofen, N., & Shing, Y. L. (2013). From perception to memory: Changes in memory systems across the lifespan. *Neurosci.Biobehav.Rev., 37*(9 Pt B), 2258-2267.

Ogg, R. J., Zou, P., Allen, D. N., Hutchins, S. B., Dutkiewicz, R. M., & Mulhern, R. K. (2008). Neural correlates of a clinical continuous performance test. *Magn.Reson.Imaging, 26*(4), 504-512.

Ohgidani, M., Kato, T. A., & Kanba, S. (2015). Introducing directly induced microglia-like (iMG) cells from fresh human monocytes: A novel translational research tool for psychiatric disorders. *Front.Cell.Neurosci., 9*, 184.

Ohuoha, D. C., Hyde, T. M., & Kleinman, J. E. (1993). The role of serotonin in schizophrenia: An overview of the nomenclature, distribution and alterations of serotonin receptors in the central nervous system. *Psychopharmacology (Berl), 112*(1 Suppl), 5.

Okada, M., Fukuyama, K., Kawano, Y., Shiroyama, T., Suzuki, D., & Ueda, Y. (2019). Effects of acute and sub-chronic administrations of guanfacine on catecholaminergic transmissions in the orbitofrontal cortex. *Neuropharmacology, 156*, 107547.

Okubo, Y., Suhara, T., Suzuki, K., Kobayashi, K., Inoue, O., et al. (1997). Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature*, *385*(6617), 634-636.

Olney, J. W., & Farber, N. B. (1995). Glutamate receptor dysfunction and schizophrenia. *Arch.Gen.Psychiatry, 52*(12), 998-1007.

Olpe, H. R., & Koella, W. P. (1977). The response of striatal cells upon stimulation of the dorsal and median raphe nuclei. *Brain Res., 122*(2), 357-360.

O'Neill, J., Fitten, L. J., Siembieda, D. W., Ortiz, F., & Halgren, E. (2000). Effects of guanfacine on three forms of distraction in the aging macaque. *Life Sci., 67*(8), 877-885.

Ong, M. L., Ball, S. G., & Vaughan, P. F. (1991). Regulation of noradrenaline release from rat occipital cortex tissue chops by alpha 2-adrenergic agonists. *J.Neurochem., 56*(4), 1387-1393.

Ongur, D., & Price, J. L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cereb.Cortex, 10*(3), 206-219.

Ordway, G. A. (1995). Effect of noradrenergic lesions on subtypes of alpha 2-adrenoceptors in rat brain. *J.Neurochem., 64*(3), 1118-1126.

Ordway, G. A., Jaconetta, S. M., & Halaris, A. E. (1993). Characterization of subtypes of alpha-2 adrenoceptors in the human brain. *J.Pharmacol.Exp.Ther., 264*(2), 967-976.

Ornstein, K., Milon, H., McRae-Degueurce, A., Alvarez, C., Berger, B., & Wurzner, H. P. (1987). Biochemical and radioautographic evidence for dopaminergic afferents of the locus coeruleus originating in the ventral tegmental area. *J.Neural Transm., 70*(3-4), 183-191.

Orsetti, M., Colella, L., Dellarole, A., Canonico, P. L., & Ghi, P. (2007). Modification of spatial recognition memory and object discrimination after chronic administration of haloperidol, amitriptyline, sodium valproate or olanzapine in normal and anhedonic rats. *Int.J.Neuropsychopharmacol., 10*(3), 345-357.

Ortega, J. E., (2007). Nuevas estrategias de pontenciación antidepresiva basadas en la interacción entre los sistemas noradrenérgico y serotonérgico centrales. Estudio mediante microdiálisis cerebral in vivo. University of the Basque Country (UPV-EHU) (Spain).

Ortega, J. E., Fernandez-Pastor, B., Callado, L. F., & Meana, J. J. (2010). In vivo potentiation of reboxetine and citalopram effect on extracellular noradrenaline in rat brain by alpha2-adrenoceptor antagonism. *Eur.Neuropsychopharmacol., 20*(11), 813-822.

Osborne, A. L., Solowij, N., Babic, I., Huang, X. F., & Weston-Green, K. (2017). Improved social interaction, recognition and working memory with cannabidiol treatment in a prenatal infection (poly I:C) rat model. *Neuropsychopharmacology, 42*(7), 1447-1457.

Otani, S., Blond, O., Desce, J. M., & Crepel, F. (1998). Dopamine facilitates long-term depression of glutamatergic transmission in rat prefrontal cortex. *Neuroscience*, *85*(3), 669-676.

Owen, A. M., Downes, J. J., Sahakian, B. J., Polkey, C. E., & Robbins, T. W. (1990). Planning and spatial working memory following frontal lobe lesions in man. *Neuropsychologia*, *28*(10), 1021-1034.

Owen, A. M., Roberts, A. C., Hodges, J. R., Summers, B. A., Polkey, C. E., & Robbins, T. W. (1993). Contrasting mechanisms of impaired attentional set-shifting in patients with frontal lobe damage or Parkinson's disease. *Brain, 116 (Pt 5)*, 1159-1175.

Ozawa, K., Hashimoto, K., Kishimoto, T., Shimizu, E., Ishikura, H., & Iyo, M. (2006). Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: A neurodevelopmental animal model of schizophrenia. *Biol.Psychiatry, 59*(6), 546-554.

Paine, T. A., & Carlezon, W. A., Jr. (2009). Effects of antipsychotic drugs on MK-801-induced attentional and motivational deficits in rats. *Neuropharmacology*, *56*(4), 788-797.

Paine, T. A., Tomasiewicz, H. C., Zhang, K., & Carlezon, W. A., Jr. (2007). Sensitivity of the five-choice serial reaction time task to the effects of various psychotropic drugs in Sprague-Dawley rats. *Biol.Psychiatry, 62*(6), 687-693.

Palacios, J. M., Hoyer, D., & Cortes, R. (1987). Alpha 1-adrenoceptors in the mammalian brain: Similar pharmacology but different distribution in rodents and primates. *Brain Res., 419*(1-2), 65-75.

Palacios, J., Waeber, C., Mengod, G., & Hoyer, D. (1991). Autoradiography of 5-HT receptors: A critical appraisal. *Neurochem.Int., 18*(1), 17-25.

Palaniyappan, L., Simmonite, M., White, T. P., Liddle, E. B., & Liddle, P. F. (2013). Neural primacy of the salience processing system in schizophrenia. *Neuron*, *79*(4), 814-828.

Pantelis, C., Yucel, M., Wood, S. J., Velakoulis, D., Sun, D., et al. (2005). Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia. *Schizophr.Bull., 31*(3), 672-696.

Pape, H. C. (1996). Queer current and pacemaker: The hyperpolarization-activated cation current in neurons. *Annu.Rev.Physiol., 58*, 299-327.

Pape, H. C., & McCormick, D. A. (1989). Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current. *Nature*, *340*(6236), 715-718.

Parasuraman, R. (1998). *The attentive brain*. Cambridge, MA, US: The MIT Press.

Parkinson Study Group. (1999). Low-dose clozapine for the treatment of drug-induced psychosis in Parkinson's disease. *N.Engl.J.Med., 340*(10), 757-763.

Parkis, M. A., & Berger, A. J. (1997). Clonidine reduces hyperpolarization-activated inward current (ih) in rat hypoglossal motoneurons. *Brain Res., 769*(1), 108-118.

Passetti, F., Chudasama, Y., & Robbins, T. W. (2002). The frontal cortex of the rat and visual attentional performance: Dissociable functions of distinct medial prefrontal subregions. *Cereb.Cortex, 12*(12), 1254-1268.

Patil, S. T., Zhang, L., Martenyi, F., Lowe, S. L., Jackson, K. A., et al. (2007). Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: A randomized phase 2 clinical trial. *Nat.Med., 13*(9), 1102-1107.

Patterson, P. H. (2007). Neuroscience. maternal effects on schizophrenia risk. *Science, 318*(5850), 576-577.

Patterson, P. H. (2009). Immune involvement in schizophrenia and autism: Etiology, pathology and animal models. *Behav.Brain Res., 204*(2), 313-321.

Pauwels, P. J. (1997). 5-HT 1B/D receptor antagonists. *Gen.Pharmacol., 29*(3), 293-303.

Pazos, A. (1988). A2-adrenoceptors in human forebrain: Autoradiographic visualization and biochemical parameters using the agonist [3H]UK-14304. *Brain Res., 475*(2), 361-365.

Pazos, A., & Palacios, J. M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. serotonin-1 receptors. *Brain Res., 346*(2), 205-230.

Pedersen, C. B., & Mortensen, P. B. (2001). Evidence of a dose-response relationship between urbanicity during upbringing and schizophrenia risk. *Arch.Gen.Psychiatry, 58*(11), 1039-1046.

Penington, N. J., & Fox, A. P. (1994). Effects of LSD on ca++ currents in central 5-HT-containing neurons: 5-HT1A receptors may play a role in hallucinogenesis. *J.Pharmacol.Exp.Ther., 269*(3), 1160-1165.

Perez-Costas, E., Melendez-Ferro, M., Rice, M. W., Conley, R. R., & Roberts, R. C. (2012). Dopamine pathology in schizophrenia: Analysis of total and phosphorylated tyrosine hydroxylase in the substantia nigra. *Front.Psychiatry., 3*, 31.

Perez-Garcia, G. S., & Meneses, A. (2005). Effects of the potential 5-HT7 receptor agonist AS 19 in an autoshaping learning task. *Behav.Brain Res., 163*(1), 136-140.

Peterhoff, M., Sieg, A., Brede, M., Chao, C. M., Hein, L., & Ullrich, S. (2003). Inhibition of insulin secretion via distinct signaling pathways in alpha2-adrenoceptor knockout mice. *Eur.J.Endocrinol., 149*(4), 343-350.

Petrash, A. C., & Bylund, D. B. (1986). Alpha-2 adrenergic receptor subtypes indicated by [3H]yohimbine binding in human brain. *Life Sci., 38*(23), 2129-2137.

Philipp, M., Brede, M., & Hein, L. (2002). Physiological significance of alpha(2)-adrenergic receptor subtype diversity: One receptor is not enough. *Am.J.Physiol.Regul.Integr.Comp.Physiol., 283*(2), 287.

Phillips, L. J., McGorry, P. D., Garner, B., Thompson, K. N., Pantelis, C., et al. (2006). Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: Implications for the development of psychotic disorders. *Aust.N.Z.J.Psychiatry, 40*(9), 725-741.

Picciotto, M. R., Zoli, M., Rimondini, R., Lena, C., Marubio, L. M., et al. (1998). Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature*, *391*(6663), 173-177.

Pichat, P., Bergis, O. E., Terranova, J. P., Urani, A., Duarte, C., et al. (2007). SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (II) efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia. *Neuropsychopharmacology, 32*(1), 17-34.

Pichlmair, A., Schulz, O., Tan, C. P., Rehwinkel, J., Kato, H., et al. (2009). Activation of MDA5 requires higher-order RNA structures generated during virus infection. *J.Virol., 83*(20), 10761-10769.

Pihlajamaki, M., Tanila, H., Kononen, M., Hanninen, T., Hamalainen, A., et al. (2004). Visual presentation of novel objects and new spatial arrangements of objects differentially activates the medial temporal lobe subareas in humans. *Eur.J.Neurosci., 19*(7), 1939-1949.

Pijnenburg, A. J., Honig, W. M., Van der Heyden, J. A., & Van Rossum, J. M. (1976). Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. *Eur.J.Pharmacol., 35*(1), 45-58.

Pijnenburg, A. J., Honig, W. M., & Van Rossum, J. M. (1975). Inhibition of d-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. *Psychopharmacologia*, *41*(2), 87-95.

Pimoule, C., Schoemaker, H., Reynolds, G. P., & Langer, S. Z. (1985). 3H]SCH 23390 labeled D1 dopamine receptors are unchanged in schizophrenia and Parkinson's disease. *Eur.J.Pharmacol., 114*(2), 235-237.

Piontkewitz, Y., Arad, M., & Weiner, I. (2011). Risperidone administered during asymptomatic period of adolescence prevents the emergence of brain structural pathology and behavioral abnormalities in an animal model of schizophrenia. *Schizophr.Bull., 37*(6), 1257-1269.

Piontkewitz, Y., Arad, M., & Weiner, I. (2012a). Tracing the development of psychosis and its prevention: What can be learned from animal models. *Neuropharmacology*, *62*(3), 1273-1289.

Piontkewitz, Y., Bernstein, H. G., Dobrowolny, H., Bogerts, B., Weiner, I., & Keilhoff, G. (2012b). Effects of risperidone treatment in adolescence on hippocampal neurogenesis, parvalbumin expression, and vascularization following prenatal immune activation in rats. *Brain Behav.Immun., 26*(2), 353-363.

Pitsikas, N., Boultadakis, A., & Sakellaridis, N. (2008). Effects of sub-anesthetic doses of ketamine on rats' spatial and non-spatial recognition memory. *Neuroscience*, *154*(2), 454-460.

Pittaluga, A., & Raiteri, M. (1988). Clonidine enhances the release of endogenous gamma-aminobutyric acid through alpha-2 and alpha-1 presynaptic adrenoceptors differentially located in rat cerebral cortex subregions. *J.Pharmacol.Exp.Ther., 245*(2), 682-686.

Pogarell, O., Koch, W., Karch, S., Dehning, S., Muller, N., et al. (2012). Dopaminergic neurotransmission in patients with schizophrenia in relation to positive and negative symptoms. *Pharmacopsychiatry*, *45 Suppl 1*, 36-41.

