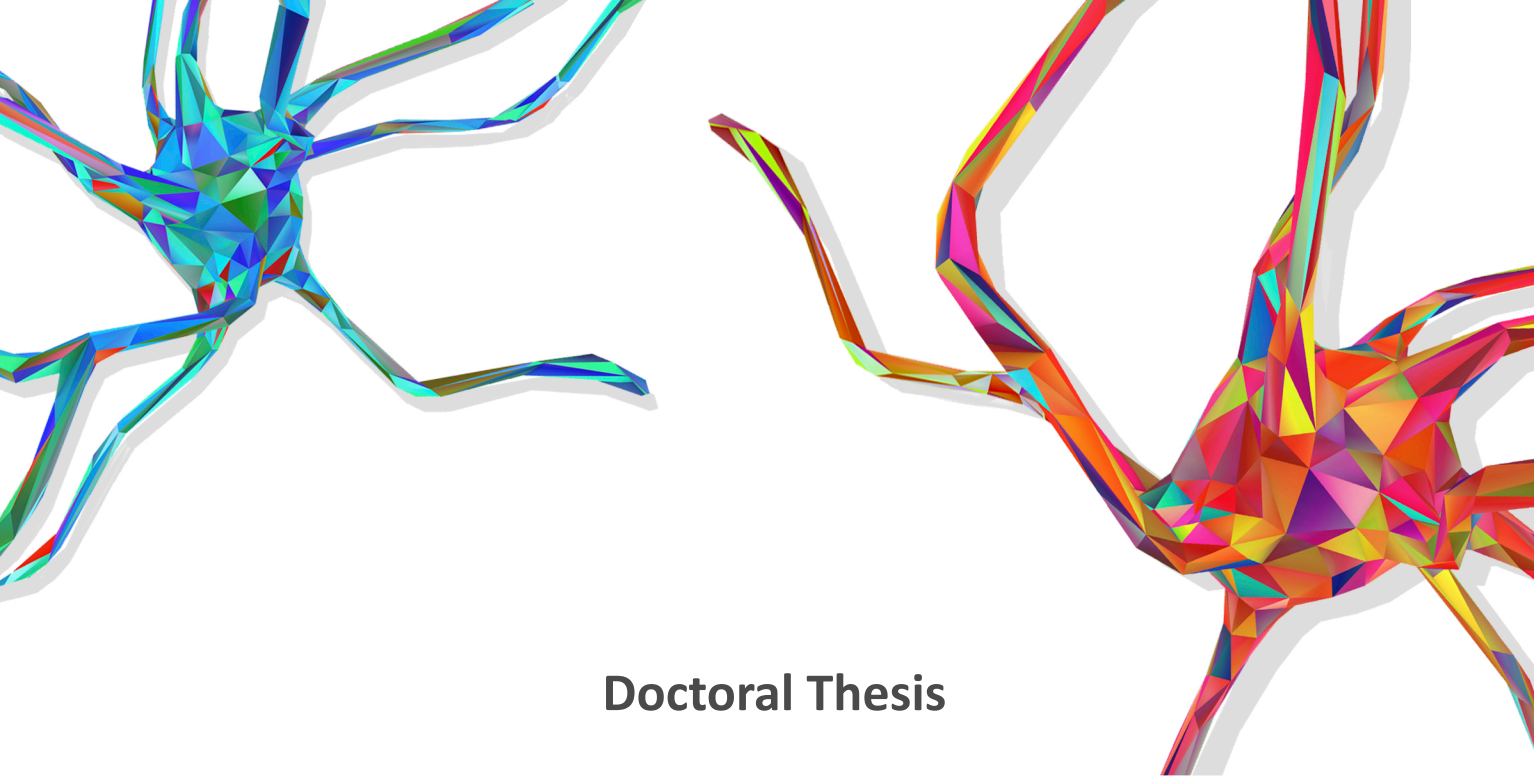
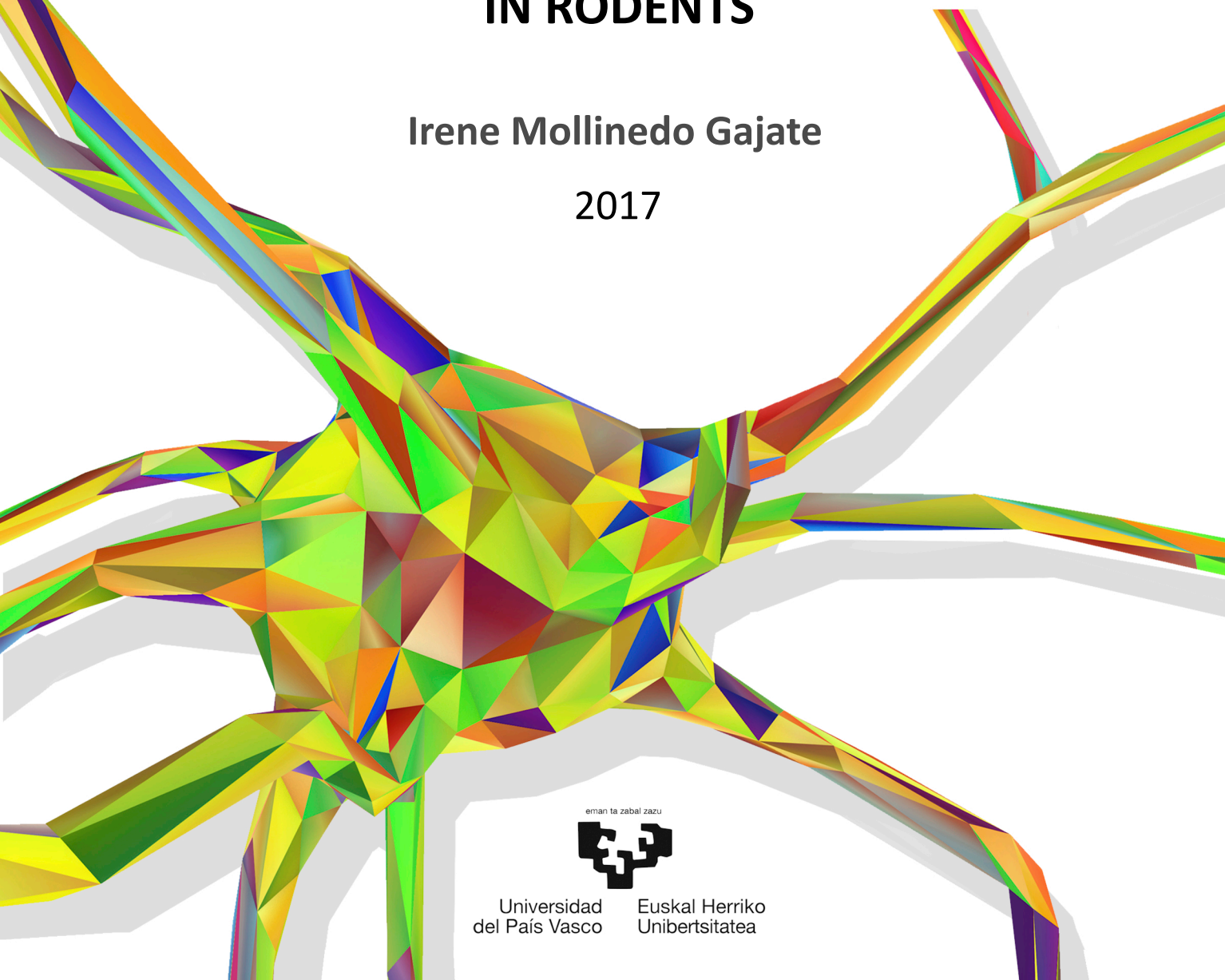


Doctoral Thesis

IN VIVO FUNCTIONAL ROLE OF THE 5-HT_{2A}/$mGlu_2$ RECEPTOR HETEROCOMPLEX IN RODENTS

Irene Mollinedo Gajate

2017



eman ta zabal zazu

Universidad del País Vasco

Euskal Herriko Unibertsitatea

eman ta zabal zazu

Universidad del País Vasco

Euskal Herriko Unibertsitatea

Doctoral Thesis

In vivo functional role of the 5-HT_{2A}/$mGlu_2$ receptor heterocomplex in rodents

Irene Mollinedo Gajate

2017

This Doctoral Thesis has been developed thanks to the financial support of a Predoctoral Training Fellowship from the University of the Basque Country (UPV/EHU), during 2013-2017.

Additionally, the present work has been funded by the Spanish Ministerio de Economía y Competitividad (SAF-2009/08460), the Instituto de Salud Carlos III, and the Basque Government (IT-616/13).

ABBREVIATION LIST

α_2	α_2-Adrenoceptor
A	Amygdala
A_1	Adenosine 1 receptor
AA	Arachidonic acid
AADC	Aromatic L-amino acid decarboxylase
AC	Adenylyl ciclase
ACC	Anterior cingulate cortex
AMPA	α-Amino-3-hydroxy-5-methyl-isoxazole-4-propionate
ANOVA	Analysis of variance
AON	Anterior olfactory nucleus
AP	Anteroposterior
AP-VAB	Ansa peduncularis–ventral amygdaloid bundle system
AT	Angiotensin receptor
ATPase	Adenosine triphosphatase
AUC	Area under the curve
BBB	Blood-brain-barrier
BDNF	Brain derived neurotrophic factor
BNST	Bed nucleus of the stria terminalus
BRET	Bioluminescence resonance energy transfer
BS	Brainstem nuclei
Ca^{2+}	Calcium
CAMK	Ca^{2+}/calmodulin-dependent protein kinase
cAMP	Cyclic adenosine monophosphate
CB	Cannabinoid receptor
CC	Corpus callosum
CER	Cerebellum
CNS	Central nervous system
CNV	Copy number variation

COMT	Catechol-*O*-methyl-transferase
CSF	Cerebrospinal fluid
CTT	Central tegmental tract
D	Dopamine receptor
DA	Dopamine
DAG	Diacylglycerol
DAT	Dopamine transporter
DB	Dorsal bundle
DBH	Dopamine-β-hydroxylase
DDC	DOPA descarboxylase
DISC-1	Disrupted in schizophrenia-1
DOB	2,5-Dimethoxy-4-bromoamphetamine
(±)-DOI	2,5-Dimethoxy-4-iodoamphetamine
DOM	2,5-Dimethoxy-4-methylamphetamine
DOPAC	3, 4- Dihydroxyphenyl-acetic acid
DPS	Dorsal periventricular system
DPX	Distyrene plasticizer xylene
DR	Dorsal raphe nucleus
D-ser	D-serine
DSP4	(N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride)
DV	Dorsoventral
E	Epinephrine
EAA	Excitatory amino acid
EC	Entorhinal cortex
ECL	Extracellular loop
EDTA	Ethylenediaminetetraacetic acid
$\mathbf{E_{max}}$	Maximal effect
EPS	Extrapyramidal side effects
EPSCs	Excitatory postsynaptic currents
ErbB4	ErbB-type tyrosine kinase receptor B

F	Fornix
FC	Frontal cortex
FISH	Fluorescent *in situ* hybridization
FITC	Fluorescein isothiocyanate
FR	Fasiculus retroflexus
FRET	Fluorescence resonance energy transfer
GABA	γ-Aminobutyric acid
GDP	Guanosine diphosphate
GFP	Green fluorescent protein
GHSR1a	Ghrelin receptor 1a
GIRK	G-protein-sensitive inwardly rectifying K^+ channel
Glu	Glutamate
GPCR	G protein-coupled receptor
GPIb	Human platelet glycoprotein Ib
GTP	Guanosine triphosphate
GTPγS	Guanosine 5'-O-[gamma-thio]triphosphate
GWAS	Genome wide association studies
H	Hypothalamus
H^+	Proton
HEK-293	Human embryonic kidney 293 cellular line
HF	Hippocampal formation
HPLC	High-performance liquid chromatography
5-HT	5-Hydroxytryptamine (serotonin)
5-HT2AR	Serotonin 2A receptor, $5\text{-}HT_{2A}$
$5\text{-}HT2AR^{-/-}$	Serotonin 2A receptor knock-out, 5-HT2AR-KO
$5\text{-}HT2AR^{+/+}$	Serotonin 2A receptor wild-type
5-HT2CR	Serotonin 2C receptor, $5\text{-}HT_{2c}$
5-HTP	5-Hydroxytryptophan
HTR	Head-twitch response
HSV	*Herpes simplex* virus

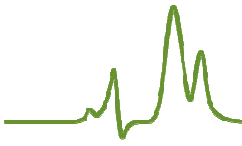
HVA	Homovanilic acid
Hz	Hertz
ICL	Intracellular loop
i.c.v.	Intracerebroventricular
iGluR	Ionotropic glutamate receptor
i.p.	Intraperitoneal
IP_3	Inositol-1,4,5-trisphosphate
K^+	Potasium
KA	Kainate
K_d	Dissociation constant
kDa	Kilodalton
kg	Kilogram
K_i	Inhibition constant
KYN	Kynurenic acid
L	Lateral
LC	Locus coeruleus
LGICR	Ligand-gated ion channel receptor
LSD	Lysergic acid diethylamide
LSD post hoc test	Fisher's Least Significant Difference
M	Molar
M100907	((R)-(2,3-Dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol; Volinanserin)
MAO	Monoamine oxidase
mCPP	Meta-chlorophenylpiperazine
mFC	Medial frontal cortex
mGluR2	Metabotropic glutamate 2 receptor, $mGlu_2$
mGluR2$^{-/-}$	Metabotropic glutamate 2 receptor knock-out, mGluR2-KO
mGluR2$^{+/+}$	Metabotropic glutamate 2 receptor wild-type
mGluR3$^{-/-}$	Metabotropic glutamate 3 receptor knock-out, mGluR3-KO
mGluR3$^{+/+}$	Metabotropic glutamate 3 receptor wild-type

µl	Microliter
ml	Milliliter
µM	Micromolar
mM	Millimolar
MOPEG	3-Methoxy-4-hydroxyphenylethyleneglycol
MOR	Mu-opioid receptor
MRI	Magnetic resonance imaging
ms	Milliseconds
MT	Melatonin receptor
3-MT	3-Methoxytyramine
Na^+	Sodium
NAcc	Nucleus accumbens
NAM	Negative allosteric modulator
NE	Norepinephrine (also termed noradrenaline, NA)
NET	Norepinephrine transporter
nM	Nanomolar
NMDA	*N*-methyl-*D*-aspartate
NR1	Subunit 1 of NMDA receptor
NRG-1	Neuregulin-1
OB	Olfactory bulb
OSA	1-Octanesulfonic acid
PAG	Periaqueductal gray area
PAM	Positive allosteric modulator
PAZ	Presynaptic active zone
PBS	Phosphate buffered saline
pc	Pars compacta
PC	Piriform cortex
PCP	Phencyclidine
PDE4B	Phosphodiesterase 4B
Peri-LC	Pericoerulear area

PET	Positron emission tomography
PFA	Paraformaldehyde
PFC	Prefrontal cortex
PGi	Paragigantocellularis nucleus
PIP_2	Phosphatidylinositol-4,5-bisphosphate
PKC	Protein kinase C
PLA_2	Phospholipase A_2
PLC	Phospholipase C
Poly(I:C)	Polyinosinic-polycytidilic acid
PPI	Prepulse inhibition
PPTg	Pedunculopontine tegmental nucleus
PrH	Prepositus hypoglossi nucleus
PSD	Postsynaptic density
PSD95	Postsynaptic density protein 95
PTX	Pertussis toxin
PV	Parvoalbumin
RF	Reticular formation
RGS	Regulators of G protein signaling
RGS4	Regulator of G protein signaling 4
Ro 60-0175	((αS)-6-Chloro-5-fluoro-a-methyl-1H-indole-1-ethanamine monofumarate)
RT	Retention time
RX821002	(2-[2-(2-Methoxy-1,4-benzodioxanyl)]imidazoline hydrochloride)
S	Septum
SB242084	(6-Chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline dihydrochloride hydrate)
SC	Spinal cord
SERT	Serotonin transporter
SN	Substantia nigra
SPA	Scintillation proximity assay
TCR	T-cell receptor complex

TH	Tyrosine hydroxylase
THC	Δ^9-Tetrahydrocannabinol
TLR4	Toll-like receptor 4
TM	Transmembrane domain
TPH2	Isoform 2 of tryptophan hydroxylase
TTX	Tetrodotoxin
UHPLC	Ultra-high performance liquid chromatography
VEH	Vehicle
VMA	Vanillylmandelic acid
VMAT	Vesicular monoamine transporter
VTA	Ventral tegmental area

INDEX



1. INTRODUCTION

1.1. SCHIZOPHRENIA

1.1.1. Epidemiology, symptoms and aetiology

Psychotic phenomena are abnormal mental states involving loss of contact with reality. The understanding of its triggering agents has been the spotlight of numerous attempts throughout recorded history, tracing back to Plato and Aristotle era. Even many observations have noted similarities between three major, still partially known, processes namely dreams, hallucinogenic drug states, and psychosis. Thus, Kant stated "The lunatic is a wakeful dreamer". Shopenhauer wrote "A dream is a short-lasting psychosis, and a psychosis is a long-lasting dream". Freud simply marked "A dream, then, is a psychosis" (Fischman, 1983). A different current of thought states that schizophrenia is the price that humans pay for language (Crow, 2000). Therefore, the understanding of the aetiology and course of psychosis has become the cornerstone for the better understanding of the disease, crucial to develop effective treatments.

The German psychiatrist Emil Kraepelin (1896) was the first to describe schizophrenia as a neurodegenerative disease, introducing the term "dementia praecox" in the book "Psychiatrie: Ein Lehrbuch für Studirende und Aertze". He undertook extensive research on psychiatric symptomatology classification, psychiatric degenerative processes and risk factors that predispose to the development of the disease. The reputable Swiss psychiatrist Paul Eugen Bleuler understood the illness as a neurodevelopmental disorder stating the term "schizophrenia" for the first time in a conference in 1908. Bleuler thought that "dementia" was an effect not directly caused by the underlying biological process and made great efforts to understand the complexity of the disease (Bleuler, 1978).

After more than a century of studying schizophrenia, the causes of the disorder remain poorly understood. Pharmacological treatments have been in wide use for nearly half a century, yet there is little evidence that these treatments have substantially improved the course of the disease for many patients with schizophrenia. Such unsatisfactory results lead to rethink schizophrenia as a neurodevelopmental disorder with psychosis as the latest stages, and that could be potentially prevented gaining insight into its aetiology.

Schizophrenia is a chronic and disabling mental illness that affects roughly 0.7% of the population worldwide (Faludi *et al*., 2011). Although the incidence is relatively low (McGrath *et al*., 2008), its chronicity makes the disease burden greater, being ranked as one of the top ten causes of long-term disability (Murray & Lopez, 1996; Carr *et al*., 2003; Mueser & McGurk, 2004). The onset of the disease is most commonly during adolescence or early adulthood (16-30 years) (Mueser & McGurk, 2004). A later age of onset in females has been reported (Hambrecht *et al*., 1992; Jablensky *et al*., 1992; Häfner *et al*., 1993; Castle *et al*., 1995; Takahashi *et al*., 2000; Venkatesh *et al.*, 2008), likely due to gonadal hormones and their interactions with neurotransmitters (Halbreich & Kahn, 2003).

To establish reliable criteria for diagnosing schizophrenia and closely related behavioral disorders, the guidelines most commonly used by psychiatrists are the Tenth Revision of the International Classification of Disease (ICD-10) (World Health Organization, 1992), and the Fifth Edition of the Statistical Manual of Mental Disorder (DSM-5) (American Psychiatric Association, 2013).

Schizophrenia symptoms are grouped into three main categories:

- Positive symptoms: visual, auditory (most frequently), olfactory or tactile hallucinations, and false beliefs (delusions). These symptoms tend to occur in episodes, separate by full or partial remissions. Neuroleptic drugs are generally more effective for positive than negative symptoms. Acute exacerbations of these symptoms might be precipitated by major life events or critical family atmosphere.

- Negative symptoms: apathy (no interest or unwilling to initiate or follow through on plans), emotional blunting (e.g. immobile facial expression, monotonous voice tone), anhedonia (lack of pleasure), social withdrawal and alogia (impoverished language). These symptoms are typically continuous rather than episodic, although do not affect all schizophrenic patients.

- Cognitive impairment: difficulties in attention and concentration, learning, memory, and executive functions (e.g. abstract thinking and problem solving).

The study of the aetiology of schizophrenia has been the focus of intensive research for decades, albeit is poorly known so far. However, both genetic and environmental factors appear to be key players in the emergence of the disease. While some researchers understand schizophrenia as a neurodevelopmental disorder, having its roots in early brain development followed by neurological and behavioral abnormalities since childhood (Bender, 1947; Watt, 1978); others converge towards a neurodegenerative hypothesis of schizophrenia as a "dementia praecox" with a progressive downhill course (Pino *et al*., 2014). Nevertheless, both theories are synergic rather than exclusive, inasmuch as they can individually explain phenomena observed in different stages of the illness (Berlim *et al*., 2003; Gupta & Kulhara, 2010).

1.1.1.1. Genetic risk factors

Kraepelin already mentioned that about 70% of his patients with dementia praecox had a family story of psychosis. Compelling evidence posits the importance of genetic factors in the development of schizophrenia. Siblings or offspring of first degree schizophrenic patients are up to ten times at higher risk than the general population, reaching up to 46% higher probability of developing the disease when both parents are affected, and 85% when a monozygotic twin is affected (McGuffin *et al.*, 1995; Cardno *et al.*, 1999). That risk decreases in second and third degree relatives of schizophrenia, but it is still higher than in the general population.

A substantial number of candidate genes underlying genetic risk for schizophrenia has been postulated using genome wide association studies (GWAS) and copy number variation studies (CNV) (Lee *et al*., 2012), including: neuregulin-1 (*NRG1*), catechol-*O*-methyl-transferase (*COMT*), brain derived neurotrophic factor (*BDNF*), disrupted in schizophrenia-1 (*DISC1*), *N*-methyl-*D*-aspartate (*NMDA*) receptor subunit (*NR1*), regulator of G protein signaling 4 (*RGS4*), Dysbindin, Reelin, phosphodiesterase 4B (*PDE4B*) (Berry *et al*., 2003; Di forti *et al*., 2007; Gray & Roth, 2007; Carpenter & Koenig, 2008; Lu *et al*., 2011) (Figure 1).

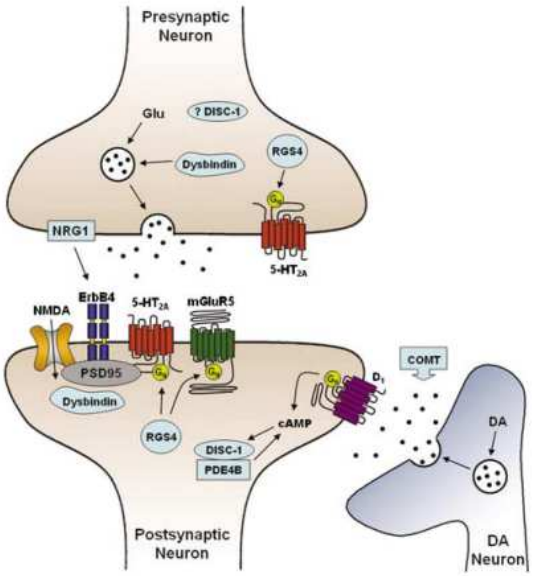


Fig. 1- Hypothetical schematic picture of various putative candidate genes, which confer susceptibility to develop schizophrenia, including DISC-1, Dysbindin, NMDA, NRG1, RGS4, and PDE4B. Other abbreviations are: Glu (glutamate), DA (dopamine), 5-HT_{2A} (serotonin 2A receptor, 5-HT2AR), mGluR5 (metabotropic glutamate 5 receptor), D_1 (dopamine receptor 1), ErbB4 (ErbB-type tyrosine kinase receptor B4), cAMP (cyclic adenosine monophosphate), G_q/G_s (G proteins), PSD95 (postsynaptic density protein 95), D-ser (D-serine) (Adapted from Gray & Roth, 2007).

However, over 60% of schizophrenic patients have no first or second degree relatives with the disorder (Bleuler, 1978), pointing out that environmental factors may also play an important role.

1.1.1.2. Environmental risk factors

Several biological and psychosocial factors have been posited to play a role in the emergence of schizophrenia (Broome *et al*., 2005). Maternal viral infections (Mednick *et al*., 1988; Barr *et al*., 1990; Shi *et al*., 2003; Brown & Derkits, 2010; Moreno *et al.*, 2011b), obstetric complications (Goodman, 1988; Thomas *et al*., 2001; Cannon *et al*., 2002), maternal nutritional deficits (Andrews, 1990; Susser *et al*., 1996; Mackay *et al*., 2017), paternal age (Messias *et al*., 2007), and cannabis use (Bangalore *et al*., 2008; Wilkinson *et al*., 2015) among others, have all been suggested to increase the risk of the illness.

In terms of sociodemographic factors, Bruce and co-workers (1991) demonstrated that lower social classes are at higher risk for a number of psychiatric disorders, including schizophrenia. People living in urban regions would also be at higher risk than those living in rural areas (Peen

& Dekker, 1997; Marcelis *et al*., 1999; McGrath *et al*., 2004; van Os, 2004; Krabbendam & van Os, 2005; Spauwen *et al*., 2006). Even some authors have reported that season of birth can influence the incidence of developing schizophrenia, positing winter and early spring as the season with higher risk to develop the disease (Mortensen *et al*., 1999; Dean & Murray, 2005).

There is controversy about the incidence variation of schizophrenia across countries. The influential study of the incidence of schizophrenia among 10 countries, the WHO 10 Nation Study, points schizophrenia as an egalitarian disorder, affecting equally to the population all over the world. However, prognosis is better in developing countries patients than in developed countries (Jablensky *et al*., 1992). Elevated rates are present in some ethnic minorities, likely attributable to the stress of being a minority, which could lead to increased vulnerability to schizophrenia in biologically predisposed individuals (Boydell *et al*., 2001). Conversely, others support that schizophrenia rates vary internationally (McGrath, 2005; Messias *et al*., 2007).

A relatively new theory in vogue to explain processes of schizohrenia links neuroinflammation with higher risk of developing schizophrenia (Müller *et al*., 2015; Pasternak *et al*., 2016). In this sense, pro-inflammatory cytokines could lead to structural neuroanatomic alterations in regions of the brain implicated in schizophrenia (Ellman *et al*., 2010). Even changes in intestinal microbiota composition, as a factor to yield inflammation, have been postulated to confer risk of schizophrenia, through a gut-brain axis. Increased incidence of gastrointestinal barrier dysfunction, food antigen sensitivity, inflammation, and metabolic syndrome found in schizophrenic patients may be influenced by the gut microbiota composition (Nemani *et al*., 2015; Rogers *et al*., 2016; Dinan & Cryan, 2017).

1.1.2. Antipsychotic treatment

Antipsychotic drugs are the keystone in the treatment and management of schizophrenia. To date, none of them cure the disease, but they greatly reduce the symptoms, especially the positive symptoms, and bestow on patients a better quality of life.

Dating back to 1950s, the French doctor Henri Laborit serendipitously discovered the drug chlorpromazine when looking for sedative drugs to treat states of shock. Later on, the French psychiatrists Jean Delay and Pierre Deniker proved its intrinsic antipsychotic effects

suppressing hallucinations and delusions in psychotic patients. That was the beginning of the modern pharmacological era. From that time on, increasing efforts have been made to discover new antipsychotic treatments. The "first-generation antipsychotics" or "typical antipsychotics", known also as "neuroleptics", encompass chlorpromazine, fluphenazine, haloperidol, loxapine, perphenazine, thioridazine, thiothixene and trifluoperazine. They can effectively treat positive symptoms, but their efficacy in treating negative and cognitive symptoms is limited. They all block dopamine D_2 receptors, even those located in the nigrostriatal core, increasing the risk of developing extrapyramidal side effects (EPS) (e.g. bradykinesia, tremor and rigidity). This generation of drugs can also impair cognitive and emotional symptoms. Thus, its use has been declined in the last few years, being replaced by the "second-generation antipsychotics".

Shortly after clozapine was approved by the Food and Drug Administration (FDA), in 1990, the era of "atypical antipsychotics" - also termed "second-generation antipsychotics" - began. Clozapine stood out as an effective psychotic treatment, ameliorating positive symptoms, even in antipsychotics non-responder patients (Carpenter & Koenig, 2008; Swartz *et al.*, 2008; Van Sant & Buckley, 2011), improving cognitive impairment (Meltzer, 1999), and sharply reducing the rate of suicide attempt, the main case of premature death in schizophrenic patients (Meltzer, 2001). However, the dark side of clozapine that restricts its prescription as a first choice is the risk of suffering fatal agranulocytosis, estimated to occur among 1-2% of treated patients in the first months under treatment (Alvir *et al*., 1993). That was how clozapine gave way to other atypical antipsychotic drugs as first choice use such as quetiapine, risperidone, olanzapine, aripiprazole, asenapine, lurasonide, paliperidone and ziprasonide, with similar pharmacological properties, but without agranulocytosis risk. Normal doses of these drugs produce little or no EPS, but other kind of side effects, mainly metabolic adverse effects which comprise weight gain, raising blood sugar, cholesterol and triglyceride levels (Melkersson & Dahl, 2004).

Meltzer (1989) postulated the hypothesis that what really confers effectiveness to antipsychotic treatments is the serotonin 2A receptor (5-HT2AR) and dopamine D_2 receptor blockade ratio (5-HT2AR:D_2), since high ratios would trigger negative symptoms, whereas low ratios would evoke positive symptoms. Atypical antipsychotic drugs are characterized by their low affinity to block D_2 receptors as well as their profile as 5-HT2AR antagonists. The optimal occupancy of D_2 receptor appears to be crucial to balance efficacy and side effects (Meltzer,

1989; Meltzer *et al*., 1989). These drugs are able to block D_2 receptor transiently (termed the "fast-off-D2 theory"), sufficient to exert antipsychotic effects with few or no EPS (Seeman, 2014). They are also antagonists at 5-HT2AR, able to modulate dopamine (DA) neuronal firing in areas such as dorsal striatum, prefrontal cortex (PFC), and nigrostriatum. The ability to enhance dopaminergic activity in the former areas might explain their efficacy for negative and cognitive symptoms (likely related to prefrontal dopaminergic hypoactivity). The increased in DA release in nigrostriatal areas may balance the D_2 antagonism, resulting in milder EPS (Kessler *et al*., 2005). Animal studies have revealed that 5-HT2AR antagonists have antipsychotic-like effects (Varty *et al*., 1999). However, a subsequent clinical trial confirmed that the selective 5-HT2AR antagonist M100907 was more effective than placebo at treating schizophrenia, but did not show significantly greater efficacy than the typical antipsychotic haloperidol in neuroleptic-responsive patients (de Paulis, 2000; Halberstadt & Geyer, 2013b). Even M100907 could worsen negative symptoms of schizophrenia and withdrawal syndrome triggered by chronic drug use (Semenova & Markou, 2010). Ritanserin, a drug with high affinity at 5-HT2AR and serotonin 2C receptor (5-HT2CR) and much lower affinity at D_1, α_1- and α_2-adrenoceptors (Leysen *et al*., 1985) has never marketed for clinical use as antipsychotic alone, but it could be useful as an adjunctive treatment strategy for the negative symptoms of schizophrenia (Akhondzadeh *et al*., 2008). Recently, a selective 5-HT2AR inverse agonist (pimavanserin, ACP-103) which does not block D_2 receptros (Stahl, 2016), has been reported to reverse psychosis-like behaviors associated with Parkinson's disease (Ballanger *et al*., 2010; McFarland *et al*., 2011; Sarva & Henchcliffe, 2016; Bozymski *et al*., 2017).

α-Adrenoceptors, α_1 and α_2, are other target receptors of some atypical antipsychotics. It has been shown that risperidone can antagonize α_2-adrenoceptors, and this antagonism could likely be a plus in its efficacy as antipsychotic (Raja, 2009). Dopamine D_1 and D_3 receptors, as well as the 5-HT2CR antagonism, confer properties that may also play a role in their efficacy, as well as the agonist effects at serotonin 1A receptor (5-HT1AR) (Horacek *et al*., 2006).

According to the Practice Guideline for the Treatment of Patients with Schizophrenia, Second Edition (American Psychiatric Association, APA, 2010), the appropriate treatment of schizophrenia depends on the phase of the disorder:

- Acute phase: the aim is to treat psychotic symptoms, prevent harm and control disturbed behavior. Frequently higher doses of antipsychotics are employed. Oral medication is mostly preferred, albeit non-adherent patients with recurrent relapses

are candidates for long-lasting antipsychotic injectables. Special care must be taken to protect patients from suicidal intentions. Lifetime risk of suicide among schizophrenic patients is 12-25 times greater than in the general population (Meltzer, 2001; Dutta *et al*., 2010). Sometimes co-treatment with other kind of drugs such as benzodiazepines is required.

- Stabilization phase: dosage is monitored to prevent adverse effects, and improves adherence to treatment in order to promote the recovery, minimize relapses and let patients return to a normal life.

- Stable phase: treatment is usually life-long maintained at a minimal effective dose, regularly monitored to ensure controlled symptom remission and adverse side effects. While most of schizophrenic patients improve with antipsychotic treatment, there is a minority of adherent patients who do not respond to medications, termed "treatment resistant patients". By contrast, a few of them seem not to need it (Harvey & Rosenthal, 2016). Adjuvant psychosocial interventions are also recommended in all phases of schizophrenia.

1.1.3. Animal models of schizophrenia

Animals are widely employed to mimic physiological or pathophysiological processes occurred in humans with the ultimate goal of gaining insight into the biology, pathophysiology and treatment of a human disease. Animal models serve as tools for studying psychiatric disorders, inasmuch as they allow the use of research methods which are impossible to undertake in humans for ethical reasons. Many approaches are employed to generate animal models, such as genetic engineering, brain lesions, environmental manipulations, prenatal drug exposures, optogenetic tools or pharmacological treatments (Nestler & Hyman, 2010; Jones *et al.*, 2011).

However, the main limitation of the use of animal models to study psychiatric disorders is the uniquely human nature of psychiatric diseases, with a strong subjective component. Thus, the effects observed in humans cannot be directly reproduced in animals. For this reason, animal models are generated as proxies for specific signs or symptoms linked to an illness.

The validity and reliability of animal models must be evaluated using established criteria guidelines. There are three types of validators (Figure 2):

- Construct validity: animals must present the etiological processes of the illness (e.g. knocking out a specific gene that potentially confers susceptibility to develop a disease in humans) (Chadman *et al.*, 2009).

- Face validity: indicates the plausibility to reproduce anatomical, behavioral, biochemical, neuropathological features and/or neurochemical abnormalities of the human disease.

- Predictive validity: ability of the pharmacological effective treatment in humans to revert the signals and symptoms evoked in that animal. It allows the discovery of potential novel therapeutic approaches.

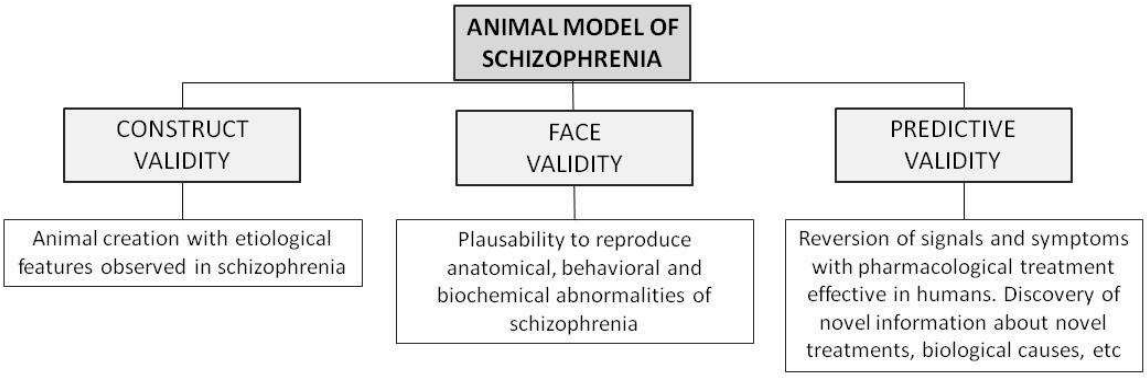


Fig. 2- Schematic diagram of the three types of validators necessary for creating animal models of schizophrenia. Based on Jones *et al.*, (2011).