Pohjanoksa, K., Jansson, C. C., Luomala, K., Marjamaki, A., Savola, J. M., & Scheinin, M. (1997). Alpha2-adrenoceptor regulation of adenylyl cyclase in CHO cells: Dependence on receptor density, receptor subtype and current activity of adenylyl cyclase. *Eur.J.Pharmacol., 335*(1), 53-63.

Pollmacher, T., Haack, M., Schuld, A., Kraus, T., & Hinze-Selch, D. (2000). Effects of antipsychotic drugs on cytokine networks. *J.Psychiatr.Res., 34*(6), 369-382.

Pompeiano, M., Palacios, J. M., & Mengod, G. (1992). Distribution and cellular localization of mRNA coding for 5-HT1A receptor in the rat brain: Correlation with receptor binding. *J.Neurosci., 12*(2), 440-453.

Pozgajova, M., Sachs, U. J., Hein, L., & Nieswandt, B. (2006). Reduced thrombus stability in mice lacking the alpha2A-adrenergic receptor. *Blood*, *108*(2), 510-514.

Prades, R., Munarriz-Cuezva, E., Urigüen, L., Gil-Pisa, I., Gomez, L., et al. (2017). The prolyl oligopeptidase inhibitor IPR19 ameliorates cognitive deficits in mouse models of schizophrenia. *Eur.Neuropsychopharmacol., 27*(2), 180-191.

Pratt, J. A., Winchester, C., Egerton, A., Cochran, S. M., & Morris, B. J. (2008). Modelling prefrontal cortex deficits in schizophrenia: Implications for treatment. *Br.J.Pharmacol., 153 Suppl 1*, 465.

Preuss, T. M. (1995). Do rats have prefrontal cortex? the rose-woolsey-akert program reconsidered. *J.Cogn.Neurosci., 7*(1), 1-24.

Pribram, K. H., & Mishkin, M. (1955). Simultaneous and successive visual discrimination by monkeys with inferotemporal lesions. *J.Comp.Physiol.Psychol., 48*(3), 198-202.

Price, J. L., Carmichael, S. T., & Drevets, W. C. (1996). Networks related to the orbital and medial prefrontal cortex; a substrate for emotional behavior? *Prog.Brain Res., 107*, 523-536.

Prickaerts, J., Sik, A., van Staveren, W. C., Koopmans, G., Steinbusch, H. W., et al. (2004). Phosphodiesterase type 5 inhibition improves early memory consolidation of object information. *Neurochem.Int., 45*(6), 915-928.

Prieto, M., & Giralt, M. T. (2001). Effects of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) on alpha2-adrenoceptors which regulate the synthesis and release of noradrenaline in the rat brain. *Pharmacol.Toxicol., 88*(3), 152-158.

Pritchard, J. K., & Cox, N. J. (2002). The allelic architecture of human disease genes: Common disease-common variant...or not? *Hum.Mol.Genet., 11*(20), 2417-2423.

Pudovkina, O. L., Cremers, T. I., & Westerink, B. H. (2003). Regulation of the release of serotonin in the dorsal raphe nucleus by alpha1 and alpha2 adrenoceptors. *Synapse, 50*(1), 77-82.

Pudovkina, O. L., Kawahara, Y., de Vries, J., & Westerink, B. H. (2001). The release of noradrenaline in the locus coeruleus and prefrontal cortex studied with dual-probe microdialysis. *Brain Res., 906*(1-2), 38-45.

Puolivali, J., Bjorklund, M., Holmberg, M., Ihalainen, J. A., Scheinin, M., & Tanila, H. (2002). Alpha 2C-adrenoceptor mediated regulation of cortical EEG arousal. *Neuropharmacology*, *43*(8), 1305-1312.

Pupo, A. S., & Minneman, K. P. (2001). Adrenergic pharmacology: Focus on the central nervous system. *CNS Spectr., 6*(8), 656-662.

Purves-Tyson, T. D., Owens, S. J., Rothmond, D. A., Halliday, G. M., Double, K. L., et al. (2017). Putative presynaptic dopamine dysregulation in schizophrenia is supported by molecular evidence from post-mortem human midbrain. *Transl.Psychiatry., 7*(1), e1003.

Puumala, T., Bjorklund, M., Ruotsalainen, S., Riekkinen, M., Jakala, P., et al. (1997). Lack of relationship between thalamic oscillations and attention in rats: Differential modulation by an alpha-2 antagonist. *Brain Res.Bull., 43*(2), 163-171.

Quarta, D., & Large, C. H. (2011). Effects of lamotrigine on PCP-evoked elevations in monoamine levels in the medial prefrontal cortex of freely moving rats. *J.Psychopharmacol., 25*(12), 1703-1711.

Ragozzino, M. E. (2002). The effects of dopamine D(1) receptor blockade in the prelimbic-infralimbic areas on behavioral flexibility. *Learn.Mem., 9*(1), 18-28.

Rainbow, T. C., Parsons, B., & Wolfe, B. B. (1984). Quantitative autoradiography of beta 1- and beta 2-adrenergic receptors in rat brain. *Proc.Natl.Acad.Sci.USA*., *81*(5), 1585-1589.

Raiteri, M., Maura, G., Gemignani, A., & Pittaluga, A. (1983). Differential blockade by (-)mianserin of the alpha 2-adrenoceptors mediating inhibition of noradrenaline and serotonin release from rat brain synaptosomes. *Naunyn Schmiedebergs Arch.Pharmacol., 322*(2), 180-182.

Rajji, T. K., Ismail, Z., & Mulsant, B. H. (2009). Age at onset and cognition in schizophrenia: Meta-analysis. *Br.J.Psychiatry, 195*(4), 286-293.

Rama, P., Linnankoski, I., Tanila, H., Pertovaara, A., & Carlson, S. (1996). Medetomidine, atipamezole, and guanfacine in delayed response performance of aged monkeys. *Pharmacol.Biochem.Behav., 55*(3), 415-422.

Ramos, B. P., & Arnsten, A. F. (2007). Adrenergic pharmacology and cognition: Focus on the prefrontal cortex. *Pharmacol.Ther., 113*(3), 523-536.

Ramos, B. P., Birnbaum, S. G., Lindenmayer, I., Newton, S. S., Duman, R. S., & Arnsten, A. F. (2003). Dysregulation of protein kinase a signaling in the aged prefrontal cortex: New strategy for treating age-related cognitive decline. *Neuron*, *40*(4), 835-845.

Ramos, B. P., Stark, D., Verduzco, L., van Dyck, C. H., & Arnsten, A. F. (2006). Alpha2A-adrenoceptor stimulation improves prefrontal cortical regulation of behavior through inhibition of cAMP signaling in aging animals. *Learn.Mem., 13*(6), 770-776.

Ranganath, C., Minzenberg, M. J., & Ragland, J. D. (2008). The cognitive neuroscience of memory function and dysfunction in schizophrenia. *Biol.Psychiatry, 64*(1), 18-25.

Rao, T. S., Kim, H. S., Lehmann, J., Martin, L. L., & Wood, P. L. (1989). Differential effects of phencyclidine (PCP) and ketamine on mesocortical and mesostriatal dopamine release in vivo. *Life Sci., 45*(12), 1065-1072.

Rao, T. S., Kim, H. S., Lehmann, J., Martin, L. L., & Wood, P. L. (1990). Selective activation of dopaminergic pathways in the mesocortex by compounds that act at the phencyclidine (PCP) binding site: Tentative evidence for PCP recognition sites not coupled to N-methyl-D-aspartate (NMDA) receptors. *Neuropharmacology*, *29*(3), 225-230.

Rapisarda, A., Kraus, M., Tan, Y. W., Lam, M., Eng, G. K., et al. (2014). The continuous performance test, identical pairs: Norms, reliability and performance in healthy controls and patients with schizophrenia in Singapore. *Schizophr.Res., 156*(2-3), 233-240.

Rapoport, J. L., Addington, A. M., Frangou, S., & Psych, M. R. (2005). The neurodevelopmental model of schizophrenia: Update 2005. *Mol.Psychiatry, 10*(5), 434-449.

Rapoport, J. L., Giedd, J. N., & Gogtay, N. (2012). Neurodevelopmental model of schizophrenia: Update 2012. *Mol.Psychiatry, 17*(12), 1228-1238.

Rapport, M. M., Green, A. A., & Page, I. H. (1948). Crystalline serotonin. *Science, 108*(2804), 329-330.

Redrobe, J. P., Bull, S., & Plath, N. (2010). Translational aspects of the novel object recognition task in rats abstinent following sub-chronic treatment with phencyclidine (PCP): Effects of modafinil and relevance to cognitive deficits in schizophrenia. *Front.Psychiatry., 1*, 146.

Reed, A. C., Harris, J. G., & Olincy, A. (2016). Schizophrenia, smoking status, and performance on the matrics cognitive consensus battery. *Psychiatry Res., 246*, 1-8.

Rees, S., den Daas, I., Foord, S., Goodson, S., Bull, D., et al. (1994). Cloning and characterization of the human 5-HT5A serotonin receptor. *FEBS Lett., 355*(3), 242-246.

Rees, S., & Harding, R. (2004). Brain development during fetal life: Influences of the intra-uterine environment. *Neurosci.Lett., 361*(1-3), 111-114.

Rees, S., & Inder, T. (2005). Fetal and neonatal origins of altered brain development. *Early Hum.Dev., 81*(9), 753-761.

Regan, J. W., Kobilka, T. S., Yang-Feng, T. L., Caron, M. G., Lefkowitz, R. J., & Kobilka, B. K. (1988). Cloning and expression of a human kidney cDNA for an alpha 2-adrenergic receptor subtype. *Proc.Natl.Acad.Sci.U.S.A., 85*(17), 6301-6305.

Reis, D. J., Granata, A. R., Joh, T. H., Ross, C. A., Ruggiero, D. A., & Park, D. H. (1984). Brain stem catecholamine mechanisms in tonic and reflex control of blood pressure. *Hypertension, 6*(5 Pt 2), II-15.

Reisinger, S., Khan, D., Kong, E., Berger, A., Pollak, A., & Pollak, D. D. (2015). The poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. *Pharmacol.Ther., 149*, 213-226.

Reith, J., Benkelfat, C., Sherwin, A., Yasuhara, Y., Kuwabara, H., et al. (1994). Elevated dopa decarboxylase activity in living brain of patients with psychosis. *Proc.Natl.Acad.Sci.U.S.A., 91*(24), 11651-11654.

Reith, M. E., Li, M. Y., & Yan, Q. S. (1997). Extracellular dopamine, norepinephrine, and serotonin in the ventral tegmental area and nucleus accumbens of freely moving rats during intracerebral dialysis following systemic administration of cocaine and other uptake blockers. *Psychopharmacology (Berl), 134*(3), 309-317.

Reneerkens, O. A., Rutten, K., Steinbusch, H. W., Blokland, A., & Prickaerts, J. (2009). Selective phosphodiesterase inhibitors: A promising target for cognition enhancement. *Psychopharmacology (Berl), 202*(1-3), 419-443.

Repovs, G., Csernansky, J. G., & Barch, D. M. (2011). Brain network connectivity in individuals with schizophrenia and their siblings. *Biol.Psychiatry, 69*(10), 967-973.

Reynolds, G. P., Mason, S. L., Meldrum, A., De Keczer, S., Parnes, H., et al. (1995). 5-hydroxytryptamine (5-HT)4 receptors in post mortem human brain tissue: Distribution, pharmacology and effects of neurodegenerative diseases. *Br.J.Pharmacol., 114*(5), 993-998.

Richtand, N. M., Ahlbrand, R., Horn, P., Stanford, K., Bronson, S. L., & McNamara, R. K. (2011). Effects of risperidone and paliperidone pre-treatment on locomotor response following prenatal immune activation. *J.Psychiatr.Res., 45*(9), 1194-1201.

Rico, B., & Marin, O. (2011). Neuregulin signaling, cortical circuitry development and schizophrenia. *Curr.Opin.Genet.Dev., 21*(3), 262-270.

Riekkinen, M., & Riekkinen, P.,vJr. (1999a). Alpha2-adrenergic agonist clonidine for improving spatial working memory in Parkinson's disease. *J.Clin.Psychopharmacol., 19*(5), 444-449.

Riekkinen, P.,Jr, & Riekkinen, M. (1999b). THA improves word priming and clonidine enhances fluency and working memory in Alzheimer's disease. *Neuropsychopharmacology, 20*(4), 357-364.

Rilling, J. K., & Insel, T. R. (1999). The primate neocortex in comparative perspective using magnetic resonance imaging. *J.Hum.Evol., 37*(2), 191-223.

Rimer, M., Barrett, D. W., Maldonado, M. A., Vock, V. M., & Gonzalez-Lima, F. (2005). Neuregulin-1 immunoglobulin-like domain mutant mice: Clozapine sensitivity and impaired latent inhibition. *Neuroreport*, *16*(3), 271-275.

Rivero, G., Martin-Guerrero, I., de Prado, E., Gabilondo, A. M., Callado, L. F., et al. (2016). Alpha2C-adrenoceptor Del322-325 polymorphism and risk of psychiatric disorders: Significant association with opiate abuse and dependence. *World J.Biol.Psychiatry., 17*(4), 308-315.

Rivett, A. J., Francis, A., & Roth, J. A. (1983). Distinct cellular localization of membrane-bound and soluble forms of catechol-O-methyltransferase in brain. *J.Neurochem., 40*(1), 215-219.

Robbins, T. W. (1984). Cortical noradrenaline, attention and arousal. *Psychol.Med., 14*(1), 13-21.

Robbins, T. W. (1996). Dissociating executive functions of the prefrontal cortex. *Philos.Trans.R.Soc.Lond.B.Biol.Sci., 351*(1346), 146-1.

Robbins, T. W. (1997). Arousal systems and attentional processes. *Biol.Psychol., 45*(1-3), 57-71.

Robbins, T. W. (2000). From arousal to cognition: The integrative position of the prefrontal cortex. *Prog.Brain Res., 126*, 469-483.

Robbins, T. W. (2002). The 5-choice serial reaction time task: Behavioural pharmacology and functional neurochemistry. *Psychopharmacology (Berl), 163*(3-4), 362-380.

Robbins, T. W. (2005). Chemistry of the mind: Neurochemical modulation of prefrontal cortical function. *J.Comp.Neurol., 493*(1), 140-146.

Robbins, T. W., & Roberts, A. C. (2007). Differential regulation of fronto-executive function by the monoamines and acetylcholine. *Cereb.Cortex, 17 Suppl 1*, 151.

Roberts, G. W. (1991). Schizophrenia: A neuropathological perspective. *Br.J.Psychiatry, 158*, 8-17.

Robinson, E. S. (2012). Blockade of noradrenaline re-uptake sites improves accuracy and impulse control in rats performing a five-choice serial reaction time tasks. *Psychopharmacology (Berl), 219*(2), 303-312.

Robinson, R. B., & Siegelbaum, S. A. (2003). Hyperpolarization-activated cation currents: From molecules to physiological function. *Annu.Rev.Physiol., 65*, 453-480.

Rodrigues, S. M., Schafe, G. E., & LeDoux, J. E. (2004). Molecular mechanisms underlying emotional learning and memory in the lateral amygdala. *Neuron*, *44*(1), 75-91.

Roenker, N. L., Gudelsky, G., Ahlbrand, R., Bronson, S. L., Kern, J. R., et al. (2011). Effect of paliperidone and risperidone on extracellular glutamate in the prefrontal cortex of rats exposed to prenatal immune activation or MK-801. *Neurosci.Lett., 500*(3), 167-171.

Roerig, B., Nelson, D. A., & Katz, L. C. (1997). Fast synaptic signaling by nicotinic acetylcholine and serotonin 5-HT3 receptors in developing visual cortex. *J.Neurosci., 17*(21), 8353-8362.

Romberg, C., Mattson, M. P., Mughal, M. R., Bussey, T. J., & Saksida, L. M. (2011). Impaired attention in the 3xTgAD mouse model of Alzheimer's disease: Rescue by donepezil (aricept). *J.Neurosci., 31*(9), 3500-3507.

Romero, E., Ali, C., Molina-Holgado, E., Castellano, B., Guaza, C., & Borrell, J. (2007). Neurobehavioral and immunological consequences of prenatal immune activation in rats. influence of antipsychotics. *Neuropsychopharmacology, 32*(8), 1791-1804.

Romero, E., Guaza, C., Castellano, B., & Borrell, J. (2010). Ontogeny of sensorimotor gating and immune impairment induced by prenatal immune challenge in rats: Implications for the etiopathology of schizophrenia. *Mol.Psychiatry, 15*(4), 372-383.

Rose, J. E., & Woolsey, C. N. (1948). The orbitofrontal cortex and its connections with the mediodorsal nucleus in rabbit, sheep and cat. *Res.Publ.Assoc.Res.Nerv.Ment.Dis., 27 (1 vol.)*(1 vol.), 210-232.

Rose, K. M., Parmar, M. S., & Cavanaugh, J. E. (2014). Dietary supplementation with resveratrol protects against striatal dopaminergic deficits produced by in utero LPS exposure. *Brain Res., 1573*, 37-43.

Rosenberg, D. R., & Lewis, D. A. (1995). Postnatal maturation of the dopaminergic innervation of monkey prefrontal and motor cortices: A tyrosine hydroxylase immunohistochemical analysis. *J.Comp.Neurol., 358*(3), 383-400.

Rosenbrock, H., Marti, A., & Koros, E. (2015). BI 409306, a novel phophodiesterase 9A inhibitor, part II: In-vivo characterization regarding target engagement and cognitive task in rodents. *Schizophr Bull.,* (41) S-S341.

Rosenkranz, J. A., & Grace, A. A. (2002). Dopamine-mediated modulation of odour-evoked amygdala potentials during pavlovian conditioning. *Nature*, *417*(6886), 282-287.

Rosenkranz, J. A., & Johnston, D. (2006). Dopaminergic regulation of neuronal excitability through modulation of ih in layer V entorhinal cortex. *J.Neurosci., 26*(12), 3229-3244.

Rosin, D. L., Talley, E. M., Lee, A., Stornetta, R. L., Gaylinn, B. D., et al. (1996). Distribution of alpha 2C-adrenergic receptor-like immunoreactivity in the rat central nervous system. *J.Comp.Neurol., 372*(1), 135-165.

Ross, C. A., Margolis, R. L., Reading, S. A., Pletnikov, M., & Coyle, J. T. (2006). Neurobiology of schizophrenia. *Neuron*, *52*(1), 139-153.

Rothman, R. B., & Baumann, M. H. (2003). Monoamine transporters and psychostimulant drugs. *Eur.J.Pharmacol., 479*(1-3), 23-40.

Rouquier, L., Claustre, Y., & Benavides, J. (1994). Alpha 1-adrenoceptor antagonists differentially control serotonin release in the hippocampus and striatum: A microdialysis study. *Eur.J.Pharmacol., 261*(1-2), 59-64.

Rouru, J., Wesnes, K., Haenninen, J., Murphy, M., Riordan, H., & Rinne, J. (2013). A 12-week proof-of-concept efficacy and safety study of ORM-12741 in patients with moderate Alzheimer's disease. *Neurology, 333*(1), E201.

Rubia, K., Overmeyer, S., Taylor, E., Brammer, M., Williams, S. C. R., et al. (1999). Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: A study with functional MRI. *Am.J.Psychiatry, 156*(6), 891-896.

Rubin, R. D., Watson, P. D., Duff, M. C., & Cohen, N. J. (2014). The role of the hippocampus in flexible cognition and social behavior. *Front.Hum.Neurosci., 8*, 742.

Rudolph, M. D., Graham, A. M., Feczko, E., Miranda-Dominguez, O., Rasmussen, J. M., et al. (2018). Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. *Nat.Neurosci., 21*(5), 765-772.

Rueckert, L., & Grafman, J. (1996). Sustained attention deficits in patients with right frontal lesions. *Neuropsychologia*, *34*(10), 953-963.

Rugg, M. D., Otten, L. J., & Henson, R. N. (2002). The neural basis of episodic memory: Evidence from functional neuroimaging. *Philos.Trans.R.Soc.Lond.B.Biol.Sci., 357*(1424), 1097-1110.

Ruiz, J., Gabilondo, A. M., Meana, J. J., & Garcia-Sevilla, J. A. (1992). Increased [3H] raclopride binding sites in postmortem brains from schizophrenic violent suicide victims. *Psychopharmacology (Berl), 109*(4), 410-414.

Rutten, K., Vente, J. D., Sik, A., Ittersum, M. M., Prickaerts, J., & Blokland, A. (2005). The selective PDE5 inhibitor, sildenafil, improves object memory in swiss mice and increases cGMP levels in hippocampal slices. *Behav.Brain Res., 164*(1), 11-16.

Sacchetti, G., Bernini, M., Bianchetti, A., Parini, S., Invernizzi, R. W., & Samanin, R. (1999). Studies on the acute and chronic effects of reboxetine on extracellular noradrenaline and other monoamines in the rat brain. *Br.J.Pharmacol., 128*(6), 1332-1338.

Sahakian, B. J., Coull, J. J., & Hodges, J. R. (1994). Selective enhancement of executive function by idazoxan in a patient with dementia of the frontal lobe type. *J.Neurol.Neurosurg.Psychiatry., 57*(1), 120-121.

Sahakian, B. J., & Owen, A. M. (1992). Computerized assessment in neuropsychiatry using CANTAB: Discussion paper. *J.R.Soc.Med., 85*(7), 399-402.

Sakurai, T., Gamo, N. J., Hikida, T., Kim, S. H., Murai, T., et al. (2015). Converging models of schizophrenia--network alterations of prefrontal cortex underlying cognitive impairments. *Prog.Neurobiol., 134*, 178-201.

Salgado-Pineda, P., Baeza, I., Perez-Gomez, M., Vendrell, P., Junque, C., et al. (2003). Sustained attention impairment correlates to gray matter decreases in first episode neuroleptic-naive schizophrenic patients. *Neuroimage, 19*(2 Pt 1), 365-375.

Sallee, F. R., McGough, J., Wigal, T., Donahue, J., Lyne, A., et al. (2009). Guanfacine extended release in children and adolescents with attention-deficit/hyperactivity disorder: A placebo-controlled trial. *J.Am.Acad.Child Adolesc.Psychiatry, 48*(2), 155-165.

Sallee, F., Connor, D. F., & Newcorn, J. H. (2013). A review of the rationale and clinical utilization of alpha2-adrenoceptor agonists for the treatment of attention-deficit/hyperactivity and related disorders. *J.Child Adolesc.Psychopharmacol., 23*(5), 308-319.

Sallinen, J., Haapalinna, A., MacDonald, E., Viitamaa, T., Lahdesmaki, J., et al. (1999). Genetic alteration of the alpha2-adrenoceptor subtype c in mice affects the development of behavioral despair and stress-induced increases in plasma corticosterone levels. *Mol.Psychiatry, 4*(5), 443-452.

Sallinen, J., Haapalinna, A., Viitamaa, T., Kobilka, B. K., & Scheinin, M. (1998). Adrenergic alpha2C-receptors modulate the acoustic startle reflex, prepulse inhibition, and aggression in mice. *J.Neurosci., 18*(8), 3035-3042.

Sallinen, J., Höglund, I., Engström, M., Lehtimäki, J., Virtanen, R., et al. (2007). Pharmacological characterization and CNS effects of a novel highly selective alpha2C-adrenoceptor antagonist JP-1302. *Br.J.Pharmacol., 150*(4), 391-402.

Sallinen, J., Holappa, J., Koivisto, A., Kuokkanen, K., Chapman, H., et al. (2013a). Pharmacological characterization of a structurally novel alpha2C-adrenoceptor antagonist ORM-10921 and its effects in neuropsychiatric models. *Basic Clin.Pharmacol.Toxicol., 113*(4), 239-249.

Sallinen, J., Link, R. E., Haapalinna, A., Viitamaa, T., Kulatunga, M., et al. (1997). Genetic alteration of alpha 2C-adrenoceptor expression in mice: Influence on locomotor, hypothermic, and neurochemical effects of dexmedetomidine, a subtype-nonselective alpha 2-adrenoceptor agonist. *Mol.Pharmacol., 51*(1), 36-46.

Sallinen, J., Rouru, J., Lehtimaki, J., Marjamaeki, P., Haaparanta-Solin, M., & Arponen, E. (2013b). ORM-12741: Receptor pharmacology of a novel alpha2c-adrenergic receptor subtype selective antagonist with multi-therapeutic potential. *Neuropsychopharmacology*, (38), 558–558.

Sams-Dodd, F. (1995). Automation of the social interaction test by a video-tracking system: Behavioural effects of repeated phencyclidine treatment. *J.Neurosci.Methods, 59*(2), 157-167.

Sanchez-Roige, S., Pena-Oliver, Y., & Stephens, D. N. (2012). Measuring impulsivity in mice: The five-choice serial reaction time task. *Psychopharmacology (Berl), 219*(2), 253-270.

Santana, N., Bortolozzi, A., Serrats, J., Mengod, G., & Artigas, F. (2004). Expression of serotonin1A and serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb.Cortex, 14*(10), 1100-1109.

Santana, N., Mengod, G., & Artigas, F. (2009). Quantitative analysis of the expression of dopamine D1 and D2 receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb.Cortex, 19*(4), 849-860.

Sanz, J. C., Gomez, V., Vargas, M. L., & Marin, J. J. (2012). Dimensions of attention impairment and negative symptoms in schizophrenia: A multidimensional approach using the conners continuous performance test in a Spanish population. *Cogn.Behav.Neurol., 25*(2), 63-71.

Sara, S., J. (1985). The locus coeruleus and cognitive function: Attempts to relate noradrenergic enhancement of signal/noise in the brain to behavior. *Physiological Psychology, 13*(3), 151-162.

Sara, S. J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nat.Rev.Neurosci., 10*(3), 211-223.

Sara, S. J., & Devauges, V. (1989). Idazoxan, an alpha-2 antagonist, facilitates memory retrieval in the rat. *Behav.Neural Biol., 51*(3), 401-411.

Sara, S. J., & Hervé-Minvielle, A. (1995). Inhibitory influence of frontal cortex on locus coeruleus neurons. *Proc.Natl.Acad.Sci.U.S.A., 92*(13), 6032-6036.

Sara, S. J., Roullet, P., & Przybyslawski, J. (1999). Consolidation of memory for odor-reward association: B-adrenergic receptor involvement in the late phase. *Learn. Mem., 6*(2), 88-96.

Sara, S. J., & Segal, M. (1991). Plasticity of sensory responses of locus coeruleus neurons in the behaving rat: Implications for cognition. *Prog.Brain Res., 88*, 571-585.

Sargent, P. A., Kjaer, K. H., Bench, C. J., Rabiner, E. A., Messa, C., et al. (2000). Brain serotonin1A receptor binding measured by positron emission tomography with [11C]WAY-100635: Effects of depression and antidepressant treatment. *Arch.Gen.Psychiatry, 57*(2), 174-180.

Sastre, M., & Garcia-Sevilla, J. A. (1994). Alpha 2-adrenoceptor subtypes identified by [3H]RX821002 binding in the human brain: The agonist guanoxabenz does not discriminate different forms of the predominant alpha 2A subtype. *J.Neurochem., 63*(3), 1077-1085.

Saunders, R. C., Kolachana, B. S., Bachevalier, J., & Weinberger, D. R. (1998). Neonatal lesions of the medial temporal lobe disrupt prefrontal cortical regulation of striatal dopamine. *Nature*, *393*(6681), 169-171.