Many different animal models of schizophrenia have been created and postulated to lend pro-psychotic properties, although modeling psychosis in animals remains controversial since is difficult to evaluate psychosis-like states in the absence of verbalization. Various genetically modified mice, knocking down candidate genes (e.g. *DISC1*, *NRG1*, *erbB4*, Dysbindin and Reelin), or overexpressing specific proteins (e.g. Munc 18-1), have been reported to resemble behavioral components of schizophrenia (Carpenter & Koenig, 2008; Jones *et al.*, 2011; Urigüen *et al.*, 2013). Other approaches that have triggered positive and negative symptoms as well as cognitive deficits of schizophrenia include: environmental-based interventions, such as

post-weaning social isolation; virus analogue administration to pregnant mice using polyinosinic-polycytidilic acid (poly(I:C)); pharmacological approaches; or brain lesions, such as in ventral hippocampus (Carpenter & Koenig, 2008; Jones *et al*., 2011; Koh *et al*., 2016; MacDowell *et al*., 2016).

The most common pharmacological approaches to recapitulate symptoms of schizophrenia in healthy humans and rodents include the use of NMDAR antagonists (e.g. PCP, ketamine and MK-801), hallucinogenic 5-HT2R agonist drugs (such as psilocybin, (±)-DOI or lysergic acid diethylamide - LSD -), monoamines uptake inhibitors (such as amphetamine and cocaine), and the primary active component of cannabis, Δ^9-tetrahydrocannabinol (THC) (Steeds *et al*., 2015). They all produce psychotic symptoms and cognitive disturbances in healthy humans and rodents (Quednow *et al*., 2012; Hanks & González-Maeso, 2013; Steeds *et al*., 2015; Talpos *et al*., 2015; De Gregorio *et al*., 2016). However, it is important to note that, in contrast to drug models, schizophrenia is chronic, neurodevelopmental, and episodic, with different symptom domains predominating at different stages. Therefore, any purely pharmacological model is likely destined to be incomplete in the extent to which it can represent the full picture of schizophrenia.

In humans, the psychostimulants amphetamine and cocaine, the dissociative anesthetics PCP and ketamine, the hallucinogenic 5-HT2AR agonists LSD and psilocybin, as well as THC have been reported to induce psychotic states in healthy individuals and exacerbate psychotic episodes in people with existing schizophrenia (Lahti *et al*., 1995; D'Souza, 2007; Koethe *et al*., 2009; Morrison *et al*., 2009; Morrison & Stone, 2011). Positive symptoms induced by these drugs include agitation, anxiety, visual hallucinations and illusions, which are similar to symptoms seen in the first psychotic episode of the illness (Hyde *et al*., 1978; Fletcher & Honey, 2006). Acute doses of ketamine in healthy volunteers induce schizophrenic-like positive and negative symptoms, and may also lead to impairments in cognitive functions that resemble schizophrenia (Morgan *et al*., 2004; Krystal *et al.,* 2005; Deakin *et al*., 2008; Stone *et al*., 2012). However, there are several dissimilarities between the ketamine-, THC- and hallucinogenic 5-HT2AR agonists-induced states and schizophrenia (Koethe *et al*., 2006; Steen *et al*., 2006). For instance, auditory hallucinations are one of the most common symptoms in this disease, but the hallucinations and illusions experienced following acute administration of the latter drugs are more commonly visual (Abi-Saab *et al*., 1998; Klosterkotter *et al*., 2001; Steen *et al*., 2006; Geyer & Vollenweider, 2008). On this basis, it has been suggested that,

rather than modeling chronic schizophrenia, acute ketamine or hallucinogenic 5-HT2AR agonist drugs administration may induce a state closer to the prodrome/early stages of schizophrenia (Klosterkotter *et al*., 2001; Corlett *et al*., 2007).

On the other hand, chronic PCP and ketamine users have persistent schizophrenia-like symptoms (Krystal *et al*., 1994; Jentsch & Roth, 1999), and such patients may display a symptomatic profile that can so closely resemble schizophrenia (Abi-Saab *et al*., 1998). Reported symptoms triggered by chronic consumption of PCP and ketamine include paranoid delusions, persistent cognitive deficits, and greater incidence of auditory than visual hallucinations (Jentsch & Roth, 1999). Depressive and dissociative symptoms also increase with persistent use of ketamine (Morgan *et al*., 2004). The chronic abuse of these substances produces biochemical brain changes that are similar to those seen in schizophrenia patients. Chronic ketamine users have reduced PFC grey matter volume (Liao *et al*., 2011) and decreased blood flow in the frontal cortex, similar to that seen in schizophrenia (Hertzmann *et al*., 1990). Moreover, dopamine D_1 receptors are upregulated in the frontal cortex of patients with schizophrenia and in chronic ketamine users (Narendran *et al*., 2005). This upregulation is associated with cognitive impairment and dopaminergic hypofunction (Narendran *et al*., 2005).

Individuals with a family history of schizophrenia appear to be at greater risk of schizophrenic symptoms following LSD consumption, and it has been suggested that LSD use may lead to an earlier onset of this disease (Vardy & Kay, 1983).

In rodents, amphetamine, hallucinogenic 5-HT2AR agonists, and NMDAR antagonists administration have been reported to yield hyperlocomotion and repeated ("stereotyped") behaviors, including sniffing, chewing, head-weaving, backpedalling and turning, that may be related to the positive symptoms of psychosis. All these families of drugs produce negative symptoms (such as delusions in terms of basic associative learning processes, immobilization in the forced swim test, and social withdrawal) (Corbett *et al*., 1995; Sams-Dodd, 1998a,b, 1999; Corlett *et al*., 2007; Jenkins *et al*., 2008; Marona-Lewicka *et al*., 2011). Acute and repeated administration of the latter recreational drugs have been reported to develop also cognitive impairments, including spatial working memory (Schneider & Koch, 2003; Beraki *et al*., 2009; Rubino *et al*., 2008; Realini *et al*., 2009), and attentional disturbances (Corlett *et al*., 2007). Acute and chronic treatment with PCP, THC and hallucinogenic 5-HT2AR agonists in rodents impair prepulse inhibition (PPI), a marker of sensory gating impairment also described as altered in patients with schizophrenia (Jackson *et al*., 1994; Kitaichi *et al*., 1994; Ogren and

Goldstein, 1994; Jentsch & Roth, 1999; Schneider & Koch, 2003; Jones *et al*., 2011; Steeds *et al*., 2015). Notably, hallucinogenic 5-HT2AR agonists disrupt PPI through direct stimulation of the 5-HT2ARs (Aghajanian & Marek, 2000; Quednow *et al*., 2012).

One of the most used behavioral proxies performed in rodents for human hallucinogen effects is the drug-induced "head twitch" response (HTR) (Halberstadt & Geyer, 2014). The first description of a drug-elicited HTR was reported in 1956 by Keller and Umbreit, following intravenous administration of LSD to mice. They described an easily observed and quantifiable response similar to the reflexive response observed when the ear is touched or pinched (the pinna reflex response), or following LSD administration (which became 'permanent' in a subset of mice following an injection of indole followed by LSD). In their words: "The response consists of a rapid and violent head shaking. . . The head-twitch response does not occur in normal mice, and with a little experience the response is easy to detect. Independent observations by different workers are remarkably consistent, so that provided a suitable tool for the behavioral studies" (Canal & Morgan, 2012). HTR is well defined as a rapid side-to-side rotational head movement that occurs in rats and mice after injection of psychedelic compounds, such as LSD, (±)-DOI, 2,5-dimethoxy-4-methylamphetamine (DOM) or 2,5-dimethoxy-4-bromoamphetamine (DOB) (serotonergic agonists with high affinity for 5-HT2AR (Halberstadt & Geyer, 2011; Canal & Morgan, 2012; Canal *et al*., 2013), PCP (Hori *et al*., 2000), and other GPCR selective agonists (Vollenweider *et al*., 1998; González-Maeso *et al*., 2003; Moreno *et al*., 2011a). Head-twitches in rats are sometimes referred to as wet-dog shakes because in that species the behavior frequently involves the head, neck, and trunk (Bedard & Pycock, 1977). There is a body of evidence aiming at the 5-HT2AR as the main responsible for the occurrence of hallucinations in humans and the associated animal behavioral paradigms as HTR (Pranzatelli, 1990; Willins & Meltzer, 1997; González-Maeso *et al*., 2003; González-Maeso *et al*., 2007; Quednow *et al*., 2012; Kometer *et al*., 2013; Halberstadt, 2015). Notably, despite the fact that lisuride is a 5-HT2AR agonist, studies indicate that lisuride does not evoke HTR (González-Maeso *et al*., 2003; González-Maeso *et al*., 2007; Halberstadt & Geyer, 2010). Based on the behavioral inactivity of lisuride in this paradigm, it appears that the HTR has utility as a behavioral screen that can distinguish hallucinogenic *versus* non-hallucinogenic 5-HT2AR agonists (Geyer & Vollenweider, 2008). In recent years, HTR has become one of the few behaviors that can reliably distinguish hallucinogenic and non-hallucinogenic 5-HT2AR agonists, a behavioral response antagonized by 5-HT2AR antagonist antipsychotic drugs (González-Maeso *et al*., 2007).

Another proxy for schizophrenia in rodents is a deficit in PPI. PPI describes the phenomenon in which a weak initial stimulus (the prepulse) inhibits the startle response that is elicited by a strong stimulus. Deficient PPI is thought to demonstrate impaired sensorimotor gating that occurs in schizophrenia, but also in several other neuropsychiatric disorders (Sipes & Geyer, 1994; Powell *et al*., 2009; Vollenweider *et al*., 2007; Halberstadt & Geyer, 2010). Hallucinogens affect PPI in humans and rodents (Halberstadt & Geyer, 2013a; Hanks & González-Maeso, 2013).

In the present Doctoral Thesis, three types of genetically modified mice, animals lacking mGluR2, mGluR3 or 5-HT2AR, were used to gain insight into the pathophysiology of schizophrenia. Pharmacological approaches administering the psychedelic drug 2,5-dimethoxy-4-iodoamphetamine ((±)-DOI) have been used to mimic psychosis-like effects in mice, evaluating HTR.

1.2. NEUROTRANSMISSION NETWORKS: FOCUS ON DOPAMINE AND NOREPINEPHRINE

1.2.1. From DA and NE synthesis to synapse: a brief understanding

DA and norepinephrine (NE) - also termed noradrenaline (NA) - belong to a group of neurotransmitters termed catecholamines, due to the presence of a catechol ring and an amine side chain. The above catecholamines together with serotonin comprise the family of monoamines, in view of the existence of a unique amine group in their structures. They are neurotransmitters, endogenous chemicals released from synaptic vesicles into the synaptic cleft, where they interact with receptors on the target cells.

Tyrosine is the precursor amino acid (AA) for the synthesis of both monoamines. It is mainly provided with the dietary intake, albeit a small proportion is derived from hydroxylation of phenylalanine by the action of the liver enzyme phenylalanine hydroxylase. Tyrosine is transported by blood, can cross the blood-brain-barrier (BBB), and is finally taken up into neurons. Dopamine is synthesized in the cytoplasm of dopaminergic neurons by the action of two enzymes, the rate-limiting factor tyrosine hydroxylase (TH), and the aromatic L-amino acid decarboxylase (AADC), in this case DOPA descarboxylase (DDC) (Figure 3). Once DA is synthesized, it is translocated by the vesicular monoamine transporter (VMAT) into storage vesicles. DA can be further turned into NE inside the vesicles by the enzyme dopamine-β-hydroxylase (DBH) (Südhof & Starke, 2008). DA and NE can be synthesized and released from dendrites, as well as from terminal regions.

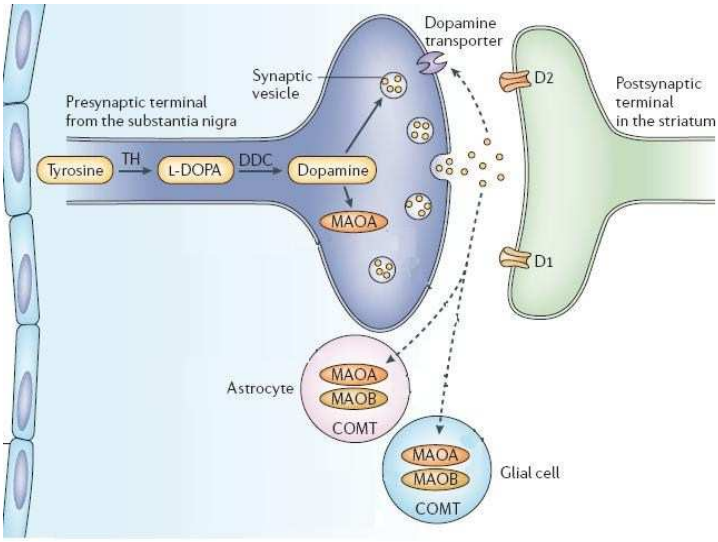


Fig. 3- Representative picture of DA synthesis, storage, release and metabolism**.** DA is synthesized from the amino acid tyrosine by two steps involving TH and DDC enzymes. Once synthesized DA is stored in axon terminals vesicles or can be degraded by action of MAOA. Once DA is released in the synaptic cleft can act at postsynaptic receptors, be metabolized by MAOA, MAOB and COMT or re-uptaken back into the presynaptic neurons through DAT.

Vesicle membrane contains an H^+-ATPase, which maintains the proton gradient that

energizes VMAT and preserves an acidic intravesicular environment. An action potential is generated by Na^+ entrance through the opening of its voltage-dependent channels, which depolarizes the membrane potential and triggers the activation of voltage-dependent K^+ channels, which leads to K^+ efflux. Upon the arrival of an action potential (input), which is initiated in a cell body and travels along the neuron to the terminal nerve, a change in membrane protein conformation allows the influx of Ca^{2+}, which is a key part to trigger the fusion of vesicles with the neuronal membrane and the neurotransmitter release (output) into the synaptic cleft, a process termed exocytosis (Hilfiker *et al*., 1999; Westfall *et al*., 2002). During this process, there is a fusion between the membranes of the vesicles and the cell membrane of the presynaptic neuron, which involves the action of certain proteins (SNARE proteins) to mediate the docking process, including: synaptobrevin, synaptotagmin, SNAP-25, syntaxin, and *N*-ethylmaleimide sensitive factor (NSF) (Südhof, 2004).

Once neurotransmitters are released and remain in the synaptic cleft they can target receptors on postsynaptic neurons, but also can fall prey of metabolic enzymes: monoamine oxidase isoforms (MAO) – MAOA located primarily in mitochondria of NE and DA neurons but also in glial cells and astrocytes, and MAOB located in glial cells and astrocytes -, and COMT - located in glia and in the external membrane of postsynaptic neurons -. Degradation metabolites of DA include: 3,4-dihydroxyphenyl-acetic acid (DOPAC), 3-methoxytyramine (3-MT), and homovanillic acid (HVA). On the other hand, when NE is degraded generates the following metabolites: normetanephrine, DOPAC, vanillylmandelic acid (VMA), 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG), and epinephrine (E) (Rang *et al*, 2007). Determination of the DOPAC/DA concentration ratio is a useful method to estimate rapid changes in neuronal activity. Released DA and NE in the synaptic cleft can be reuptake into synapses vesicles in the presynaptic neuron by the DA transporter (DAT) or the NE transporter (NET), respectively (Südhof & Starke, 2008).

1.2.2. Main DA-containing nuclei and projection pathways

DA has been largely associated with physiological and neuropathological processes such as arousal, stress, reward, social behavior, drug addiction, depression, and schizophrenia, among others (Graybiel, 2000; Vetulani, 2001; Dunlop & Nemeroff, 2007; Kress *et al*., 2013; Cho & Sohal, 2014; Laman-Maharg & Trainor, 2017).

The majority of the DA-containing neurons are clustered in two principal areas in the midbrain: the substantia nigra (SN) and the ventral tegmental area (VTA) (Tritsch & Sabatini, 2012). DA neurons appear to be capable of storing and releasing DA from somatodendritic areas in the SN (Cheramy *et al*., 1981) and in the VTA (Kalivas & Duffy, 1991) in a calcium-dependent, tetrodotoxin (TTX)-sensitive manner. DA can be released by axonal and somatodendritic sites and both mechanisms can be regulated by similar factors, although somatodendritic release is less influenced by action potential generation and less responsive to some releasing agents (Kalivas & Duffy, 1991).

Ascending dopaminergic projections constitute the main DA pathways: the nigrostriatal pathway, which arises from the SN and innervates predominantly the striatum (caudate-putamen); and the mesocorticolimbic pathway, which originates in the VTA and projects to the frontal cortex (FC) through limbic areas such as amygadala (A), nucleus accumbens (NAcc) and hippocampus (Albanese & Minciacchi, 1983; Sesack *et al*., 1995; Taylor *et al*., 2014) (Figure 4). The mesocorticolimbic system can be further subdivided into two subsystems, depending on the location of the dopaminergic cell bodies within the VTA, and the projecting areas. Thus, dopaminergic cells arising from the VTA projecting to the NAcc comprise the mesolimbic system, which is largely associated with reward, motivation and social behavior (Kim *et al*., 2016; Laman-Maharg & Trainor, 2017); whereas projections arising from the VTA to cortical structures comprise the mesocortical system, thought to be involved in the modulation of cognitive functions (Tzschentke, 2001; Alex & Pehek, 2007). The nigrostriatal dopaminergic system has been classically associated with the control of voluntary movement.

Another important dopaminergic loop in the brain is the hypothalamo-hypophyseal pathway, which transmits DA from the arcuate nucleus (infundibular nucleus) of the hypothalamus to the pituitary gland via DA release into the median eminence and subsequent circulation through the hypophyseal portal system (Björklund *et al*., 1968). This pathway regulates the secretion of certain hormones, such as prolactin, from the pituitary gland. Hypothalamus acts as a negative regulator of prolactin secretion in the pituitary systems, through the release of the neurotransmitter DA. Moreover, prolactin itself regulates the secretion of the inhibitory factor, DA (Grattan, 2015). MacLeod and co-workers (1970) demonstrated that dopaminergic agonists were effective at suppressing prolactin secretion *in vivo*, as well as the ability of DA as an inhibitor of prolactin secretion from pituitary glands. Supporting evidence were later found when DA receptors were identified on lactotroph cells in the anterior pituitary (Mansour *et al*., 1990), and after observing that mice lacking D_2 receptors are hyperprolactinemic (Kelly *et al*.,

1997; Saiardi *et al*., 1997). Altogether, clearly demonstrates the crucial role of DA in suppressing endogenous prolactin secretion.

Dopamine exerts its effects by interaction with the dopamine D_1 family receptors (comprising the D_1 and D_5 subtypes) and the D_2 family receptors (comprising the D_2, D_3, and D_4 subtypes). While activation of D_1 family receptors are thought to trigger excitatory effects, D_2 family receptors appear to display a pharmacological inhibitory profile. Presynaptic somatodendritic D_2 receptors located on dopaminergic neurons in the VTA play an important role as autoreceptors. Activation of these receptors inhibits the cell firing and the DA release in the VTA. Conversely, their blockade enhances DA activity (Adell & Artigas, 2004).

DA neurons exhibit at least three electrophysiological patterns of activity along which they are capable of activating postsynaptic transmitter release: a change from inactive to spontaneously discharging state, an increase in the rate of spontaneous spike discharge, and an alteration from single-spiking to a burst-discharge mode of activity. An additional state, involving depolarization block, occurs when an abnormally large demand, typically mediated by an exogenous pharmacological agent, causes the system to be overdriven to the inactivity state. DA neurons have been shown to be under a number of complex regulatory influences. Firstly, the membrane properties that drive spike activity in the DA neuron enable it to fire spontaneously in the absence of afferent activation, and to accommodate rapidly to maintained excitatory influences and regain its basal level of activity. Secondly, dendrodendritic interactions appear to underlie synchronization of activity as well as provide autoreceptor-mediated local feedback modulation of the neuron activity. Thirdly, the influences of afferent and feedback processes apparently play a minor role when the system is in a basal state, but provide a substantial impact on the discharge pattern and level of activity when the system is challenged pharmacologically or physiologically.

These multiple homeostatic factors appear to act in concert to enable the DA neuron to maintain a basal level of activity and compensate for maintained changes. It seems that the discharge of DA neurons is not involved in the maintenance of long-term changes in the midbrain, but instead only alters its activity state phasically in response to short-term demand (Hollerman & Grace, 1990). Hence, by preserving basal states of electrophysiological activity, the DA neuron conserves the dynamic range of electrophysiological response that can be rapidly drawn upon when required by the system. In fact, the ability of the DA system to maintain basal activity levels when faced with a tonic demand but respond rapidly with a massive phasic increase when required is consistent with the behavioral studies showing that

DA neuron discharge is only altered when the system is changing state, as occurs with the learning of a novel stimulus paradigm. On the other hand, when DA system is driven in a manner such that the biochemical compensations are inadequate to respond to the demand (resulting in a tonic activation of DA cell firing - for instance, with chronic neuroleptic treatment or near maximal DA depletions - or if one of the primary regulatory responses is thwarted (- for instance, administering a DA blocker to a system recovered from a lesion -), the ultimate consequence appears to be depolarization blockade of DA cell discharge and a reinstatement of the deficit state (Hollerman *et al*., 1992). However, under conditions in which the DA system appears to be showing an abnormal increased responsivity, such as schizophrenia (Grace, 1991), the induction of depolarization blockade may result the most effective way to refrain behaviorally mediated activation of this system (Grace, 1991; Hollerman *et al*., 1992; Grace, 1993).

Immunohistochemistry assays have also revealed that some DA neurons, especially in the hypothalamus, co-express γ-aminobutyric acid (GABA) and glutamate receptors, and might thus operate with more than one neurotransmitter or neuropeptide (Yamaguchi *et al*., 2015).

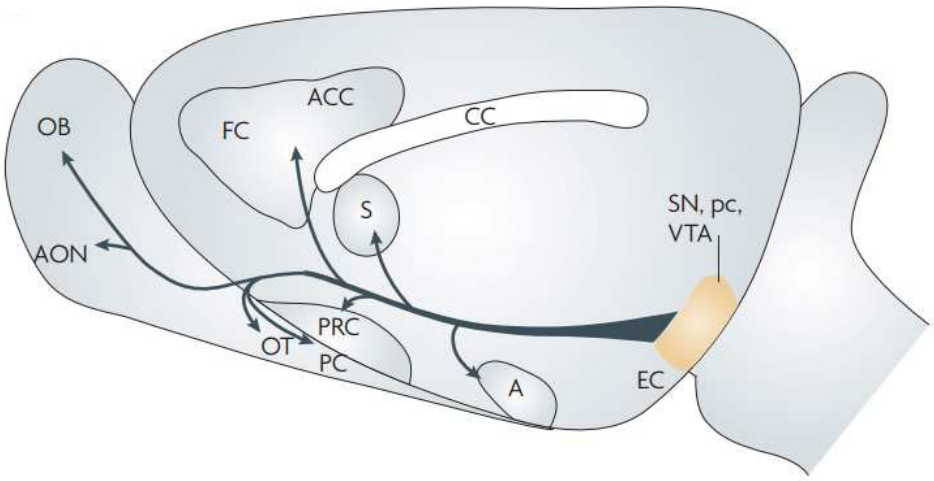


Fig.4- Schematic distribution of dopaminergic efferent projections in the rat brain. A: amygdala, S: septum, VTA: ventral tegmental area, SN: substantia nigra, EC: entorhinal cortex, PC: piriform cortex, ACC: anterior cingulate cortex, AON: anterior olfactory nucleus, CC: corpus callosum, FC: frontal cortex, OB: olfactory bulb, OT: olfactory tract, pc: pars compacta, PRC: perirhinal cortex (from Sara, 2009).

Although most of the neurons in the VTA are dopaminergic, this nucleus also contains GABAergic and glutamatergic neurons (Geisler & Wise, 2008; Stuber *et al*., 2012). Many afferent projections converge in the VTA displaying regulatory roles in the activity of this nucleus. Optogenetic approaches have allowed the identification of excitatory projections to the VTA arising from lateral hypothalamus, medial frontal cortex (mFC), lateral habenula,

pedunculopontine tegmental nucleus (PPTg), and inhibitory afferents arising from NAcc, amygdala and the bed nucleus of the stria terminalus (BNST) (Stuber & Mason, 2013; Yau *et al*., 2016). Dopamine cells also receive convergent afferents from the dorsal raphe nucleus (DR) (Phillipson, 1979), and 5-HT axon terminals can synapse onto DA neurons modulating its activity (Hervé *et al*., 1987). Indeed, the VTA contains one of the highest levels of 5-HT of the limbic system (Pessia *et al*., 1994).

1.2.3. Main NE-containing nucleus and projection pathways

The locus coeruleus (LC) - also termed A6 pontine noradrenergic cell group - is a small nucleus (comprising roughly 1500 neurons in rats) considered as the main source of noradrenergic neurons in the mammalian brain. Immunohistochemistry assays have revealed a vast amount of TH enzyme containing neurons mainly in the posterior levels of the LC, and most of these neurons also contain DBH, presumably being noradrenergic neurons (Swanson, 1976; Debure *et al*., 1992). LC is depicted adjacent to the fourth ventricle in the pontine brainstem (Maeda, 2000). The region surrounding the LC is termed pericoerulear area (peri-LC), which is densely populated by NE neuron dendrites from the LC (Luppi *et al*., 1995) and is a convergence site of terminal projections arising from various brain areas (Aston-Jones *et al*., 1991). LC neurons have long branched dendrites which can make axo-somatic, axo-dendritic and dendro-dendritic, and rarely axo-axonic synapses (Shimizu & Imamoto, 1970; Groves & Wilson, 1980). Even the axon collaterals of a single noradrenergic neuron can reach separated areas of the brain.

LC innervates and supplies NE throughout the central nervous system (CNS). It can modulate the activity of a high number of brain areas, standing out as a critical component of the neural architecture. Thus, it has been considered to play a role in a variety of physiological processes (e.g. sleeping, arousal, memory, and attention), behavioral impairments, mental diseases (e.g. attention-deficit hyperactivity disorder, depression, insomnia, narcolepsy, pain modulation, and stress-related disorders) or opiate withdrawal, among others (Singewald & Philippu, 1998; Vandergriff & Rasmussen, 1999; Berridge & Waterhouse, 2003; Llorca-Torralba *et al*., 2016).

NE neurons can fire following tonic and phasic activity patterns. Tonic activity is characterized by relatively low-frequency (between 1 Hz-15 Hz), sustained and regular discharge patterns.

On the contrary, phasic discharge rates are observed (generally in response to salient sensory stimuli) with a relatively short latency (15-70 ms), and are comprised of a brief burst of 2-3 action potentials followed by a more prolonged period of suppression of discharge activity (300-700 ms). Phasic discharge has been associated with sustained attention in tests of vigilance (Berridge & Waterhouse, 2003).

Other putative neuropeptides have been found in LC neurons, and they might also play a role as modulators acting in this area. Some of them display stimulatory effects, such as vasopressin, substance P, adrenocorticotropin hormone and corticotropin-releasing factor; whereas galanin, somatostatin, neuropeptide Y and enkephalin exert depressant effects (Olpe & Steinmann, 1991).

Although LC is the most important noradrenergic area, there are also some noradrenergic nuclei in the medulla oblongata involved in autonomic and endocrine regulations (termed A1, A2 and A5), which together with the A7 pontine cluster constitute the lateral tegmental group of NE cells (Guyenet, 1991).

NE neurons have ramified axons that project to a number of brain areas, such as neocortex - including FC -, raphe nuclei, thalamus, amygdala, hippocampus, hypothalamus, spinal cord and other nuclei (Austin & Takaori, 1976; Foote *et al*., 1983; Florin-Lechner *et al*., 1996) (Figure 5). LC is posited as the sole provider of NE in the FC and hippocampus, both areas highly implicated in cognitive and affective processes (Conrad *et al*., 1974; Foote *et al*., 1983; Berridge & Waterhouse, 2003).

Studies in monkeys have revealed a stratified distribution within the neocortex. While all six cortical layers possess NE innervation, the densest DBH-labelled fibers have being found in cortical layers III and IV, whereas layer I receives sparse innervation (Lewis *et al*., 1987). In rats, all cortical layers seem to be enriched in DBH-immunoreactive axons, although there are some morphological and geometrical distinct patterns in each layer (Grzanna *et al*., 1978). Layers IV and V appears to be terminal fields of NE neurons (Grzanna *et al*., 1978; Morrison *et al*., 1978). Electron microscopic histochemical techniques have provided new data on the presence of numerous monoaminergic axo-dendritic synapses in layer IV of somatosensory cortex in immature rats (Molliver & Kristt, 1975).

NE released in terminal areas can bind α- (α_1 and α_2) and β-adrenoceptors. Activation of α_1 and β-adrenoceptors generally leads to excitatory responses in the follower cells, whereas α_2 triggers inhibitory effects. α_2-Adrenoceptors are densely located in the LC acting as autoreceptors. Its activation evokes decreases in the firing rate of LC neurons and in the release of NE (Fernández-Pastor & Meana, 2002; Samuels & Szabadi, 2008). $GABA_A$, NMDA, non-NMDA glutamate, and muscarinic receptors in the LC also play roles in controlling the activity of NE neurons (Kawahara *et al*., 1999).

A wide array of areas projecting to the LC has been reported so far. Tract-tracing and electrophysiology studies have allowed a better understanding of the afferent fibers projecting to the LC. The two major afferent inputs arise from the excitatory nucleus paragigantocellularis (PGi) and the inhibitory nucleus prepositus hypoglossi (PrH), both located in the rostral medulla, which project toward peri-LC zones and to the LC proper (Aston-Jones *et al*., 1991; Luppi *et al*., 1995). Additionally, other minor projections mainly to the pericoerulear area have been found, arising from forebrain structures such as the cortex, the central nucleus of the amygdala, the medial, lateral and magnocellular preoptic areas, the BNST, and the dorsomedial, paraventricular and lateral hypothalamic areas.

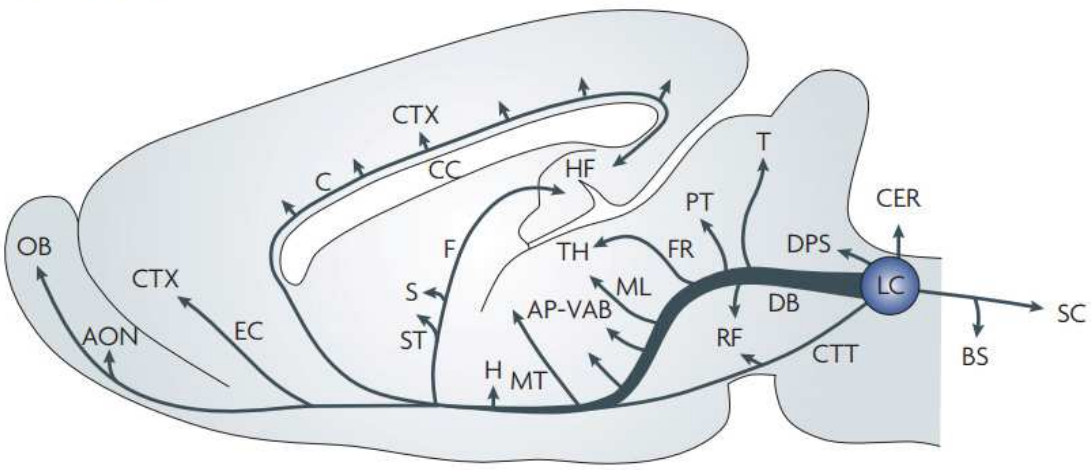


Fig. 5- Schematic distribution of noradrenergic efferent projections in the rat brain. LC: Locus coeruleus, S: septum, EC: entorhinal cortex, AON: anterior olfactory nucleus, AP-VAB: ansa peduncularis–ventral amygdaloid bundle system, BS: brainstem nuclei, CC: corpus callosum, CER: cerebellum, CTT: central 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. (from Sara, 2009).

From the brainstem, afferent projections have been found arising from the periaqueductal gray area (PAG), the raphe nuclei, the VTA, the reticular formation, vestibular, solitary tract and lateral reticular nuclei. In particular, the areas of catecholamine cell groups A1, A2 and A5 appeared to contain many reactive cells. Labeled neurons were also observed in the fastigial nuclei and in the marginal zones of the dorsal horns of the spinal cord (Cedarbaum & Aghajanian, 1978; Luppi *et al*., 1995).

LC neurons receive vast excitatory amino acid (EAA) inputs, primarily originating from the PGi, (Aston-Jones *et al*., 1991). The EAA antagonist kynurenic acid is able to block PGi-induced activation of the LC neurons (Ennis & Aston-Jones, 1988). The NMDAR antagonists AP5 or AP7 were not effective in either response, indicating that PGi-induced EAA activation of LC neurons probably takes place at a non-NMDAR in the LC. There are also some fibers arising from the FC to the peri-LC region (Luppi *et al*., 1995). Stimulation of neurons in the FC leads to an activation of LC neurons through EAA inputs acting at NMDAR and non-NMDAR, such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors (Jodo & Aston-Jones, 1997). Another region implicated in LC neurons activation is the PAG in the midbrain. Electrical stimulation in PAG was found to be able to vastly stimulate neurons in the peri-LC (Ennis *et al*., 1991).

Conversely, low frequency stimulation of the other major input to the LC, the GABAergic nucleus PrH, potently inhibit LC neurons discharge, presumably via $GABA_A$ receptors located in the LC. Microinfusion with the $GABA_A$ receptor antagonist bicuculline antagonizes this effect (Ennis & Aston-Jones, 1989).

The LC also receives noradrenergic inputs from the A1, A2, A5 catecholaminergic cells groups as well as the contralateral LC (Cedarbaum & Aghajanian, 1978), and there are extensive recurrent axon collaterals within the nucleus (Aghajanian *et al*., 1977). Any of these structures may be capable of releasing NE.

Noradrenergic activity has been largely proven to be regulated by α_2-adrenoceptors located on NE neurons in the LC, controlling NE release and cell firing (Cedarbaum & Aghajanian, 1976; Jorm & Stamford, 1993; Van Gaalen *et al*., 1997; Mateo *et al*., 1998; Kawahara *et al*., 1999) in terminal areas. The peri-LC zone, but not the LC proper, is densely innervated by 5-HT projections, presumably arising from the DR (Aston-Jones *et al*., 1991). α_2-Adrenoceptors may act in synergy with 5-HT1ARs on serotonergic neurons located in the peri-LC, since the

inhibition of the LC firing rate by an agonist at α_2-adrenoceptors is potentiated by the simultaneous administration of a 5-HT1AR agonist, having no effect on the firing rate when the serotonergic agonist is administered (Ruiz-Ortega & Ugedo, 1997). α_2-Adrenoceptors are widely expressed in the LC, FC, thalamus, hippocampus, striatum, amygdala, hypothalamus, vestibular nuclei and spinal cord, among others (Pazos *et al*., 1988; Meana *et al*., 1989; Pascual *et al*., 1992; Nicholas *et al*., 1993; Talley *et al*., 1996), concretely in dendrites, presynaptic axon terminals, and astrocytes (Lee *et al*., 1998). The α-adrenoceptor subtype α_{2A}, and not the closely related $\alpha_{2B/C}$-adrenoceptors, seems to be the one that modulate NE release and cell firing in the rat LC (Callado & Stamford, 1999; Fernández-Pastor & Meana, 2002).