Savolainen, K., Ihalainen, J., Jalkanen, A. J., & Forsberg, M. M. (2019). Selective adrenergic alpha2C receptor antagonist ameliorates acute phencyclidine-induced schizophrenia-like social interaction deficits in rats. *Psychopharmacology (Berl), 236*(4), 1245-1253.

Sawaguchi, T. (1998). Attenuation of delay-period activity of monkey prefrontal neurons by an alpha2-adrenergic antagonist during an oculomotor delayed-response task. *J.Neurophysiol., 80*(4), 2200-2205.

Sawaguchi, T., & Goldman-Rakic, P. S. (1991). D1 dopamine receptors in prefrontal cortex: Involvement in working memory. *Science, 251*(4996), 947-950.

Sawaguchi, T., Matsumura, M., & Kubota, K. (1990). Catecholaminergic effects on neuronal activity related to a delayed response task in monkey prefrontal cortex. *J.Neurophysiol., 63*(6), 1385-1400.

Sawamura, S., Kingery, W. S., Davies, M. F., Agashe, G. S., Clark, J. D., et al. (2000). Antinociceptive action of nitrous oxide is mediated by stimulation of noradrenergic neurons in the brainstem and activation of a2B adrenoceptors. *J.Neurosci., 20*(24), 9242-9251.

Saykin, A. J., Shtasel, D. L., Gur, R. E., Kester, D. B., Mozley, L. H., et al. (1994). Neuropsychological deficits in neuroleptic naive patients with first-episode schizophrenia. *Arch.Gen.Psychiatry, 51*(2), 124-131.

Scahill, L., Aman, M. G., McDougle, C. J., McCracken, J. T., Tierney, E., et al. (2006). A prospective open trial of guanfacine in children with pervasive developmental disorders. *J.Child Adolesc.Psychopharmacol., 16*(5), 589-598.

Scahill, L., Chappell, P. B., Kim, Y. S., Schultz, R. T., Katsovich, L., et al. (2001). A placebo-controlled study of guanfacine in the treatment of children with tic disorders and attention deficit hyperactivity disorder. *Am.J.Psychiatry, 158*(7), 1067-1074.

Scarpace, P. J., & Abrass, I. B. (1988). Alpha- and beta-adrenergic receptor function in the brain during senescence. *Neurobiol.Aging, 9*(1), 53-58.

Scatton, B., Javoy-Agid, F., Rouquier, L., Dubois, B., & Agid, Y. (1983). Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease. *Brain Res., 275*(2), 321-328.

Scatton, B., Simon, H., Le Moal, M., & Bischoff, S. (1980). Origin of dopaminergic innervation of the rat hippocampal formation. *Neurosci.Lett., 18*(2), 125-131.

Scheibner, J., Trendelenburg, A. U., Hein, L., & Starke, K. (2001a). Alpha2-adrenoceptors modulating neuronal serotonin release: A study in alpha2-adrenoceptor subtype-deficient mice. *Br.J.Pharmacol., 132*(4), 925-933.

Scheibner, J., Trendelenburg, A. U., Hein, L., & Starke, K. (2001b). Stimulation frequency-noradrenaline release relationships examined in alpha2A-, alpha2B- and alpha2C-adrenoceptor-deficient mice. *Naunyn Schmiedebergs Arch.Pharmacol., 364*(4), 321-328.

Scheinin, M., Lomasney, J. W., Hayden-Hixson, D. M., Schambra, U. B., Caron, M. G., et al. (1994). Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain. *Brain Res.Mol.Brain Res., 21*(1-2), 133-149.

Scheinin, M., Sallinen, J., & Haapalinna, A. (2001). Evaluation of the alpha2C-adrenoceptor as a neuropsychiatric drug target studies in transgenic mouse models. *Life Sci., 68*(19-20), 2277-2285.

Schendzielorz, N., Oinas, J. P., Myohanen, T. T., Reenila, I., Raasmaja, A., & Mannisto, P. T. (2013). Catechol-O-methyltransferase (COMT) protein expression and activity after dopaminergic and noradrenergic lesions of the rat brain. *PLoS One, 8*(4), e61392.

Schmidt, C. J., & Fadayel, G. M. (1996). Regional effects of MK-801 on dopamine release: Effects of competitive NMDA or 5-HT2A receptor blockade. *J.Pharmacol.Exp.Ther., 277*(3), 1541-1549.

Schmidt, M. J., & Mirnics, K. (2015). Neurodevelopment, GABA system dysfunction, and schizophrenia. *Neuropsychopharmacology, 40*(1), 190-206.

Schoeffter, P., & Hoyer, D. (1991). Interaction of the alpha-adrenoceptor agonist oxymetazoline with serotonin 5-HT1A, 5-HT1B, 5-HT1C and 5-HT1D receptors. *Eur.J.Pharmacol., 196*(2), 213-216.

Schoepp, D. D. (2001). Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *J.Pharmacol.Exp.Ther., 299*(1), 12-20.

Schoepp, & Marek, G. J. (2002). Preclinical pharmacology of mGlu2/3 receptor agonists: Novel agents for schizophrenia? *Curr.Drug Targets CNS Neurol.Disord., 1*(2), 215-225.

Schotte, A., Janssen, P. F., Gommeren, W., Luyten, W. H., Van Gompel, P., et al. (1996). Risperidone compared with new and reference antipsychotic drugs: In vitro and in vivo receptor binding. *Psychopharmacology (Berl), 124*(1-2), 57-73.

Schretlen, D. J., Cascella, N. G., Meyer, S. M., Kingery, L. R., Testa, S. M., et al. (2007). Neuropsychological functioning in bipolar disorder and schizophrenia. *Biol.Psychiatry, 62*(2), 179-186.

Schroeter, S., Apparsundaram, S., Wiley, R. G., Miner, L. H., Sesack, S. R., & Blakely, R. D. (2000). Immunolocalization of the cocaine- and antidepressant-sensitive l-norepinephrine transporter. *J.Comp.Neurol., 420*(2), 211-232.

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *J.Neurophysiol., 80*(1), 1-27.

Schultz, W. (2002). Getting formal with dopamine and reward. *Neuron*, *36*(2), 241-263.

Schultz, W., Apicella, P., & Ljungberg, T. (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J.Neurosci., 13*(3), 900-913.

Schultz, W., Tremblay, L., & Hollerman, J. R. (2003). Changes in behavior-related neuronal activity in the striatum during learning. *Trends Neurosci., 26*(6), 321-328.

Schwartz, T. L., Sachdeva, S., & Stahl, S. M. (2012). Glutamate neurocircuitry: Theoretical underpinnings in schizophrenia. *Front.Pharmacol., 3*, 195.

Schwendener, S., Meyer, U., & Feldon, J. (2009). Deficient maternal care resulting from immunological stress during pregnancy is associated with a sex-dependent enhancement of conditioned fear in the offspring. *J.Neurodev Disord., 1*(1), 15-32.

Seamans, J. K., Floresco, S. B., & Phillips, A. G. (1998). D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J.Neurosci., 18*(4), 1613-1621.

Seeman, P. (1987). Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse, 1*(2), 133-152.

Seeman, P., Lee, T., Chau-Wong, M., & Wong, K. (1976). Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature*, *261*(5562), 717-719.

Seguela, P., Watkins, K. C., Geffard, M., & Descarries, L. (1990). Noradrenaline axon terminals in adult rat neocortex: An immunocytochemical analysis in serial thin sections. *Neuroscience*, *35*(2), 249-264.

Selemon, L. D., & Goldman-Rakic, P. S. (1999). The reduced neuropil hypothesis: A circuit based model of schizophrenia. *Biol.Psychiatry, 45*(1), 17-25.

Selten, J. P., Frissen, A., Lensvelt-Mulders, G., & Morgan, V. A. (2010). Schizophrenia and 1957 pandemic of influenza: Meta-analysis. *Schizophr.Bull., 36*(2), 219-228.

Semkovska, M., Bedard, M. A., Godbout, L., Limoge, F., & Stip, E. (2004). Assessment of executive dysfunction during activities of daily living in schizophrenia. *Schizophr.Res., 69*(2-3), 289-300.

Sesack, S. R., & Carr, D. B. (2002). Selective prefrontal cortex inputs to dopamine cells: Implications for schizophrenia. *Physiol.Behav., 77*(4-5), 513-517.

Sesack, S. R., Deutch, A. Y., Roth, R. H., & Bunney, B. S. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: An anterograde tract-tracing study with phaseolus vulgaris leucoagglutinin. *J.Comp.Neurol., 290*(2), 213-242.

Sesack, S. R., Hawrylak, V. A., Matus, C., Guido, M. A., & Levey, A. I. (1998). Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J.Neurosci., 18*(7), 2697-2708.

Shahid, M., Walker, G. B., Zorn, S. H., & Wong, E. H. (2009). Asenapine: A novel psychopharmacologic agent with a unique human receptor signature. *J.Psychopharmacol., 23*(1), 65-73.

Sharma, S., Kedrowski, J., Rook, J. M., Smith, R. L., Jones, C. K., et al. (2009). Discovery of molecular switches that modulate modes of metabotropic glutamate receptor subtype 5 (mGlu5) pharmacology in vitro and in vivo within a series of functionalized, regioisomeric 2- and 5-(phenylethynyl)pyrimidines. *J.Med.Chem., 52*(14), 4103-4106.

Sharma, T. (1999). Cognitive effects of conventional and atypical antipsychotics in schizophrenia. *Br.J.Psychiatry Suppl.,* (38), 44-51.

Sharp, T., Zetterstrom, T., Ljungberg, T., & Ungerstedt, U. (1987). A direct comparison of amphetamine-induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis. *Brain Res., 401*(2), 322-330.

Shekhar, A., Potter, W. Z., Lightfoot, J., Lienemann, J., Dube, S., et al. (2008). Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *Am.J.Psychiatry, 165*(8), 1033-1039.

Shi, L., Fatemi, S. H., Sidwell, R. W., & Patterson, P. H. (2003). Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J.Neurosci., 23*(1), 297-302.

Shi, L., Smith, S. E., Malkova, N., Tse, D., Su, Y., & Patterson, P. H. (2009). Activation of the maternal immune system alters cerebellar development in the offspring. *Brain Behav.Immun., 23*(1), 116-123.

Siddiqui, S.,Veqar, Chatterjee, U., Kumar, D., Siddiqui, A., & Goyal, N. (2008). Neuropsychology of prefrontal cortex. *Indian J.Psychiatry*, *50*(3), 202-208.

Siiteri, P. K., & Stites, D. P. (1982). Immunologic and endocrine interrelationships in pregnancy. *Biol.Reprod., 26*(1), 1-14.

Simon, H., Scatton, B., & Moal, M. L. (1980). Dopaminergic A10 neurones are involved in cognitive functions. *Nature*, *286*(5769), 150-151.

Simpson, E. H., Kellendonk, C., & Kandel, E. (2010). A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. *Neuron*, *65*(5), 585-596.

Simpson, E. H., Kellendonk, C., Ward, R. D., Richards, V., Lipatova, O., et al. (2011). Pharmacologic rescue of motivational deficit in an animal model of the negative symptoms of schizophrenia. *Biol.Psychiatry, 69*(10), 928-935.

Singh-Curry, V., Malhotra, P., Farmer, S. F., & Husain, M. (2011). Attention deficits following ADEM ameliorated by guanfacine. *J.Neurol.Neurosurg.Psychiatry., 82*(6), 688-690.

Sirvio, J., Riekkinen, P.,Jr, Vajanto, I., Koivisto, E., & Riekkinen, P. J. (1991). The effects of guanfacine, alpha-2 agonist, on the performance of young and aged rats in spatial navigation task. *Behav.Neural Biol., 56*(1), 101-107.

Siuta, M. A., Robertson, S. D., Kocalis, H., Saunders, C., Gresch, P. J., et al. (2010). Dysregulation of the norepinephrine transporter sustains cortical hypodopaminergia and schizophrenia-like behaviors in neuronal rictor null mice. *PLoS Biol., 8*(6), e1000393.

Sjoholm, H., Bratlid, T., & Sundsfjord, J. (2004). 123I-beta-CIT SPECT demonstrates increased presynaptic dopamine transporter binding sites in basal ganglia in vivo in schizophrenia. *Psychopharmacology (Berl), 173*(1-2), 27-31.

Skarsfeldt, T. (1996). Differential effect of antipsychotics on place navigation of rats in the morris water maze. A comparative study between novel and reference antipsychotics. *Psychopharmacology (Berl), 124*(1-2), 126-133.

Slifstein, M., van de Giessen, E., Van Snellenberg, J., Thompson, J. L., Narendran, R., et al. (2015). Deficits in prefrontal cortical and extrastriatal dopamine release in schizophrenia: A positron emission tomographic functional magnetic resonance imaging study. *JAMA Psychiatry., 72*(4), 316-324.

Smith, S. E., Li, J., Garbett, K., Mirnics, K., & Patterson, P. H. (2007). Maternal immune activation alters fetal brain development through interleukin-6. *J.Neurosci., 27*(40), 10695-10702.

Smith, T., Weston, C., & Lieberman, J. (2010). Schizophrenia (maintenance treatment). *Am.Fam.Physician, 82*(4), 338-339.

Snell, L. D., & Johnson, K. M. (1986). Characterization of the inhibition of excitatory amino acid-induced neurotransmitter release in the rat striatum by phencyclidine-like drugs. *J.Pharmacol.Exp.Ther., 238*(3), 938-946.

Snitz, B. E., Macdonald, A. W.,3rd, & Carter, C. S. (2006). Cognitive deficits in unaffected first-degree relatives of schizophrenia patients: A meta-analytic review of putative endophenotypes. *Schizophr.Bull., 32*(1), 179-194.