Extracellular NE in the LC seems to be released from somatodendritic areas of NE neurons (Fernández-Pastor *et al*., 2005), but also from noradrenergic afferents from other nuclei such as the A5 and the A7 nuclei (Luppi *et al*., 1995) and from collateral axons (Aghajanian *et al*., 1977; Ennis & Aston-Jones, 1986). LC expresses a high density of NE reuptake binding sites (Tejani-Butt, 1992; Ordway *et al*., 1997), which leads to increased extracellular NE concentrations in the LC after the administration of the NE reuptake inhibitor antidepressant desipramine (Mateo *et al*., 1998). NE concentrations in the LC decrease after local infusion of Ca^{2+}-free medium, and with the infusion of the sodium channel blocker TTX, whereas increased via KCl-induced depolarization. Altogether points out that the extracellular NE released in the LC seems to follow an impulse flow-dependent vesicular exocytosis pattern (Fernández-Pastor *et al*., 2005). The presence of NE release in somatodendritic areas plays a crucial role in regulating noradrenergic transmission in terminal areas via feedback inhibition trough α_{2A}-adrenoceptors.

1.3. NEUROBIOLOGY OF SCHIZOPHRENIA

Several neurotransmitter systems and functional networks within the brain, as well as brain structures, have been found to be altered in patients with schizophrenia. The threshold question is whether these alterations cause the development of the disease or are consequences of the illness progression or treatment. Below, the most important findings and hypotheses in schizophrenia neurobiology are briefly presented.

1.3.1. Morphological brain alterations

PFC has long been implicated in the neuropathology of schizophrenia, especially in the manifestation of negative and cognitive symptoms, since PFC plays an important executive role in working memory, attention, motivation and impulse control (Callicott *et al*., 2000; Selemon & Zecevic, 2015). In fact, lesions of this brain region in animals and humans produce the latter impairments (disruption in working memory, reduced impulsive choice related to reward, enhancing environmental stimuli, and behavioral restraint) (Fritts *et al*., 1998; Eagle *et al*., 2008; Barbey *et al*., 2013). However, unilateral frontal lesions retain some working memory function, likely due to functional compensation by the intact hemisphere (D'Esposito *et al*., 2006). It is thus not surprising that abnormalities in PFC have been largely associated with schizophrenia and other psychiatric disorders (Aghajanian & Marek, 2000; Alex & Pehek, 2007).

It is well-established that schizophrenic patients exhibit enlarged ventricles, especially in the lateral and third ventricles (Johnstone *et al*., 1976; Weinberger *et al*., 1979; Murray *et al*., 1985; Buckley, 2005; Kempton *et al*., 2010; Olabi *et al*., 2011; Sayo *et al*., 2012). The size of the ventricles increase progressively after the onset of the illness (Kempton *et al*., 2010), as the whole brain gray matter is reduced over time in patients with schizophrenia (Olabi *et al*., 2011).

Grey matter comprises neuronal cell bodies, dendrites lacking myelin, glial cells, synapse processes and capillaries. It is distributed in various areas of the brain, such as the surface of the cerebral cortex, cerebellum, thalamus, hypothalamus, basal ganglia-putamen, globus pallidus, NAcc, and SN. Magnetic resonance imaging (MRI) and voxel-based morphometry studies have disclosed grey matter loss in cortical areas, and impaired neuronal networks in

schizophrenic patients, although no differences in total brain volume were found (Andreasen *et al*., 1994; Buchanan *et al*., 1998; Crespo-Facorro *et al*., 2000; Honea *et al*., 2005; Narr *et al*., 2005; Gutiérrez-Galve *et al*., 2010; Rosso *et al*., 2010; Torres *et al*., 2013; Yang *et al*., 2014). Hippocampus, amygdala, thalamus and insula are other regions shown to be reduced in schizophrenia (Wright *et al*., 2000; Shepherd *et al*., 2012).

Positron emission tomography (PET) studies have unmasked abnormalities in FC, thalamus and cerebellum in patients with schizophrenia while performing cognition tasks, likely reflecting disturbances between cortical and subcortical networks (Andreasen, 1997). Increases in regional cerebral blood flow and glucose metabolism in the FC of acute unmedicated patients have been found (Cleghorn *et al*., 1989; Parellada *et al*., 1994). Morphological studies in *postmortem* brains of schizophrenic patients have revealed differences in cellular distribution likely attributed to altered neuronal migration early in brain development, loss of pyramidal cells, malformed cell structure, and decreased number of GABA interneurons (Faludi *et al*., 2011; Schmidt & Mirnics, 2015). The FC is an area entrusted with cognitive and emotional functions. Thus, these alterations might explain the functional disturbances in thinking, socializing and behaving that schizophrenic patients face.

1.3.2. Impaired neurotransmission systems in schizophrenia

Albeit the aetiology of schizophrenia remains still obscure, many lines of evidence aims at abnormalities in neurotransmission systems as agents thereof. DA has long been studied and associated with the disease; however, over the past few decades other systems such as serotonergic, noradrenergic, GABAergic, glutamatergic or cholinergic, have been suggested to play a role in the disease.

1.3.2.1. Dopaminergic system in schizophrenia

The most lasting neurotransmission hypothesis to explain the aetiology of schizophrenia is the dopaminergic hypothesis. In 1957, Arvid Carlsson and co-workers aimed at DA as the main neurotransmitter involved in this disease. Initially, an overall hyperdopaminergia in the brain was stated, thought to be responsible for the psychotic symptoms. This model emerges mainly due to the observations that the clinical effects of typical antipsychotics (chlorpromazine, haloperidol and similar drugs) were derived from the postsynaptic D_2 receptors blockade,

positioning DA and the dopaminergic hypothesis in the spotlight as the main neurotransmitter involved in schizophrenia. Moreover, some recreational drugs which increase DA activity, such as amphetamine or cocaine, can induce psychotic symptoms (Carlsson & Lindqvist, 1963; Davis *et al*., 1991; Howes & Kapur, 2009; Faludi *et al*., 2011).

Soon afterwards, the theory was reformulated, aiming at subcortical hyperdopaminergia (mostly in striatum and NAcc, based on the high concentrations of DA terminals and D_2 receptors in these regions) and cortical hypodopaminergia. According to this explanation, there could be an increased activity of mesolimbic DA pathways potentially responsible for the positive symptoms of the disease, whereas insufficient DA signaling in frontal cortical regions may explain the negative symptoms (Carlsson, 1959; Graybiel, 2000; Carr *et al*., 2002; Carlsson & Carlsson, 2006; Brisch *et al*., 2014).

It has been posited that there could exist two different subpopulations of D_2 receptors, so that D_2 receptors are not the same in the striatum, responsible of extrapyramidal effects, and in the PFC, predicting as antipsychotic effects. The overall occupancy of D_2 receptors by antipsychotic drugs is higher in temporal cortex than in striatum (Xiberas *et al*., 2001).

The reason to explain the above differences could be that D_2 receptors in striatum could be synaptically located and in the cortex the distribution could be predominantly extrasynaptic (Carlsson & Carlsson, 2006). Extrasynaptic receptors are those not activated during low-frequency synaptic events, and can be found in the cell body, the dendritic shaft, the neck of the dendritic spine and also adjacent to the postsynaptic density (perisynaptic). It has been reported that the same receptor can lead to opposite effects depending on its synaptic or extrasynaptic location by the activation of different intracellular signaling pathways (Hardingham & Bading, 2010). Extrasynaptic D_2 receptors are located on the dopaminergic neuron itself and they have high capacity for up- and downregulation. Moreover, extrasynaptic receptors are more responsive than postsynaptic receptors (Carlsson & Carlsson, 2006).

Elevated DA synthesis, higher tonic and phasic DA release and increased postsynaptic D_2 receptor binding in striatum of drug-naïve patients are described. Conversely, diminished extrastriatal D_2 receptor binding has been observed in patients with schizophrenia, especially in the thalamus, an area highly implicated in this disease (Hirvonen & Hietala, 2011). Recently, it has been reported by PET studies that schizophrenic patients have lower extracellular DA concentrations in the PFC and other extrastriatal regions, due to a deficit in the capacity for DA

release (Slifstein *et al*., 2015). Furthermore, *postmorten* brains of schizophrenic patients have reduced DA innervations in the dorsolateral PFC (Laruelle, 2014). Studies in schizophrenics have suggested a relationship between low cerebrospinal fluid (CSF) homovanillic acid, a measure of DA activity in the PFC, and poor working memory. Negative and cognitive symptoms seem to be resistant to D_2 blockers. Imaging techniques revealed that the PFC impairment might be responsible of these symptoms. It seems that D_1 receptor is upregulated in the FC of schizophrenic patients, likely due to chronic DA depletion. Such upregulation was correlated with a poor achievement on a working memory assignment. However, D_1 receptor antagonists do not have antipsychotic activity, even worsen positive symptoms (Laruelle, 2014). Indeed, some of the key benefits of clozapine to improve cognition and negative symptoms are attributed to the ability to increase dopaminergic activity in the PFC and its potent D_1 receptor occupancy (Meltzer, 1989).

The dopaminergic hypothesis supports that excessive stimulation of D_2 receptors in the striatum could be responsible of positive symptoms. Moreover, the blockade of D_2 receptors in the anterior striatum, but not those in the ventral striatum, is correlated with therapeutic effects. However, the continuous blockade of D_2 receptors in the ventral striatum could worsen negative symptoms (Laruelle, 2014).

However, this DA hypothesis seems not to be sufficient to explain the disease, inasmuch as negative and cognitive symptoms have been shown to be resistant to DA receptor blockers. In addition, 30% of schizophrenic patients do not adequately respond to typical antipsychotic drugs, suggesting that other neurotransmitter systems must be also involved in the pathophysiology of the illness (Faludi *et al*., 2011). In fact, DA synthesis is under the inhibitory control of D_2 receptors in neuron terminals (el Mestikawy *et al*., 1986; Santiago & Westerink, 1991). Ichikawa and Meltzer (1995) suggest that 5-HT2Rs in the striatum may have a facilitatory effect on the amphetamine-induced DA release, at least in part, via their ability to modulate DA synthesis regulated by D_2 autoreceptors.

The latest versions of the dopaminergic hypothesis recognize an indirect associative connection between DA and glutamate. Preclinical and clinical data suggest that dysfunction of DA systems in schizophrenia may be triggered by a deficit in NMDA receptor function. Psychoactive drugs, such as ketamine, a NMDA blocker, are able to indirectly increase DA release. It has been found that co-administration of ketamine and amphetamine in healthy

humans, can induce a striatal DA release similar to that exhibit in schizophrenia patients (Kegeles *et al*., 2000; Howes & Kapur 2009). It is now understood that DA has a modulatory role on glutamate performance. Thus, changes in DA function might affect NMDA activity, and vice versa (Laruelle, 2014).

Administration of NMDAR antagonists, cocaine and amphetamine, modulates neurotransmission systems in the brain toward increased DA release in the rat FC, striatum, and VTA (Quarta & Large, 2011; Matsumoto *et al*., 2014; Paasonen *et al*., 2017). Whereas Matsumoto and co-workers (2014) did not observe changes in extracellular DA concentrations after (±)-DOI injection (5 mg/kg i.p.) in rats, others have reported enhanced DA release (2.5 mg/kg i.p.) in rat FC (Pehek *et al*., 2001). NMDA blockers have direct effects on the D_2 receptor and 5-HT2R, since it has been reported that ketamine displays very similar affinity at the NMDA receptor and D_2 sites with a slightly lower affinity for 5-HT2R (0.5 μM, 0.5 μM and 15 μM respectively), whereas PCP shows similar affinity for the NMDA and 5-HT2R sites, with a slightly lower affinity for the D_2 site (2 μM, 5 μM and 37 μM respectively) (Kapur & Seeman, 2002).

Interestingly, acute administration of antipsychotic drugs exerts similar pattern of DA release than propsychotic drugs. Microdialysis experiments have shown that systemic administration of the antipsychotic drugs haloperidol and clozapine enhance DA outflow in the NAcc, striatum, and rat FC (Moghaddam & Bunney, 1990). Likewise, chronic treatment with clozapine increases extracellular basal concentrations in the FC (Yamamoto & Cooperman, 1994).

1.3.2.2. Noradrenergic system in schizophrenia

This catecholamine has received only marginal attention as an aetiologic factor of schizophrenia throughout decades. It was as early as 1971 when NE was posited to be involved in the pathophysiology and long-term downhill course of the disease (Stein & Wise, 1971; Tissot, 1975). Nevertheless, introduction of atypical antipsychotics appears to have awakened interest in NE dysfunction again. Practically all clinically effective neuroleptic drugs act on more than a single type of neurotransmitter receptor, including DA, NE, 5-HT, acetylcholine or histamine, among others. Regarding NE receptors, the majority of antipsychotics display antagonism at α-adrenoceptors (Minzenberg & Yoon, 2011).

Neuromelanin is a dark pigment structurally related to melanin, expressed in large quantities in catecholaminergic cells of the substantia nigra pars compacta and LC, giving dark color to the structures. This compound is assumed to be an indicator of cathecolaminergic neuronal activity, though it was thought to serve no pivotal functions until recently (Usunoff *et al*., 2002). In 1980, Kaiya discussed the possibility of an interaction between DA and NE in schizophrenia patients after seeing that they displayed a high negative correlation in melanin content between the substantia nigra and the LC.

Yamamoto and Hornykiewicz (2004) provided compelling evidence of the implication of the noradrenergic system in the pathophysiology of the illness. Studies in *postmortem* brain have shown increased levels of NE in schizophrenic patients. Likewise, plasma and CSF of these patients exhibit higher NE concentrations. NE controls a wide range of brain functions, being crucial in sleep-wakefulness cycles, arousal, attention, novelty-oriented behavior, anxiety, fear, aggressiveness, stress, memory, learning, psychomotor behavior and many neuroendocrine functions.

The noradrenergic theory supports the involvement of a noradrenergic system overactivity to be responsible for positive symptoms; whereas negative and cognitive symptoms would be the result of noradrenergic hypoactivity. In this sense, positive symptoms could be associated with hypervigilant states of consciousness, while 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).

It has been described that the blockade of catecholaminergic activity improves positive symptoms, whereas drugs that facilitates catecholaminergic transmission would worsen these psychotic states. On the other hand, catecholaminergic agonists ameliorate negative and cognitive symptoms, whilst antagonists would worsen them (Yamamoto & Hornykiewicz, 2004).

Animal models subjected to acute injection of NMDAR antagonist drugs have been shown to increase NE outflow in rat FC (Kubota *et al*., 1999; Quarta & Large, 2011). Likewise, the recreational drug amphetamine enhances DA release in the FC following systemic and cortical administration (Géranton *et al*., 2003). Antipsychotic drugs such as risperidone (Kaminska *et al*., 2013) and clozapine (Devoto *et al*., 2003b) also induces NE release in the FC.

NE also plays an important role in brain development and neuronal differentiation, potentially linked to the developmental process of the pathology of schizophrenia (Lechin & van der Dijs, 2005). Noradrenergic system also controls cerebral blood flow and metabolic rate, suggesting that long-term NE dysfunction could take part in the altered blood flow and metabolic rate in the cortex, and the abnormalities seen in the ventricles and grey matter of schizophrenic patients, which may lead to brain atrophy (Yamamoto & Hornykiewicz, 2004).

1.3.2.3. Serotonergic system in schizophrenia

Serotonin (5-HT) is synthesized both in peripheral tissues and in the CNS. Enterochromaffin cells in the gastrointestinal tract are the major producers of this monoamine. However, 5-HT cannot cross the BBB. Therefore, 5-HT is also synthesized in the brain, in serotonergic neurons, where acts as a neurotransmitter. L-Tryptophan is the AA precursor of 5-HT. It is provided with the dietary intake and can cross the BBB, entering into the serotonergic neurons. Tryptophan is catalysed to 5-hydroxytryptophan (5-HTP) by the isoform 2 of tryptophan hydroxylase (TPH2), which is a rate-limiting step of the synthesis of 5-HT (Walther *et al*., 2003). The enzyme L-aromatic amino acid decarboxylase (DDC) then convert 5-HTP to 5-HT. Once synthesized, 5-HT is taken from cytoplasm and transported into synaptic vesicles by VMATs, where they remain stored until exocytosis. 5-HT is released in synapses through a Ca^{2+}-dependent mechanism, and can then be reuptake from the synaptic cleft by the serotonin transporter (SERT) (Yun & Rhim, 2011).

The hypothesis regarding the involvement of 5-HT in schizophrenia emerged after the observation of the psychotic states that LSD and other indolamine compounds produced in healthy humans (Wooley & Shaw, 1954; Pletscher *et al*., 1955; Iqbal & van Praag, 1995). Natural hallucinogens, such as mescaline of psilocybin, and synthetic hallucinogens, such as LSD and diethylamine, cause their neuropsychological effects by activating 5-HT2AR (Vollenweider *et al*., 1998; Sealfon & González-Maeso, 2008). This hypothesis was further supported when observed that atypical antipsychotics such as clozapine, risperidone and olanzapine, show roughly 20-fold higher affinity for 5-HT2AR than for D_2 receptors (Arnt & Skarsfeldt, 1998).

Studies on human *postmortem* brains from schizophrenia patients show some inconsistencies in the variation of 5-HT and 5-HT2AR levels, so it is not clear enough so far whether an increase or a decrease in those levels occurs in the disease (Lovett Doust *et al*., 1975; Crow *et al*., 1979; Bleich *et al*., 1988; Iqbal *et al*., 1991; Ohuoha *et al*., 1993; Muguruza *et al*., 2014).

The connection between dreams and endogenous psychosis could be explained by a serotonergic hypothesis. 5-HT structure-related hallucinogens (LSD, mescaline) trigger mainly visual hallucinations. On the other hand, chronic schizophrenic states show primarily auditory perceptions, whereas acute schizophrenic states can entail visual hallucinations, as LSD does. Such visual perceptions resemble states shown while dreaming. Neurotransmission system activities, especially 5-HT, NE and D, also show some homologies between dreams and hallucinations (Fischman, 1983).

Psychedelic drugs such as PCP or ketamine, substantially increase 5-HT release in rat FC (Quarta & Large, 2011). Likewise, cocaine, amphetamine, and amphetamine-related compounds, with the exception of (±)-DOI, increase 5-HT output in rat FC (Matsumoto *et al*., 2014). Martin-Ruiz and co-workers (2001) observed decreased 5-HT release following acute administration of (±)-DOI (1 mg/kg i.p.) in rat FC.

Antipsychotics can also modulate 5-HT output in animal models. Thus, risperidone induces 5-HT release in rat FC (Ichikawa *et al*., 1998; Huang *et al*., 2006), whereas clozapine, olanzapine, and haloperidol are able to diminish 5-HT release in rat FC (Amargós-Bosch *et al*., 2006), and co-administration with PCP and ketamine blunt the high 5-HT release evoke by the latter recreational drugs (Amargós-Bosch *et al*., 2006). However, discrepancies have been found since Ichikawa and co-workers (1998) observed that clozapine augments 5-HT ouput in the FC, while olanzapine, sulpiride, haloperidol and M100907 have no effect on extracellular 5-HT concentrations in that region.

1.3.2.4. Glutamatergic and GABAergic systems in schizophrenia

Glutamate is the principal EAA neurotransmitter in the brain. Apart from its role in neurotransmission, glutamate fulfills a broad array of functions in the CNS. It serves as a precursor for peptide and protein synthesis, is involved in fatty acids synthesis, contributes to the regulation of ammonia levels, serves as a precursor for GABA, and is involved in carbohydrate metabolism (Westerink *et al*., 2007). There is considerable body of evidence

indicating that dysfunction of the glutamate system may contribute to the pathophysiology of schizophrenia (Fusar-Poli *et al*., 2011). It is well-established that administration of NMDAR antagonists, such as PCP or ketamine, can induce psychosis-like states in healthy humans, resembling positive, negative and cognitive symptoms seen in schizophrenic patients (Insel, 2010; Faludi *et al*., 2011). Even subanesthetic doses of ketamine can exacerbate these symptoms when administered to schizophrenic patients (Krystal *et al*., 1994). Psychoactive drugs modulate glutamatergic systems in the brain. NMDA receptor blockade, 5-HT2A agonism, THC and amphetamine administration, have all been reported to increase synaptic glutamate concentrations in PFC (Moghaddam *et al*., 1997; Pistis *et al*., 2002; Scruggs *et al*., 2003; Muschamp *et al*., 2004; Rowland *et al*., 2005; Stone *et al*., 2012). Although it has not been established whether the increased glutamate concentrations are associated with the psychosis-like effects of these drugs, it is interesting to note that glutamine, a marker of increased prefrontal glutamate release, has been reported in patients in the early phase of psychosis (Marsman *et al*., 2013) and, moreover, that prefrontal glutamate concentrations appear to be associated with failure to achieve remission following dopaminergic antipsychotic drug treatment (Egerton *et al*., 2012; Szulc *et al*., 2013; Demjaha *et al*., 2014).

Acute and chronic administration of PCP has been posited as an experimental animal model of schizophrenia, which evokes hyperlocomotion and stereotyped behaviors in rodents (Steinpreis, 1996). Furthermore, increased glutamate release in the PFC seems to be a common feature shared by non-competitive NMDAR antagonists and hallucinogenic drugs, both of which mimic some of the symptoms of acute psychosis (Aghajanian & Marek, 1999). NMDAR antagonists increase prefrontal glutamate concentrations, demonstrated both in animal microdialysis studies and in human proton magnetic resonance spectroscopy studies (Moghaddam *et al*., 1997; Rowland *et al*., 2005; Stone *et al*., 2012). Moreover, it has been shown that NMDA receptor antagonism leads to a reduction in GABAergic interneuron function, possibly through the blockade of NMDAR expressed on GABAergic cells (Homayoun & Moghaddam, 2007).

On the other hand, antipsychotic drugs as clozapine, whereas at a dose of 15 mg/kg s.c. do not exert any response on extracellular glutamate concentrations, at 25 mg/kg s.c. dose significantly increase its release in rat FC (Daly & Moghaddam, 1993). Quetiapine activates glutamatergic projections from the mediodorsal thalamic nucleus and enhances glutamate release in the FC (Yamamura *et al*., 2009). The typical antipsychotic haloperidol does not exert any change (Daly & Moghaddam, 1993).

Substantial evidence suggests that cortical GABAergic neurotransmission is altered in schizophrenia, especially including alterations of parvalbumin (PV)-positive cells (Lewis, 2011). Alterations in PV neuron-mediated inhibition could cause an impairment of gamma band synchrony - known to play a crucial role in cognitive functions - and contribute to cognitive deficits in schizophrenia (Gonzalez-Burgos *et al*., 2010; Lewis, 2011). It has been suggested that NMDAR hypofunction is an upstream cause of alterations of PV neurons in schizophrenia (Coyle, 2006). NMDAR hypofunction could lead to PV neuron abnormalities through various direct and indirect mechanisms (Lewis & Gonzalez-Burgos, 2008). Systemic NMDA receptor antagonists increase pyramidal cell firing, apparently by producing disinhibition, and repeated exposure to NMDA antagonists leads to changes in the GABAergic markers that mimic the impairments found in schizophrenia (Olney & Farber, 1995; Rotaru *et al*., 2012). Some researchers assert that PV neuron deficits in schizophrenia are secondary to NMDAR hypofunction at glutamatergic synapses onto these cells (Seamans, 2008). However, Rotaru and co-workers (2012) demonstrated that cortical disihibition and GABAergic impairment produced by NMDAR antagonists are unlikely to be mediated via NMDARs at glutamatergic synapses onto mature cortical PV neurons (Rotaru *et al*., 2012).

It has been demonstrated that NMDA antagonists, while acting ubiquitously, seem to only evoke morphological changes to interneurons. Particularly, NMDA antagonist treatment diminished the expression of PV, a key calcium-binding protein in some GABAergic interneurons, and GAD67, a main synthesizing enzyme for GABA. Schizophrenics display a similar decrease in GAD67, in particular in the subgroup of PV-containing fast-spiking interneurons (Seamans, 2008).

As mentioned above, the onset of schizophrenia usually occurs during adolesce or early adulthood. It is thought that disturbed maturation of the adolescent brain is highly involved in the pathophysiology of the disease. Significant developmental changes during adolescence occur in cortical GABAergic neurotransmission (Hoftman & Lewis, 2011). Development of innervations by PV neurons is dependent on GAD67 levels. Thus, environmemtal insults affecting GAD67 expression may lead to abnormal formation of synaptic contact by these interneurons (Rotaru *et al*., 2012).

Moreover, previous studies have found an important relationship between glutamate and 5-HT receptors. In that sense, indoleamine and phenethylamine hallucinogens which activate 5-HT2AR, rather than 5-HT2CR, can increase the frequency of “spontaneous” (non-electrically

evoked) glutamatergic excitatory postsynaptic currents (EPSCs) in apical dendrites of layer V cortical pyramidal cells (Aghajanian & Marek, 1997; Aghajanian & Marek, 1999; Marek & Aghajanian, 1999), as well as glutamate release in the FC (Scruggs *et al*., 2003; Muschamp *et al*., 2004). Such EPSCs increase was fully blocked by the AMPA/kainate antagonist LY293558 (Aghajanian & Marek, 1997).

Vast preclinical data have led to the hypothesis that NMDA receptor hypofunction can play a role in the pathophysiology of schizophrenia (Coyle, 2006; Javitt, 2010; Rubeša *et al*., 2011). Decreasing tone at this receptor, moreover, may ultimately cause a dysregulation of thalamocortical circuitry by altering the balance of excitation and inhibition (Walker & Conn, 2015). Research has suggested that hypofunction of NMDA receptors may potentially account for the hyper- and hypoactivity of DA pathways in regions highly implicated in limbic and cortical areas, respectively. That could explain positive, negative and cognitive symptoms of schizophrenia. Cortical areas are connected with mesolimbic dopaminergic pathways through GABA interneurons in the VTA. Stimulation of NMDA receptors, located on such interneurons, triggers the release of the inhibitory amino acid GABA, which evokes diminished activity of DA release in mesolimbic pathways. Therefore, hypoactivity of NMDA receptors on GABAergic cells would impair the inhibitory tonic state, resulting in a hyperactivity of dopaminergic pathways that could enhance DA release in mesolimbic areas, which in turn might evoke positive symptoms of psychosis. The cortex-brainstem projection communicates directly with mesocortical dopaminergic pathway in VTA (no GABAergic interneurons present) and causes tonic excitation in basal conditions. The hypofunctionality of NMDA receptors in the cortex-brainstem projection could make mesocortical dopaminergic pathways hypoactive, potentially producing cognitive and negative symptoms of schizophrenia (Pralong *et al*., 2002; Rubeša *et al*., 2011; Marsman *et al*., 2014).

Many aspects of synaptic development, plasticity, and neurotransmission are critically influenced by NMDARs. Dysfunction of NMDARs has been implicated not only in schizophrenia, but in a broad array of neurological disorders (e.g. stroke, epilepsy and neuropathic pain). Classically, NMDARs were thought to be exclusively postsynaptic. However, substantial evidence in the last 10 years demonstrates that NMDARs also exist presynaptically, and that presynaptic NMDA receptors modulate synapse function and have critical roles in plasticity at many synapses (Corlew *et al*., 2008).

Furthermore, mGluR2/3 receptors have been shown to influence a variety of glutamatergic dependent processes by either suppressing postsynaptic neuronal activity or inhibiting presynaptic release of glutamate (Vandergriff & Rasmussen, 1999). Preclinical findings in rodents have demonstrated that mGluR2/3 agonists block both the locomotor hyperactivity induced by PCP-like drugs (Moghaddam & Adams, 1998), and the HTR induced by LSD-like drugs (Gewirtz & Marek, 2000).

1.4. G PROTEIN-COUPLED RECEPTORS (GPCRs)

1.4.1. GPCR: Overview

G protein-coupled receptors are one of the largest super families of cell-surface protein receptors in vertebrate animals, which mediate important physiological functions and are target for myriad of modern drugs (González-Maeso & Sealfon, 2009a; Salon *et al.*, 2011; Munk *et al*., 2016). These receptors are ubiquitously expressed and respond to a diverse range of physical and chemical compounds that include photons, enzymatic processes, soluble small molecules such as neurotransmitters and ions, peptides, and large proteins outside the cell, activating internal signaling pathways and subsequent cellular responses (Palczewski & Orban, 2013). These receptors and their signaling have been studied extensively and breakthroughs in how they work have received various Noble prizes (Lin, 2013). GPCRs have been clustered in the following classes based on structural homology of amino acid sequence, location of the orthosteric binding site, and functional similarities: class A (rhodopsin-like), class B (secretin-receptor), class C (metabotropic glutamate), class D (fungal mating pheromone receptors), class E (cyclic AMP receptors) and class F (frizzled/smoothened) (Figure 6). Classes D and E are not found in vertebrates (Schiöth & Fredriksson, 2005; Alexander *et al*., 2015).

All GPCRs converge on a common structure, consisting of a single polypeptide with an extracellular amino terminus, an intracellular carboxy terminus, and seven hydrophobic transmembrane domains (TM1-TM7) linked by three extracellular loops (ECL1-ECL3) and three intracellular loops (ICL1-ICL3). Approximately 800 GPCRs have been described in the human genome, roughly half of which have been posited to mediate olfactory, tasty or visual functions, whereas the other half mediate intersignaling by ligands that differ in size, from small molecules to large peptides and serve as targets for the majority of drugs (Alexander *et al*., 2015). Studies on crystal structures have revealed highly conserved characteristics of the orthosteric ligand-binding sites across members of GPCRs, which sometimes make it difficult to achieve high selectivity for different GPCR subtypes. Alternatively, allosteric modulators have been proven highly successful for ligand-gated ions channels, and interest is growing for the development of selective allosteric modulators of specific receptor subtypes. These small molecules do not bind to the orthosteric ligand binding site but instead act at an alternatively located binding site (allosteric site), which is distinct from the orthosteric site, to either potentiate or inhibit activation of the receptor by an orthosteric agonist (Conn *et al*., 2009).

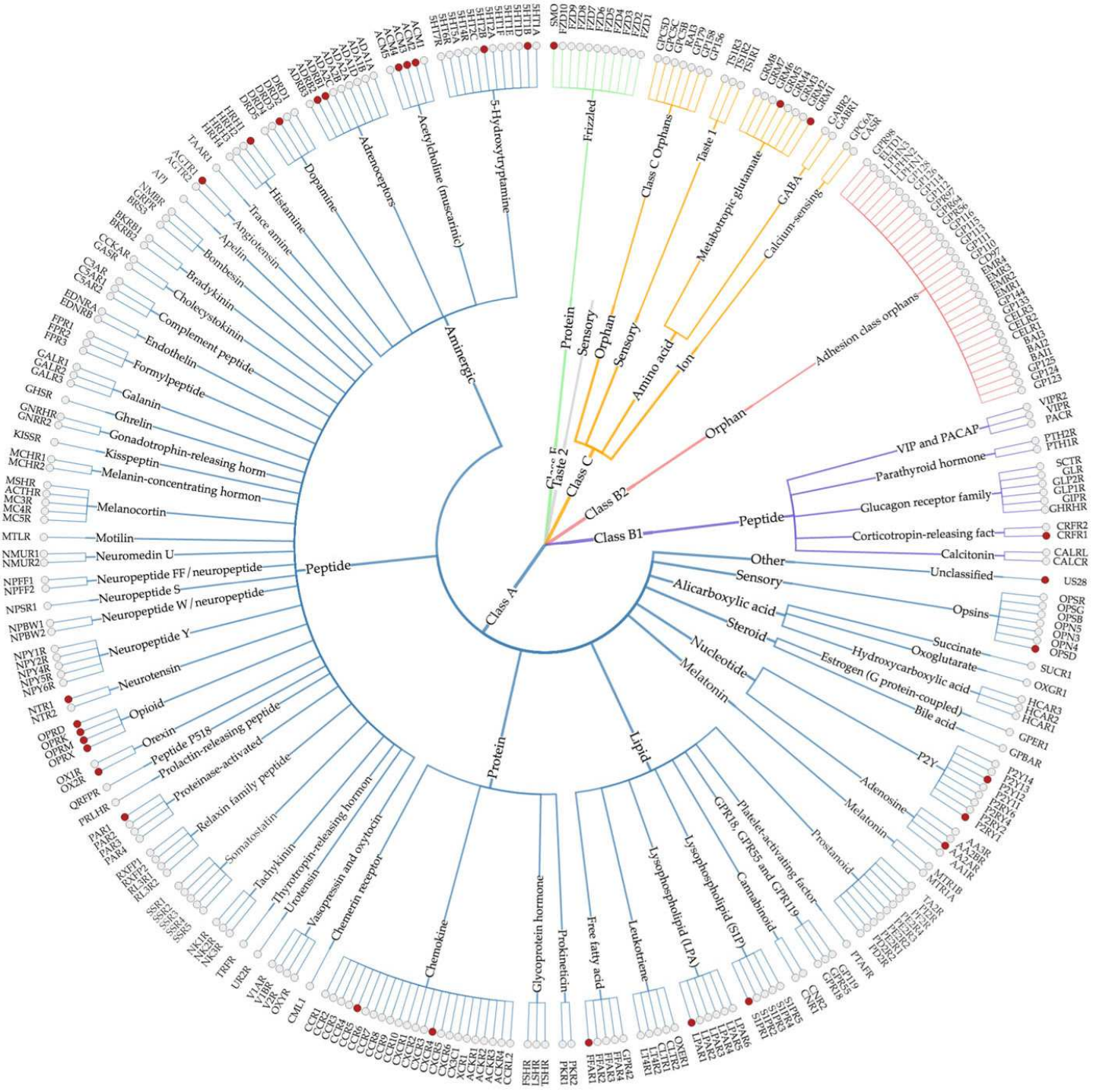


Fig. 6- Tree from the structure statistics page depicting crystallized receptors with a red circle. The tree can be navigated from its centre on the levels of class (endogenous) ligand type, receptor family and receptor. The receptor families are listed as defined by the nomenclature committee of the International Union of Pharmacology (from Munk *et al.*, 2016).

In recent years, increasing efforts have being made to discover new positive allosteric modulators (PAMs), which increase the response of the receptor of study; or negative allosteric modulators (NAMs), which reduce receptor responsiveness, and neutral allosteric ligands, which bind to the allosteric site but display no effects on the responses to the orthosteric ligand.

In class A GPCRs, the orthosteric binding site is present within the cavity formed by the transmembrane segments, able to bind small molecules as neurotransmitters, peptides and hormones. However, in class B GPCRs the orthosteric binding site is present on the extracellular loops and can bind large peptide ligands. Interestingly, class C GPCRs presents a large bi-lobed N-terminal "Venus fly trap" domain within the orthosteric binding site for neurotransmitters binding (Kolakowski, 1994; Chun *et al.*, 2012; Munk *et al.*, 2016) (Figure 7).

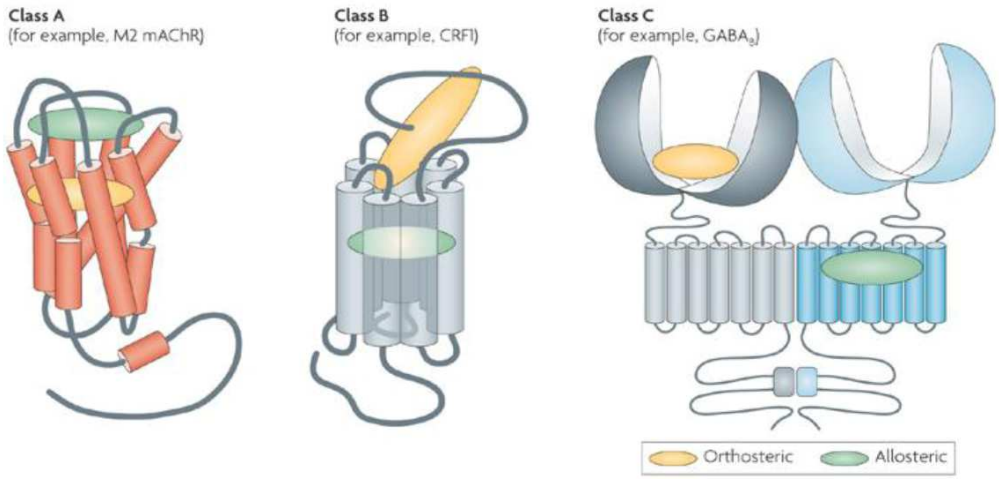


Fig. 7- Structural topology of typical orthosteric and allosteric binding sites of families A, B and C of GPCRs (from Conn *et al.*, 2009).