Snyder, S. H. (1972). Catecholamines in the brain as mediators of amphetamine psychosis. *Arch.Gen.Psychiatry, 27*(2), 169-179.

Sokoloff, P., & Schwartz, J. C. (1995). Novel dopamine receptors half a decade later. *Trends Pharmacol.Sci., 16*(8), 270-275.

Sørensen, H.,J., Mortensen, E.,L., Reinisch, J.,M., & Mednick, S.,A. (2009). Association between prenatal exposure to bacterial infection and risk of schizophrenia. *Schizophr.Bull., 35*(3), 631-637.

Sorkin, E. M., & Heel, R. C. (1986). Guanfacine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in the treatment of hypertension. *Drugs*, *31*(4), 301-336.

Spanagel, R., & Weiss, F. (1999). The dopamine hypothesis of reward: Past and current status. *Trends Neurosci., 22*(11), 521-527.

Spencer, K. M., Nestor, P. G., Niznikiewicz, M. A., Salisbury, D. F., Shenton, M. E., & McCarley, R. W. (2003). Abnormal neural synchrony in schizophrenia. *J.Neurosci., 23*(19), 7407-7411.

Spooren, W., Riemer, C., & Meltzer, H. (2005). NK3 receptor antagonists: The next generation of antipsychotics? *Nat.Rev.Drug Discov., 4*(12), 967-975.

Squire, L. R., Wixted, J. T., & Clark, R. E. (2007). Recognition memory and the medial temporal lobe: A new perspective. *Nat.Rev.Neurosci., 8*(11), 872-883.

St Clair, D., Xu, M., Wang, P., Yu, Y., Fang, Y., et al. (2005). Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961. *Jama, 294*(5), 557-562.

Starke, K. (1987). Presynaptic alpha-autoreceptors. *Rev.Physiol.Biochem.Pharmacol., 107*, 73-146.

Starke, K., Gothert, M., & Kilbinger, H. (1989). Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol.Rev., 69*(3), 864-989.

Staton, D.,M., & Solomon, P.,R. (1984). Microinjections of d-amphetamine into the nucleus accumbens and caudate-putamen differentially affect stereotypy and locomotion in the rat. *Physiological Psychology, 12*(2), 159-162.

Stauffer, V. L., Millen, B. A., Andersen, S., Kinon, B. J., Lagrandeur, L., et al. (2013). Pomaglumetad methionil: No significant difference as an adjunctive treatment for patients with prominent negative symptoms of schizophrenia compared to placebo. *Schizophr.Res., 150*(2-3), 434-441.

Steckler, T., Weis, C., Sauvage, M., Mederer, A., & Holsboer, F. (1999). Disrupted allocentric but preserved egocentric spatial learning in transgenic mice with impaired glucocorticoid receptor function. *Behav.Brain Res., 100*(1-2), 77-89.

Steeds, H., Carhart-Harris, R. L., & Stone, J. M. (2015). Drug models of schizophrenia. *Ther.Adv.Psychopharmacol., 5*(1), 43-58.

Steere, J. C., & Arnsten, A. F. (1997). The alpha-2A noradrenergic receptor agonist guanfacine improves visual object discrimination reversal performance in aged rhesus monkeys. *Behav.Neurosci., 111*(5), 883-891.

Stein, L., & Wise, C. D. (1972). Possible etiology of schizophrenia: Progressive damage to the noradrenergic reward system by endogenous 6-hydroxydopamine. *Res.Publ.Assoc.Res.Nerv.Ment.Dis., 50*, 298-314.

Steinpreis, R. E., Sokolowski, J. D., Papanikolaou, A., & Salamone, J. D. (1994). The effects of haloperidol and clozapine on PCP- and amphetamine-induced suppression of social behavior in the rat. *Pharmacol.Biochem.Behav., 47*(3), 579-585.

Stephan, K. E., Friston, K. J., & Frith, C. D. (2009). Dysconnection in schizophrenia: From abnormal synaptic plasticity to failures of self-monitoring. *Schizophr.Bull., 35*(3), 509-527.

Stern, Y., Tetrud, J. W., Martin, W. R., Kutner, S. J., & Langston, J. W. (1990). Cognitive change following MPTP exposure. *Neurology*, *40*(2), 261-264.

Sternberg, D. E., van Kammen, D. P., Lake, C. R., Ballenger, J. C., Marder, S. R., & Bunney, W. E.,Jr. (1981). The effect of pimozide on CSF norepinephrine in schizophrenia. *Am.J.Psychiatry, 138*(8), 1045-1050.

Sterpenich, V., D'Argembeau, A., Desseilles, M., Balteau, E., Albouy, G., et al. (2006). The locus coeruleus is involved in the successful retrieval of emotional memories in humans. *J.Neurosci., 26*(28), 7416-7423.

Stevens, D. R., Kuramasu, A., Eriksson, K. S., Selbach, O., & Haas, H. L. (2004). Alpha 2-adrenergic receptor-mediated presynaptic inhibition of GABAergic IPSPs in rat histaminergic neurons. *Neuropharmacology, 46*(7), 1018-1022.

Stilo, S. A., & Murray, R. M. (2010). The epidemiology of schizophrenia: Replacing dogma with knowledge. *Dialogues Clin.Neurosci., 12*(3), 305-315.

Stober, G., Franzek, E., Beckmann, H., & Schmidtke, A. (2002). Exposure to prenatal infections, genetics and the risk of systematic and periodic catatonia. *J.Neural Transm.(Vienna), 109*(5-6), 921-929.

Stober, G., Nothen, M. M., Porzgen, P., Bruss, M., Bonisch, H., et al. (1996). Systematic search for variation in the human norepinephrine transporter gene: Identification of five naturally occurring missense mutations and study of association with major psychiatric disorders. *Am.J.Med.Genet., 67*(6), 523-532.

Stone, E. A., Rosengarten, H., Lin, Y., & Quartermain, D. (2001). Pharmacological blockade of brain alpha1-adrenoceptors as measured by ex vivo [3H]prazosin binding is correlated with behavioral immobility. *Eur.J.Pharmacol., 420*(2-3), 97-102.

Stone, E. A., Zhang, Y., Rosengarten, H., Yeretsian, J., & Quartermain, D. (1999). Brain alpha 1-adrenergic neurotransmission is necessary for behavioral activation to environmental change in mice. *Neuroscience*, *94*(4), 1245-1252.

Sugita, S., Shen, K. Z., & North, R. A. (1992). 5-hydroxytryptamine is a fast excitatory transmitter at 5-HT3 receptors in rat amygdala. *Neuron*, *8*(1), 199-203.

Summers, R. J., Papaioannou, M., Harris, S., & Evans, B. A. (1995). Expression of beta 3-adrenoceptor mRNA in rat brain. *Br.J.Pharmacol., 116*(6), 2547-2548.

Susser, E. S., & Lin, S. P. (1992). Schizophrenia after prenatal exposure to the dutch hunger winter of 1944-1945. *Arch.Gen.Psychiatry, 49*(12), 983-988.

Susser, E., Neugebauer, R., Hoek, H. W., Brown, A. S., Lin, S., et al. (1996). Schizophrenia after prenatal famine. further evidence. *Arch.Gen.Psychiatry, 53*(1), 25-31.

Susser, E., & St Clair, D. (2013). Prenatal famine and adult mental illness: Interpreting concordant and discordant results from the dutch and chinese famines. *Soc.Sci.Med., 97*, 325-330.

Suzuki, Y., Jodo, E., Takeuchi, S., Niwa, S., & Kayama, Y. (2002). Acute administration of phencyclidine induces tonic activation of medial prefrontal cortex neurons in freely moving rats. *Neuroscience*, *114*(3), 769-779.

Sved, A. F., Cano, G., & Card, J. P. (2001). Neuroanatomical specificity of the circuits controlling sympathetic outflow to different targets. *Clin.Exp.Pharmacol.Physiol., 28*(1-2), 115-119.

Svensson, T. H. (2003). Alpha-adrenoceptor modulation hypothesis of antipsychotic atypicality. *Prog.Neuropsychopharmacol.Biol.Psychiatry, 27*(7), 1145-1158.

Svensson, T. H., Bunney, B. S., & Aghajanian, G. K. (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res., 92*(2), 291-306.

Swanson, C. J., Perry, K. W., Koch-Krueger, S., Katner, J., Svensson, K. A., & Bymaster, F. P. (2006). Effect of the attention deficit/hyperactivity disorder drug atomoxetine on extracellular concentrations of norepinephrine and dopamine in several brain regions of the rat. *Neuropharmacology, 50*(6), 755-760.

Swartz, B. E., Kovalik, E., Thomas, K., Torgersen, D., & Mandelkern, M. A. (2000). The effects of an alpha-2 adrenergic agonist, guanfacine, on rCBF in human cortex in normal controls and subjects with focal epilepsy. *Neuropsychopharmacology, 23*(3), 263-275.

Swerdlow, N. R., Braff, D. L., Taaid, N., & Geyer, M. A. (1994). Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Arch.Gen.Psychiatry, 51*(2), 139-154.

Swerdlow, N. R., & Geyer, M. A. (1998). Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr.Bull., 24*(2), 285-301.

Swerdlow, N. R., Lipska, B. K., Weinberger, D. R., Braff, D. L., Jaskiw, G. E., & Geyer, M. A. (1995). Increased sensitivity to the sensorimotor gating-disruptive effects of apomorphine after lesions of medial prefrontal cortex or ventral hippocampus in adult rats. *Psychopharmacology (Berl), 122*(1), 27-34.

Szoke, A., Schurhoff, F., Meary, A., Mathieu, F., Chevalier, F., et al. (2006). Lack of influence of COMT and NET genes variants on executive functions in schizophrenic and bipolar patients, their first-degree relatives and controls. *Am.J.Med.Genet.B.Neuropsychiatr.Genet., 141B*(5), 504-512.

Szoke, A., Trandafir, A., Dupont, M. E., Meary, A., Schurhoff, F., & Leboyer, M. (2008). Longitudinal studies of cognition in schizophrenia: Meta-analysis. *Br.J.Psychiatry, 192*(4), 248-257.

Szot, P., Lester, M., Laughlin, M. L., Palmiter, R. D., Liles, L. C., & Weinshenker, D. (2004). The anticonvulsant and proconvulsant effects of alpha2-adrenoreceptor agonists are mediated by distinct populations of alpha2A-adrenoreceptors. *Neuroscience*, *126*(3), 795-803.

Tait, D. S., Brown, V. J., Farovik, A., Theobald, D. E., Dalley, J. W., & Robbins, T. W. (2007). Lesions of the dorsal noradrenergic bundle impair attentional set-shifting in the rat. *Eur.J.Neurosci., 25*(12), 3719-3724.

Takeuchi, O., & Akira, S. (2007). Recognition of viruses by innate immunity. *Immunol.Rev., 220*, 214-224.

Talaia, C., Queiroz, G., Pinheiro, H., Moura, D., & Goncalves, J. (2006). Involvement of G-protein betagamma subunits on the influence of inhibitory alpha2-autoreceptors on the angiotensin AT1-receptor modulation of noradrenaline release in the rat vas deferens. *Neurochem.Int., 49*(7), 698-707.

Tallerico, T., Novak, G., Liu, I. S., Ulpian, C., & Seeman, P. (2001). Schizophrenia: Elevated mRNA for dopamine D2(longer) receptors in frontal cortex. *Brain Res.Mol.Brain Res., 87*(2), 160-165.

Talley, E. M., Rosin, D. L., Lee, A., Guyenet, P. G., & Lynch, K. R. (1996). Distribution of alpha 2A-adrenergic receptor-like immunoreactivity in the rat central nervous system. *J.Comp.Neurol., 372*(1), 111-134.

Talpos, J., & Steckler, T. (2013). Touching on translation. *Cell Tissue Res., 354*(1), 297-308.

Tamminga, C. (1999). Glutamatergic aspects of schizophrenia. *Br.J.Psychiatry Suppl., (37)*(37), 12-15.

Tanda, G., Carboni, E., Frau, R., & Di Chiara, G. (1994). Increase of extracellular dopamine in the prefrontal cortex: A trait of drugs with antidepressant potential? *Psychopharmacology (Berl), 115*(1-2), 285-288.

Tandon, R., & Jibson, M. D. (2002). Extrapyramidal side effects of antipsychotic treatment: Scope of problem and impact on outcome. *Ann.Clin.Psychiatry, 14*(2), 123-129.

Tanila, H., Mustonen, K., Sallinen, J., Scheinin, M., & Riekkinen, P.,Jr. (1999). Role of alpha2C-adrenoceptor subtype in spatial working memory as revealed by mice with targeted disruption of the alpha2C-adrenoceptor gene. *Eur.J.Neurosci., 11*(2), 599-603.

Tanila, H., Rama, P., & Carlson, S. (1996). The effects of prefrontal intracortical microinjections of an alpha-2 agonist, alpha-2 antagonist and lidocaine on the delayed alternation performance of aged rats. *Brain Res.Bull., 40*(2), 117-119.

Tanoue, A., Koshimizu, T. A., Shibata, K., Nasa, Y., Takeo, S., & Tsujimoto, G. (2003). Insights into alpha1 adrenoceptor function in health and disease from transgenic animal studies. *Trends Endocrinol.Metab., 14*(3), 107-113.

Tao, R., & Auerbach, S. B. (2000). Regulation of serotonin release by GABA and excitatory amino acids. *J.Psychopharmacol., 14*(2), 100-113.

Tao, R., & Hjorth, S. (1992). A2-adrenoceptor modulation of rat ventral hippocampal 5-hydroxytryptamine release in vivo. *Naunyn Schmiedebergs Arch.Pharmacol., 345(2), 137-143.*

Tavares, A., Handy, D. E., Bogdanova, N. N., Rosene, D. L., & Gavras, H. (1996). Localization of alpha 2A- and alpha 2B-adrenergic receptor subtypes in brain. *Hypertension, 27*(3 Pt 1), 449-455.