Although this kind of proteins is often described as "bimodal switches with inactive and active states", in reality there are more than those two conformational states, even a ligand for a specific GPCR can display diverse effects by stabilization of specific conformations of the extracellular part that lead to activation of distinct G-protein signaling pathways downstream (Kenakin, 2003; González-Maeso & Sealfon, 2009a). Thus, the term "agonist" is defined as a ligand that can fully activate the receptor; "partial agonist" is the one that induce a submaximal activation of the G protein, even at saturating concentrations; "inverse agonist"

inhibits basal activity; and "antagonist" while having no effect on basal activity, competitively block the access of other ligands. Allosteric modulator drugs can target a GPCR with a specific conformation (Kobilka & Deupi, 2007; Bokoch *et al*., 2010).

1.4.2. Heterotrimeric guanine nucleotide-binding proteins

Heterotrimeric guanine nucleotide-binding proteins (G-proteins) represent an ancient protein family that has been highly conserved over evolution. GPCRs associate with heterotrimeric G proteins to transduce ligand binding of the receptor to downstream effectors. Bacterial exotoxins (e.g. cholera toxin or pertussis toxin) were observed to modify the α-subunit of many heterotrimeric G-proteins and subsequently alter their function. Those toxins were key tools in the discovery, understanding and classification of the G-proteins. These cytosolic proteins bind guanine nucleotides and are coupled to transmembrane receptors and mediate a wide variety of cellular functions, including synthesis of second messengers such as cAMP, mobilize internal calcium stores, modulate ion channel function, control neurotransmitter release and influence gene expression (Milligan & Kostenis, 2006; Johnson & Lovinger, 2016). G-proteins are composed of a nucleotide-binding α subunit (Gα), of 39-52 kDa, and a dimer consisting of the β-subunit, of 35-36 kDa, and γ-subunit, of 8-10 kDa (Gβγ) (Vuoristo *et al*., 2000). Twenty different Gα, five different Gβ, and twelve different Gγ isoforms associate in distinct combinations with GPCRs (Milligan & Kostenis, 2006).

In their inactive state, Gα-subunits are bound to GDP and tightly associated with Gβγ. When a ligand binds to and activates the receptor, a conformational change in the receptor is produced, which in turn triggers a dramatic conformational change in the G-protein α subunit. Upon activation of a GPCR, the affinity of the receptor for the agonist is enhanced and the specificity of the G protein for guanine nucleotides changes in favor of GTP over GDP. Agonist binding to the receptor promotes its interaction with the GDP-bound Gαβα heterotrimer, leading to the exchange of GDP for GTP and the heterotrimeric G protein dissociates into G_{α}-GTP and Gβγ subunits, both of which are biologically active and can regulate the functional activity of a diverse array of effector proteins to simultaneously influence many cellular functions. The system returns to its resting state when the ligand is released from the receptor and the GTPase activity that resides in the α-subunit hydrolyzes GTP to GDP. The latter action leads to re-association of the free α subunit with the βγ subunit complex to restore the original

heterodimers (Gilman, 1995; Nestler & Duman, 1999; Latek *et al*., 2012; Johnson & Lovinger, 2016) (Figure 8).

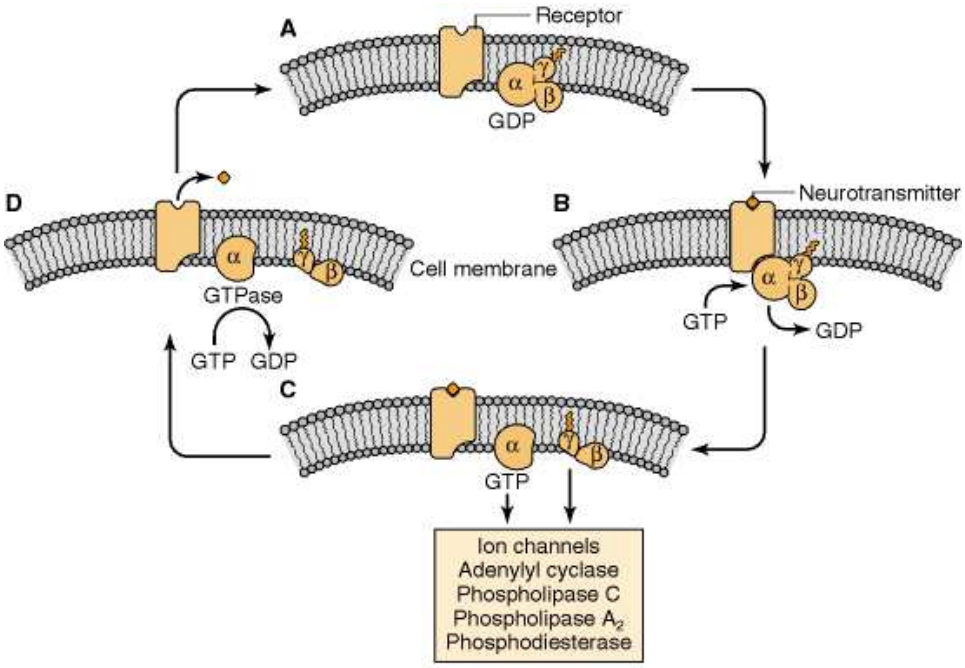


Fig. 8- Functional cycle of heterotrimeric G proteins. A: basal conditions, G proteins exist in cell membranes as heterotrimers composed of α, β, and γ subunits, loosely associated with the receptor. GDP is bound to the α subunit. B: upon activation of the receptor by its agonist, such as a neurotransmitter, the receptor associates with the α subunit, which leads to the dissociation of GDP from the subunit and the binding of GTP instead. C: GTP binding induces the generation of free α subunit by causing the dissociation of the α subunit from β and γ subunits and the receptor. Free α subunits (bound to GTP) and free βγ subunit dimers are functionally active and directly regulate a number of effector proteins, which, depending on the type of subunit and cell involved, can include ion channels, adenylyl cyclase, phospholipase C, phospholipase A_2 and phosphodiesterase. D: GTPase activity intrinsic to the α subunit degrades GTP to GDP. This leads to the reassociation of the α and βγ subunits, which, along with the dissociation of ligand from receptor, leads to restoration of the basal state (from Nestler & Duman, 1999).

Gα proteins are divided into four families (G_s, $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$) based on the structural properties and function of α subunits, and each family is further subdivided into several subtypes (González-Maeso & Meana, 2006). The G_s class is involved in activating adenylyl cyclase (AC), the $G_{i/o}$ in inhibiting AC and regulating ion channels, the $G_{q/11}$ in activating phospholipase C (PLC) and $G_{12/13}$ in activating the Na^+/H^+ exchanger pathway (Nestler & Duman, 1999).

Stimulatory Gα (G_s) protein family couples the receptor activation with the stimulation of all isoforms of transmembrane AC activity, leading to cAMP formation. All $G\alpha_s$ isoforms can be

activated by cholera toxin through the ADP-ribosylation on an arginine residue, leading to a constitutive activation of the Gα subunit.

Conversely, inhibitory Gα ($G_{i/o}$) proteins receive its name due to its ability for inhibiting AC activity and consequently diminish the cAMP accumulation. All members of this family - with the exception of G_z - contain a conserved cysteine residue on its carboxy-terminus susceptible of being ADP-ribosylated by pertussis toxin (PTX) from *Bordetella pertussis.* In fact, $G\alpha_i$ proteins are also called PTX-sensitive G proteins; whereas the rest of Gα proteins are called PTX-insensitive G proteins (Merritt & Hol, 1995; Nestler & Duman, 1999; González-Maeso & Meana, 2006; Milligan & Kostenis, 2006).

Finally, the activation of $G_{q/11}$ protein family members stimulates the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) to diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3) via PLC. The catalytic action of PLC leads to an IP_3-induced release of calcium ions from intracellular stores and because of it, to a calcium-mediated downstream signaling. Moreover, calcium ions and diacylglycerol activate some isoforms of protein kinase C (PKC).

Less is known about the non-dissociated $G_{\beta\gamma}$ protein heterodimer. There are eight G_β and twelve G_γ subunits and despite the initial thoughts, the $G_{\beta\gamma}$ dimer also mediates signalling through GPCRs activation, including G protein-gated potassium channels, N-type calcium channels and also phospholipase Cβ (PLC-β) (Nestler & Duman, 1999; Chen & Lambert, 2000; González-Maeso & Meana, 2006; Milligan & Kostenis, 2006).

Regulators of G protein signaling (RGS) are a family of proteins that has been found to play a role in desensitization of G_q and $G_{i/o}$ proteins, by accelerating the rate of GTP hydrolysis (Diversé-Pierluissi *et al*., 1999).

It has also been found that certain GPCR, besides activation of their canonical pathways, are able to activate other signal transduction cascades in a ligand-dependent manner. In this sense, ligands induce unique, ligand-specific receptor conformations that eventually can trigger differential activation of signal transduction pathways associated with that particular receptor. This ligand-induced distinct activation of downstream signaling pathways is termed

"functional selectivity", "biased agonism", "agonist-directed trafficking of receptor stimulus" (to name just a few). Functional selectivity allows some GPCRs to preferentially signal through a signaling pathway over another when they interact with certain ligands, namely biased agonists (Berg *et al*., 1998; Kenakin, 2007; Urban *et al*., 2007; Diez-Alarcia *et al*., 2016).

1.4.2.1. Influence of pertussis toxin on $G\alpha_{i/o}$ proteins

PTX is an exotoxin secreted by the Gram-negative, aerobic coccobacillus *Bordetella pertussis*, a pathogen that causes pertussis disease, also known as whooping cough. It comprises respiratory tract infections that most commonly affect immunocompromised patients (Zlamy, 2016).

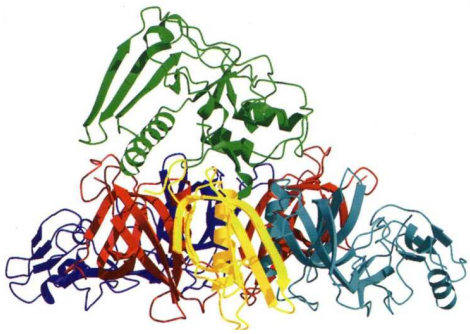


Fig. 9- Schematic perpendicular view picture of PTX. A-helix is shown as a spiral (green). B-strands are shown as arrows (Modified from Stein *et al*., 1994).

The crystal structure of PTX was solved for the first time by Stein and co-workers (1994). PTX belongs to the AB_5 toxin family, a hexameric assembling comprising a single catalytic active A-subunit and a pentamer of B-subunits. A-fragment is responsible for the catalytic activity, whereas B-fragment has the function of binding and delivery of the toxin to the target cells (Figure 9). PTX modifies cell responses by two different mechanisms.

- A-promoter: once the catalytic A-subunit of PTX is released from B-oligomer into the cytoplasm, it can irreversibly ribosylate cysteine residues of the Gα subunit of $G\alpha_{i/o}$ proteins, through its ADP-ribosyltransferase activity, locking the α subunit into a GDP-bound inactivate state. The inhibitory effect of $G\alpha_{i/o}$ protein on AC activity is then halted, accumulating cAMP levels intracellularly (Figure 10). Such accumulation of cAMP results in pathological effects where the second messenger takes part in, including insulin increase secretion by pancreatic cells, hypoglycemia, lipid degradation in adipocytes, lymphocitosis, neutrophil migration and vascular permeability. In fact, PTX-induced lipolysis has been used as a sensitive assay for the action of PTX. Restoration of $G\alpha_{i/o}$ function in cells is dependent on the synthesis of new $G\alpha_{i/o}$ proteins (Merritt & Hol, 1995; Mangmool & Kurose, 2011).

- B-oligomer: its effects mediate $G\alpha_{i/o}$ protein-independent actions. Most of the biological effects are related to an enhancement of immune responses through several receptors such as toll-like receptor 4 (TLR4), human platelet glycoprotein Ib (GPIb) and T-cell receptor complex (TCR). TLR4 activation generates a Rac-mediated signaling cascade that ends with the activation of NF-κB and the increase of interleukin IL-1β, whereas GPIb-PTX interaction leads to a downstream activation of platelet aggregation. Other effects ascribed to the B-oligomer of PTX are an induction of dendritic cell maturation, inhibition of growth cone guidance, induction of myelomonocytic cell adhesion, induction of ERK1/2 activation in endothelial cells, activation of T lymphocytes, induction of Th1/Th17 immune response through MAPK and IL-10 activation (Carbonetti, 2010).

$G\alpha_{i/o}$ protein-dependent effects of PTX display a slow onset (at least 1-2 h) and require low concentrations of the toxin. Conversely, $G\alpha_{i/o}$ protein-independent responses of PTX have a rapid onset but demand higher concentrations of the toxin to induce the effects described above (Mangmool & Kurose, 2011).

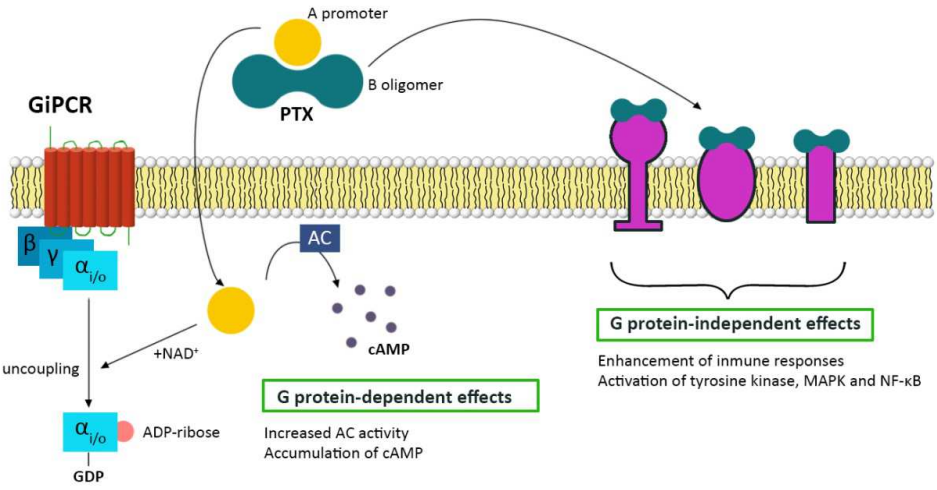


Fig. 10- Schematic picture of $G\alpha_{i/o}$ protein-dependent and -independent effects of A-promoter and B-oligomer subunits of PTX.

1.4.3. Serotonin 2 receptors: focus on 5-HT2AR and 5-HT2CR

5-HT mediates its effects through the activation of 14 different receptors, grouped in seven families; each family being further subdivided into distinct subclasses, namely: 5-HT1 (5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, and 5-HT1F), 5-HT2 (5-HT2A, 5-HT2B, and 5-HT2C), 5-HT3, 5-HT4, 5-HT5 (5-HT5A, 5-HT5B), 5-HT6, and 5-HT7. The complexity of the 5-HT system is further increased by alternative splicing and mRNA editing of several 5-HT receptors. All these receptors are GPCRs, except for 5-HT3 that is a ligand-gated ion channel receptor (LGICR). So far, a vast number of studies have recognized the fundamental role of these receptors in behavior, mood control, anxiety disorders, cognitive impairment, circadian rhythm, reproductive behaviors, thermoregulation, food intake, reward processes, suicide, schizophrenia, depression, autism, and other psychiatric disorders (Tseng & Atzori, 2007; Millan *et al*., 2008; Yun & Rhim, 2011).

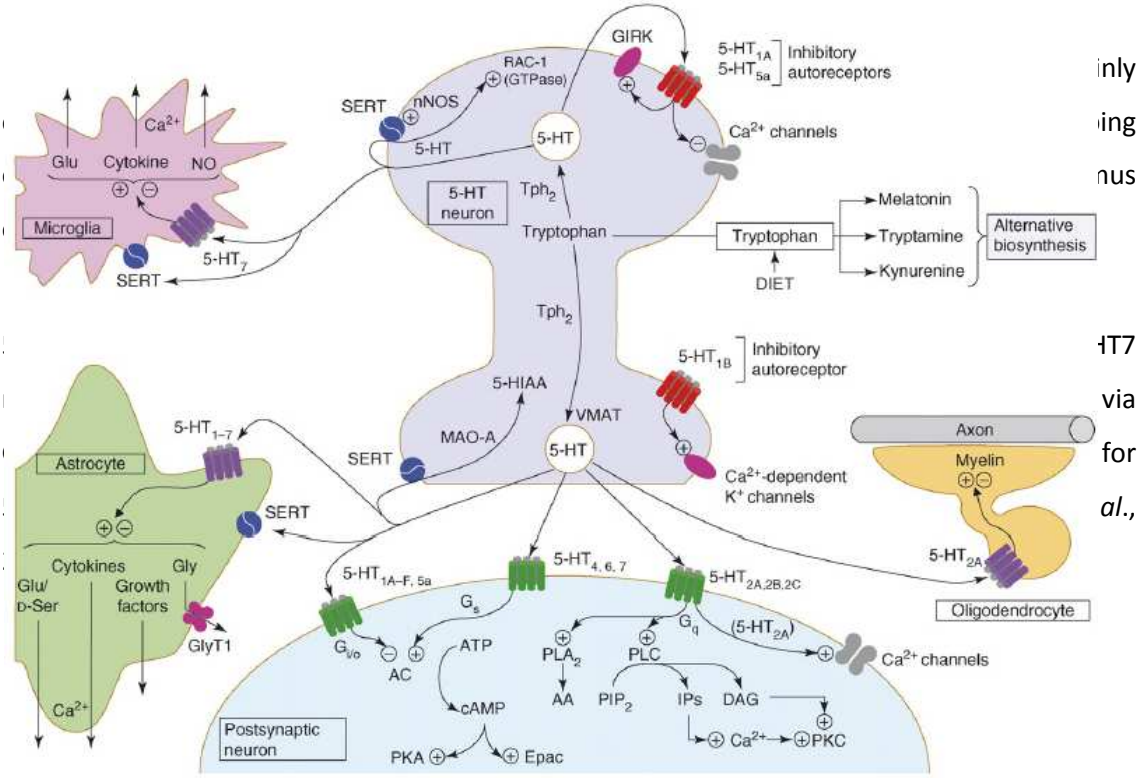


Fig. 11- An integrated view of signaling at serotonergic neurons. 5-HT is derived from tryptophan by an action of tryptophan hydroxylase 2 (TPH2), and it is deactivated by MAOA after release and reuptake via 5-HT transporters (SERT). 5-HT receptors are localized both pre- and post-synaptically to serotonergic neurons, but all subtypes are not necessarily co-localized at the same postsynaptic location. 5-HT1A and 5-HT1B inhibitory autoreceptors are localized on cell bodies and terminals, respectively, and 5-HT5A autoreceptors might also be present on the former. Characterization of non-neuronal 5-HT receptors is far from complete. Note that ligand-gated ion-channel 5-HT3 receptors, not considered herein, are present postsynaptically both on neurons and on non-neuronal cells. The major modes of signal transduction are shown at the postsynaptic level and many receptors converge on specific signaling pathways. Moreover, individual subtypes recruit multiple cascades, for example 5-HT2A receptors couple to Ca^{2+} channels, PLC and PLA2. Ion currents are an important mode for autoreceptor-feedback inhibition of serotonergic transmission. Abbreviations: 5-HIAA, 5-hydroxyindole amino acid; DAG, diacylglycerol; Gly, glycine; GlyT1, glycine transporter; IP, inositol phosphate; PIP2, phosphoinositol bisphosphate; D-Ser, D-serine; VMAT, vesicular monoamine transporter (from Millan *et al*., 2008).

Receptor		Major signal pathway	Other G-proteins	Main signal pathways	Agonists**	Antagonists**
5-HT_1	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}	Gi/o	Gz	↓ cAMP	8-OH-DPAT, Buspirone Anpirtoline, CP 94253	WAY-100635 SB-224289, GR-127935
5-HT_2	5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}	Gq/11	Gi/o, G12, and G13	PLC, Ca^{2+}, and PKC (+)*	DOI, m-CPP, R0 600175	Ketanserin, M100907 Mesulergine, SB-200907
5-HT_3	5-HT_{3A}, 5-HT_{3B}, 5-HT_{3C}, 5-HT_{3D}, 5-HT_{3E}	Ion channel	–	Depolarization	mCPBG, 2-CH3-5-HT	Ondansetron, Tropisetron
5-HT_4	5-HT_{4A} - 5-HT_{4H}, 5-HT_{4HB}	Gs	G13	↑ cAMP	BIMU-8, RS 67333, Cisapride	GR-113808, SB-204070
5-HT_5	5-HT_{5A}, 5-HT_{5B}	Gi/o	NT	↓ cAMP	–	–
5-HT_6	–	Gs	NT	↑ cAMP	EMDT, WAY 181187, WAY 208466	SB-399885
5-HT_7	5-HT_{7A}, 5-HT_{7B}, 5-HT_{7C}, 5-HT_{7D}	Gs	G12	↑ cAMP	8-OH-DPAT	Amisulpiride, SB-269970

*(+), stimulation; NT, not tested; PLC, phospholipase C; PKC, protein kinase C. **Agonists and antagonists were adapted from Carr and Lucki [3].

Table 1- 5-HT receptor classification, signaling pathways, agonist and antagonist drugs. (+): stimulation; NT: not tested; PLC: phospholipase C; PKC: protein kinase C. (from Yun & Rhim, 2011).

PET studies have revealed an overactivity of the FC in acute states of psychosis as well as in drug-induced hallucinations, potentially mediated by hyperactivation of 5-HT2 receptors (Vollenweider *et al*., 1997). The binding rate of the endogenous neurotransmitter ligand serotonin varies across 5-HTR subtypes, for example, 5-HT1ARs (K_i= 0.2-400 nM), 5-HT1BR (K_i= 1-40 nM), 5-HT7R (K_i= 0.3-8 nM) and 5-HT2AR (K_i= 4-1000 nM).

The 5-HT2Rs family comprises 5-HT2AR, 5-HT2BR and 5-HT2CRs, all of them are coupled preferentially to $G_{q/11}$ proteins, thus leading to a hydrolysis of PIP_2 that increase levels of IP_3, and elevate cytosolic Ca^{2+} that eventually modulate excitatory neurotransmission. 5-HT2R family members display a high degree of structural homology between them. Thus, 5-HT2BR has approximately a 70% homology to 5-HT2AR and 5-HT2CR, while 5-HT2AR and 5-HT2CR subtypes share 80% amino acid sequence homology (Hartig *et al*., 1990; Baez *et al*., 1995). While, as already mentioned, the main signal transduction mechanism for both receptors is the same, they can have opposite effects downstream. For instance, 5-HT has higher affinity (K_d=10 nM) for the low affinity state of the 5-HT2CR than for the low affinity state of the 5-HT2AR (K_d=100-1000 nM) (Zifa & Fillion, 1992).

At present, it is no easy matter to pharmacologically discriminate between the 5-HT2R family members, due to the lack of truly selective 5-HT2AR or 5-HT2CR ligands. There are a number of 5-HT2 agonists and antagonists on the market, however all of them display at most about two orders of magnitude more affinity for one subtype rather than for others. (±)-DOI, and the

analogues DOM and DOB, are psychedelic drugs with amphetamine-related structure, which display preferential affinity for 5-HT2ARs, but at higher doses can also bind at 5-HT2B and 5-HT2CRs (May *et al*., 2003; Pigott *et al*., 2012). Ro 60-0175 has been raised as preferential 5-HT2CR agonist (Porter *et al*., 1999; Higgins *et al*., 2001), whereas M100907 has been posited as a potent and selective antagonist of the 5-HT2ARs with much lower affinity for the 5-HT2CR or other receptors (Schreiber *et al*., 1994; Kehne *et al*., 1996; Pehek *et al*., 2006). Likewise, SB 242084 is a 5-HT2CR antagonist with 100-fold higher affinity for 5-HT2C than for the other two receptors of the family (Bromidge *et al*., 1997; Kennett *et al*., 1997).

1.4.3.1. 5-HT2ARs: localization, triggering effects, and signaling pathways

Localization

5-HT2AR belongs to the class A of GPCRs. This receptor is coupled to $G_{q/11}$ protein subtypes, which mediate excitatory neurotransmission (Millan *et al*., 2008). It has been located on human chromosome 13q14-q21 and comprises 471 amino acids in humans, mice and rats.

It is widely distributed in peripheral and central tissues. Autoradiographic approaches as well as *in situ* hybridization and histochemistry studies in rats have revealed a high presence of 5-HT2AR mRNA coding as well as receptor itself in FC, piriform cortex, CA3 subfield of the hippocampus, medial mammilary nucleus, the pontine nuclei, the motor cranial nerve nuclei in the brainstem, and the ventral horn of the spinal cord. Mild presence was also found in some areas of the basal ganglia, such as caudate-putamen, NAcc, SN, and amygdala; some areas in the thamalus, such as the reticular nucleus, lateral geniculate nucleus and zona incerta; some regions in the hypothalamus; some areas in the brainstem, such as raphe nuclei, subcoeruleus nucleus, vestibuar nuclei and PrH; and some regions of the reticular formation, such as PGi and lateral reticular nucleus (Pazos *et al*., 1985; Appel *et al*., 1990; Pompeiano *et al*., 1994; Willins *et al*., 1997; Jakab & Goldman-Rakic, 1998; Fay & Kubin, 2000; Miner *et al*., 2003; Weber & Andrade, 2010). It should be noted that a negligible presence of 5-HT2AR mRNA was found in LC (Pompeiano *et al*., 1994). Although previous studies did not observe the presence of 5-HT2ARs in VTA, subsequent immunohistochemical studies indicated its existence (Doherty & Pickel, 2000; Nocjar *et al*., 2002).

Within the FC there is expression of 5-HT2AR in layers II, III, V and VI (Willins *et al*., 1997; Puig, 2011). Strikingly, layer V is the layer which shows the highest density of this receptor, as

assessed by using autoradiography with [^{3}H]M100907 and FISH approaches in rodents (López-Giménez *et al*., 1997; González-Maeso *et al*., 2007).

The two major cell types in the FC are glutamate-containing pyramidal neurons and GABAergic interneurons. Pyramidal neurons represent approximately 70-80% of the neurons in the cortex, while the remaining 20-30% are mostly inhibitory interneurons (Markram *et al*., 2004; Espuny-Camacho *et al*., 2013). While both 5-HT2AR and 5-HT2CR are widely expressed throughout the mFC, the overall density of 5-HT2ARs seems to be higher (Pompeiano *et al*., 1994; Clemett *et al*., 2000). Immunohistochemical and electrophysiological approaches using genetically modified mice revealed that the vast majority of 5-HT2ARs corresponds to layer V pyramidal cells, concretely on apical dendrites of pyramidal cells of the anterior cortex, and to a lesser extent, on parvalbumin expressing fast-spiking GABAergic interneurons of the middle layers (Willins *et al*., 1997; Jakab & Goldman-Rakic, 1998; Hamada *et al*., 1998; Cornea-Hebert *et al*., 1999; Miner *et al*., 2003; Weber & Andrade, 2010; Puig & Gulledge, 2011). In fact, both 5-HT2AR and 5-HT2CR expression has been found on both GABAergic and pyramidal cells within the prelimbic FC (Willins *et al*., 1997; Jakab & Goldman-Rakic, 2000; Carr *et al*., 2002; Santana *et al*., 2004; Liu *et al*., 2007; Puig & Gulledge, 2011). A small percentage of 5-HT2CRs are co-localizing with 5-HT2AR on pyramidal cells, being the latter receptor highly expressed on these cells (Nocjar *et al*., 2015). This co-localization in pyramidal neurons is further supported by the finding that nearly all 5-HT2CR-expressing pyramidal shaped cells co-expressed 5-HT2AR mRNA, though only 28%-53% of 5-HT2AR-expressing pyramidal cells co-express 5-HT2CR mRNA (Vysokanov *et al*., 1998; Carr *et al*., 2002). Recently, a heterodimer formation between 5-HT2AR and 5-HT2CR has been reported (Moutkine *et al*., 2017).

Inside the neurons, 5-HT2ARs are located both intracellularly and on the plasma membranes of nerve cell bodies and axons, albeit there is a predominant somatodendritic presence (López-Giménez *et al*., 1997; Fiorica-Howells *et al*., 2002). Such a synaptic localization is consistent with previous findings reporting that 5-HT2A receptor expression is reduced in PSD-95 knockout mice (Abbas *et al*., 2009). Moreover, it is suggested that 5-HT2ARs are also located on cortical axon terminals of projecting neurons (Marek *et al*., 2001; Scruggs *et al*., 2003), and in astrocytes (Barnes & Sharp, 1999). Light microscopic studies in the rat frontal cortex have localized 73% of 5-HT2ARs on postsynaptic dendritic shafts, or dendritic spine heads and necks; 24% on presynaptic axons and varicosities, most of these with morphological features characteristic of monoamine axons, and the remaining 4% on glial cells (Miner *et al*., 2003).

Regarding the potential presence of 5-HT2AR presynaptically located on monoaminergic axons in the FC reported by Miner and co-workers (2003), it is unlikely that the 5-HT2AR-labeled monoaminergic profiles are noradrenergic, as it has been reported that the LC is devoid of mRNA for 5-HT2AR (Pompeiano *et al*., 1994, López-Giménez *et al*., 2001a). On the contrary, it is plausible that such terminal axons belong to dopaminergic neurons. Moderate mRNA expression for the 5-HT2AR has been shown in DA-rich areas of the rat midbrain (Pompeiano *et al*., 1994) that include cells with projections to the FC (Berger *et al*., 1976). Supporting findings reveal, using dual immunocytochemical localization, that midbrain dopamine cell bodies express 5-HT2ARs (Nocjar *et al*., 2002). Pehek and co-workers (2001) also stand up for a presynaptic presence of 5-HT2ARs on dopamine axon terminals that modulate the functioning of DA pathways. Microdialysis studies have suggested the potential presence of 5-HT2ARs presynaptically on glutamatergic terminals in the FC arising from the mediodorsal thalamus (Martin-Ruiz *et al*., 2001), although no substantial number of 5-HT2AR immunoreactive axon terminals whose features are characteristic of glutamate profiles were observed in the study performed by Miner and co-workers (2003).

5-HT2AR is not merely co-expressed with 5-HT2CR within cells in the mFC. It has been revealed that 5-HT2AR is also co-expressed with 5-HT1AR on the majority of pyramidal neurons, both receptors triggering opposing crosstalk (Celada *et al*., 2004, 2013).

5-HT2AR-dependent effects

5-HT2AR is coupled to $G_{q/11}$ proteins, activates downstream PLC isoforms that leads to the production of IP_3, enhances intracellular Ca^{2+} release and activates PKC (Carr *et al*., 2002; Yun & Rhim, 2011).

In vitro slice recordings have reported that 5-HT2ARs activation increases the excitability of neurons in the FC (Araneda & Andrade, 1991). Moreover, 5-HT2ARs stimulation enhances spontaneous glutamatergic EPSCs in layer V, a layer enriched with pyramidal neurons (Aghajanian & Marek, 1997). That effect was partially attributed to a boost in voltage-dependent Na^+ currents, likely as a consequence of 5-HT2 receptor-mediated activation of PKC, which finally increases dendritic excitability and thereby reverses the activity-dependent reduction in dendritic action-potential propagation (Astman *et al*., 1998; Franceschetti *et al*., 2000).

Intracortical administration of 5-HT2AR agonists enhances local pyramidal cell excitation in a dose-dependent manner (Ashby *et al*., 1990; Arvanov *et al*., 1999; Nocjar *et al*., 2015), while local 5-HT2CR agonism triggers GABAergic excitation and transmitter release that are thought to conversely inhibit cortical pyramidal activity (Abi-Saab *et al*., 1999; Leggio *et al*., 2009; Zhang *et al*., 2010; Nocjar *et al*., 2015). GABAergic stimulation elicited by (±)-DOI is partially blocked by 5-HT2CR antagonists, yet fully blocked by dual 5-HT2A/2C receptor antagonism (Zhang *et al*., 2010).

Other responses likely mediated by the 5-HT2AR include hyperthermia, hyperlocomotion, drug discrimination, and neuroendocrine responses such as increased secretion of cortisol, ACTH, rennin and prolactin (Barnes & Sharp, 1999). Even activation of 5-HT2AR in cortical areas has been linked with DA release induced by mild stress, as that produced by gentle handling (Pehek *et al*., 2006). A variety of biological functions such as mood, cognition, sleep, pain, motor function and endocrine secretion are under control of 5-HT2ARs. Correspondingly, disruptions of serotonergic transmission are implicated in the pathogenesis of depression, anxiety, schizophrenia and chronic pain, among others (Millan *et al*., 2008). Particularly, several lines of evidence underscore the crucial role of 5-HT2AR in the pathophysiology and therapeutics of neuropsychiatric disorders, including schizophrenia (Gray & Roth, 2007).

Hallucinations: molecular and behavioral effects

Despite behavioral animal models of acute hallucinations cannot mimic perturbations of perception, cognition, and mood evoked by hallucinogens in humans, head twitch response in rodents and wet-dog shakes in rats have emerged as a robust and reliable proxy of human hallucinogenic effects.

Certain behavioral responses have been attributed to activation of 5-HT2A receptors. Head twitches in mice and wet dog shakes in rats induced by drugs such as (±)-DOI and structural analogues, as well as 5-HT releasing agents and precursors like 5-HTP, have been largely proven to be 5-HT2AR-mediated. The potency which 5-HT2R antagonists inhibit agonist-induced head shakes closely correlates with their affinity for the 5-HT2A binding site rather than the 5-HT2C binding site. Furthermore, 5-HT2AR antagonists such as ketanserine and M100907 inhibit the head shake response (Hoyer *et al*., 2002), while 5-HT2B/2C receptor selective antagonists do not (Barnes & Sharp, 1999). Furthermore, genetically modified mice

lacking 5-HT2AR do not display such HTR (González-Maeso *et al*., 2003). Genetic restoration of 5-HT2AR signaling in cortical neurons of *htr2A*$^{-/-}$ mice is sufficient to rescue hallucinogenic-specific signaling signature and behavioral response. The sole expression of 5-HT2AR in cortex is sufficient to mediate the signaling pattern and behavioral response to hallucinogens. Subcortical populations of 5-HT2AR appear not to be required for hallucinogenic actions (González-Maeso *et al*., 2007).

Mescaline (found in peyote cactus), psilocybin (found in magic mushrooms) and LSD are all hallucinogenic drugs which act via agonism at 5-HT2AR. However, not all 5-HT2ARs agonists trigger hallucinations. The closely related 5-HT2AR agonists lisuride or ergotamine lack comparable psychoactive properties, although their agonist activity at 5-HT2AR is similar (González-Maeso *et al.,* 2007). Moreover, pharmacological or genetic inactivation of 5-HT2AR signaling blocks the behavioral effects of hallucinogens (González-Maeso *et al*., 2007).

While all the 5-HT2AR agonists induce *c-fos* expression, the induction of *egr-2* and *egr-1* most robustly predict behavioral activity. c-*Fos* and *egr* are transcription factors that control the expression of genes, being in this way master regulators of every cell's development and functioning (Herdegen & Leah, 1998). In primary cultures of cortical neurons from *htr2A*$^{+/+}$ and *htr2A*$^{-/-}$ mouse embryos, using fluorescent *in situ* hybridization (FISH) approaches, both LSD and lisuride were found to induce *c-fos* expression in neurons expressing 5-HT2AR mRNA, while only LSD induced *egr-2* expression in 5-HT2AR-expressing neurons. FISH studies of primary cultures from *htr2A*$^{-/-}$ mice showed no induction of these transcripts. The presence of TTX, which suppresses action potentials propagations, does not affect the induction of gene transcripts in cortical primary culture, thus indicating that neuronal circuitry is unlikely involved in the induction of *egr-1* and *egr-2* mRNAs. LSD-mediated activation of *egr-1* and *egr-2* is suggested to be intrinsic to neurons with 5-HT2AR signaling capacity (González-Maeso *et al*., 2007).

5-HT2AR antagonists have been demonstrated to induce the internalization of 5-HT2ARs *in vitro* and a redistribution of 5-HT2ARs *in vivo* in apical dendrites of rat cortical pyramidal neurons. Chronic treatment with the atypical antipsychotics clozapine and olanzapine enhances intracellular 5-HT2AR-like immunoreactivity, while causing a decrease in labeling of apical dendrites in the mFC. On the contrary, the typical antipsychotic drug haloperidol did not induce the latter effect. It is plausible that the loss of 5-HT2ARs from the apical dendrites of

pyramidal neurons is important for the beneficial effects of atypical antipsychotic drugs in schizophrenia (Willins *et al*., 1999).

Functional selectivity of 5-HT2AR

Evidence in cellular recombinant systems as well as in human brain tissue has reported the existence of multiple conformations/states of the 5-HT2AR (López-Giménez *et al*., 2001b). The 5-HT2ARs are primarily coupled to $G_{q/11}$ subtype proteins. The activation of $G_{q/11}$ proteins stimulates PLC-β, which catalyzes the cleavage of membrane-bound PIP_2 into the second messengers IP_3 and DAG (Raote *et al*., 2007). IP_3 elicits Ca^{2+} release from intracellular stores, while DAG diffuses along the plasma membrane and mediates protein kinase C (PKC) activation (Berridge *et al*., 2003; Becker & Hannun, 2005). Elevated intracellular Ca^{2+} binds and allosterically activates calmodulins proteins, which in turn go on to activate enzymes such as Ca^{2+}/calmodulin-dependent kinases (CAMKs).

It is well-established that drugs with affinity at 5-HT2ARs (both agonists and antagonists) can stabilize distinct receptor conformations leading to biased interactions or functional selectivity with various downstream effectors, which can include the canonical $G_{q/11}$ protein and different non-canonical signaling (Roth, 2011; Martí-Solano *et al*., 2015). The agonist functional selectivity has been proposed to explain the mechanism of action of hallucinogens, an explanation that posits that different receptor agonists stabilize distinct conformations of the receptor that preferentially recruit and activate specific signaling pathways, leading to distinct behavioral responses (Kenakin, 2003; Urban *et al*., 2007).

One of the first evidence of functional selectivity of 5-HT2AR arose from the findings of Berg and co-workers, regarding the ability of this receptor to signal not only via PLC bus also via phospholipase A_2 (PLA_2), mediating arachidonic acid (AA) release (Berg *et al*., 1998)

Hallucinogenic and non-hallucinogenic 5-HT2AR agonists also differentially influence FC patterns of gene expression (González-Maeso *et al*., 2007). The relationship of multiple signaling pathways to function at 5-HT2AR is complex, but these observations help explain the paradox of why some 5-HT2AR agonists elicit hallucinations whereas structurally related compounds do not (Millan *et al*., 2008). While the signaling pathways activated by both the non-hallucinogenic 5-HT2AR agonist lisuride, and the hallucinogenic agonist LSD, involve $G_{q/11}$ and phospholipase C, only LSD effects involve PTX-sensitive $G_{i/o}$ proteins (González-Maeso *et*

al., 2007). The hallucinogenic-type transcriptome response to LSD depends on its specific regulation of $G_{i/o}$ proteins (González-Maeso *et al*., 2007).

Additionally, 5-HT2AR activates other signal transduction cascades, besides G protein mediated PLC-β pathway, in a ligand-dependent manner. β-Arrestins are ubiquitous intracellular scaffolding proteins that can diminish or facilitate GPCR signaling and may represent a key point at which receptor signaling may diverge in response to particular ligands (Gurevich & Gurevich, 2006). 5-HT and (±)-DOI can differentially activate the 5-HT2AR in cellular models and *in vivo*. The precursor of 5-HT, 5-HTP, requires β-arrestin2-mediated signaling cascade for inducing HTR, whereas (±)-DOI does not (Schmid *et al*., 2008). In this way, β-arrestin2-KO mice display HTR following 5-HT2AR activation by (±)-DOI and *N*-methyltryptamines - psychoactive metabolites of 5-HT -, but not in response to 5-HT. Using mice and cortical neuron cultures from these animals, 5-HT was shown to stimulate Akt phosphorylation through the activation of a β-Arrestin2, Src and Akt complex. Moreover, the signaling through this complex was demonstrated to be necessary to evoke 5-HT-induced HTR (Schmid & Bohn, 2010). Conversely, endogenous *N*-methyltryptamines, produced by tryptamines and 5-HT degradation by *N*-methyltransferases, evoked HTR in the absence of β-arrestin2 and thus, in the absence of recruitment of the β-arrestin2-Scr-Akt complex (Schmid & Bohn, 2010).

5-HT, the cognate neurotransmitter at the 5-HT2AR, is able to induce 5-HT2AR internalization dependently of β-arrestin2 (Schmid *et al*., 2014). On the other hand, the atypical antipsychotic clozapine inhibits 5-HT2AR signaling through G protein-dependen mechanisms, induces 5-HT2AR internalization and Akt phosphorylation independently of receptor interactions with β-arrestin2 (Schmid *et al*., 2014). Thus, the pharmacologically oppositional ligands 5-HT and clozapine use distinct molecular mechanisms to achieve the same 5-HT2AR-mediated downstream events: Akt phosphorylation and receptor internalization. Albeit β-arrestin2 has no effect on clozapine's actions *in vivo*, Akt phosphorylation is required for clozapine's efficacy in suppressing schizophrenic behaviors in mice induced by MK-801 and PCP administration (Schmid *et al*., 2014).

This functional selectivity arises independently of differences in efficacy and/or potency at the different signaling pathways (Urban *et al*., 2007; Martí-Solano *et al*., 2015). Moreover, 5-HT2AR activation could also lead to phospholipase A-2 activation and to subsequent release of arachidonic acid (Berg *et al*., 1998).

1.4.3.2. 5-HT2CRs: localization, triggering effects, and signaling pathways

Localization

The 5-HT2CR has been mapped to human chromosome Xq24 (Hoyer *et al*., 2002). *In situ* hybridization histochemistry studies in rats have revealed 5-HT2CR presence in choroid plexus, the FC, anterior olfactory nucleus, lateral septal nucleus, subthalamic nucleus, amygdala, substantia nigra pars compacta, several nuclei in the brainstem, PGi, DR, and the whole gray matter of the spinal cord. Moderate presence of 5-HT2CR mRNA was found in many nuclei of the basal ganglia, thalamus, VTA, and LC (Molineaux *et al*., 1989; Pompeiano *et al*., 1994; Clemett *et al*., 2000; López-Giménez *et al*., 2001a). Within the cortex, moderate presence was found in neocortex - restricted to layer V - and cingulate cortex (Pompeiano *et al*., 1994; López-Giménez *et al*., 2001a).

At a cellular level, receptor autoradiography and *in situ* hybridization histochemistry studies suggest a predominantly somatodendritic presence of 5-HT2CR, according to the high levels of mRNA for 5-HT2CRs and the high density of binding sites. However, 5-HT2CRs are also suggested to be presynaptically located in axon terminals of some brain areas, since receptor labeling has been found with no presence of 5-HT2CR mRNA, suggesting its synthesis in septal and diagonal band cell bodies and its subsequent transport to axon terminals (López-Giménez *et al*., 2001a). Supporting these findings, a study carried out in humans showed a predominant presence of 5-HT2CRs in layer V of cortical areas (Pasqualetti *et al*., 1999), contrarily to the final disposition in layer III where these receptors are commonly found employing radioligand binding techniques. This could indicate that 5-HT2CRs are present either in dendrite or axon terminals of the same cells located in layer V (Pazos *et al*., 1987).

Many neuronal populations have been shown to express 5-HT2CR mRNA and/or protein, including serotonergic, cholinergic, GABAergic, neuropeptidergic, glutamatergic and dopaminergic neurons (Mengod, 2011). In that sense, 5-HT2CRs can be key players on modulating the activity of other neurotransmission systems.

As mentioned above, 5-HT2CR co-expresses with 5-HT2AR on both GABAergic and pyramidal cells within the prelimbic FC (Willins *et al*., 1997; Jakab & Goldman-Rakic, 2000; Carr *et al*., 2002; Santana *et al*., 2004; Liu *et al*., 2007; Puig & Gulledge, 2011). However, there is a larger

presence of 5-HT2AR than 5-HT2CR on pyramidal cells (Vysokanov *et al*., 1998; Carr *et al*., 2002; Nocjar *et al*., 2015). Studies employing immunofluorescence with confocal microscopy in rat prelimbic mFC have revealed that the majority of neurons expressing 5-HT2CR immunoreactivity in layers V-VI also co-express the 5-HT2AR. These cells are likely GABAergic for the most part, since 73% of the 5-HT2CR-IR cells in this region co-express the GABA cell marker GAD-67, suggesting that a cellular subpopulation within the deep layers of the FC could be directly co-regulated by 5-HT2CR and 5-HT2AR. Thus, both receptors could be mediating the inhibitory control over efferent pyramidal projections from the FC (Nocjar *et al*., 2015).

5-HT2CR-dependent effects

5-HT2CRs are coupled to $G_{q/11}$ proteins, activate downstream PLC isoforms, leading to the IP_3, enhance intracellular Ca^{2+} release and activate PKC (Carr *et al*., 2002; Yun & Rhim, 2011).

5-HT2CR has long been associated with the regulation of food seeking behavior (Aulakh *et al*., 1992; Higgins *et al*., 2016) and addictive disorders including drug reward (Higgins & Fletcher, 2003; Filip *et al*., 2012; Higgins & Fletcher, 2015). Characteristic behavioural responses evoked by 5-HT2CRs agonist agents, such as meta-chlorophenylpiperazine (mCPP) and Ro 60-0175, attribute to central 5-HT2CR activation an important role in hypoactivity, hypophagia, increased penile grooming/erections, and oral dyskinesia (Hoyer *et al*., 2002). 5-HT2CR stimulation has been shown to exert a tonic inhibitory influence upon frontocortical dopaminergic and adrenergic, but not serotonergic, transmission (Millan *et al*., 2003) and, in part, to play a role in the neuroendocrine function. Consistent with its action as a 5-HT2CR antagonist, RS 102221 increased food intake and weight gain in rats, yet it failed to reverse the hypolocomotion induced by mCPP, possibly due to restricted brain penetration. Moreover, the 5-HT2CR KO mouse suffers from spontaneous convulsions, cognitive impairment, increased food intake and obesity, although similar effects are not reproduced by selective antagonists, suggesting that these changes may result from neuroadaptation (Hoyer *et al*., 2002).

5-HT2CRs have received growing attention as a target for the treatment of depressive and anxious states (Greenwood *et al*., 2012; Chagraoui *et al*., 2016), even for depressive episodes in schizophrenia (Englisch *et al*., 2016).

1.4.4. Metabotropic glutamate receptors

Glutamate receptors are divided into two main families: voltage-gated ion channels, commonly referred as ionotropic glutamate receptors (iGluRs), and G protein-coupled glutamate receptors, also termed metabotropic glutamate receptors (mGluRs) (Traynelis *et al*., 2010; Julio-Pieper *et al*., 2011). The ionotropic receptor family includes NMDA, AMPA, and kainate receptor subfamilies, so-called due to the chemical agonist that selectively binds to each subfamily. While iGluRs regulate rapid responses upon activation, mGluRs modulate signal transduction cascades.

mGluRs belong to the C family of GPCRs. On the basis of their intracellular signal transduction mechanisms, pharmacological characteristics, and structural homology, mGluRs are split into three families (Figure 12): group I (comprising mGluR1 and mGluR5), group II (comprising mGluR2 and mGluR3), and group III (comprising mGluR4, mGluR6, mGluR7, and mGluR8) (Cryan & Dev, 2008; Willard & Koochekpour, 2013). Receptors belonging to the same group exhibit roughly 70% sequence homology, whereas the homology between receptors from different groups is about 45% (Pin & Duvoisin, 1995). Alternatively, spliced receptor variants have been reported and confirmed for mGluR1, mGluR3, mGluR5, mGluR6, mGluR7, and mGluR8 (Pin & Duvoisin, 1995; Flor *et al*., 1997; Corti *et al*., 1998; Zhu *et al*., 1999; Valerio *et al*., 2001; Malherbe *et al*., 2002; Sartorius *et al*., 2006)

Group I mGluR are coupled to G_q proteins, thus its activation leads to stimulation of PLC and PKC, stimulates phosphoinositide hydrolysis with subsequent formation of IP_3 and DAG, and causes the release of Ca^{2+} from intracellular stores. Group II and group III in contrast are coupled to G_i proteins, and inhibit AC activity, decreasing levels of cAMP formation (Julio-Pieper *et al*., 2011) (Figure 13), and are thought to function as inhibitory presynaptic autoreceptors that may play a role in synaptic plasticity (Conn & Pin, 1997).

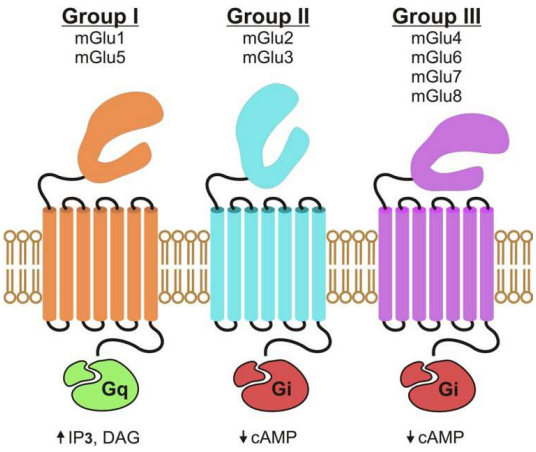


Fig. 12- mGlu receptor families and signaling pathways (from Julio-Pieper *et al*., 2011).

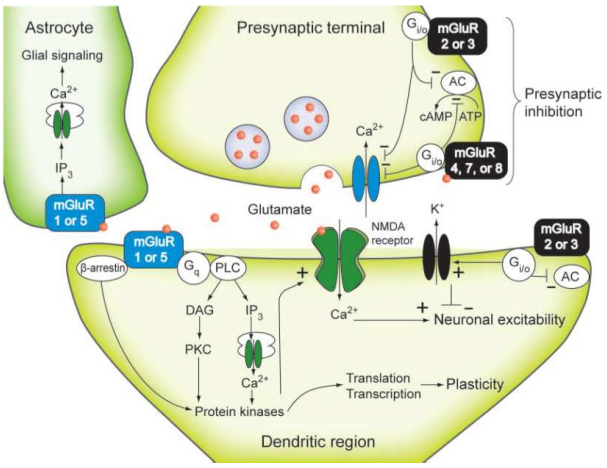


Fig. 13- Schematic representation of group I, group II, and group III mGluRs pre- and post-synaptically located (from Benarroch, 2008).

mGluRs are expressed in the CNS, mainly located on neurons and glial cells, adjacent to the synaptic cleft. They can regulate not only the effect of glutamate in postsynaptic neurons, but also the release of glutamate and other neurotransmitters in presynaptic locations. These receptors are widely distributed in the brain and are implicated in a number of diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease and schizophrenia, among others (Ribeiro *et al*., 2017). They are also expressed in peripheral tissues where they play a role in a variety of physiological and pathological processes, including hormone production in the adrenal gland and pancreas, regulation of mineralization in the developing cartilage, modulation of lymphocyte cytokine production, direction of the state of differentiation in embryonic stem cells, and modulation of gastrointestinal secretory function (Julio-Pieper *et al*., 2011).

1.4.4.1. Metabotropic glutamate II receptors: localization and functioning

Localization

The gene encoding the human mGluR2 has been mapped to chromosome 3p21.1-p21.2, and no splicing variants have been reported so far (Muguruza *et al*., 2016). mGluR2/3 receptors are presynaptically and postsynaptically located in the neurons, primarily away from the site of release in various regions of the brain, such as cortex, mainly in FC, amygdala, basal ganglia, thalamus, hypothalamus, and LC, among other many regions (Petralia *et al*., 1996; Ohishi *et al*., 1998; Cartmell & Shoepp, 2000; Wright *et al*., 2001; Gu *et al*., 2008). While mGluR2 expression is restricted to neurons, mGluR3 has been found on glial cells throughout brain regions (Tamaru *et al*., 2001). In mouse FC, the largest density of mGluR2/3 binding has been shown in layers I and Va (Marek *et al*., 2000), and both pre- and post-synaptically located (Venkatadri & Lee, 2014). Thus, glutamate can act potentially as an inhibitory neurotransmitter, by binding to

postsynaptic group II mGluRs. mGluR2s are known to function as autoreceptors on terminal thalamorcortical neurons (Marek *et al*., 2001).

Group II mGluRs functioning and schizophrenia

Psychomimetic agents increase activity of glutamatergic synapses (Aghajanian & Marek, 1999; Anneken *et al*., 2013) in the FC, a feature that has been postulated to be critical in the pathophysiology of schizophrenia. Effects of psychomimetic agents on glutamatergic transmission in the FC are blocked by group II mGluR agonists (Conn & Jones, 2009).

Intense efforts in targeting mGluR2/3 to treat psychosis in schizophrenia have been made for decades. In fact, preclinical data demonstrated promising antipsychotic potential of orthosteric mGluR2/3 agonists (such as LY354740, LY404039, LY2140023 and related compounds) by blocking the hyperlocomotive, glutamate release in FC and NAcc, and stereotypy inducing effects of the NMDA receptor antagonist PCP in rats (Moghaddam & Adams, 1998). Subsequent studies confirmed the latter results and also demonstrated that these compounds additionally block excessive release of DA, glutamate, and NE, triggered by NMDA antagonists by *in vivo* microdialysis (Cartmell *et al*., 1999; Lorrain *et al*., 2003a; Lorrain *et al*., 2003b; Winter *et al*., 2004; Swanson *et al*., 2004). Despite the promising therapeutic benefits in preclinical studies (Patil *et al*., 2007), clinical trials were eventually not successful (Delille *et al*., 2012; Li *et al*., 2015; Walker & Conn, 2015).

Development of highly selective agonists and antagonists that act at the orthosteric binding site of mGluR2 is difficult, inasmuch as the glutamate binding site is highly conserved across mGluR subtypes. Recently, tremendous advances have been made in the creation of new drugs that interact with allosteric sites on the receptors, which is a region less highly conserved. This novel class of compounds, known as positive allosteric modulators (PAMs), that are selective for mGluR2 have shown exciting potential as an alternative approach to mGluR2/3 agonists. mGluR2-selective PAMs, possessing distinct structural properties, have displayed efficacy in animal models of psychosis and anxiety disorders (Conn & Jones, 2009). These compounds do not activate mGluR2 directly but bind to a site distinct from the glutamate-binding site to enhance responses of mGluR2 to glutamate. Various mGluR2 PAMs have been identified, all of which are structurally related to two prototypical mGluR2 PAMs, termed LY487379, and BINA (Conn & Jones, 2009). Furthermore, some PAMs, frequently

named “ago-PAMs” can also produce allosteric agonist activity by generating receptor signaling in the absence of glutamate besides potentiating glutamate response (Walker & Conn, 2015).

1.5. GPCRs HETEROMERS

There is an increasing body of evidence suggesting that GPCRs exist and function as monomeric entities and coupled with others to create dimers or even higher-order oligomers. These heterocomplexes can display alternative ligand-binding properties and signaling pathways compared to the individual monomeric units (George *et al*., 2000; Gomes *et al*., 2000; Angers *et al*., 2002; Milligan, 2007; Moreno *et al*., 2013).

Experimental evidence indicates that family C GPCRs, including $GABA_B$ and mGlu receptors, exist and function as constitutive dimers. For instance, $GABA_B$ receptors need the assembly of $GABA_B$-R1 and $GABA_B$-R2 to function properly. mGluRs are thought to be present at the plasma membrane as strict dimers, and not higher-order oligomers, that are covalently linked through a disulfide bond (Maurel *et al*., 2008). Class A GPCRs likely exist as homomers, dimers and potentially as higher-order oligomers.

GPCR heteromer formation can affect functional outcomes such as ligand binding profiles, patterns of G-protein coupling, and subcellular location (Figure 14). They have gained importance inasmuch as they can modulate mechanisms underlying physiological and behavioral responses induced by signaling pathways downstream GPCRs. As various DA, 5-HT, glutamate, NE, and adenosine receptors have been shown to form GPCR heteromers *in vitro*, in tissue culture and *in vivo*, in animal models, a better understanding of their structure, neuroanatomical location, and physiological function may represent a new target for the design of new drugs for the treatment of neuropsychiatric disorders (Moreno *et al*., 2013).

Co-immunoprecipitation, bioluminescence resonance energy transfer (BRET), and fluorescence resonance energy transfer (FRET) assays have enabled the identification of a wide range of GPCR heteromers potentially implicated in neuropsychiatric disorders, all of them in cell lines, and others in further tissues: 5-HT2A-D_2 in striatum, A_1-A_{2A} in striatum, A_1-D_1 in striatum, A_1-mGluR1 still only in cell lines, A_1-5-HT2A in cortex, A_{2A}-D_2 in striatum, A_{2A}-mGluR5 in striatum, A_{2A}-D_2-mGluR5 in striatum, A_{2A}-CB1 in striatum, A_{2A}-CB1-D_2 still only in cell lines, α_{2A}-MOR in striatum, CB1-D_2 in striatum, CB1-5-HT2A in cortex, striatum and hippoccampus, D_1-D_2 in striatum, D_1-D_3 in cell lines, D_2-D_3 in striatum, D_2-H_3 in striatum, D_4-MOR in striatum, GHS-R1a-D2 hypothalamus, GHS-R1a-5-HT2CR in cell lines, mGluR2-5-HT2AR in cortex, mGluR2-5-HT2B

in cells, and mGluR2-mGluR5 in cells (Albizu *et al*., 2011; Delille *et al*., 2012; Moreno *et al*., 2013; Schellekens *et al*., 2013; González-Maeso, 2014; Viñals *et al*., 2015).

The present work focuses on 5-HT2AR/mGluR2 heteromer or heteroxomplex, its formation, signaling pathways and functionality.

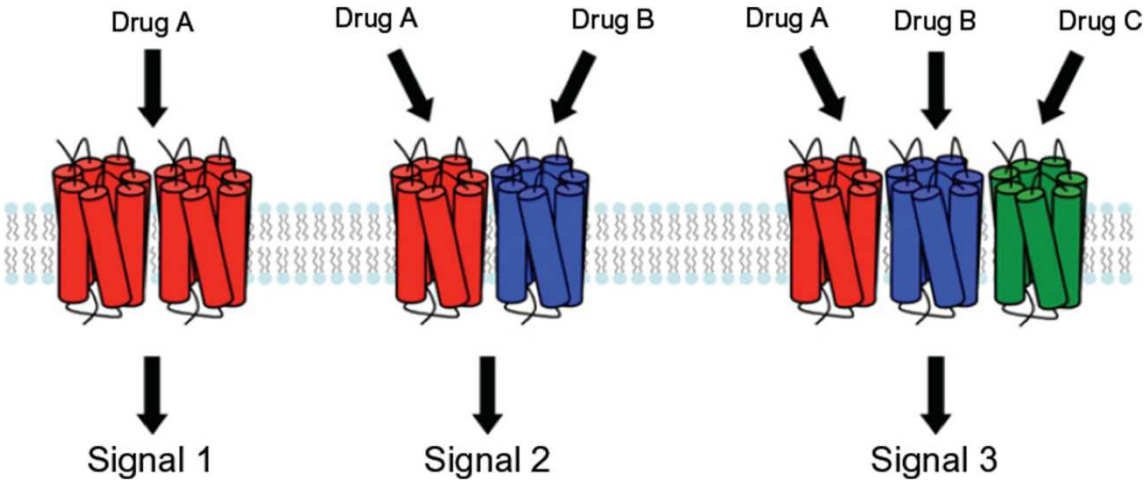


Fig. 14- Schematic model of functional responses induced by GPCR homomers compared to GPCR heteromers. Combinations of drugs modulate different signaling pathways, as well as a cross-talk between the components of the GPCR heteromeric complex leads to unique signaling properties (Moreno *et al*., 2013).

1.5.1. 5-HT2AR/mGluR2heterocomplex formation

Marek and co-workers (2000) were the first to describe a functional interaction between mGluR2/3 and 5-HT2AR in the medial FC (mFC). They previously reported stimulated glutamatergic activity in the FC, subsequent to the activation of 5-HT2ARs in such area (Aghajanian & Marek, 1997; Aghajanian & Marek, 1999; Marek & Aghajanian, 1999). Shortly after, it was discovered that the selective mGluR2/mGluR3 agonist LY354740 attenuated the 5-HT2AR agonism-induced EPSCs and glutamate release recorded in layer V pyramidal cells of the mFC. In fact, (±)-DOI binding was enhanced by thalamic lesions (Marek *et al*., 2001). Moreover, autoradiography studies show a laminar overlapping for 5-HT2AR and mGluR2/3 receptor binding in the mFC, compared to other cortical regions (Marek *et al*., 2000). Thus, Marek and co-workers state that mGluR2/3 function as inhibitory autoreceptors in cortical glutamatergic terminals whose transmitter release is positively regulated by 5-HT2AR activation. Further studies have shown that pharmacological activation of mGluR2/3 can

inhibit behavioral and electrophysiological effects derived from hallucinogenic drugs (Zhai *et al*., 2003; Benneyworth *et al*., 2007), and prevent the down-regulation of 5-HT2ARs in FC induced by repeated administration of (±)-DOI (Marek *et al*., 2006).

González-Maeso and co-workers (2008) demonstrated for the first time that the $G_{q/11}$-coupled 5-HT2AR and the $G_{i/o}$-coupled mGluR2, but not the closely related $G_{i/o}$-coupled mGluR3, form a GPCR heteromer in mouse and human FC (Snyder, 2008). Studies using mGluR2 and mGluR3 chimeric constructs enable to discern the ability of mGluR2 and not mGluR3 to co-express in close proximity with 5-HT2ARs. The three amino acid residues Ala-$677^{4.40}$, Ala-$681^{4.44}$, and Ala-$685^{4.48}$ located at the intracellular end of TM4 of mGluR2 seem to be necessary to form a GPCR heteromer with 5-HT2AR in HEK293 cells. Supporting evidence was found when performed immunoprecipitation assays using mGluR3 chimeras containing TM4 and TM5 of mGluR2 receptor (mGluR3ΔTM4,5) showed the formation of a heterodimer with the 5-HT2AR (Moreno *et al*., 2012). A recent report performing immunoprecipitation assays with chimeras of 5-HT2AR and 5-HT2CR demonstrated that TM4 of the 5-HT2AR was the key domain to form heterocomplex with mGluR2 (Moreno *et al*., 2016). Assays with subcellular purified fractions enriched in presynaptic active zone (PAZ) and postsynaptic density (PSD) proteins have revealed that the exact localization of this heterocomplex in mouse is in postsynaptic cortical neurons (Moreno *et al*., 2016).

It has been shown, in *postmortem* brains of antipsychotic-free schizophrenic subjects, increased 5-HT2AR and decreased mGluR2 densities, and such increase in 5-HT2AR negatively correlated with aging (González-Maeso *et al*., 2008). However, there are some discrepancies in this field, since some studies have shown decrease or unchanged 5-HT2AR densities in schizophrenia patients (Dean, 2003). On the other hand, atypical antipsychotic drugs have been shown to decrease the density of 5-HT2AR and mGluR2 (Dean, 2003; González-Maeso *et al*., 2008). Later, it was discovered that the allosteric binding crosstalk between 5-HT2AR and mGluR2 as a GPCR heterocomplex is upregulated in *postmortem* prefrontal cortex of schizophrenia brains (Moreno *et al*., 2012; Muguruza *et al*., 2013). In contrast, no changes in expression and density of both 5-HT2AR and mGluR2/3 in the *postmortem* prefrontal cortex of subjects with major depressive disorder under basal conditions have been reported, reinforcing the assumption that the latter imbalances are characteristic of schizophrenia (Muguruza *et al*., 2014).

The same profile showed in *postmortem* brains of schizophrenic subjects was shown in the offspring of pregnant mice infected with influenza virus during pregnancy. Cortical 5-HT2AR was up-regulated, as well as *c-fos*, *egr-1* and *egr-2*, whereas mGluR2 was down-regulated (Moreno *et al*., 2011b). Likewise, prenatal stress leads to a similar pattern of receptor expression, increased 5-HT2AR and decreased mGluR2 densities in FC, as well as to a higher sensitivity to evoke HTR induced by (±)-DOI (Holloway *et al*., 2013).

1.5.2. Signaling pathways of 5-HT2AR/mGluR2 heterocomplex and its involvement in behavioral responses

To determine whether the formation of the 5-HT2AR/mGluR2 heterocomplex has functional consequences, the effects of an mGluR2/3 agonist on the competition binding of several hallucinogenic 5-HT2AR agonists, and vice versa, were examined in mouse cortex (González-Maeso *et al*., 2008). Radioligand binding assays showed that activation of mGluR2 increased the affinity of hallucinogenic drugs for the 5-HT2AR, whereas conversely activation of 5-HT2AR decreased the affinity of agonists for the mGluR2 (González-Maeso *et al*., 2008). Hallucinogenic and non-hallucinogenic drugs activate the same population of 5-HT2ARs in cortical pyramidal neurons, but differ in the 5-HT2AR-dependent pattern of G protein regulation and gene expression that they elicit (González-Maeso *et al*., 2003; González-Maeso *et al*., 2007). In brain cortical neurons, the signaling elicited by hallucinogenic and non-hallucinogenic drugs causes the induction of *c-fos* and requires $G_{q/11}$-dependent activation of PLC. However, the signaling of hallucinogens such as (±)-DOI and LSD acting at the 5-HT2AR also induces *egr-2* expression, which is $G_{i/o}$-dependent (González-Maeso *et al*., 2008). Studies of ion channels in *Xenopus* oocytes as markers of $G_{q/11}$-dependent and $G_{i/o}$-dependent G proteins signaling demonstrate that both serotonergic and glutamatergic ligands balance the pattern of G protein signaling downstream of the 5-HT2AR/mGluR2 heteromer in a way that predicts their pro- or anti-psychotic potential (Fribourg *et al*., 2011). Supporting this theory, Molinaro and co-workers (2009) reported that in the FC mGluR2/3 receptors negatively regulate the canonical G_q and PLC pathway activated by 5-HT2ARs, unless PLC is activated by $G_{i/o}$ proteins in response to (±)-DOI.

The implication of 5-HT2AR/mGluR2 heterocomplex in behavioral effects has been demonstrated using hallucinogenic 5-HT2AR agonists as a mouse model of psychosis. As mentioned above, hallucinogenic 5-HT2AR agonists, such as LSD, mescaline or (±)-DOI, induce

head twitch behavior in mice, and this behavior is absent in 5-HT2AR knockout mice (González-Maeso *et al*., 2003; González-Maeso *et al*., 2007). Moreover, head twitch response is reduced in mGluR2 knockout mice, as is the high-affinity binding site of (±)-DOI at 5-HT2AR in mouse FC membranes, despite the 5-HT2AR level of expression is unaffected (Moreno *et al*., 2011a) (Figure 15). Remarkably, viral-mediated overexpression of mGluR2 in FC rescues the effects of LSD-like hallucinogenic drugs in mGluR2-null mice (Moreno *et al*., 2011a; Moreno *et al*., 2012). Importantly, overexpression of mGluR2ΔTM4N, a construct lacking TM4 of the mGluR2 which therefore cannot form heterocomplex with 5-HT2AR, does not rescue the LSD-like-mediated HTR (Moreno *et al*., 2012). Taken together, these data suggest that the 5-HT2AR/mGluR2 heterocomplex, and not 5-HT2AR alone, is the molecular target responsible for the psychosis-like behavioral effects of LSD-like hallucinogenic drugs (Fribourg *et al*., 2011; Moreno *et al*., 2011a; Moreno *et al*., 2012).

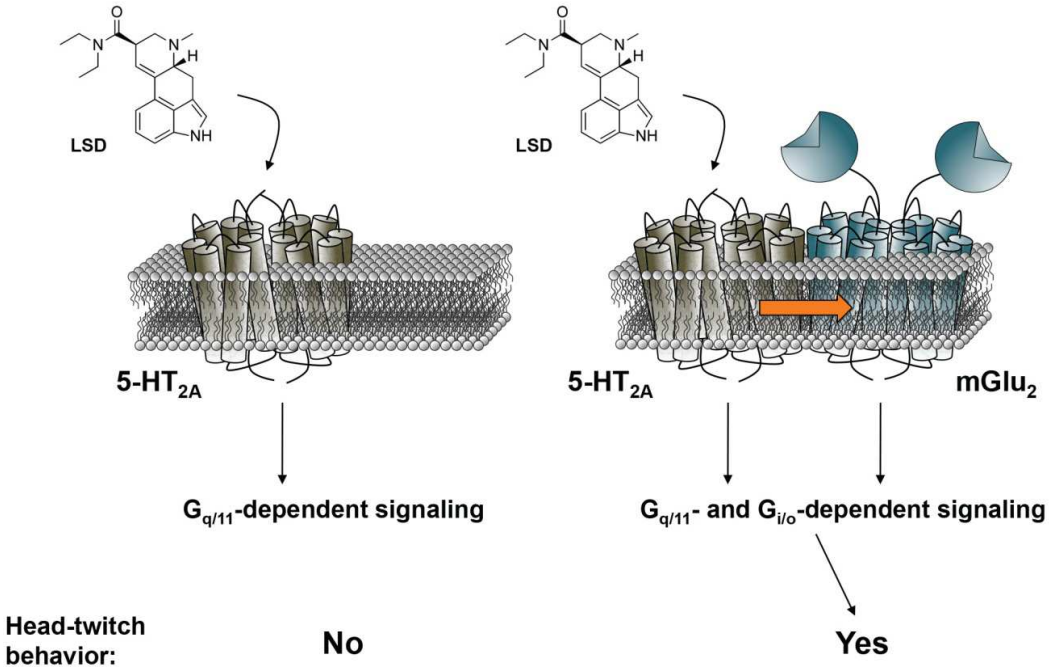


Fig. 15- G protein-dependent signaling and behavioral responses that require a GPCR heterocomplex. When 5-HT2AR and mGluR2 are prevented from forming a receptor heterocomplex, activation of 5-HT2AR by LSD-like compounds elicits signaling characteristic of $G_{q/11}$ protein subtypes. In contrast, LSD-like compounds acting at the 5-HT2AR/mGluR2 receptor heterocomplex activate both $G_{q/11}$- and $G_{i/o}$-dependent signaling. Head twitch behavior is reliably and robustly elicited by hallucinogenic 5-HT2AR agonists, and is absent in mGluR2 knockout mice (from González-Maeso, 2011).

The most recent findings in this field show that activation of the mGluR2 (with the mGluR2/3 agonist LY379268) activates $G_{q/11}$-dependent signaling leading to stimulation of PLC and increased Ca^{2+} intracellularly through a molecular mechanism that requires the functional

presence of both 5-HT2AR and mGluR2 forming a heterocomplex. mGluR2 coupled to an activation of $G_{i/o}$ proteins is necessary for the 5-HT2AR/mGluR2 heterocomplex-dependent effects of LY379268, since this drug failed to increase intracellular Ca^{2+} after pre-treatment with PTX. [^{35}S]GTPγS binding assays followed by immunoprecipitation with antibodies against $G\alpha_{i1,2,3}$ proteins or $G\alpha_{q/11}$ proteins in plasma membrane preparations from the mouse FC suggest that the canonical coupling of mGluR2 receptors is activation of $G\alpha_{i1,2,3}$ proteins, and such activation is unaffected by the loss of 5-HT2AR. However, the existence of 5-HT2AR is an essential condition for mGluR2 to couple to $G_{q/11}$ proteins (Moreno *et al*., 2016).

As mentioned above, *postmortem* frontal cortex of schizophrenic subjects have higher density of 5-HT2AR. The extent of activation of $G_{q/11}$ proteins by LY379268 in such frontal cortices is markedly reduced compared to controls, whereas the ability of LY379268 to activate $G_{i1,2,3}$ proteins was unaffected (Moreno *et al*., 2016). Moreover, an apparent supersensitivity to activate inhibitory G proteins ($G\alpha_{i1,3}$ and $G\alpha_{z}$) has been observed in *postmortem* drug-naïve human brains of schizophrenic patients (unpublished data).