Taylor, F. B., & Russo, J. (2001). Comparing guanfacine and dextroamphetamine for the treatment of adult attention-deficit/hyperactivity disorder. *J.Clin.Psychopharmacol., 21*(2), 223-228.

Taylor, J. R., Birnbaum, S., Ubriani, R., & Arnsten, A. F. (1999). Activation of cAMP-dependent protein kinase A in prefrontal cortex impairs working memory performance. *J.Neurosci., 19*(18), RC23.

Tejani-Butt, S. M. (1992). 3H]nisoxetine: A radioligand for quantitation of norepinephrine uptake sites by autoradiography or by homogenate binding. *J.Pharmacol.Exp.Ther., 260*(1), 427-436.

Tek, C., Gold, J., Blaxton, T., Wilk, C., McMahon, R. P., & Buchanan, R. W. (2002). Visual perceptual and working memory impairments in schizophrenia. *Arch.Gen.Psychiatry, 59*(2), 146-153.

Tellioglu, T., & Robertson, D. (2001). Genetic or acquired deficits in the norepinephrine transporter: Current understanding of clinical implications. *Expert Rev.Mol.Med., 2001*, 1-10.

Terry, A. V., Jr, Buccafusco, J. J., Herman, E. J., Callahan, P. M., Beck, W. D., et al. (2011). The prototypical ranitidine analog JWS-USC-75-IX improves information processing and cognitive function in animal models. *J.Pharmacol.Exp.Ther., 336*(3), 751-766.

Terry, A. V., Jr, Buccafusco, J. J., & Wilson, C. (2008). Cognitive dysfunction in neuropsychiatric disorders: Selected serotonin receptor subtypes as therapeutic targets. *Behav.Brain Res., 195*(1), 30-38.

Terry, A. V., Jr, Gearhart, D. A., Warner, S., Hohnadel, E. J., Middlemore, M. L., et al. (2007). Protracted effects of chronic oral haloperidol and risperidone on nerve growth factor, cholinergic neurons, and spatial reference learning in rats. *Neuroscience*, *150*(2), 413-424.

Tervo, D. G., Hwang, B. Y., Viswanathan, S., Gaj, T., Lavzin, M., et al. (2016). A designer AAV variant permits efficient retrograde access to projection neurons. *Neuron*, *92*(2), 372-382.

Thermenos, H. W., Keshavan, M. S., Juelich, R. J., Molokotos, E., Whitfield-Gabrieli, S., et al. (2013). A review of neuroimaging studies of young relatives of individuals with schizophrenia: A developmental perspective from schizotaxia to schizophrenia. *Am.J.Med.Genet.B.Neuropsychiatr.Genet., 162B*(7), 604-635.

Thomas, D. N., & Holman, R. B. (1991). A microdialysis study of the regulation of endogenous noradrenaline release in the rat hippocampus. *J.Neurochem., 56*(5), 1741-1746.

Thompson-Schill, S. L., Jonides, J., Marshuetz, C., Smith, E. E., D'Esposito, M., et al. (2002). Effects of frontal lobe damage on interference effects in working memory. *Cogn.Affect.Behav.Neurosci., 2*(2), 109-120.

Timmermans, P. B., & van Zwieten, P. A. (1982). Alpha 2 adrenoceptors: Classification, localization, mechanisms, and targets for drugs. *J.Med.Chem., 25*(12), 1389-1401.

Tinsley, J. H., Chiasson, V. L., South, S., Mahajan, A., & Mitchell, B. M. (2009). Immunosuppression improves blood pressure and endothelial function in a rat model of pregnancy-induced hypertension. *Am.J.Hypertens., 22*(10), 1107-1114.

To, Z. P., Bonhaus, D. W., Eglen, R. M., & Jakeman, L. B. (1995). Characterization and distribution of putative 5-ht7 receptors in guinea-pig brain. *Br.J.Pharmacol., 115*(1), 107-116.

Tobler, P. N., Dickinson, A., & Schultz, W. (2003). Coding of predicted reward omission by dopamine neurons in a conditioned inhibition paradigm. *J.Neurosci., 23*(32), 10402-10410.

Toda, M., & Abi-Dargham, A. (2007). Dopamine hypothesis of schizophrenia: Making sense of it all. *Curr.Psychiatry Rep., 9*(4), 329-336.

Toide, K., Iwamoto, Y., Fujiwara, T., & Abe, H. (1995). JTP-4819: A novel prolyl endopeptidase inhibitor with potential as a cognitive enhancer. *J.Pharmacol.Exp.Ther., 274*(3), 1370-1378.

Tork, I. (1990). Anatomy of the serotonergic system. *Ann.N.Y.Acad.Sci., 600*, 5.

Torrey, E. F. (1991). A viral-anatomical explantation of schizophrenia. *Schizophr.Bull., 17*(1), 15-18.

Tose, R., Kushikata, T., Yoshida, H., Kudo, M., Furukawa, K., et al. (2009). Interaction between orexinergic neurons and NMDA receptors in the control of locus coeruleus-cerebrocortical noradrenergic activity of the rat. *Brain Res., 1250*, 81-87.

Trendelenburg, A. U., Philipp, M., Meyer, A., Klebroff, W., Hein, L., & Starke, K. (2003). All three alpha2-adrenoceptor types serve as autoreceptors in postganglionic sympathetic neurons. *Naunyn Schmiedebergs Arch.Pharmacol., 368*(6), 504-512.

Trendelenburg, A. U., Starke, K., & Limberger, N. (1994). Presynaptic alpha 2A-adrenoceptors inhibit the release of endogenous dopamine in rabbit caudate nucleus slices. *Naunyn Schmiedebergs Arch.Pharmacol., 350*(5), 473-481.

Tritsch, N. X., & Sabatini, B. L. (2012). Dopaminergic modulation of synaptic transmission in cortex and striatum. *Neuron, 76*(1), 33-50.

Tsai, G. E., & Lin, P. Y. (2010). Strategies to enhance N-methyl-D-aspartate receptor-mediated neurotransmission in schizophrenia, a critical review and meta-analysis. *Curr.Pharm.Des., 16*(5), 522-537.

Tseng, H. H., Chen, K. C., Chen, P. S., Lee, I. H., Chang, W. H., et al. (2017). Dopamine transporter availability in drug-naive patients with schizophrenia and later psychotic symptoms severity. *Schizophr.Res., 190*, 185-186.

Tseng, K. Y., & O'Donnell, P. (2004). Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. *J.Neurosci., 24*(22), 5131-5139.

Tsuang, M. (2000). Schizophrenia: Genes and environment. *Biol.Psychiatry, 47*(3), 210-220.

Turetsky, B. I., Moberg, P. J., Roalf, D. R., Arnold, S. E., & Gur, R. E. (2003). Decrements in volume of anterior ventromedial temporal lobe and olfactory dysfunction in schizophrenia. *Arch.Gen.Psychiatry, 60*(12), 1193-1200.

Turner, J. A., Smyth, P., Macciardi, F., Fallon, J. H., Kennedy, J. L., & Potkin, S. G. (2006). Imaging phenotypes and genotypes in schizophrenia. *Neuroinformatics*, *4*(1), 21-49.

Twarog, B. M., & Page, I. H. (1953). Serotonin content of some mammalian tissues and urine and a method for its determination. *Am.J.Physiol., 175*(1), 157-161.

Uematsu, A., Tan, B. Z., Ycu, E. A., Cuevas, J. S., Koivumaa, J., et al. (2017). Modular organization of the brainstem noradrenaline system coordinates opposing learning states. *Nat.Neurosci., 20*(11), 1602-1611.

Uhl, G. R., & Johnson, P. S. (1994). Neurotransmitter transporters: Three important gene families for neuronal function. *J.Exp.Biol., 196*, 229-236.

Uhlen, S., Dambrova, M., Nasman, J., Schioth, H. B., Gu, Y., et al. (1998). 3H]RS79948-197 binding to human, rat, guinea pig and pig alpha2A-, alpha2B- and alpha2C-adrenoceptors. comparison with MK912, RX821002, rauwolscine and yohimbine. *Eur.J.Pharmacol., 343*(1), 93-101.

Uhlen, S., Muceniece, R., Rangel, N., Tiger, G., & Wikberg, J. E. (1995). Comparison of the binding activities of some drugs on alpha 2A, alpha 2B and alpha 2C-adrenoceptors and non-adrenergic imidazoline sites in the guinea pig. *Pharmacol.Toxicol., 76*(6), 353-364.

Uhlen, S., Porter, A. C., & Neubig, R. R. (1994). The novel alpha-2 adrenergic radioligand [3H]-MK912 is alpha-2C selective among human alpha-2A, alpha-2B and alpha-2C adrenoceptors. *J.Pharmacol.Exp.Ther., 271*(3), 1558-1565.

Uhlen, S., & Wikberg, J. E. (1991). Delineation of rat kidney alpha 2A- and alpha 2B-adrenoceptors with [3H]RX821002 radioligand binding: Computer modelling reveals that guanfacine is an alpha 2A-selective compound. *Eur.J.Pharmacol., 202*(2), 235-243.

Uhlen, S., Xia, Y., Chhajlani, V., Felder, C. C., & Wikberg, J. E. (1992). 3H]-MK 912 binding delineates two alpha 2-adrenoceptor subtypes in rat CNS one of which is identical with the cloned pA2d alpha 2-adrenoceptor. *Br.J.Pharmacol., 106*(4), 986-995.

Uhlhaas, P. J. (2011). High-frequency oscillations in schizophrenia. *Clin.EEG Neurosci., 42*(2), 77-82.

Uhlhaas, P. J. (2013). Dysconnectivity, large-scale networks and neuronal dynamics in schizophrenia. *Curr.Opin.Neurobiol., 23*(2), 283-290.

Uhlhaas, P. J., Linden, D. E., Singer, W., Haenschel, C., Lindner, M., et al. (2006). Dysfunctional long-range coordination of neural activity during gestalt perception in schizophrenia. *J.Neurosci., 26*(31), 8168-8175.

Umbricht, D., Alberati, D., Martin-Facklam, M., Borroni, E., Youssef, E. A., et al. (2014). Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: A randomized, double-blind, proof-of-concept study. *JAMA Psychiatry., 71*(6), 637-646.

Ungless, M. A., Magill, P. J., & Bolam, J. P. (2004). Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science, 303*(5666), 2040-2042.

Unnerstall, J. R., Kopajtic, T. A., & Kuhar, M. J. (1984). Distribution of alpha 2 agonist binding sites in the rat and human central nervous system: Analysis of some functional, anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agents. *Brain Res., 319*(1), 69-101.

Unschuld, P. G., Buchholz, A. S., Varvaris, M., van Zijl, P. C., Ross, C. A., et al. (2014). Prefrontal brain network connectivity indicates degree of both schizophrenia risk and cognitive dysfunction. *Schizophr.Bull., 40*(3), 653-664.

U'Prichard, D. C., Bechtel, W. D., Rouot, B. M., & Snyder, S. H. (1979). Multiple apparent alpha-noradrenergic receptor binding sites in rat brain: Effect of 6-hydroxydopamine. *Mol.Pharmacol., 16*(1), 47-60.

Urigüen, L., Garcia-Fuster, M. J., Callado, L. F., Morentin, B., La Harpe, R., et al. (2009). Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: Effect of antipsychotic treatment. *Psychopharmacology (Berl), 206*(2), 313-324.

Uylings, H. B., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex? *Behav.Brain Res., 146*(1-2), 3-17.

Uylings, H. B., & van Eden, C. G. (1990). Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans. *Prog.Brain Res., 85*, 31-62.

Uys, M. M., Shahid, M., & Harvey, B. H. (2017). Therapeutic potential of selectively targeting the alpha2C-adrenoceptor in cognition, depression, and schizophrenia-new developments and future perspective. *Front.Psychiatry., 8*, 144.

Uys, M. M., Shahid, M., Sallinen, J., & Harvey, B. H. (2017). The alpha2C-adrenoceptor antagonist, ORM-10921, exerts antidepressant-like effects in the flinders sensitive line rat. *Behav.Pharmacol., 28*(1), 9-18.

Uys, M., Shahid, M., Sallinen, J., Dreyer, W., Cockeran, M., & Harvey, B. H. (2016). The alpha2C-adrenoceptor antagonist, ORM-10921, has antipsychotic-like effects in social isolation reared rats and bolsters the response to haloperidol. *Prog.Neuropsychopharmacol.Biol.Psychiatry, 71*, 108-116.

Vaidya, V. A., Marek, G. J., Aghajanian, G. K., & Duman, R. S. (1997). 5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J.Neurosci., 17*(8), 2785-2795.

van den Heuvel, M. P., & Fornito, A. (2014). Brain networks in schizophrenia. *Neuropsychol.Rev., 24*(1), 32-48.

van den Heuvel, M. P., & Hulshoff Pol, H. E. (2010). Exploring the brain network: A review on resting-state fMRI functional connectivity. *Eur.Neuropsychopharmacol., 20*(8), 519-534.

van den Heuvel, M. P., Sporns, O., Collin, G., Scheewe, T., Mandl, R. C., et al. (2013). Abnormal rich club organization and functional brain dynamics in schizophrenia. *JAMA Psychiatry., 70*(8), 783-792.

van Dongen, P. A. (1981). The central noradrenergic transmission and the locus coeruleus: A review of the data, and their implications for neurotransmission and neuromodulation. *Prog.Neurobiol., 16*(2), 117-143.