Therefore, it is possible that 5-HT2AR/mGluR2 heteromer-dependent signaling effects may involve 5-HT and glutamate signaling, thus contributing to the abnormalities of thought and behavior observed in schizophrenic patients.

2. AIMS

Schizophrenia is a severe and complex brain disorder that is the result of multiple interactions between genetic, environmental, and social risk factors. Despite great efforts have been made so far, the basic understanding of the etiopathogenesis of schizophrenia, in which multiple and insufficiently known molecular pathways are likely to be involved, is still incomplete. In this sense, the tools for treatment or prevention of this disease are urgently needed. Currently, antipsychotic treatment focuses primarily on the antagonism of the dopaminergic D_2 receptors and serotonergic 5-HT2A receptors. However, these treatments do not work effectively in a substantial number of patients, and lead to serious side effects. A deeper understanding of schizophrenia at a neurobiological level would enable the identification of new molecular targets which could act as therapeutic targets for new drugs. In this context, the generation of animal models would be a useful tool to gain insight into the neurobiological and molecular aspects related to the disease, allowing more effective treatments to be designed.

There is extensive evidence to support the involvement of the glutamatergic system with the pathophysiology of schizophrenia, linking the dysfunction of the ionotropic glutamate receptors with the disease. Moreover, drugs acting through the mGluR2 are emerging as new possible therapeutic approaches due to their regulatory capacity over the glutamatergic system. Noradrenergic and dopaminergic systems are also altered in this disease. The existence of the 5-HT2AR/mGluR2 complex in cellular and animal models, as well as in human brain is convincingly demonstrated from a structural, molecular, biochemical, and cellular point of view, but only partially from a neurochemical, pharmacological and behavioral outlook. It is suggested that the 5-HT2AR/mGluR2 complex must be intact to allow the proper cortical modulation of NE and DA release. A heterocomplex dysfunction (knockout mice for each receptor), or the use of selective drugs acting specifically through each receptor result in a neurochemical and behavioral dysbalance that alters the effects mediated by the other receptor that comprises the complex. In schizophrenic patients and animal models of this disease, the functional activity of the heterocomplex is dysbalanced, likely toward a hyperactivation of the 5-HT2AR and a hypoactivation of mGluR2. The mechanism of action of antipsychotic drugs requires the 5-HT2AR/mGluR2 heterocomplex integrity and would act toward the 5-HT2AR hyperactivation blockade (atypical antipsychotics) or restoring the hypoactivation of mGluR2.

In this framework, the specific goals of this research work were to study:

1. The *in vivo* evaluation of the effect induced by the 5-HT2AR/2CR agonist (±)-DOI on cortical DA and NE release.

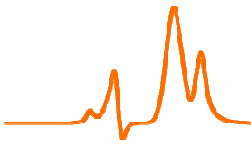
2. The comparison of the (±)-DOI-induced effect on cortical DA and NE release between intra-cortical and systemic administration.

3. The *in vivo* neurochemical characterization of the influence of the mGluR2/5-HT2AR heterocomplex on cortical DA and NE release evoked by (±)-DOI administration.

4. The influence of iGluRs located on the LC and/or the FC in the (±)-DOI-induced effect on NE release.

5. The influence of α_2-adrenoceptors located in the LC on the modulation of cortical NE release induced by (±)-DOI.

6. The *in vivo* characterization of the influence of the mGluR2/5-HT2AR heterocomplex on head-twitch responses to (±)-DOI administration.



3. MATERIALS & METHODS

3. 1. ANIMALS

3.1.1. Genetically modified mice

Wild-type and genetically modified mice weighting between 25-40 g were housed 5-10 per cage, until the beginning of the experiment, on a 12/12-h light/dark cycle at 22±2°C and 65-70% humidity. Food and water was provided *ad libitum*.

Animal husbandry, housing as well as experimental procedures were carried out at the University of the Basque Country, Leioa, Spain. Animal care and experimental protocols 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), 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). Procedures were approved by the Ethics Committee for Animal Welfare (CEBA) of the University of the Basque Country (UPV/EHU) (CEBA/107/2010/MEANA MARTINEZ, CEBA/181/2011/MEANA MARTINEZ, CEBA/182/2011/MEANA MARTINEZ, CEBA/203/2011/MEANA MARTINEZ, CEEA/345/2013/MEANA MARTINEZ).

3.1.1.1. Metabotropic glutamate 2 receptor knock-out mice (mGluR2$^{-/-}$, mGluR2-KO)

mGluR2-KO animals were kindly donated by Dr. Javier González-Maeso (Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Virginia, USA).

This animal model corresponds to a mouse (*Mus Musculus*) generated in a 129/Sv strain background by directed mutagenesis of embryonic stem cells. The 15.3-kb DNA fragment containing exon 2 of the gene encoding mGluR2 was isolated from a 129/Sv mouse genomic library. For gene targeting, the translation initiation site and its downstream sequence in exon 2 were disrupted by replacement of this exon with the neomycin resistance gene (NEO) (Masu *et al*., 1995).

The lack of mGluR2 expression has been largely verified by DNA, RNA assays and protein expression in southern, northern and western blot experiments. These mGluR2-deficient mice showed neither behavioral abnormalities nor any gross anatomical changes in the brain compared to the wild-type littermates (Yokoi *et al.*, 1996).

Knockout (mGluR2$^{-/-}$) and wild-type (mGluR2$^{+/+}$) mice were regularly genotyped by using conventional PCR coupled to an electrophoresis procedure (data not shown) to verify the lack or presence of mGlu2 receptor.

3.1.1.2. Serotonin 2A receptor knock-out mice (5-HT2AR$^{-/-}$, 5-HT2AR-KO)

These animals were kindly donated by Dr. Rafael Maldonado (Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona, Spain).

The 5-HT2AR$^{-/-}$ mice were originally generated at Columbia University (USA) in a 129S6/SvEv background mice. A transcriptional termination sequence (NEO-STOP) was placed between the promoter and the coding sequence blocking transcription and translation of the *htr2a* gene (Figure 16). Subsequently, animals were backcrossed over at least ten generations onto the inbred C57BL/6 line (González-Maeso *et al*., 2003; Weisstaub *et al*., 2006; González-Maeso *et al*., 2007; Orejarena *et al*., 2011). 5-HT2AR-KO and WT mice were further genotyped by conventional PCR analyses of tissue samples from the tails of the animals (data not shown), as described by Fiorica-Howells and co-workers (2002) to verify the lack or presence of 5-HT2AR.

5-HT2AR$^{-/-}$ mice had no detectable changes in brain development or cytoarchitecture (González-Maeso *et al*., 2003).

Fig. 16- Schematic representation of the generation of the genetically modified mice with global disruption of 5-HT2AR signaling capacity (5-HT2AR$^{-/-}$). Filled blue boxes represent exons of *htr2a* gene. The narrow box labeled with *phtr2a* represents the endogenous promoter for the gene. Serpentine symbol indicates the *htr2a* gene product. Left panel: Schematic of the wild-type *htr2a* locus. Right panel: Lox-p (triangles)-flanked cassette (red box) inserted upstream from the first initiation codon of the *htr2a* gene blocks transcription and translation. Modified from Weisstaub *et al*., (2006).

3.1.1.3. Metabotropic glutamate 3 receptor knock-out mice (mGluR3$^{-/-}$, mGluR3-KO)

These animals were kindly donated by Dr. Pedro Grandes (Neuroscience Department, University of the Basque Country UPV/EHU, Leioa, Spain).

These mGlu3 receptor knock-out mice (mGluR3$^{-/-}$) were generated in a C57BL/6J X 129/Sv background. Similarly as for breeding mGlu2R KO animals, mGluR3 mice were generated by directed mutagenesis using embryonic stem cells. Briefly, targeted disruption of exon 2 of the *Grm3* gene and further cloning into different vectors were performed. Then, the final *Grm3* targeting construct was injected into a line of mouse embryonic stem cells, and isolated recombinant cells were injected into murine C57BL/6J blastocysts (Masu *et al*., 1995) (Figure 17).

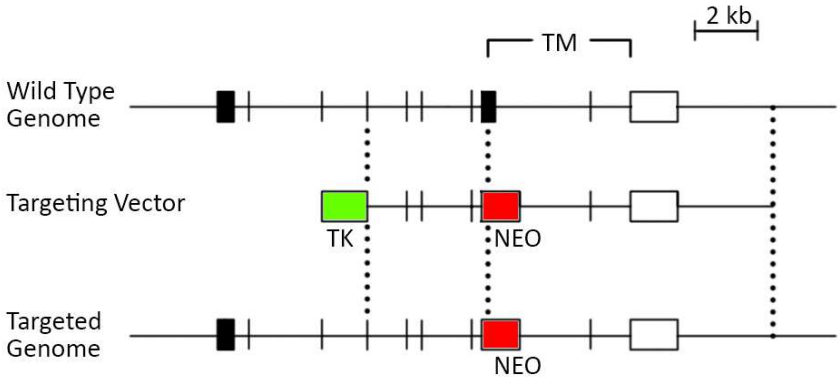


Fig. 17- Schematic representation of a targeted disruption of the mGluR3 gene. Homologous recombination resulted in replacement of a fragment encoding a part of the mGluR3 transmembrane TM region with the neomycin resistance gene (NEO). The *herpes simplex* virus thymidine kinase gene (TK) was attached to the 5'end of the targeting vector for negative selection. Modified from Masu *et al*., (1995).

3.1.2. Sprague-Dawley rats

Male Sprague-Drawley rats weighting 245-300 g at the time of surgery were used. Rats were housed 5 per cage in a controlled temperature room with free access to food and water, on a 12/12-h light/dark schedule and 60% humidity. Animals were obtained from the Animal Facility of the University of the Basque Country (SGIKER), Leioa, Spain.

Animal care and all experimental protocols were performed in agreement with European Union Regulations for the protection of animals used for scientific purposes, housing, caring and good laboratory practice (European Union Directive 2010/63/UE) and National Regulations

for animals welfare, transportation, experimentation, slaughter, as well as basic safety standards for the protection of animals used for scientific purposes (Royal Decree 53/2013). All protocols were approved by the Ethics Committee for Animal Welfare (CEBA) of the University of the Basque Country (CEBA/90/2010/MEANA MARTINEZ, CEBA/106/2010/MEANA MARTINEZ).

3.2. MATERIALS

3.2.1. Drugs

- **Desipramine** (*Desmethylimipramine hydrochloride*): Tricyclic antidepressant inhibitor of the norepinephrine transporter (NET) (Sigma Aldrich®, St. Louis, MO, USA. Batch: 86H0942).

- **Chloral hydrate** (*Trichloroacetaldehyde hydrate)*: general anesthetic (Sigma Aldrich®, St. Louis, MO, USA. Batch: 1173065).

- **(±)-DOI** (*(±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride; (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride*): potent 5-HT2 receptor agonist with higher affinity for 5-HT2A than for 5-HT2C receptors (Sigma Aldrich®, St. Louis, MO, USA. Batches: 078K4625, 012M4612V).

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

- **Isoflurane** (*1-chloro-2,2,2-trifluoroethyl difluoromethyl ether*): general inhaled anesthetic drug. Two-pore potassium 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. Batches: 50003XN, 50016XN).

- **Kynurenic acid** (*4-Hydroxyquinoline-2-carboxylic acid*): non-selective antagonist NMDA and AMPA/kainate receptors and nicotinic acetylcholine receptors (Tocris®, Ellisville, MO, USA. Batch: 40A/50307).

- **M100907** ((R)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol; Volinanserin): selective 5-HT2AR antagonist (Sigma Aldrich®, St. Louis, MO, USA. Batch: 082M4604V).

- **Norepinephrine** (*L-(−)-Noradrenaline (+)-bitartrate salt monohydrate*): neurotransmitter (Sigma Aldrich®, St. Louis, MO, USA. Batches: 035K0960, SLBC1882V).

- **Pertussis Toxin** (PTX): Toxin from *Bordetella Pertussis* bacteria (Merck Millipore®, Darmstadt, Germany. Batch: D00079735).

- **Ro 60-0175** (*(aS)-6-Chloro-5-fluoro-a-methyl-1H-indole-1-ethanamine monofumarate*): 5-HT2C receptor agonist (Sigma Aldrich®, St. Louis, MO, USA. Batch: 030M4621V).

- **RX821002** (*2-[2-(2-Methoxy-1,4-benzodioxanyl)]imidazoline hydrochloride*): Selective α_2-adrenoceptor antagonist (LASA Laboratory, Barcelona, Spain).

- **Serotonin** (*3-(2-Aminoethyl)-5-hydroxyindole hydrochloride; 5-HT; 5-Hydroxy-tryptamine hydrochloride*): neurotransmitter (Sigma Aldrich®, St. Louis, MO, USA. Batch: 061M5178).

- **SB 242084** (*6-Chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline dihydrochloride hydrate*): selective 5-HT2C receptor antagonist (Sigma Aldrich®, St. Louis, MO, USA. Batch: 3A/130042)

3.2.2. Antibodies

Antibodies used for immunohistochemical assays were:

- Primary polyclonal antibody: Rabbit policlonal antibody anti-GFP (Santa Cruz Biotechnology, Dallas, TX, USA. Batch: C2614).

- Fluorescent secondary antibody: Alexa 488 fluor donkey anti-rabbit (Invitrogen, Waltham, MA, USA. Batch: 51811A).

3.2.3. Gases

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

3.2.4. Reagents

Supply company	Reagent
Carlo Erba Reagents (Val de Reouil, France)	Methanol
Thermo Fisher Scientific (Waltham, MA, USA)	Citric acid monohydrate, 99.5%
	Ethylenediaminetetraacetic acid, disodium salt dihydrate, +99%
	1-Octanesulfonic acid, sodium salt, HPLC grade
Merck (Darmstadt, Germany)	Calcium chloride dihydrate
	Ethylenediaminetetraacetic acid disodium salt (EDTA)
	Magnesium chloride hexahydrate
	Paraformaldehyde
	Potassium chloride
	Sodium dihydrogen phosphate monohydrate
	Sodium chloride
	Sodium hydroxide
	Sodium hydroxide solution
Panreac Química S.A.U. (Barcelona, Spain)	Acetonitrile
	Glacial Acetic acid (HPLC)
	Hydroxypropyl-beta-cyclodextrin
	Ortho-Phosphoric acid 85%
	D(+)-Sucrose
Probus S.A. (Badalona, Spain)	Perchloric acid 60%
Sigma Aldrich® (St. Louis, MO, USA)	Bovine Serum Albumin (BSA)
	Sodium azide
	Sodium octyl sulfate
	Triton® x-100
	Tween® 20

Table 2- Summary table of the reagents employed for the development of the procedures and providers.

3.3. DRUG ADMINISTRATION

3.3.1. Systemic administration

Acute systemic administrations containing the drug of interest dissolved were injected intraperitoneally. Injections volumes were between 0.15-0.20 ml for mice and 0.25-0.30 ml for rats. Dissolution procedures were as follows:

- **(±)-DOI** was dissolved in saline solution (0.9% NaCl).

- **M100907** was dissolved in acidified saline (0.9% NaCl) - with HCl, pH 0.6 approx. -, by steady heating and swirling until the solid compound had dissolved completely. Solution was neutralized with NaOH up to pH 6.25-7. Aliquots of 600 µl were made and stored at -80 °C until used. M100907 was injected 35 minutes before (±)-DOI.

- **Ro 60-0175** was dissolved in saline solution (0.9% NaCl).

- **SB 242084** was dissolved as previously described (Deurwaerdère *et al*., 2004) in a mixture of physiological saline (0.9% NaCl) containing hydroxypropyl-beta-cyclodextrin (8% by weight) plus citric acid (25 mM), constantly swirling until dissolution was complete. Solution was neutralized with HCl and NaOH up to pH 6.25-7. SB 242084 was injected 35 minutes before (±)-DOI.

3.3.2. Local administration

One of the main advantages of brain microdialysis is the ability to administer a drug through a probe accurately in a specific cerebral nucleus, allowing getting insight into the effects triggered by a drug in that nucleus. Drugs were dissolved to a desired concentration in CSF and pH was neutralized to physiological pH of 7.2-7.4. CSF comprised: 148 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$, pH 7.4 - neutralized with 1 mM K_2HPO_4 -.

Dissolved drugs can easily flow through the dialysis probe to the tip, where osmotic exchange makes the drug pass through the pores of the membrane (6-kDa size) to the brain. Likewise,

neurotransmitters pass from the brain to the membrane and finally are collected in the dialysates.

Basal neurotransmitter concentration values in mouse brain dialysates were under the detection limits of our high-performance liquid chromatography (HPLC) equipment. Due to the inability to detect basal levels of DA and NE, desipramine was dissolved in the CSF (1.5 µM) of all the experiments carried out in mice. However, the presence of this drug was no longer necessary in experiments performed in rats, since basal concentrations of these neurotransmitters were higher than threshold values.

Drugs, except for M100907, were prepared immediately before perfusion:

(±)-DOI was dissolved in CSF pH 7.4 and vortexed until the solution was homogeneous and transparent. Concentrations of 100 µM and 300 µM were prepared.

Kynurenic acid was suspended at 1 mM concentration in CSF and sonicated for 7 min. Solution with small particles suspended turned transparent after alkalizing with NaOH up to pH 7.2-7.4 and continuous agitation (Legault *et al*., 2000).

M100907 was dissolved in 5 µl of glacial acetic acid and CSF. The pH was controlled and neutralized with NaOH up to 7.4. In co-perfusion experiments combining M100907 + (±)-DOI, M100907 solution was prepared and subsequently (±)-DOI was added. Final solution was aliquoted and stored at -80ºC.

RX821002 was dissolved in CSF as a stock solution (1 mM) and then the solution was further diluted with CSF (pH 7.4) to reach a final concentration of 1 µM.

3.3.3. I.c.v. administration of Pertussis Toxin

Lyophilized pertussis toxin was reconstituted with Milli-Q® water to a final concentration of 0.1 µg/µl and distributed in aliquots of 25 µl. Accordingly, vehicle was prepared (50 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0) and also distributed in aliquots of 25 µl. Both vehicle and pertussis toxin were kept refrigerated at 4°C until administration.

3.4. INTRACEREBRAL MICRODIALYSIS IN FREELY MOVING ANIMALS

The principle of microdialysis technique is based on the balance between the release of neurotransmitters and its re-uptake. To study neurotransmitter levels more directly related to synaptic transmission, it is necessary to sample specifically the content of the extracellular space, which is the site of exchanges between neurons, glial cells and blood vessels (Chefer *et al*., 2009; Gardier, 2014).

Brain microdialysis requires the insertion of a small dialysis catheter, termed microdialysis probe, into a specific brain region. Through that probe, CSF is perfused. This technique is based on the law of passive diffusion of low molecular weight compounds (neurotransmitters in this case) through a porous membrane from the compartment with the highest concentration (the synaptic extracellular space) to the less concentrated compartment (the dialysis probe perfused with a buffer solution at physiological pH that does not contain neurotransmitters). The dialysate samples, obtained from the probe outlet, can be analyzed using HPLC with electrochemical detection for the quantification of oxidizable molecules recovered from the extracellular space. DA and NE neurotransmitters were analyzed in this project.

3.4.1. Microdialysis probes manufacture

Handmade probes used for microdialysis experiments comprised the following materials:

- 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).
- Araldit 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.

Probes consisted of the assembly of silica piping systems covered by steel tubes, which provide the formers with robustness and resistance (Figure 18). This "Y" shape system was fixed with Araldit adhesive and TAB 2000 dental cement at the insertion point.

Two- or four-mm length of silica tube leaned out from the bottom of the probe, depending on 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 18).

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.

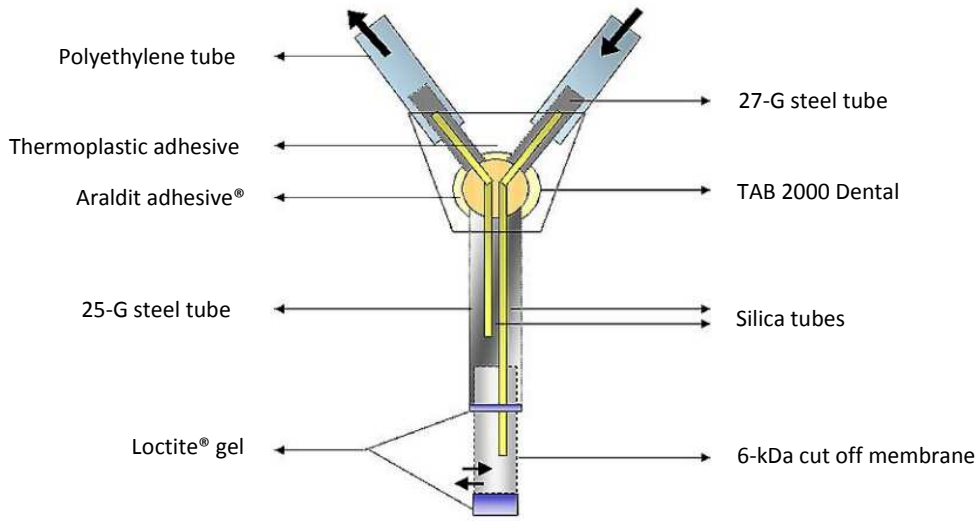


Fig. 18- Representative image of a microdialysis probe (Modified from M. Victoria Puig Velasco, 2004, Doctoral Thesis, University of Barcelona, IDIBAPS).

3.4.2. Stereotaxic surgery

3.4.2.1. Anesthesia

The general advantages to the use of inhalation agents are that the procedure is precise, rapidly adjustable, safe and effective. Moreover, 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 19), composed of:

- **Flowmeter**: to adjust medical oxygen flow rate.
- **Vaporizer**: chamber where volatile liquid anesthetic is deposited. Gas flux coming from the flowmeter flows through the vaporizer and anesthetic is added to this flux in the desired proportion.
- **Emergency valve of O_2**: after pressing allows oxygen to flow directly without anesthetic agent.
- **Safety pressure valve**: does not allow exceeding the maximum system working pressure.
- **Manifold**: it has 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 fluosorber canister**: Active vacuum to eliminate excesses of anesthetic gas.

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

Fig. 19- Left panel: inhalation anesthesia system. Right panel: mouse mask for anesthesia maintenance coupled to the inhalation anesthesia system shown on the left.

3.4.2.2. Stereotaxic surgery equipment

Surgery was performed using a Kopf™ stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The system possesses two arms to allow accurate probe positioning in specific brain areas by following three coordinates: anteroposterior (AP), lateral (L) - both relative to bregma -, and dorsoventral (DV) - relative to skull surface -. Head immobilization was assured by adjustment of the skull inserting two earbars in both ears, model 957 18º (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 (Figures 19 and 20).

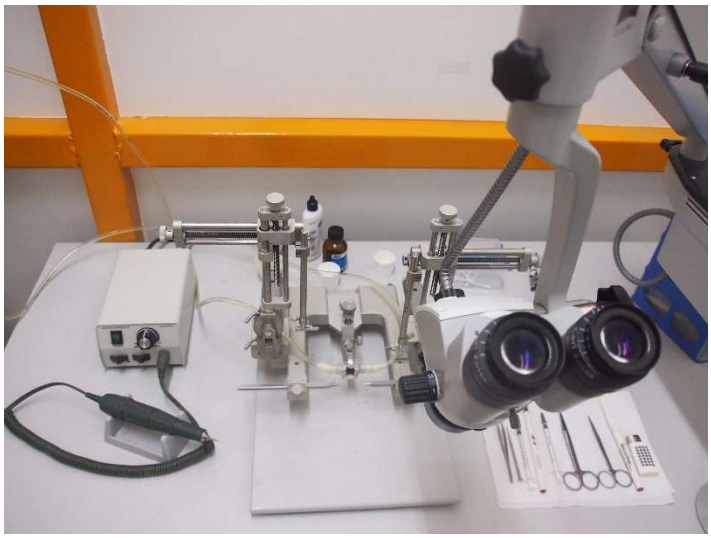


Fig. 20- Surgery equipment.

3.4.2.3. Microdialysis probes implantation

Mice

Animals were anesthetized in an induction chamber breathing isoflurane (4%) for 1-2 min, until they were utterly anesthetized. Unconscious mice were placed on the stereotaxic frame under constant flow of isoflurane (1.5%) for anesthesia maintenance. To avoid hypothermia, animals laid over a heating pad during surgery.

Once the head was horizontally immobilized - *bregma* and *lambda* in the same plane -, a sagittal 1 cm length 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, 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 3-mm membrane length, were stereotaxically implanted into the brain (comprising cingulate, prelimbic and infralimbic cortex). Implantation coordinates were selected following the guidelines set by Franklin and Paxinos atlas (2008) and strain-comparative atlas by Hof and co-workers (2000). Insertion coordinates were as follows (Figure 21):

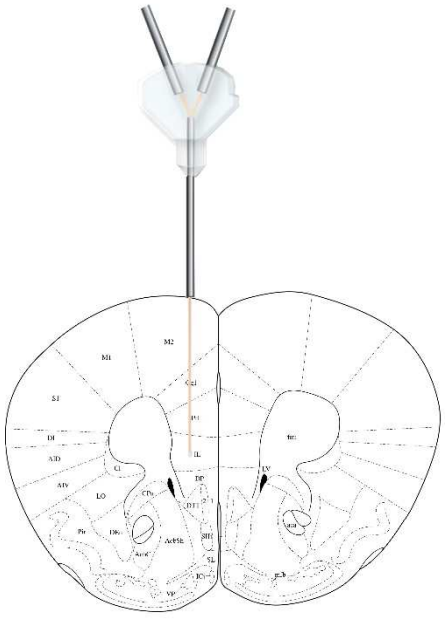


Fig. 21- Representative picture of microdialysis probe location in the frontal cortex of mice.

AP: +1.6 mm
L: +0.3 mm
DV: -3.3 mm
} 129/Sv strain FC

AP: +2.0 mm
L: +0.3 mm
DV: -3.3 mm
} C57BL/6 strain FC

Bregma was the benchmark to obtain AP and L coordinates, meanwhile skull surface was for DV coordinate. Artificial cerebrospinal fluid solution was steadily perfused through the probe during the insertion time to gain strength and firmness, avoiding breakage of

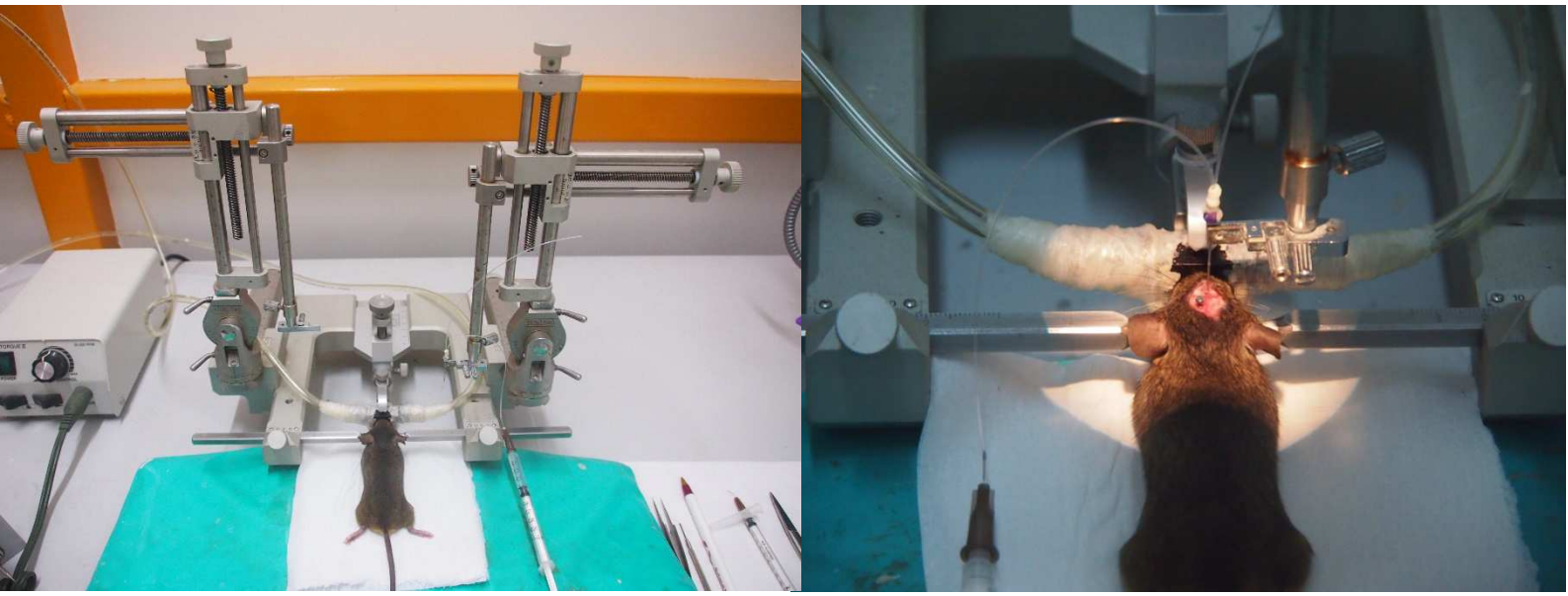


Fig. 22- Left image: skull fixation by earbars inside both ears prior to mouse surgery. Right image: intra-cortical implantation of a dialysis probe and anchor screw.

the dialysis membrane and facilitating a vertical inlet to ensure an accurate implantation. Probes were fixed in place with an anchor screw, a plastic housing as reinforcement and dental cement (Figure 22). Once the surgery was completed, the proper functioning of the probes was checked out and the ends of the polyethylene tubes were sealed.

After surgery, animals were left to recover for at least 18 hours, housed in single cages provided with free access to water and food. A major concern with intra-cerebral microdialysis is that the implantation of the probe could evoke inflammation and subsequent healing which could affect the results of the experiments. Benveniste and co-workers (1987) investigated local cerebral blood flow (LCBF) and local cerebral glucose metabolism (LCGM), being sensitive indicators of regional damage, following implantation of a microdialysis probe into the hippocampus. During the first 2 hours after implantation, rats showed disturbances in LCGM and LCBF, accompanied by a general decrease in blood flow. Around the probe small spots with increased glucose phosphorylation and decreased blood flow were detected. 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 hours post-implantation (Imperato & Di Chiara, 1984; De Lange *et al.*, 1997). 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.

Correct placement of the probes was histologically verified at the end of the experiments by staining the membrane with Fast Green solution (Figure 23).

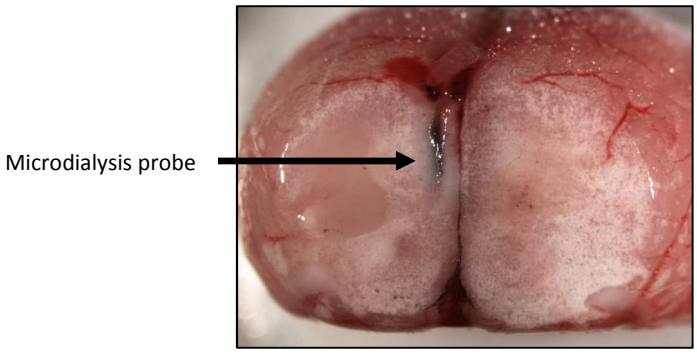


Fig. 23- Representative photograph of microdialysis probe location in mouse FC.

Rats

Rats were anesthetized in an induction chamber breathing isoflurane (4%) for 1-2 min, until they were utterly anesthetized. Unconscious animals were placed on the stereotaxic frame under constant flow of isoflurane for anesthesia maintenance (2%). To avoid hypothermia, animals laid over a heating pad during surgery. The general procedure was the same as mentioned for mouse surgery with some differences explained below.

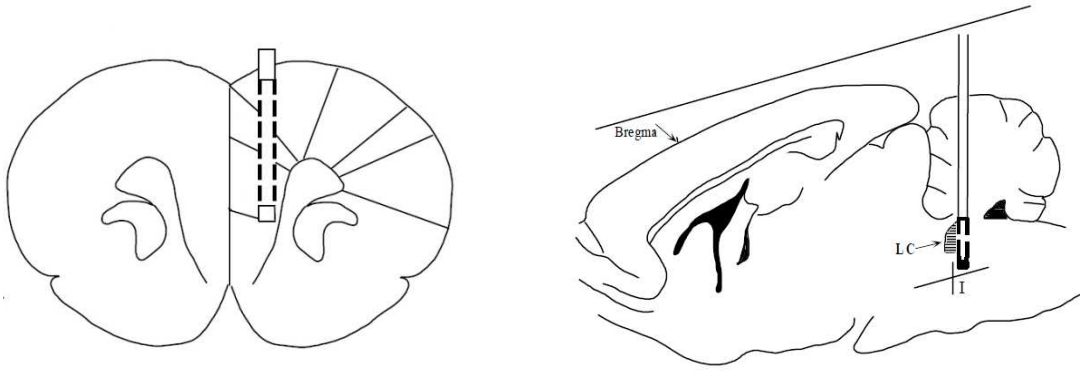
Once the head was immobilized, a sagittal incision was performed to expose the skull surface. The head was oriented downward at an angle of 10° - *bregma* 2 mm below *lambda* -. The skull was drilled four times: two holes - 3 mm approx. - were for probes implantation in the FC and LC, whereas the other two - around 0.5-1 mm diameter - were for the anchor screws placement. Prior to probe implantation, meninges were carefully removed to allow vertical insertion of the probe.

Intra-cerebral probes were stereotaxically implanted into the brain to allow positioning of dialysis probe tip in the FC and LC (Figure 24). Implantation coordinates were selected following the guidelines set by Paxinos & Watson atlas (1986).

A 2-mm membrane length was implanted into the LC vicinity. *Lambda* suture was the benchmark to obtain AP and L coordinates. Brain surface was the reference point to calculate DV coordinate.

A 4-mm membrane length was implanted into the FC. *Bregma* suture was the reference point to obtain AP and L coordinates, whereas brain surface was for DV coordinate.

Insertion coordinates were as follows:



AP: +2.8 mm
L: +1.0 mm
DV: -5.0 mm
} FC

AP: -3.7 mm
L: -1.3 mm
DV: -8.2 mm
} LC

Fig. 24- Representative picture of microdialysis probes location in the frontal cortex (left) and locus coeruleus (right) of rats.

CSF was steadily perfused through the probe during the insertion process to gain strength and firmness, avoiding breakage of the dialysis membranes and facilitating a vertical inlet to ensure an accurate implantation. Probes were fixed in place with two anchor screws (Figure 25), plastic housing as reinforcement, and dental cement. Once the surgery was completed, the proper functioning of the probes was checked out, and the ends of the polyethylene tubes were sealed.

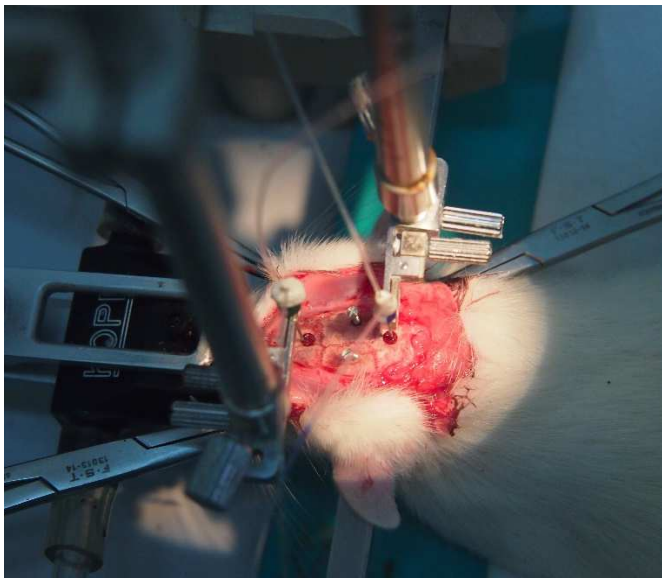


Fig. 25- Picture of a rat subjected to cerebral surgery, where microdialysis probes are being implanted into the FC and LC.

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

3.4.3. Collecting dialysate samples

3.4.3.1. Microdialysis equipment for awake mice

Sampling was carried out using a BASi equipment (BASi, West Lafayette, IN, USA), which allowed a constant flow rate throughout the probes (Figure 26). The equipment comprised the following components:

- Methacrylate cage covered by sawdust, where animal remained from the inception to the end of the experiment (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.