Van Gaalen, M., Kawahara, H., Kawahara, Y., & Westerink, B. H. (1997). The locus coeruleus noradrenergic system in the rat brain studied by dual-probe microdialysis. *Brain Res., 763*(1), 56-62.

van Haren, N. E., Bakker, S. C., & Kahn, R. S. (2008). Genes and structural brain imaging in schizophrenia. *Curr.Opin.Psychiatry., 21*(2), 161-167.

van Heesch, F., Prins, J., Konsman, J. P., Korte-Bouws, G. A., Westphal, K. G., et al. (2014). Lipopolysaccharide increases degradation of central monoamines: An in vivo microdialysis study in the nucleus accumbens and medial prefrontal cortex of mice. *Eur.J.Pharmacol., 725*, 55-63.

van Veldhuizen, M. J., Feenstra, M. G., Heinsbroek, R. P., & Boer, G. J. (1993). In vivo microdialysis of noradrenaline overflow: Effects of alpha-adrenoceptor agonists and antagonists measured by cumulative concentration-response curves. *Br.J.Pharmacol., 109*(3), 655-660.

van Zwieten, P. A., & Chalmers, J. P. (1994). Different types of centrally acting antihypertensives and their targets in the central nervous system. *Cardiovasc.Drugs Ther., 8*(6), 787-799.

Verbanac, J. S., Commissaris, R. L., & Pitts, D. K. (1996). An electrophysiological evaluation of serotonergic dorsal raphe neurons in Maudsley rats. *Life Sci., 58*(3), 245-250.

Versijpt, J., Van Laere, K. J., Dumont, F., Decoo, D., Vandecapelle, M., et al. (2003). Imaging of the 5-HT2A system: Age-, gender-, and Alzheimer's disease-related findings. *Neurobiol.Aging, 24*(4), 553-561.

Vidal, R., Pilar-Cuellar, F., dos Anjos, S., Linge, R., Treceno, B., et al. (2011). New strategies in the development of antidepressants: Towards the modulation of neuroplasticity pathways. *Curr.Pharm.Des., 17*(5), 521-533.

Vieira-Coelho, M. A., Serrao, M. P., Afonso, J., Pinto, C. E., & Moura, E. (2009). Catecholamine synthesis and metabolism in the central nervous system of mice lacking alpha-adrenoceptor subtypes. *Br.J.Pharmacol., 158*(3), 726-737.

Vijayraghavan, S., Wang, M., Birnbaum, S. G., Williams, G. V., & Arnsten, A. F. (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat.Neurosci., 10*(3), 376-384.

Vincent, S. L., Khan, Y., & Benes, F. M. (1993). Cellular distribution of dopamine D1 and D2 receptors in rat medial prefrontal cortex. *J.Neurosci., 13*(6), 2551-2564.

Vishnoi, S., Raisuddin, S., & Parvez, S. (2015). Modulatory effects of an NMDAR partial agonist in MK-801-induced memory impairment. *Neuroscience*, *311*, 22-33.

Vogel-Ciernia, A., & Wood, M. A. (2014). Examining object location and object recognition memory in mice. *Curr.Protoc.Neurosci., 69*, 8.31.-17.

Vogt, B. A., & Paxinos, G. (2014). Cytoarchitecture of mouse and rat cingulate cortex with human homologies. *Brain Struct.Funct., 219*(1), 185-192.

Volkow, N. D., Gur, R. C., Wang, G. J., Fowler, J. S., Moberg, P. J., et al. (1998). Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. *Am.J.Psychiatry, 155*(3), 344-349.

Vollenweider, F. X., Vollenweider-Scherpenhuyzen, M. F., Babler, A., Vogel, H., & Hell, D. (1998). Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport*, *9*(17), 3897-3902.

Voon, V., Irvine, M. A., Derbyshire, K., Worbe, Y., Lange, I., et al. (2014). Measuring "waiting" impulsivity in substance addictions and binge eating disorder in a novel analogue of rodent serial reaction time task. *Biol.Psychiatry, 75*(2), 148-155.

Voorn, P., Jorritsma-Byham, B., Van Dijk, C., & Buijs, R. M. (1986). The dopaminergic innervation of the ventral striatum in the rat: A light- and electron-microscopical study with antibodies against dopamine. *J.Comp.Neurol., 251*(1), 84-99.

Vorhees, C. V., Graham, D. L., Braun, A. A., Schaefer, T. L., Skelton, M. R., et al. (2015). Prenatal immune challenge in rats: Effects of polyinosinic-polycytidylic acid on spatial learning, prepulse inhibition, conditioned fear, and responses to MK-801 and amphetamine. *Neurotoxicol.Teratol., 47*, 54-65.

Voruganti, L., Slomka, P., Zabel, P., Costa, G., So, A., et al. (2001). Subjective effects of AMPT-induced dopamine depletion in schizophrenia: Correlation between dysphoric responses and striatal D(2) binding ratios on SPECT imaging. *Neuropsychopharmacology, 25*(5), 642-650.

Vuillermot, S., Weber, L., Feldon, J., & Meyer, U. (2010). A longitudinal examination of the neurodevelopmental impact of prenatal immune activation in mice reveals primary defects in dopaminergic development relevant to schizophrenia. *J.Neurosci., 30*(4), 1270-1287.

Wade, S. M., Lim, W. K., Lan, K. L., Chung, D. A., Nanamori, M., & Neubig, R. R. (1999). G(i) activator region of alpha(2A)-adrenergic receptors: Distinct basic residues mediate G(i) versus G(s) activation. *Mol.Pharmacol., 56*(5), 1005-1013.

Wadenberg, M. L., Wiker, C., & Svensson, T. H. (2007). Enhanced efficacy of both typical and atypical antipsychotic drugs by adjunctive alpha2 adrenoceptor blockade: Experimental evidence. *Int.J.Neuropsychopharmacol., 10*(2), 191-202.

Wallis, J. D., Anderson, K. C., & Miller, E. K. (2001). Single neurons in prefrontal cortex encode abstract rules. *Nature*, *411*(6840), 953-956.

Waltrip, R. W.,2nd, Buchanan, R. W., Summerfelt, A., Breier, A., Carpenter, W. T.,Jr, et al. (1995). Borna disease virus and schizophrenia. *Psychiatry Res., 56*(1), 33-44.

Wang, M., Gamo, N. J., Yang, Y., Jin, L. E., Wang, X. J., et al. (2011a). Neuronal basis of age-related working memory decline. *Nature*, *476*(7359), 210-213.

Wang, M., Ramos, B. P., Paspalas, C. D., Shu, Y., Simen, A., et al. (2007). Alpha2A-adrenoceptors strengthen working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. *Cell*, *129*(2), 397-410.

Wang, M., Tang, Z. X., & Li, B. M. (2004). Enhanced visuomotor associative learning following stimulation of alpha 2A-adrenoceptors in the ventral prefrontal cortex in monkeys. *Brain Res., 1024*(1-2), 176-182.

Wang, Q., Sporns, O., & Burkhalter, A. (2012). Network analysis of corticocortical connections reveals ventral and dorsal processing streams in mouse visual cortex. *J.Neurosci., 32*(13), 4386-4399.

Wang, R., Macmillan, L. B., Fremeau, R. T.,Jr, Magnuson, M. A., Lindner, J., & Limbird, L. E. (1996). Expression of alpha 2-adrenergic receptor subtypes in the mouse brain: Evaluation of spatial and temporal information imparted by 3 kb of 5' regulatory sequence for the alpha 2A AR-receptor gene in transgenic animals. *Neuroscience*, *74*(1), 199-218.

Wang, S. H., Hsiao, P. C., Yeh, L. L., Liu, C. M., Liu, C. C., et al. (2018). Polygenic risk for schizophrenia and neurocognitive performance in patients with schizophrenia. *Genes Brain Behav., 17*(1), 49-55.

Wang, Y., Liu, J., Gui, Z. H., Ali, U., Fan, L. L., et al. (2011b). Alpha2-adrenoceptor regulates the spontaneous and the GABA/glutamate modulated firing activity of the rat medial prefrontal cortex pyramidal neurons. *Neuroscience*, *182*, 193-202.

Ward, R. D., Kellendonk, C., Simpson, E. H., Lipatova, O., Drew, M. R., et al. (2009). Impaired timing precision produced by striatal D2 receptor overexpression is mediated by cognitive and motivational deficits. *Behav.Neurosci., 123*(4), 720-730.

Warden, M. R., Selimbeyoglu, A., Mirzabekov, J. J., Lo, M., Thompson, K. R., et al. (2012). A prefrontal cortex-brainstem neuronal projection that controls response to behavioural challenge. *Nature*, *492*(7429), 428-432.

Wedzony, K., Klimek, V., & Golembiowska, K. (1993). MK-801 elevates the extracellular concentration of dopamine in the rat prefrontal cortex and increases the density of striatal dopamine D1 receptors. *Brain Res., 622*(1-2), 325-329.

Weed, M. R., Taffe, M. A., Polis, I., Roberts, A. C., Robbins, T. W., et al. (1999). Performance norms for a rhesus monkey neuropsychological testing battery: Acquisition and long-term performance. *Brain Res.Cogn.Brain Res., 8*(3), 185-201.

Weinberger, D. R. (1987). Implications of normal brain development for the pathogenesis of schizophrenia. *Arch.Gen.Psychiatry, 44*(7), 660-669.

Weinberger, D. R. (1995). From neuropathology to neurodevelopment. *Lancet, 346*(8974), 552-557.

Weinberger, D. R. (1996). On the plausibility of "the neurodevelopmental hypothesis" of schizophrenia. *Neuropsychopharmacology, 14*(3 Suppl), 1-11S.

Weinberger, D. R., Aloia, M. S., Goldberg, T. E., & Berman, K. F. (1994). The frontal lobes and schizophrenia. *J.Neuropsychiatry Clin.Neurosci., 6*(4), 419-427.

Weinberger, D. R., Berman, K. F., & Daniel, D. G. (1992a). Mesoprefrontal cortical dopaminergic activity and prefrontal hypofunction in schizophrenia. *Clin.Neuropharmacol., 15 Suppl 1 Pt A*, 568-569A.

Weinberger, D. R., Berman, K. F., Suddath, R., & Torrey, E. F. (1992b). Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: A magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. *Am.J.Psychiatry, 149*(7), 890-897.

Weiner, D. M., Meltzer, H. Y., Veinbergs, I., Donohue, E. M., Spalding, T. A., et al. (2004). The role of M1 muscarinic receptor agonism of N-desmethylclozapine in the unique clinical effects of clozapine. *Psychopharmacology (Berl), 177*(1-2), 207-216.

Weiner, I. (2003). The "two-headed" latent inhibition model of schizophrenia: Modeling positive and negative symptoms and their treatment. *Psychopharmacology (Berl), 169*(3-4), 257-297.

Weinstock, M. (2007). Gender differences in the effects of prenatal stress on brain development and behaviour. *Neurochem.Res., 32*(10), 1730-1740.

Weir, R. K., Forghany, R., Smith, S. E., Patterson, P. H., McAllister, A. K., et al. (2015). Preliminary evidence of neuropathology in nonhuman primates prenatally exposed to maternal immune activation. *Brain Behav.Immun., 48*, 139-146.

Weisbrod, M., Kiefer, M., Marzinzik, F., & Spitzer, M. (2000). Executive control is disturbed in schizophrenia: Evidence from event-related potentials in a go/NoGo task. *Biol.Psychiatry, 47*(1), 51-60.

Weiss, E. M., Bilder, R. M., & Fleischhacker, W. W. (2002). The effects of second-generation antipsychotics on cognitive functioning and psychosocial outcome in schizophrenia. *Psychopharmacology (Berl), 162*(1), 11-17.

Westlund, K. N., Denney, R. M., Kochersperger, L. M., Rose, R. M., & Abell, C. W. (1985). Distinct monoamine oxidase A and B populations in primate brain. *Science, 230*(4722), 181-183.

Whishaw, I. Q., Fiorino, D., Mittleman, G., & Castaneda, E. (1992). Do forebrain structures compete for behavioral expression? evidence from amphetamine-induced behavior, microdialysis, and caudate-accumbens lesions in medial frontal cortex damaged rats. *Brain Res., 576*(1), 1-11.

Whitfield-Gabrieli, S., & Ford, J. M. (2012). Default mode network activity and connectivity in psychopathology. *Annu.Rev.Clin.Psychol., 8*, 49-76.

Whitfield-Gabrieli, S., Thermenos, H. W., Milanovic, S., Tsuang, M. T., Faraone, S. V., et al. (2009). Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. *Proc.Natl.Acad.Sci.U.S.A., 106*(4), 1279-1284.

Wilkinson, R. T. (1963). Interaction of noise with knowledge of results and sleep deprivation. *J.Exp.Psychol., 66*(4), 332-337.

Wilson, C., & Terry, A. V.,Jr. (2010). Neurodevelopmental animal models of schizophrenia: Role in novel drug discovery and development. *Clin.Schizophr.Relat.Psychoses, 4*(2), 124-137.

Wilson, F. A., O'Scalaidhe, S. P., & Goldman-Rakic, P. S. (1994). Functional synergism between putative gamma-aminobutyrate-containing neurons and pyramidal neurons in prefrontal cortex. *Proc.Natl.Acad.Sci.U.S.A., 91*(9), 4009-4013.

Winter, C., Djodari-Irani, A., Sohr, R., Morgenstern, R., Feldon, J., et al. (2009). Prenatal immune activation leads to multiple changes in basal neurotransmitter levels in the adult brain: Implications for brain disorders of neurodevelopmental origin such as schizophrenia. *Int.J.Neuropsychopharmacol., 12*(4), 513-524.

Winter, C., Reutiman, T. J., Folsom, T. D., Sohr, R., Wolf, R. J., et al. (2008). Dopamine and serotonin levels following prenatal viral infection in mouse--implications for psychiatric disorders such as schizophrenia and autism. *Eur.Neuropsychopharmacol., 18*(10), 712-716.