- Liquid switch (MD-1508, BASi, West Lafayette, IN, USA) 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 fraction collector (MD-1201, BASi, West Lafayette, IN, USA) kept the samples refrigerated at 4°C from the sample 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.

Fig. 26- Left photograph: BASi equipment used for microdialysis experiments in mice. Right photograph: 129/Sv strain mouse during microdialysis experiment.

3.4.3.2. Microdialysis equipment for awake rats

Sampling was carried out using a CMA/Microdialysis AB equipment (Stockholm, Sweden), which allowed a constant flow rate through the probes (Figure 27). The equipment is composed of the appliances below:

- Methacrylate cage covered by sawdust, where animal remained from inception to the end of the experiment.

- Precision pump (CMA/102, CMA/Microdialysis AB, Stockholm, Sweden) which guarantees working at constant flow rates from 0.1 to 20 µl/min with two separate syringes.

- Liquid switch (CMA/110, CMA/Microdialysis AB, Stockholm, Sweden) permits 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.

- Swivel (CMA/120, CMA/Microdialysis AB, Stockholm, Sweden) enables free movement of the animal. The swivel brace holds a wire with a collar connector and two holders to place plastic vials for sampling. The wire attached to the animal collar, turns the swivel and supports the tubing.

- Tubing system interconnecting all the components of the microdialysis equipment with the implanted probes in the experimental animals (FEP-tubing, Metalant AB, Stockholm, Sweden). Tubes have a dead volume of 1.2 µl/10 cm and opaque red color to protect neurotransmitters from light degradation. Special adaptors enable tubing interconnection (Tubing Adapters Metalant AB, Stockholm, Sweden).

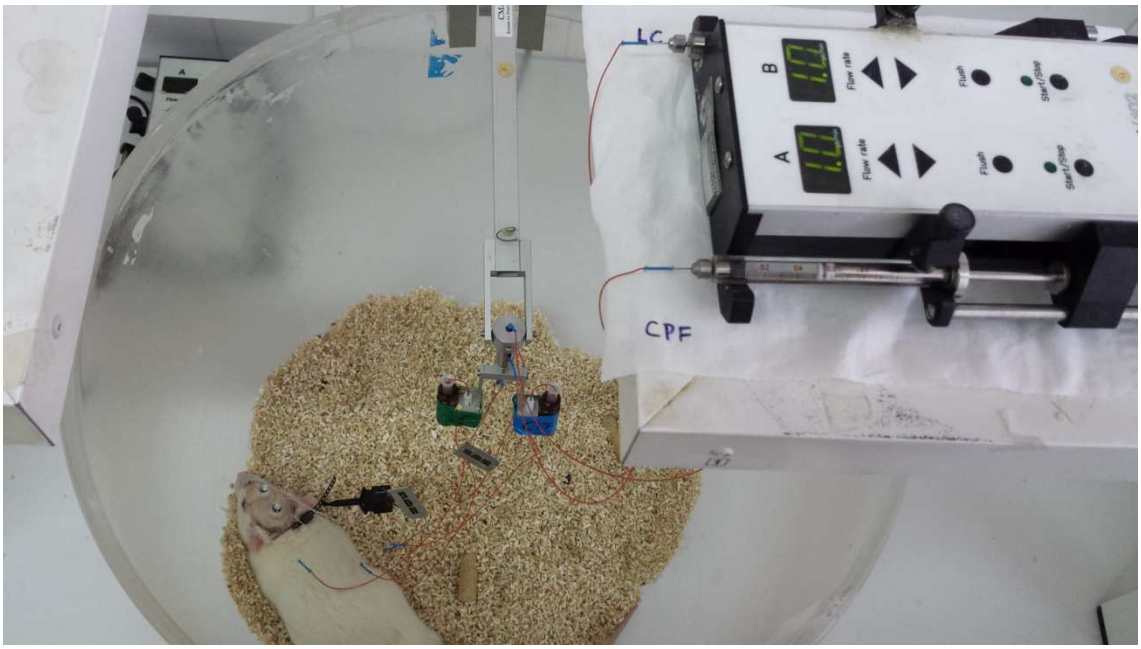


Fig. 27- Equipment used for microdialysis experiments in rats.

3.4.3.3. Conventional microdialysis

After the recovery period, probes were connected to the system and CSF solution was perfused at a rate of 1 µl/min through the microdialysis probes incessantly during the entire trial. CSF 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.

Mice

Cortical administration

Following 1 hour of acclimatization, 35-min samples were collected into a fraction collector. Time points were corrected for lag time of the perfusate from the microdialysis site to the probe outlet. The first six 35-min fractions - CSF alone or in combination with M100907 -, were used to establish monoamine baseline concentrations. The mean of the basal values was considered 100%, and the pharmacological effect was analyzed using that basal value as reference. Subsequently, the drug of interest was administered for 175 minutes (5 samples), after which CSF alone or combined with M100907 was perfused again (Figure 28). As previously mentioned, switching between syringes with no risk of air entrance or changes in pressure was possible by a Liquid switch (CMA/110, CMA/Microdialysis AB, Stockholm, Sweden).

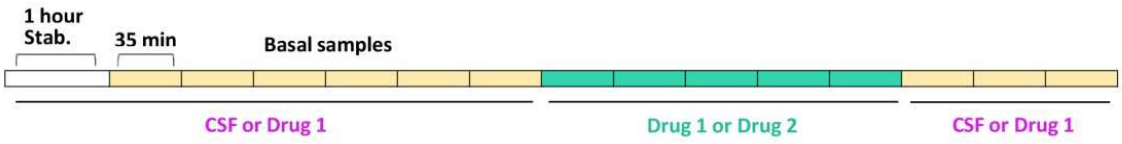


Fig. 28- Representative time-course scheme of intra-cortical administration of drugs in mice. Purple Drug 1: M100907 (300 µM); Green Drug 1: (±)-DOI (300 µM); Green Drug 2: M100907 (300 µM) + (±)-DOI (300 µM).

A key factor able to modulate the amount of drug that can reach the tissue is the flow rate at which CSF is being flushed. The lower the flow-rate, the higher the recovery is (De Lange *et al*., 1997; Stahle *et al*., 2000). The dead volume is the time spent since the drug is flushed from the inlet tube, until it reaches the microvial at the outlet tube. Thirty-five µl were calculated to be the dead volume of the BASi equipment. As the flow rate was 1 µl/min, eventually the dead volume corresponded to 35 minutes. In order to cover the time corresponding to that dead volume, an extra basal sample was collected in experiments using the BASi equipment.

Systemic administration

Following 1 hour of acclimatization, 35-min samples were collected into a fraction collector. The first five 35-min fractions with CSF were used to establish monoamine baseline concentrations. Subsequently, the drug of interest was intraperitoneally injected and 7 additional fractions were collected the following 245 minutes (Figure 29). In double-injection experiments, the first four fractions were collected to calculate basal concentrations. Afterwards, the first drug was injected and 35 min later the second one. As above, 7 additional 35-min samples were collected the following 245 minutes (Figure 30).

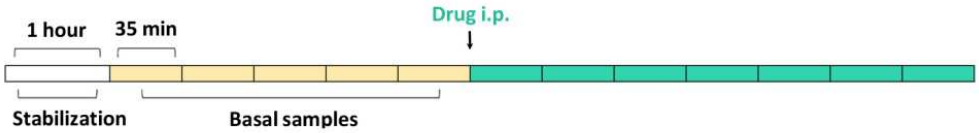


Fig. 29- Representative time-course scheme of a single systemic administration of drug or saline in mice. Drug: (±)-DOI (0.5-5 mg/kg) or Ro 60-0175 (3 mg/kg).

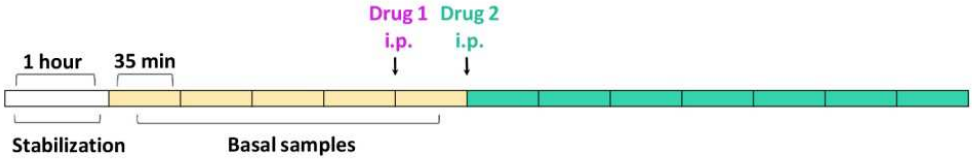


Fig. 30- Representative time-course scheme of a double systemic administration of drugs in mice. Drug 1: saline, M100907 (0.5 mg/kg) or SB242084 (1 mg/kg); Drug 2: (±)-DOI (1 mg/kg).

Rats

After 1 hour of acclimatization, and collection of three 35-min fractions to determine basal values of monoamines, drugs were delivered systemically or locally into the FC or LC. The mean of the basal values was considered 100% and the pharmacological effect was analyzed using that basal value as reference. To minimize inter-experimental error, pharmacological treatments were crossed, administering the same day control solution to some of the animals and drugs to others. Each animal was its own control, receiving one day vehicle or treatment and the second day the opposite.

Dead volumes were calculated in the equipment allocated for experiments in rats. In this case, a 10-minutes waiting time between drugs administration was enough to get rid of such dead volume.

Experimental designs of local administrations of drugs into the FC (Figure 31) or LC (Figure 32), as well as systemic administrations (Figure 33) are explained below:

Local administration in frontal cortex and simultaneous drug perfusion in the locus coeruleus

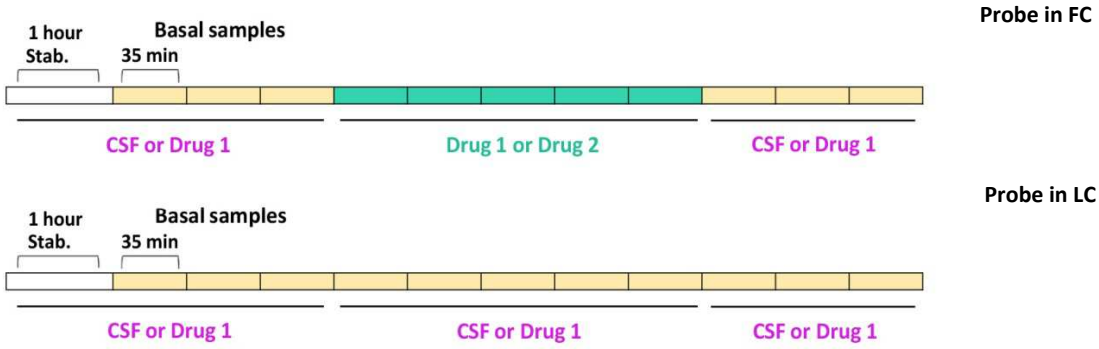


Fig. 31- Representative time-course scheme of cortical administration of drugs in rats. Purple Drug 1: KYN (1 mM); Green Drug 1: (±)-DOI (300 µM); Green Drug 2: KYN (1 mM) + (±)-DOI (300 µM).

Local administration in locus coeruleus

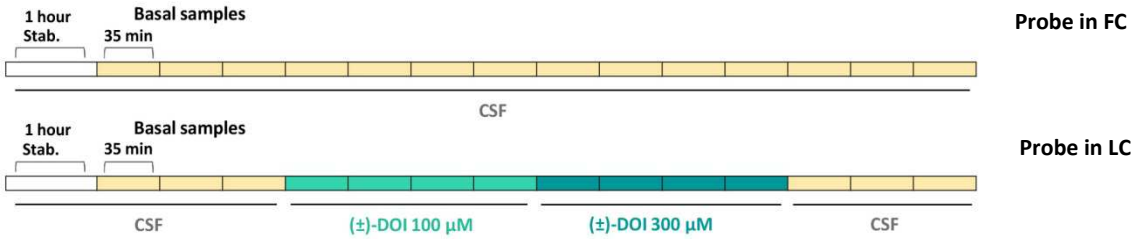


Fig. 32– Representative time-course scheme of intra-locus coeruleus administration of (±)-DOI (100 and 300 µM) in rats.

Systemic administration

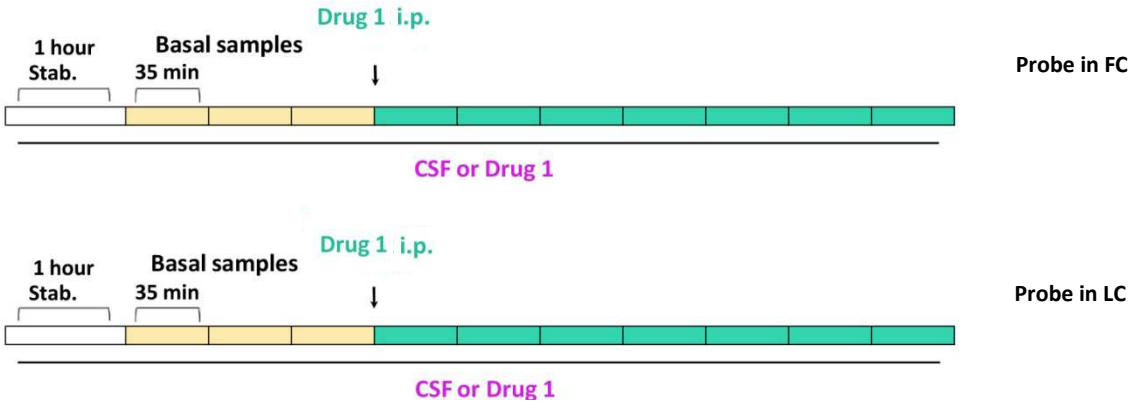


Fig. 33- Representative time-course scheme of systemic administration of drugs alone or in co-administration with other drugs locally into the rat FC or LC. Purple Drug 1: KYN (1 mM), RX821002 (1µM); Green Drug 1: (±)-DOI (1 mg/kg).

All fractions were stored at 0-10°C until their chromatographic analysis. To prevent NE and DA degradation from dialysate samples when using HPLC equipment, 5 µl of 0.6% $HClO_4$ were added to each vial. When using Alexys UHPLC equipment to quantify NE, DA and 5-HT, 5 µl of 0.1 M acetic acid were added to the vials instead of $HClO_4$ to prevent neurotransmitters from degradation.

Substances diffusion was carried out by concentration gradient. Substances transport is a slow process, and the concentration reaches in the dialysate does not correspond to the concentration that exists in the extracellular matrix. To get an approximation of the real extracellular concentrations is necessary to calculate the percentage of probe recovery of neurotransmitter of interest by *in vitro* assays. This parameter depends on the flow-rate, the pore size of the membrane and the molecular weight of the substance of interest. *In vitro* studies carried out in our own laboratory determined that this percentage is about 15-20% of the real concentration in the case of the neurotransmitters of interest, using a 2-mm length membrane (data not shown). Results displayed in this study are not corrected for probe recovery.

3.4.3.4. Histological verification of probe placement

At the end of the experiments, mice were slaughtered by cervical dislocation. Rats were culled by overdose of chloral hydrate solution. A dye solution was perfused through the probe for a few seconds to color the adjacent area where the probe was set. After removing the probe, brain was extracted and kept frozen at -20°C or -80°C. Coronal brain sections were obtained and checked to ensure correct location of the probe (see Figure 23).

3.4.4. Chromatographic analysis of the samples

3.4.4.1. Fundamentals of the HPLC 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, 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.

HPLC is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.

The sort of chromatography carries out in this study was reversed phase HPLC. In this case, the column is filled with long hydrocarbon chains (C_1-C_{18}) 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 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. The difference in potentials is directly proportional to the concentration of the analyte. Subsequently, a chromatogram was obtained, which is the record of the chromatographic separation, where each peak corresponds to a specific *redox* component in the sample.

3.4.4.2. Chromatographic equipments

Two different HPLC equipments were used. The first one was used to carry out experiments in mice and, as Figure 34 shows, it consists of:

- Degasser system for the mobile phase that avoids air entrance (Agilent HP 1100 Series, Santa Clara, CA, USA).
- A quaternary pump to push 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 stationary phase (Antec Scientific, Zoeterwoude, The Netherlands).
- Thermostat that allows to keep column temperature constant (Agilent HP 1100 Series, Santa Clara, CA, USA).
- Electrochemical detector Decade II (Antec Scientific, Zoeterwoude, The Netherlands).
- Control station to obtain chromatograms (DELL Optiplex 760, Round Rock, TX, USA).

The other chromatographic system used for experiments in rats, was an ALEXYS™ UHPLC Neurotransmitter Analyzer (Antec Scientific, Zoeterwoude, The Netherlands). It consists of (Figure 34):

- Degasser (OR 110, Antec Scientific, Zoeterwoude, The Netherlands), double-channel degasser with an integrated pulse snubber.

- 2 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.
- 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).

Fig. 34- Left panel: High-Performance Liquid Chromatography (HPLC) equipment coupled to an electrochemical detector. Right panel: Ultra-High Performance Liquid Chromatography (UHPLC) equipment coupled to two electrochemical detectors. 1=degasser; 2=pump; 3=autosampler; 4=chromatographic column; 5=electrochemical detector; 6=control station.

3.4.4.3. Monoamines detection

Samples from mice

Samples with a final volume of 40 µl: 35 µl dialysate + 5 µl of $HClO_4$ (0.1 M) were allocated in the Agilent HP 1100 Series autosampler. The injection volume for chromatographic analysis was 30 µl. The mobile phase used allowed the detection and measure of NE and DA and it comprised the following reagents: 50 mM phosphoric acid, 0.1 mM EDTA, 8 mM NaCl, 500 mg/l sodium octyl sulfate and methanol (12-20%). The pH 6.0 was adjusted with 85% H_3PO_4.

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.

The mobile phase flowed through the system at a 0.2 ml/min rate. To detect and quantify DA and NE levels, a VT-03 (Antec Scientific, Zoeterwoude, The Netherlands) electrochemical cell was used (Figure 35). *Redox* potential was 0.300 mV, and ALF-215, 2.1 x 150 mm, C18 (Antec Scientific, Zoeterwoude, The Netherlands) chromatographic column was used. The oven temperature was 35°C.

The peak areas of DA and NE were integrated by a Chemstation plus software (Hewlett Packard Ltd., Morain, OH, USA). A calibration line was calculated by the integration of peak areas from 6 standard concentration solutions of DA and NE in CSF (0.1, 0.5, 1, 3, 5, 10 nM).

Samples from rats

40 µl samples: 35 µl dilysate + 5 µl acetic acid (0.1 M) were placed in the AS 110, Antec autosampler. The injection volume in this UHPLC equipment was 10 µl. The mobile phase conditions used allowed the detection of DA, NE and 5-HT. However, only NE results obtained from these assays are discussed in the present study. DA and 5-HT levels were also measured and integrated for its use as internal controls. The mobile phase components were as follows: 100 mM phosphoric acid, 100 mM citric acid, 0.1 mM EDTA, 950 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 solution. The mobile phase was degassed for 10-15 minutes in a sonic bath.

The mobile phase flowed through the system at a rate of 75 µl/min. The flow cell SenCell (Antec Scientific, Zoeterwoude, The Netherlands), with a *redox* potential of 0.46 V, and an Acquity UPLC BEH C 18, 1.7 µm, 1 x 100 mm (Waters, Milford, MA, USA) chromatographic column were able to separate and detect NE, DA and 5-HT neurotransmitters (Figure 35). The oven temperature was 37°C.

DA, NE and 5-HT peaks were integrated by Clarity software (DataApex, Prague, Czech Republic). A calibration line was calculated by the integration of peak areas from 8 standard concentration solutions of DA, NE and 5-HT in CSF (0.01, 0.05, 0.1, 0.5, 1, 3, 5, 10 nM).

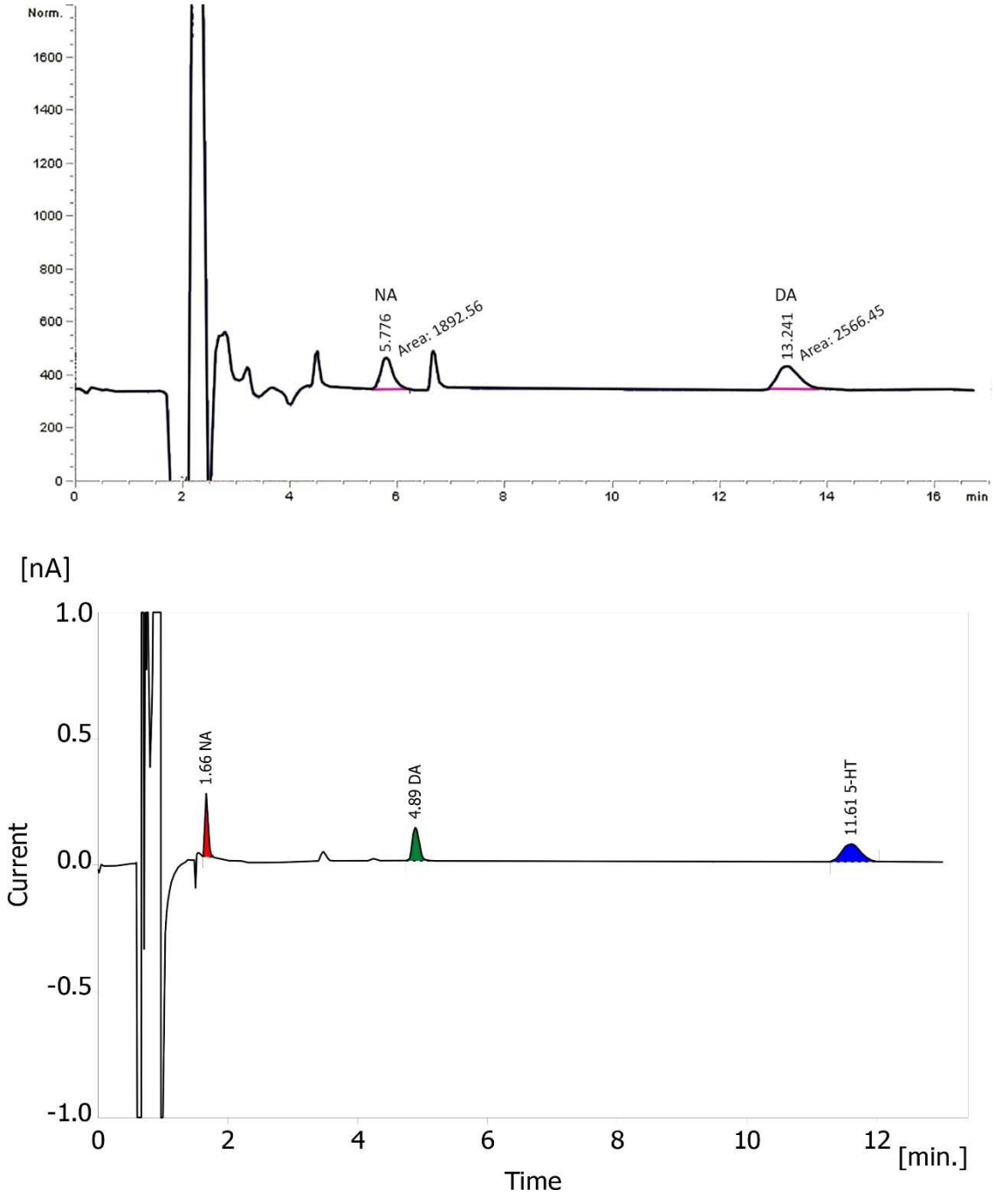


Fig. 35- Upper panel: Representative chromatogram of NE (RT: 5.776 min) and DA (RT: 13.241 min) in a 3 nM standard sample from HPLC system. Lower panel: Representative chromatogram of NE (RT: 1.66 min), DA (RT: 4.89 min) and 5-HT (RT: 11.61 min) in a 3 nM standard sample from UHPLC system.

3.5. VIRUS-MEDIATED GENE TRANSFER AND VIRAL DIFFUSION

3.5.1. Virus administration

The primers BamHI-hG2 (5'-TTTTGGATCCATGGTCCTTCTGTTGATCC-3') were used to amplify the sequence using the pcDNA3.1-HA-mGlu2 as template. The PCR product (2658 bp) was digested using BamHI and subcloned into the same restriction sites of a published bicistronic p1005+ *herpes simplex* virus (HSV) plasmid (Barrot *et al*., 2002; Tsankova *et al*., 2006; Kurita *et al*., 2012; Moreno *et al*., 2012). Viral particles were then packaged (Clark *et al*., 2002). HSV-mGluR2 or control HSV-GFP virus were injected into the mice FC by stereotaxic surgery. Animals were anesthetized with isoflurane (4% for induction and 1.5% for maintenance). The virus was delivered unilaterally with a Hamilton syringe (65459-01 2 µl Neuros model 7002 KH, point style 3, Reno, NV, USA), coupled to a Kopf® microinjector unit (Model 500, David Kopf Instruments, Tujunga, CA, USA), at a rate of 0.1 µl/min for a total volume of 0.5 µl. The following coordinates were used for virus delivery: AP= +1.6 mm; L= +0.3 mm; DV= -3.3 mm (AP and L relative to *bregma* and DV relative to the skull surface), with a 10° lateral angle.

Immediately after viral injection, a cannula (MAB 4.15.IC, Microbiotech/se AB, Stockholm, Sweden) was implanted in the insertion point location and was fixed to the skull using dental cement (liquid and powder TAB 2000, Kerr, Orange, CA, USA). Animals were housed 2 per cage during the following days, until microdialysis experiments were performed, in which animals were single housed. Three days after virus delivery, a commercial probe was implanted into the cannula (MAB 4.15.2.Cu, Microbiotech/se AB, Stockholm, Sweeden). The day after, four days after viral injection, microdialysis experiments were carried out. It has been reported that transgene expression is maximal 3-4 days after virus injection (Barrot *et al*., 2002; Kurita *et al*., 2012). Virus-mediated mGluR2 overexpression level in the FC was confirmed by Western blotting (Moreno *et al*., 2012).

3.5.2. Immunohistochemistry

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.

Immunofluorescence relies on the use of antibodies to label a specific target antigen with a fluorescent dye (also called fluorophores or fluorochromes) - such as fluorescein isothiocyanate (FITC) -. A fluorophore is a functional group in a molecule which absorbs energy of a particular wavelength and emits energy at a different but specific wavelength. Antibodies that are chemically conjugated to fluorophores are commonly used in immunofluorescence. The fluorophore allows visualization of the target distribution in the sample under a fluorescent microscope.

The method used in this study was indirect immunofluorescence. Two antibodies were used. The primary antibody is unconjugated and a fluorophore-conjugated secondary antibody directed against the primary antibody is used for detection (Figure 36).

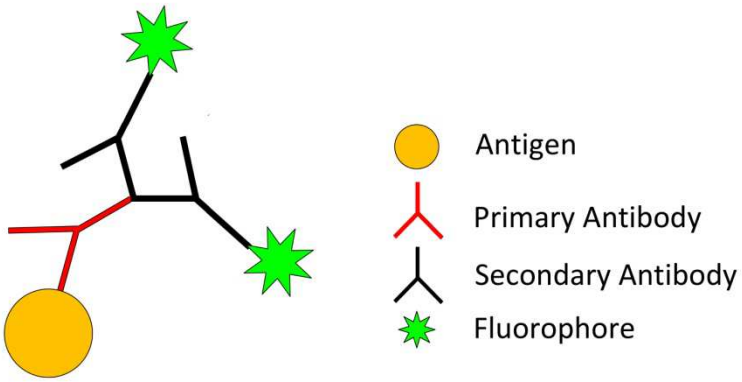


Fig. 36- Schematic picture of indirect immunofluorescence.

3.5.2.1. 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.

Prior to surgery, animals were deeply anesthetized via intraperitoneal injection of chloral hydrate (8%). 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, this secured the needle and prevented leakage. After making an incision to the right atrium to create as large an outlet as possible without damaging descending aorta, animals were perfused for 30 minutes at a pressure of 35 mmHg with a saline solution - 0.9% NaCl - to remove all the blood in vascular system. Subsequently, the fixation was performed using a freshly prepared solution of 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) (42.58 g Na_2HPO_4 and 13.79 g $NaH_2PO_4 \cdot H_2O$ in 1 l of water). The pump's pressure was also 35 mmHg and fixation lasted for 30 minutes.

Afterwards, the brain was removed from the skull and the fixation process was extended by incubation with 4 ml of 4% PFA for 24 additional hours in 10-ml tubes at 4°C. After this time, PFA was replaced by 4 ml of 30% sucrose solution (30 g sucrose, 5 µl sodium azide dissolved in 100 ml PBS at pH 7.4) for 3-7 days at 4°C, until its precipitation. Once precipitated, the brain was stored at 4°C until sectioning.

3.5.2.2. Brain sectioning

Frozen brains were embedded in a cryogenic gel (Tissue-Tek, Radnor, PA, USA) on mounting plates for cryostat sectioning (Thermo Fisher Scientific, Waltham, MA, USA). 40 µm-thick coronal slices were sectioned and preserved in 1 ml PBS (1x) at 4°C to perform immunofluorescence assays in 12-well plates.

3.5.2.3. Immunofluorescence

Immunofluorescence procedure was carried out following a modified method used by Fribourg and co-workers (2011). Cerebral sections were incubated for 2 hours at room temperature in 1 ml/well blocking buffer (5% BSA, 0.2% Triton X-100 in 1x PBS) with agitation (75 rpm), to reduce nonspecific binding and to permeabilize the cell membrane to antibodies. Subsequently, the slices were incubated overnight at 4°C in PBS containing 2.5% BSA, 0.2% Triton X-100 and (1:100 dilution) rabbit anti-GFP primary antibody (sc-8334, Santa Cruz Biotechnology, Dallas, TX, USA) with agitation (75 rpm).

The following day, after washing with blocking buffer (3 times x 1 ml, 3 minutes each), the slices were further incubated with the secondary antibody (1:500 dilution) Alexa 488-conjugated donkey anti-rabbit (A21206, Invitrogen, Carlsbad, CA, USA) for 1 hour at room temperature in the dark, with agitation (110 rpm). After washing (PBS, 3 times x 1 ml), the slices were mounted onto a glass slide to dry at 4°C in the dark. Once dried, a few drops of DPX (Electron Microscopy Sciences, Hatfield, PA, USA) were added to the slices and a coverslip was attached. Fluorescence was measured using a Nikon DS-Ri1microscope (Nikon, Tokyo, Japan) equipped with a 488 nm laser to observe FITC (Figure 37). Photographs at 4, 10, 20 and 40 times magnification were taken.

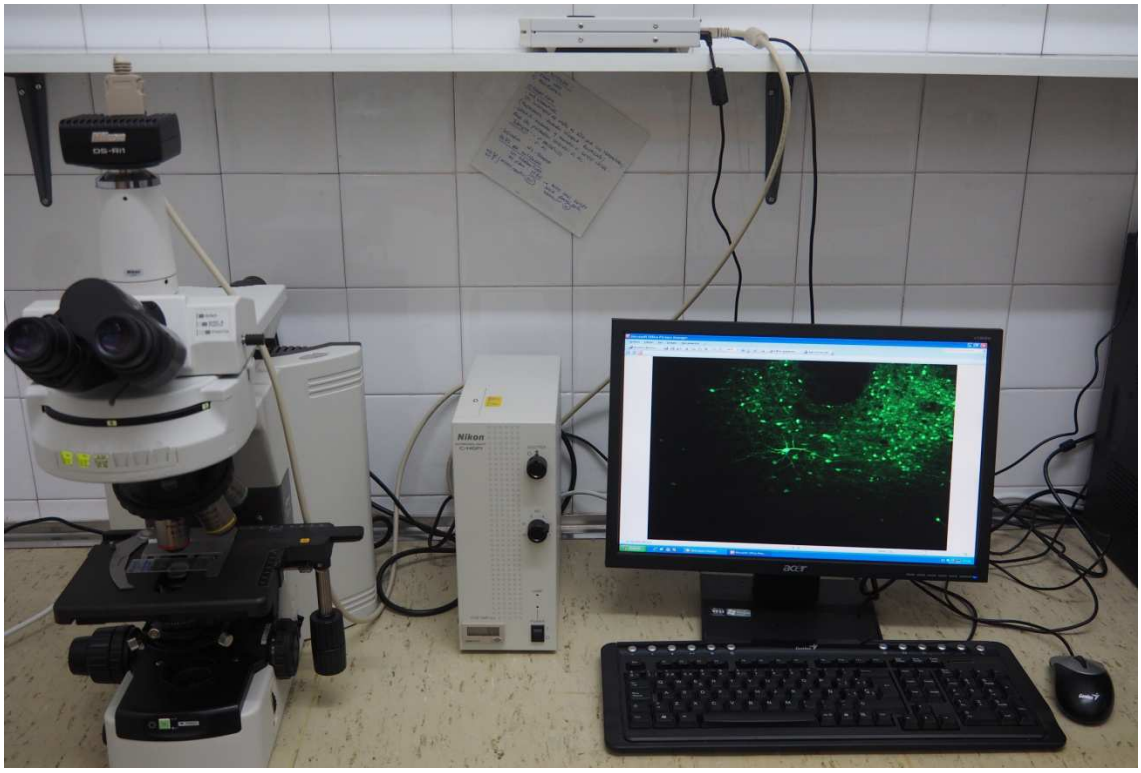


Fig. 37- Immunofluorescence microscope used for immunofluorescence assays.

3.6. G_i PROTEINS INHIBITION THROUGH PTX INJECTION

3.6.1. Intracerebroventricular administration of pertussis toxin

Pre-treatment with pertussis toxin (1 µl/min) or vehicle was intracerebroventricularly administered in a final volume of 4 µl. Once animals were immobilized by the researcher, injections were made into the right lateral ventricle by a 10 µl Hamilton syringe with a 26-gauge needle (Model 701 N SYR, 26 s gauge, point style 2, Hamilton, Reno, NV, USA), as previously described (Sánchez-Blázquez *et al*., 1995).

3.6.2. Behavioral study: Head twitch response to (±)-DOI

The head twitch response (HTR) is a rapid side-to-side rotational head movement that occurs in rats and mice after administration of serotonergic 5-HT2AR agonist hallucinogens. The HTR is widely used as a behavioral assay for 5-HT2AR activation and psychotic response (Moreno *et al*., 2012; Halberstadt & Geyer, 2013a).

To carry out these assays, mice were transferred individually into plastic cages in the behavior room, at least one hour before testing. Twenty minutes after the intraperitoneal administration of (±)-DOI (0.5 mg/kg or 1.0 mg/kg) or saline, animals were recorded using SMART software (SMART v3.0, Panlab Harvard Apparatus, Barcelona, Spain) for 30 minutes. Afterwards, the recordings were examined for head twitch events quantification.

3.6.3. Temperature variation induced by (±)-DOI

Animals were transferred into the behavioral room at least one hour before the experiment performance. The room was pre-heated with a stove up to 26°C. Saline and (±)-DOI solution were heated up to body temperature to prevent hypothermia mediated by liquid injection.

Rectal temperature was measured and monitored every 30 minutes using a digital thermometer with flexible probe (PiC solution, Artsana, Grandate, Italy) and Vaginesil gel (Combe International Ltd, Leatherhead, United Kingdom) to lubricate the rectal area. Three basal temperatures were taken, and the average of these values was considered the basal

temperature. Temperature was measured 4 times (120 min) after intraperitoneal injections of saline or (±)-DOI 0.5 mg/kg. Variations of temperature were represented as the difference in temperature over the basal value every 30 minutes.

3.7. DATA AND STATISTICAL ANALYSIS

Graphs and statistical analysis were carried out using GraphPad Prism v5.01™ software (GraphPad Software, San Diego, CA, USA). In all cases, results were represented as mean values ± standard error of the mean (SEM) and the minimum statistical significance was set at $p<0.05$.

3.7.1. Intracerebral microdialysis in freely moving animals

In order to establish DA and NE basal concentrations, the first 5 or 6 sample fractions in mice, and 3 in rats, were collected. Mean basal values were considered 100%. The effect triggered by the administration of the drugs of interest was calculated and represented as the percentage of baseline. The statistical analysis undertook to measure differences between groups were as follows:

- The analysis of basal levels between groups was performed by two-tailed unpaired Student's *t*-test.