Winter, J. C., & Rabin, R. A. (1992). Yohimbine as a serotonergic agent: Evidence from receptor binding and drug discrimination. *J.Pharmacol.Exp.Ther., 263*(2), 682-689.

Winterer, G., & Weinberger, D. R. (2004). Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. *Trends Neurosci., 27*(11), 683-690.

Winters, B. D., & Bussey, T. J. (2005). Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *J.Neurosci., 25*(1), 52-61.

Winters, B. D., Forwood, S. E., Cowell, R. A., Saksida, L. M., & Bussey, T. J. (2004). Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: Heterogeneity of function within the temporal lobe. *J.Neurosci., 24*(26), 5901-5908.

Winters, B. D., Saksida, L. M., & Bussey, T. J. (2008). Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. *Neurosci.Biobehav.Rev., 32*(5), 1055-1070.

Wisden, W., Parker, E. M., Mahle, C. D., Grisel, D. A., Nowak, H. P., et al. (1993). Cloning and characterization of the rat 5-HT5B receptor. evidence that the 5-HT5B receptor couples to a G protein in mammalian cell membranes. *FEBS Lett., 333*(1-2), 25-31.

Wise, S. P. (2008). Forward frontal fields: Phylogeny and fundamental function. *Trends Neurosci., 31*(12), 599-608.

Witte, E. A., & Marrocco, R. T. (1997). Alteration of brain noradrenergic activity in rhesus monkeys affects the alerting component of covert orienting. *Psychopharmacology (Berl), 132*(4), 315-323.

Wolff, A. R., & Bilkey, D. K. (2008). Immune activation during mid-gestation disrupts sensorimotor gating in rat offspring. *Behav.Brain Res., 190*(1), 156-159.

Wolff, A. R., Cheyne, K. R., & Bilkey, D. K. (2011). Behavioural deficits associated with maternal immune activation in the rat model of schizophrenia. *Behav.Brain Res., 225*(1), 382-387.

Wolkin, A., Sanfilipo, M., Angrist, B., Duncan, E., Wieland, S., et al. (1994). Acute d-amphetamine challenge in schizophrenia: Effects on cerebral glucose utilization and clinical symptomatology. *Biol.Psychiatry, 36*(5), 317-325.

Wong, S., Flanagan, E., Savage, G., Hodges, J. R., & Hornberger, M. (2014). Contrasting prefrontal cortex contributions to episodic memory dysfunction in behavioural variant frontotemporal dementia and Alzheimer's disease. *PLoS One, 9*(2), e87778.

Wong, Y. H., Conklin, B. R., & Bourne, H. R. (1992). Gz-mediated hormonal inhibition of cyclic AMP accumulation. *Science, 255*(5042), 339-342.

Woo, T. U., Whitehead, R. E., Melchitzky, D. S., & Lewis, D. A. (1998). A subclass of prefrontal gamma-aminobutyric acid axon terminals are selectively altered in schizophrenia. *Proc.Natl.Acad.Sci.U.S.A., 95*(9), 5341-5346.

Wood, S. J., Proffitt, T., Mahony, K., Smith, D. J., Buchanan, J. A., et al. (2002). Visuospatial memory and learning in first-episode schizophreniform psychosis and established schizophrenia: A functional correlate of hippocampal pathology? *Psychol.Med., 32*(3), 429-438.

Woodward, T. S., Thornton, A. E., Ruff, C. C., Moritz, S., & Liddle, P. F. (2004). Material-specific episodic memory associates of the psychomotor poverty syndrome in schizophrenia. *Cogn.Neuropsychiatry, 9*(3), 213-227.

Woolley, M. L., Marsden, C. A., Sleight, A. J., & Fone, K. C. (2003). Reversal of a cholinergic-induced deficit in a rodent model of recognition memory by the selective 5-HT6 receptor antagonist, ro 04-6790. *Psychopharmacology (Berl), 170*(4), 358-367.

Worbe, Y., Savulich, G., Voon, V., Fernandez-Egea, E., & Robbins, T. W. (2014). Serotonin depletion induces 'waiting impulsivity' on the human four-choice serial reaction time task: Cross-species translational significance. *Neuropsychopharmacology, 39*(6), 1519-1526.

Wortley, K. E., Heal, D. J., & Stanford, S. C. (1999). Modulation of sibutramine-induced increases in extracellular noradrenaline concentration in rat frontal cortex and hypothalamus by alpha2-adrenoceptors. *Br.J.Pharmacol., 128*(3), 659-666.

Wright, D. E., Seroogy, K. B., Lundgren, K. H., Davis, B. M., & Jennes, L. (1995). Comparative localization of serotonin1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J.Comp.Neurol., 351*(3), 357-373.

Wright, P., Nimgaonkar, V. L., Donaldson, P. T., & Murray, R. M. (2001). Schizophrenia and HLA: A review. *Schizophr.Res., 47*(1), 1-12.

Xing, B., Li, Y. C., & Gao, W. J. (2016). Norepinephrine versus dopamine and their interaction in modulating synaptic function in the prefrontal cortex. *Brain Res., 1641*(Pt B), 217-233.

Xu, M. Q., Sun, W. S., Liu, B. X., Feng, G. Y., Yu, L., et al. (2009). Prenatal malnutrition and adult schizophrenia: Further evidence from the 1959-1961 chinese famine. *Schizophr.Bull., 35*(3), 568-576.

Xu, M. Y., & Wong, A. H. C. (2018). GABAergic inhibitory neurons as therapeutic targets for cognitive impairment in schizophrenia. *Acta Pharmacol.Sin., 39*(5), 733-753.

Yagi, J., & Sumino, R. (1998). Inhibition of a hyperpolarization-activated current by clonidine in rat dorsal root ganglion neurons. *J.Neurophysiol., 80*(3), 1094-1104.

Yamada, S., Harano, M., Annoh, N., Nakamura, K., & Tanaka, M. (1999). Involvement of serotonin 2A receptors in phencyclidine-induced disruption of prepulse inhibition of the acoustic startle in rats. *Biol.Psychiatry, 46*(6), 832-838.

Yamaguchi, K., Sawada, T., Naraki, T., Igata-Yi, R., Shiraki, H., et al. (1999). Detection of borna disease virus-reactive antibodies from patients with psychiatric disorders and from horses by electrochemiluminescence immunoassay. *Clin.Diagn.Lab.Immunol., 6*(5), 696-700.

Yamamoto, B. K., & Novotney, S. (1998). Regulation of extracellular dopamine by the norepinephrine transporter. *J.Neurochem., 71*(1), 274-280.

Yamamoto, K., & Hornykiewicz, O. (2004). Proposal for a noradrenaline hypothesis of schizophrenia. *Prog.Neuropsychopharmacol.Biol.Psychiatry, 28*(5), 913-922.

Yamauchi, M., Imanishi, T., & Koyama, T. (2012). A combination of mirtazapine and milnacipran augments the extracellular levels of monoamines in the rat brain. *Neuropharmacology*, *62*(7), 2278-2287.

Yan, T. C., Dudley, J. A., Weir, R. K., Grabowska, E. M., Pena-Oliver, Y., et al. (2011). Performance deficits of NK1 receptor knockout mice in the 5-choice serial reaction-time task: Effects of d-amphetamine, stress and time of day. *PLoS One, 6*(3), e17586.

Yang, C. R., & Seamans, J. K. (1996). Dopamine D1 receptor actions in layers V-VI rat prefrontal cortex neurons in vitro: Modulation of dendritic-somatic signal integration. *J.Neurosci., 16*(5), 1922-1935.

Yasuno, F., Suhara, T., Okubo, Y., Ichimiya, T., Takano, A., et al. (2005). Abnormal effective connectivity of dopamine D2 receptor binding in schizophrenia. *Psychiatry Res., 138*(3), 197-207.

Yi, F., Liu, S. S., Luo, F., Zhang, X. H., & Li, B. M. (2013). Signaling mechanism underlying alpha2A -adrenergic suppression of excitatory synaptic transmission in the medial prefrontal cortex of rats. *Eur.J.Neurosci., 38*(3), 2364-2373.

Yizhar, O., Fenno, L. E., Davidson, T. J., Mogri, M., & Deisseroth, K. (2011). Optogenetics in neural systems. *Neuron*, *71*(1), 9-34.

Yolken, R. H., & Torrey, E. F. (1995). Viruses, schizophrenia, and bipolar disorder. *Clin.Microbiol.Rev., 8*(1), 131-145.

Yoshioka, M., Matsumoto, M., Togashi, H., Smith, C. B., & Saito, H. (1992). Effect of clonidine on the release of serotonin from the rat hippocampus as measured by microdialysis. *Neurosci.Lett., 139*(1), 57-60.

Yoshito Takano, Manami Takano, & Yaksh, T. L. (1992). The effect of intrathecally administered imiloxan and WB4101: Possible role of α2-adrenoceptor subtypes in the spinal cord. *Eur.J.Pharmacol*., *219*(3), 465-468.

Youdim, M. B. (1988). Platelet monoamine oxidase B: Use and misuse. *Experientia*, *44*(2), 137-141.

Young, J. W., Crawford, N., Kelly, J. S., Kerr, L. E., Marston, H. M., et al. (2007). Impaired attention is central to the cognitive deficits observed in alpha 7 deficient mice. *Eur.Neuropsychopharmacol., 17*(2), 145-155.

Young, J. W., Finlayson, K., Spratt, C., Marston, H. M., Crawford, N., et al. (2004). Nicotine improves sustained attention in mice: Evidence for involvement of the alpha7 nicotinic acetylcholine receptor. *Neuropsychopharmacology, 29*(5), 891-900.

Young, J. W., Powell, S. B., Risbrough, V., Marston, H. M., & Geyer, M. A. (2009). Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. *Pharmacol.Ther., 122*(2), 150-202.

Young, J. W., Powell, S. B., Scott, C. N., Zhou, X., & Geyer, M. A. (2011). The effect of reduced dopamine D4 receptor expression in the 5-choice continuous performance task: Separating response inhibition from premature responding. *Behav.Brain Res., 222*(1), 183-192.

Young, P., Berge, J., Chapman, H., & Cawthorne, M. A. (1989). Novel alpha 2-adrenoceptor antagonists show selectivity for alpha 2A- and alpha 2B-adrenoceptor subtypes. *Eur.J.Pharmacol., 168*(3), 381-386.

Yu, A. J., & Dayan, P. (2005). Uncertainty, neuromodulation, and attention. *Neuron*, *46*(4), 681-692.

Zalesky, A., Fornito, A., Seal, M. L., Cocchi, L., Westin, C. F., et al. (2011). Disrupted axonal fiber connectivity in schizophrenia. *Biol.Psychiatry, 69*(1), 80-89.

Zavitsanou, K., Dalton, V. S., Walker, A. K., Weickert, C. S., Sominsky, L., & Hodgson, D. M. (2013). Neonatal lipopolysaccharide treatment has long-term effects on monoaminergic and cannabinoid receptors in the rat. *Synapse, 67*(6), 290-299.

Zhang, F., Sarginson, J., Crombie, C., Walker, N., St Clair, D., & Shaw, D. (2006). Genetic association between schizophrenia and the DISC1 gene in the Scottish population. *Am.J.Med.Genet.B.Neuropsychiatr.Genet., 141B*(2), 155-159.

Zhang, W., Klimek, V., Farley, J. T., Zhu, M. Y., & Ordway, G. A. (1999). alpha2C adrenoceptors inhibit adenylyl cyclase in mouse striatum: Potential activation by dopamine. *J.Pharmacol.Exp.Ther., 289*(3), 1286-1292.

Zhang, Y., Cazakoff, B. N., Thai, C. A., & Howland, J. G. (2012). Prenatal exposure to a viral mimetic alters behavioural flexibility in male, but not female, rats. *Neuropharmacology*, *62*(3), 1299-1307.

Zheng, P., Zhang, X. X., Bunney, B. S., & Shi, W. X. (1999). Opposite modulation of cortical N-methyl-D-aspartate receptor-mediated responses by low and high concentrations of dopamine. *Neuroscience*, *91*(2), 527-535.

Zheng, & Hasegawa, H. (2017). Pharmacological intervention of brain neurotransmission affects exercise capacity. *Physical Activity and the aging brain*.,53-64.

Zhong, H., & Minneman, K. P. (1999). Alpha1-adrenoceptor subtypes. *Eur.J.Pharmacol., 375*(1-3), 261-276.

Zhou, F. C., Tao-Cheng, J. H., Segu, L., Patel, T., & Wang, Y. (1998). Serotonin transporters are located on the axons beyond the synaptic junctions: Anatomical and functional evidence. *Brain Res., 805*(1-2), 241-254.

Zhou, J. (2004). Norepinephrine transporter inhibitors and their therapeutic potential. *Drugs Future, 29*(12), 1235-1244.

Zhou, Y., Guo, M., Wang, X., Li, J., Wang, Y., et al. (2013). TLR3 activation efficiency by high or low molecular mass poly I:C. *Innate Immun., 19*(2), 184-192.

Zoccali, R., Muscatello, M. R., Cedro, C., Neri, P., La Torre, D., et al. (2004). The effect of mirtazapine augmentation of clozapine in the treatment of negative symptoms of schizophrenia: A double-blind, placebo-controlled study. *Int.Clin.Psychopharmacol., 19*(2), 71-76.

Zuckerman, L., Rehavi, M., Nachman, R., & Weiner, I. (2003). Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: A novel neurodevelopmental model of schizophrenia. *Neuropsychopharmacology, 28*(10), 1778-1789.

Zuckerman, L., & Weiner, I. (2005). Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring. *J.Psychiatr.Res., 39*(3), 311-323.