- Two-way repeated measures ANOVAs, with drug or genotype groups as the independent variables, and time as the repeated-measure factor, were used to evaluate whether an interaction between these two variables on the dependent variable existed (e.g. % of neurotransmitter release, AUC). Bonferroni's post hoc test was employed for individual sample comparison. *F* values are expressed as: $F_{\text{treatment}}$ expresses the ability of the treatment to exert an effect, F_{genotype} expresses differences between genotypes, $F_{\text{interaction}}$ (F_i) expresses the magnitude of difference in the response between the two independent variables on the dependent variable (e.g. genotype or treatment over time).

- Areas under curve (AUC) were calculated from the drug administration time point until the end of the assay. Prior to calculate AUC values from each animal, every single percentage value was subtracted 100. Afterwards, net area AUCs were calculated and differences between treatment and genotype groups were assessed by two-way ANOVA followed by Least Significant Difference (LSD)'s post hoc test (InVivoStat software, Cambridge, United Kingdom).

- One-way ANOVA followed by Dunnett's post hoc test was used to analyze the effect of a drug over time, from AUC data of specific drug treatment groups.

3.7.2. Head-twitch response and Temperature variation

Differences between two groups in HTR experiments were assessed using unpaired two-tailed Student's *t*-test. Two-way ANOVA analyses followed by Bonferroni's post hoc test were calculated to evaluate interactions between an independent variable (pre-treatment or genotype) on a dependent variable (treatment).

Weight and temperature variation was evaluated using two-way ANOVA, regarding time as the dependent variable, and genotype or pre-treatment as the independent variable. In experiments undertaken to determine whether an interaction effect between three independent variables (e.g. genotype, treatment and time course) on a continuous dependent variable (temperature variation) exists, three-way ANOVA was performed. All data were represented as means ± SEM. These ANOVA analyses were followed by Bonferroni's post hoc test. As above, the level of significance was chosen at $p<0.05$.

4. RESULTS

4.1. FUNCTIONAL EVALUATION OF THE 5-HT2AR/mGluR2 HETEROCOMPLEX BY *IN VIVO* BRAIN MICRODIALYSIS IN AWAKE, FREELY MOVING MICE

4.1.1. Influence of the 5-HT2AR/mGluR2 heterocomplex located in mice frontal cortex in the responses triggered by the cortical administration of the hallucinogenic 5-HT2A/2C receptor agonist (±)-DOI

4.1.1.1. Effect of the cortical infusion of (±)-DOI on DA and NE release in the frontal cortex of mGluR2$^{+/+}$ and mGluR2$^{-/-}$ mice

At least 18 hours after the implantation of the probes, 1.5 µM desipramine dissolved in CSF was perfused into the FC during the entire procedure. Desipramine is a NET inhibitor that blocks NE reuptake into the nervous terminal, increasing the concentration of this neurotransmitter in the synaptic cleft. NET is also able to reuptake extracellular DA. According to a substantial number of studies, both acute and chronic desipramine administration increase cortical NE and DA concentrations (Carboni *et al.*, 1990; Devoto *et al.*, 2003a; Devoto *et al.*, 2003b; Pan *et al*., 2004; Bongiovanni *et al*., 2005; Higashino *et al.*, 2014). The presence of desipramine enabled to raise NE and DA basal extracellular concentrations, facilitating the detection of these monoamines in the HPLC.

(±)-DOI is a 5-HT2A/2C receptor agonist showing nearly 10-fold higher affinity for 5-HT2AR than for 5-HT2CR (Hemrick-Luecke & Evans, 2002; May *et al*., 2003; Pigott *et al*., 2012). According to Bortolozzi and co-workers (2003), a concentration of 300 µM of (±)-DOI would be optimal to exert the maximum extracellular 5-HT release in mice FC after its cortical perfusion. Therefore, this concentration was selected to be infused into the FC in the following experiments.

Dopamine

After 1 hour period of stabilization, basal extracellular DA concentrations in dialysates were measured. DA basal dialysate concentrations were collected from five-six consecutive samples from the FC without considering probe recovery and pooling from all the experiments

undertook with these mice. Basal values did not differ across genotype: 1.637 ± 0.115 nM (*n*=99) in mGluR2$^{+/+}$ mice and 1.799 ± 0.204 (*n*=71) in mGluR2$^{-/-}$ mice (*t*=0.738; *p*=0.461).

Perfusion of (±)-DOI (300 µM) through FC microdialysis probes markedly increased DA output in control mGluR2$^{+/+}$ animals, displaying a maximal effect (E_{max}) of 163 ± 16% of basal values (Figure 38). Two-way repeated measures ANOVA revealed a significant (±)-DOI-induced effect over time, when compared to the group treated with vehicle (VEH) ($F_i[13, 221]=5.397$; $p<0.0001$). In mGluR2$^{-/-}$ mice, (±)-DOI triggered a lower DA stimulation (Figure 38), but still significant with respect to VEH (E_{max} = 134 ± 11%) ($F_i[13, 234]=3.498$; $p<0.0001$). The maximal concentration was yielded at the same time point as for its wild-type counterparts.

Despite both genotypes displayed a rise in extracellular DA concentration after (±)-DOI administration, the magnitude of the effect was significantly higher in mGluR2$^{+/+}$ mice. Thus, two-way repeated measures ANOVA revealed a significant genotype x time interaction ($F_i[13, 325]= 3.267$; $p<0.0001$) (Figure 38).

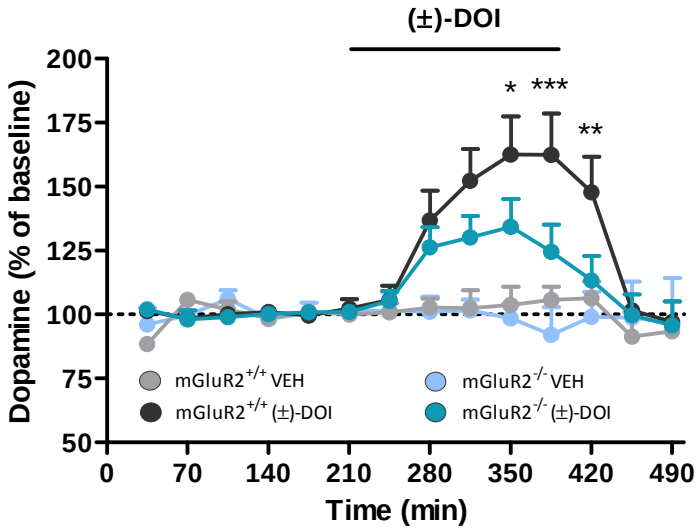


Fig. 38- Effect of cortical administration of VEH or 300 µM (±)-DOI on extracellular concentrations of DA in the FC of mGluR2$^{+/+}$ (● *n*=6, ● *n*=13) and mGluR2$^{-/-}$ mice (● *n*=6, ● *n*=14). Data are means ± SEM of DA expressed as a percentage of basal values (100%). Line represents drug infusion time. **p<0.05, **p<0.01, ***p<0.001 versus* (±)-DOI-treated mGluR2$^{+/+}$ mice (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

In order to evaluate the magnitude of the effects after local VEH or (±)-DOI administration, DA AUC values after drug infusion were analyzed (Figure 39). In both genotypes (±)-DOI evoked a rise of DA outflow comparing to the respective group where CSF was administered ($F_{treatment}[1,$

35]=12.26; p<0.01). Wild-type animals displayed a higher stimulatory effect after (±)-DOI infusion when compared to their knock-out counterparts. Nonetheless, differences between genotypes did not but were close to reach significance ($F_{genotype}$*[1, 35]=3.60; p=0.066*).

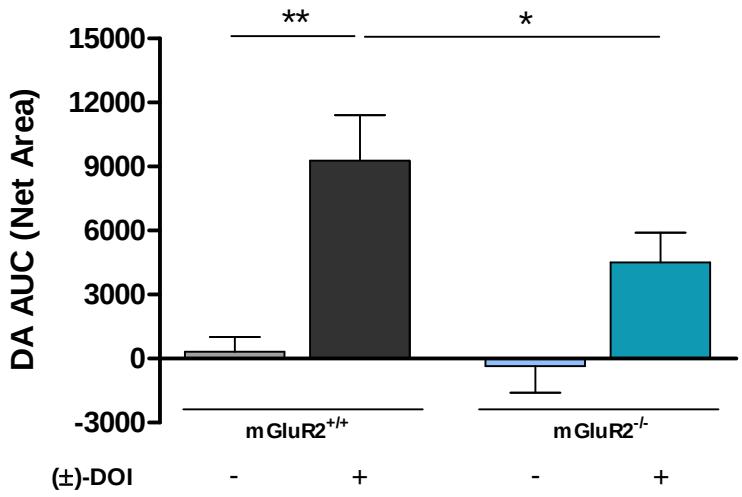


Fig. 39- Cortical DA AUC values 210 minutes after the beginning of the procedure to the end, where drug was perfused in the FC of mGluR2$^{+/+}$ (● *n*=6, ● *n*=13) and mGluR2$^{-/-}$ mice (● *n*=6, ● *n*=14). All results are expressed as means ± SEM. **p<0.05, **p<0.01* (two-way ANOVA followed by LSD's post hoc test).

Norepinephrine

Basal extracellular concentrations of NE in the FC were obtained by pooling values from all the experiments regarding cortical infusion of CSF and without considering probe recovery. Basal values were significantly higher in mGluR2$^{-/-}$ mice when compared to mGluR2$^{+/+}$ group: 1.690 ± 0.106 nM (*n*=77), 1.420 ± 0.065 nM (*n*=125), respectively (*t=2.302; p<0.05*). Intra-cortical CSF infusion did not alter cortical extracellular NE concentrations in either genotype.

As previously reported for DA, the intra-cortical administration of 300 µM (±)-DOI elicited a significant enhancement of NE outflow in the FC of mGluR2$^{+/+}$ control animals, when compared to VEH-treated group (F_i*[13, 260]=6.372; p<0.0001*). The (±)-DOI-induced increase of extracellular NE concentrationss in mGluR2$^{-/-}$ mice was roughly half of the magnitude as produced by wild-type animals, but still significant with respect to the VEH-treated group (F_i*[13, 234]=5.223; p<0.001*). The maximal (±)-DOI-induced increase in extracellular NE concentrationss of mGluR2$^{-/-}$ animals was 128 ± 5%, whereas the hallucinogen increased extracellular NE concentrationss in mGluR2$^{+/+}$ up to 156 ± 13% (Figure 40). Two-way repeated

measures ANOVA revealed a significant interaction based on the drug response yielded between genotypes over time ($F_i[13, 351]=3.579; p<0.0001$).

In terms of the (±)-DOI-induced effect magnitude, the drug exerted an enhancement of NE release in both genotypes, compared to the respective VEH-treated groups ($F_{treatment}[1, 38]=20.68; p<0.0001$). Moreover, wild-type animals displayed a substantially higher stimulatory effect compared to their knock-out counterparts ($F_{genotype}[1, 38]=6.70; p<0.05$), as shown in Figure 41.

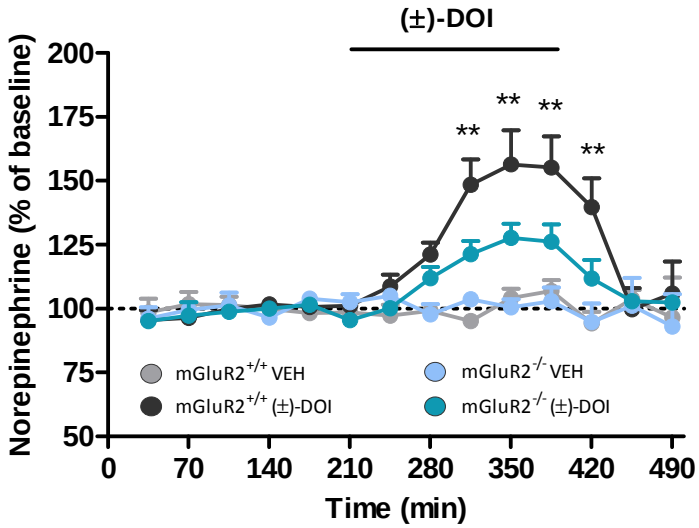


Fig. 40- Effect of cortical administration of VEH or 300 µM (±)-DOI on extracellular concentrations of NE in the FC of mGluR2 $^{+/+}$ (● *n*=6, ● *n*=16) and mGluR2$^{-/-}$ mice (● *n*=7, ● *n*=13). Data are means ± SEM of NE expressed as a percentage of basal values (100%). Line represents drug infusion time. ***p<0.01 versus* (±)-DOI-treated mGluR2$^{+/+}$ mice (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

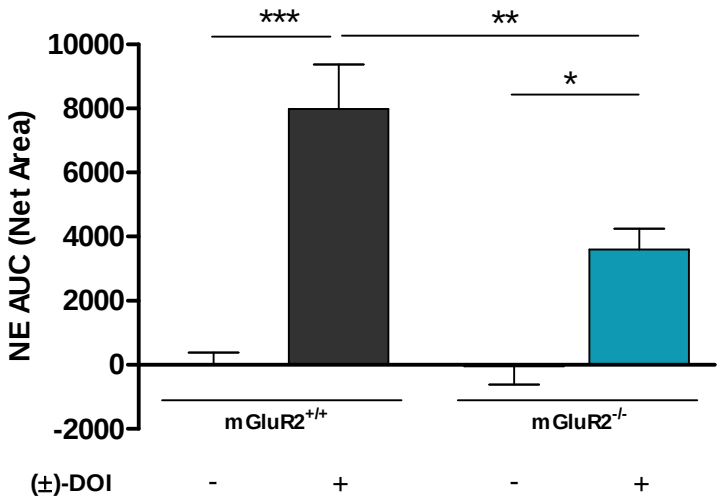


Fig. 41- Cortical NE AUC values 210 minutes after the beginning of the procedure to the end, where drug was perfused in the FC of mGluR2$^{+/+}$ (● *n*=6, ● *n*=16) and mGluR2$^{-/-}$ mice (● *n*=7, ● *n*=13). All results are expressed as means ± SEM. **p<0.05, **p<0.01, ***p<0.001* (two-way ANOVA followed by LSD's post hoc test).

4.1.1.2. Effect of intra-cortical co-perfusion of the 5-HT2AR antagonist M100907 on (±)-DOI-induced rise of extracellular DA and NE in the cortex of mGluR2$^{+/+}$ and mGluR2$^{-/-}$ mice

M100907 is a potent and primarily 5-HT2AR antagonist that displays approximately 100-fold higher affinity for 5-HT2AR than for other receptors, including 5-HT2CR and sigma receptors (Sorensen *et al*., 1993; Schreiber *et al*., 1994; Kehne *et al*., 1996; Gobert *et al*., 2000; Knight *et al*., 2004; Kristiansen *et al*., 2005; Pehek *et al*., 2006). Substantial scientific evidence suggests that M100907 can abolish effects derived by (±)-DOI (Pehek *et al*., 2001; Szabo *et al*., 2001; Body *et al*., 2006; Pehek *et al*., 2006).

Dopamine

The sole administration of M100907 (300 µM) into the FC was able to significantly decrease cortical DA release in both mGluR2$^{+/+}$ and mGluR2$^{-/-}$ genotype groups (E_{max}= -65 ± 2% and E_{max}= -66 ± 7%, respectively) (Figure 42). Two-way repeated measures ANOVA showed a strong effect of treatment over time, when compared to VEH-treated groups, in both genotypes ($F_i[13, 117]=26.09$; $p<0.0001$ in mGluR2$^{+/+}$ and $F_i[13, 117]= 8.905$; $p<0.0001$ in mGluR2$^{-/-}$). There was no significant differences between genotypes in the response triggered by the drug ($F_{genotype}[1, 104]=0.011$; $p=0.920$), neither a significant interaction genotype x treatment over time ($F_i[13, 104]=1.323$; $p=0.211$).

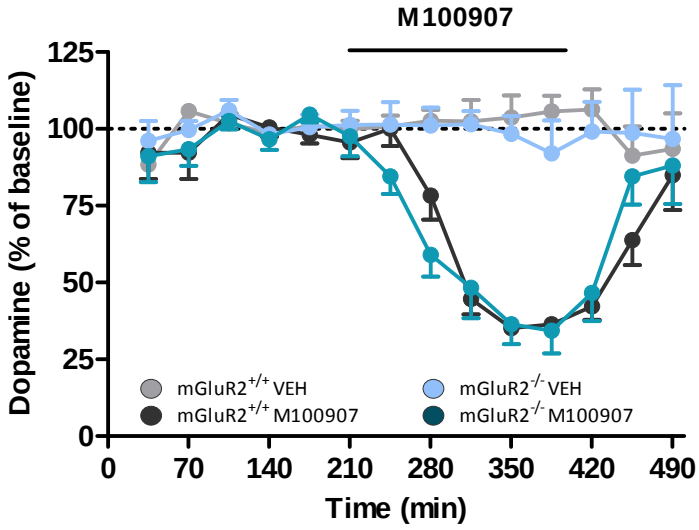


Fig. 42- Effect of cortical administration of VEH or 300 µM M100907 on extracellular DA concentrations in the FC of mGluR2 $^{+/+}$ (● *n*=6, ● *n*=5) and mGluR2$^{-/-}$ mice (● *n*=6, ● *n*=5). Data are means ± SEM of DA expressed as a percentage of basal values (100%). Line represents drug infusion time.

Before M100907 administration, there was not significant differences in basal values compared to the control (VEH) group, neither in mGluR2$^{+/+}$ nor in mGluR2$^{-/-}$ mice. mGluR2$^{+/+}$ mice subjected to M100907 perfusion (*n*=5), displayed a basal values reduction of up to 57% with respect to VEH-treated group (*n*=24) (*t=1.90; p=0.068*). Likewise, in mGluR2$^{-/-}$ mice, basal concentrations also decreased when perfused with M100907 (*n*=5), showing a reduction of 70% of basal values obtained with VEH perfusion (*n*=25) (*t=1.96; p=0.06*). Here again, no differences in DA basal values between genotypes were shown in VEH-treated (*t=1.820; p=0.07*) nor in M100907-treated groups (*t=1.488; p=0.175*).

Perfusion of 300 µM M100907, administered since the beginning of the experiment, significantly attenuated the (±)-DOI-induced increase in extracellular DA concentrations in the FC of mGluR2$^{+/+}$ mice (Figure 43). Statistical analysis using two-way repeated measures ANOVA showed a significant interaction between treatment and time (F_i*[13, 208]=2.780; p<0.01*). Conversely, M100907 did not block the (±)-DOI-induced rise of DA outflow in mGluR2$^{-/-}$ mice (F_i*[13, 221]=0.866; p=0.589*) (Figure 44).

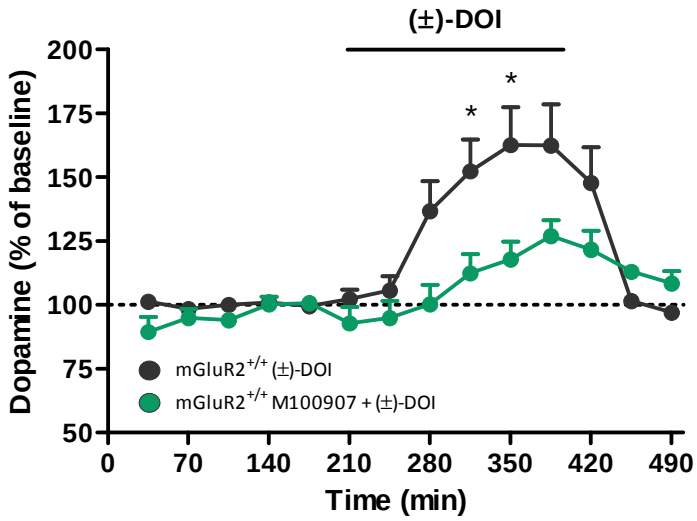


Fig. 43- Influence of the presence or absence of the 5-HT2AR antagonist M100907 (300 µM) on the modulation by the 5-HT2A/2C receptor agonist (±)-DOI (300 µM) of extracellular concentrations of DA in the FC of mGluR2$^{+/+}$ mice (● *n*=13, ● *n*=5). Data are means ± SEM of DA levels expressed as a percentage of basal values (100%). M100907 perfusion was initiated since the beginning of the experiment. The line represents (±)-DOI infusion time. **p<0.05* (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

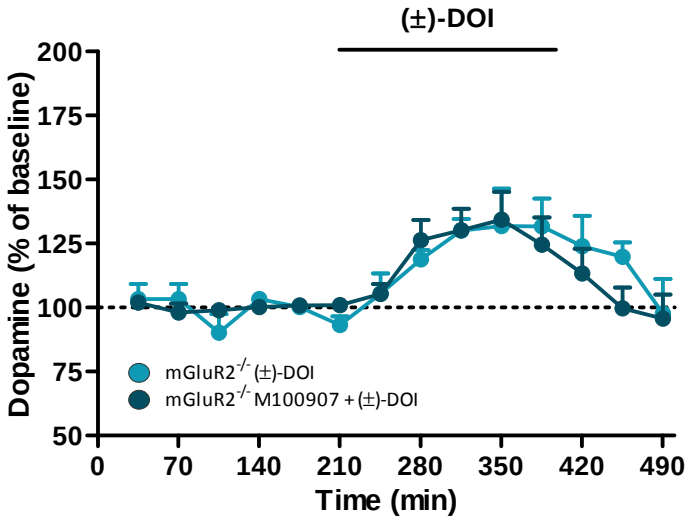


Fig. 44- Influence of the presence or absence of the 5-HT2AR antagonist M100907 (300 µM) on the modulation by the 5-HT2A/2C receptor agonist (±)-DOI (300 µM) of extracellular concentrations of DA in the FC of mGluR2$^{-/-}$ mice (● *n*=14, ● *n*=5). Data are means ± SEM of DA concentrations expressed as a percentage of basal values (100%). M100907 perfusion was initiated since the beginning of the experiment. The line represents (±)-DOI infusion time.

As shown in Figure 45, CSF perfusion did not modify AUC of DA efflux either in mGluR2$^{+/+}$, nor in mGluR2$^{-/-}$ mice. The figure also reports the magnitude of the overall increase in cortical DA efflux induced by 300 µM (±)-DOI (AUC mean=9271 ± 2132) in mGluR2$^{+/+}$ mice. This response was prevented by the continuous infusion of the 5-HT2AR antagonist M100907 (300 µM) in mGluR2$^{+/+}$ mice (AUC mean=3299 ± 1109). In contrast, the low DA stimulation generated by the hallucinogen in mGluR2$^{-/-}$ mice (AUC mean=4507 ± 1401) was not reverted by the presence of the antagonist (AUC mean=5520 ± 1413), showing a carry-over effect potentially unresponsive to 5-HT2AR antagonism. In that sense, two-way ANOVA analysis unmasked a significant effect of treatment ($F_{treatment}[2, 43]=7.07; p<0.01$).

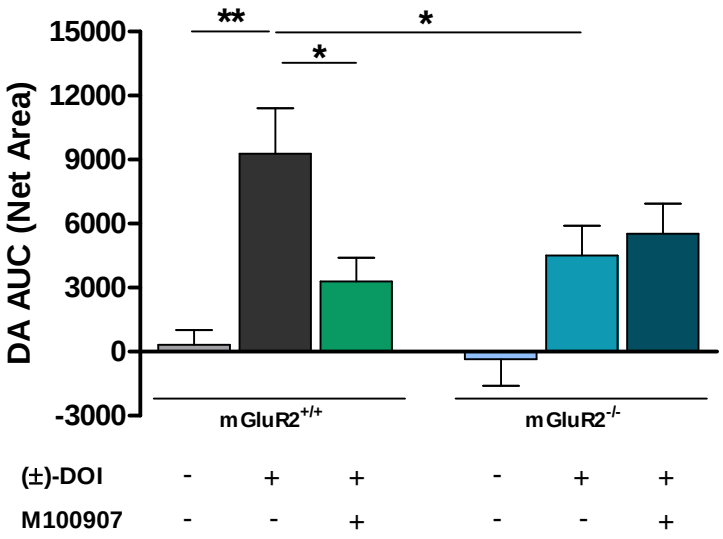


Fig. 45- Cortical DA AUC values 210 minutes after the beginning of the procedure to the end. Effect of intra-cortical infusion of vehicle, (±)-DOI (300 µM) and M100907 (300 µM) + (±)-DOI (300 µM) in mGluR2$^{+/+}$ (● *n*=6, ● *n*=13, ● *n*=5) and mGluR2$^{-/-}$ mice (● *n*=6, ● *n*=14, ● *n*=5). All results are expressed as means ± SEM. **p<0.05, **p<0.01* (two-way ANOVA followed by LSD's post hoc test).

Norepinephrine

Local administration of the antagonist 5-HT2AR M100907 (300 µM) slightly but significantly declined extracellular NE outflow in mGluR2$^{+/+}$ animals, compared to vehicle-treated group (F_i*[13, 130]=3.332; p<0.001*). The maximal inhibitory effect reached was E_{max}= -13 ± 8% (Figure 46). Likewise, an inhibitory effect after intra-cortical M100907 administration was exhibited in their knock-out counterparts (E_{max}= -24 ± 9%) (F_i*[13, 143]=5.067; p<0.0001*) (Figure 46). Statistical analysis established no differences among genotypes in the inhibitory response triggered by the local administration of 300 µM M100907 over time (F_i*[13, 130]=1.095; p=0.369*).

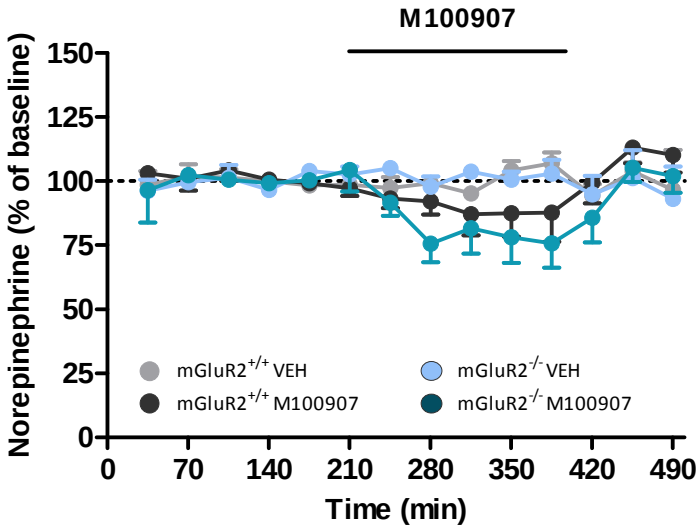


Fig. 46- Effect of cortical administration of VEH or M100907 (300 µM) on extracellular NE concentrations in the FC of mGluR2 $^{+/+}$ (● *n*=6, ● *n*=6) and mGluR2$^{-/-}$ mice (● n=6, ● n=6). Data are means ± SEM of NE expressed as a percentage of basal values (100%). M100907 perfusion was initiated after collecting the sixth sample. Line represents drug infusion time.

Despite the local administration of M100907 yielded a statistically significant inhibitory effect (Figure 46), in the following experiments pre-treatment with M100907 (300 µM) showed a downward trend in basal extracellular NE, but not sufficient to reach statistical significance in either mGluR2$^{+/+}$ and mGluR2$^{-/-}$ groups, compared to groups pre-treated with vehicle. Basal values in mGluR2$^{+/+}$ mice were 11% lower in M100907-treated group (*n*=5) than they were in vehicle-treated group (*n*=28) (*t=0.441; p=0.662*). mGluR2$^{-/-}$ mice undergone to M100907 perfusion (*n*=6) displayed a reduction of 35% in basal concentrations, compared to the vehicle-treated group (*n*=26) (*t=1.738; p=0.092*). No significant differences between genotypes were found in vehicle-treated groups (*t=0.237; p=0.814*), or in M100907-treated groups (*t=1.568; p=0.151*).

Local administration of M100907 since the beginning of the experiment was able to fully block the NE stimulation induced by intra-cortical (±)-DOI in both mGluR2$^{+/+}$ animals (Figure 47) (F_i*[13, 247]=5.461; p<0.0001*) as well as in mGluR2$^{-/-}$ mice (Figure 48) (F_i*[13, 221]=5.834; p<0.0001*).

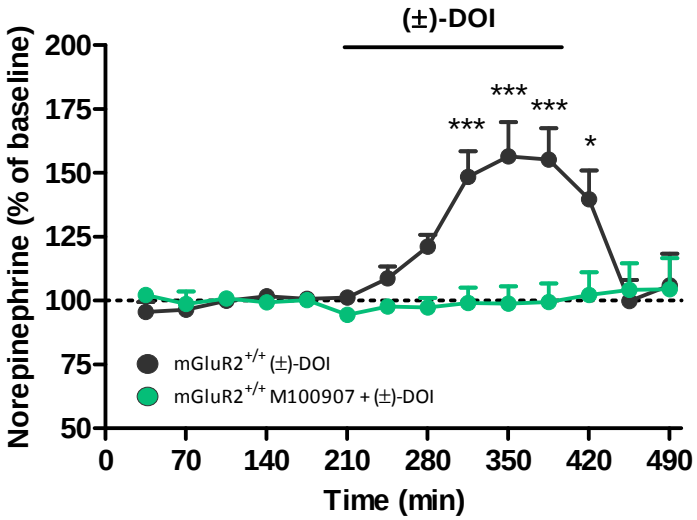


Fig. 47- Influence of the cortical presence or absence of the 5-HT2AR antagonist M100907 (300 µM) on the modulation by the 5-HT2A/2C receptor agonist (±)-DOI (300 µM) of extracellular concentrations of NE in the FC of mGluR2$^{+/+}$ mice (● *n*=16, ● *n*=5). Data are means ± SEM of NE levels expressed as a percentage of basal values (100%). M100907 perfusion was initiated since the beginning of the experiment. The line represents (±)-DOI infusion time. **p<0.05, ***p<0.001* (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

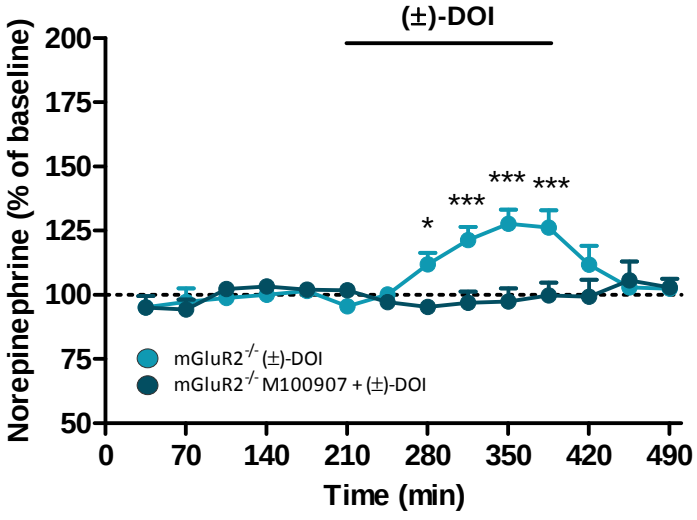


Fig. 48- Influence of the cortical presence or absence of the 5-HT2AR antagonist M100907 (300 µM) on the modulation by the 5-HT2A/2C receptor agonist (±)-DOI (300 µM) of extracellular concentrations of NE in the FC of mGluR2$^{-/-}$ mice (● *n*=13, ● *n*=6). Data are means ± SEM of NE levels expressed as a percentage of basal values (100%). M100907 perfusion was initiated since the beginning of the experiment. The line represents (±)-DOI infusion time. **p<0.05, ***p<0.001* (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

In terms of AUCs magnitudes, Figure 49 reports that there were not alterations in NE concentrations consequently to CSF perfusion. However, as already described, extracellular NE release as a result of local (±)-DOI administration was significantly higher in wild-type animals (AUC mean=7980 ± 1391), compared to their knock-out littermates (AUC mean=3608 ± 633). Interestingly, continuous intra-cortical infusion of M100907 prior to (±)-DOI perfusion in the same brain region, was successfully able to block that stimulation in both mGluR2$^{+/+}$ (AUC mean=66 ± 1374) and mGluR2$^{-/-}$ (AUC mean=-186 ±794) animal groups. Two-way ANOVA showed significant genotype-dependent response to drugs ($F_{genotype}[1, 47]=6.2; p<0.05$) and that treatments were able to modulate NE release ($F_{treatment}[2, 47]=17.33; p<0.0001$).

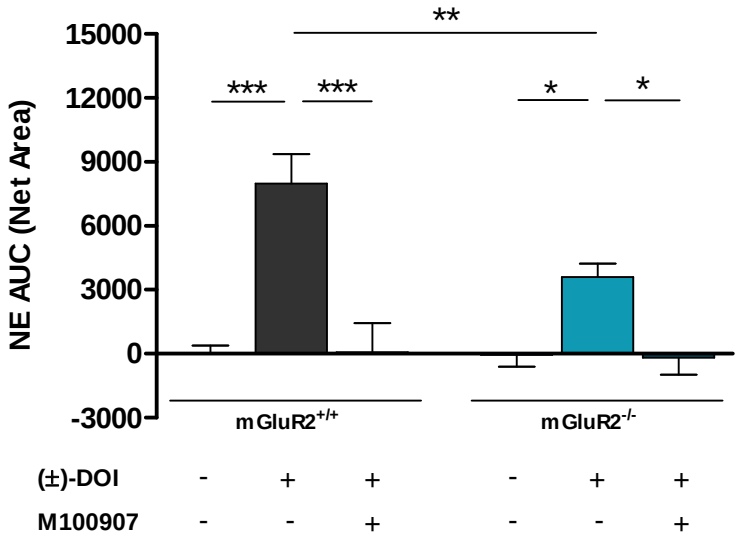


Fig. 49- Cortical NE AUC values 210 minutes after the beginning of the procedure to the end. Effect of intra-cortical infusion of vehicle, (±)-DOI (300 µM) and M100907 (300 µM) + (±)-DOI (300 µM) in mGluR2$^{+/+}$ (● *n*=6, ● *n*=16, ● *n*=5) and mGluR2$^{-/-}$ mice (● *n*=6, ● *n*=13, ● *n*=5). All results are expressed as means ± SEM. **p<0.05, **p<0.01, ***p<0.001* (two-way ANOVA followed by LSD's post hoc test).

4.1.1.3. Effect of cortical infusion of (±)-DOI on DA and NE release in the frontal cortex of 5-HT2AR$^{+/+}$ and 5-HT2AR$^{-/-}$ mice

As mentioned above, (±)-DOI is not a selective 5-HT2AR agonist drug, but possesses to some extent higher affinity at 5-HT2AR than at 5-HT2CR (Hemrick-Luecke & Evans, 2002; May *et al*., 2003; Pigott *et al*., 2012). To assess the influence of the existence of 5-HT2AR on the boost in cortical DA release after intra-cortical administration of (±)-DOI, the same procedure was carried out using 5-HT2AR$^{+/+}$ and 5-HT2AR$^{-/-}$ mice.

Dopamine

In 5-HT2AR$^{+/+}$ control mice, absolute basal concentrations of dialysate DA content, without considering probe recovery, were significantly lower compared to their 5-HT2AR$^{-/-}$ counterparts: 1.499 ± 0.179 nM (*n*=30) in 5-HT2AR$^{+/+}$ mice *versus* 2.022 ± 0.160 nM (*n*=28) in 5-HT2AR$^{-/-}$ mice (*t=2.167; p<0.05*).

Intra-cortical (±)-DOI (300 µM) infusion elicited a significant rise in extracellular DA in the FC of 5-HT2AR$^{+/+}$ mice when compared to VEH (F_i*[13, 156]=25.38; p<0.0001*) (Figure 50). Interestingly, the hallucinogenic drug elicited a more acute DA stimulation in C57BL/6 control mice (5-HT2AR$^{+/+}$) (E_{max}= 225 ± 21%) than experienced in 129/Sv control mice (mGluR2$^{+/+}$) (E_{max}= 163±16%).

In 5-HT2AR$^{-/-}$ mice there was a mild but significant increase of extracellular DA as a result of (±)-DOI infusion, when compared to VEH (E_{max}=155 ± 16%) (F_i*[13, 156]=6.60; p<0.0001*) (Figure 50). As expected, the lack of 5-HT2AR significantly impaired the stimulatory effect elicited by 300 µM (±)-DOI in the cortex, compared to wild-type animals (F_i*[13, 169]=7.932; p<0.0001*) (Figure 50).

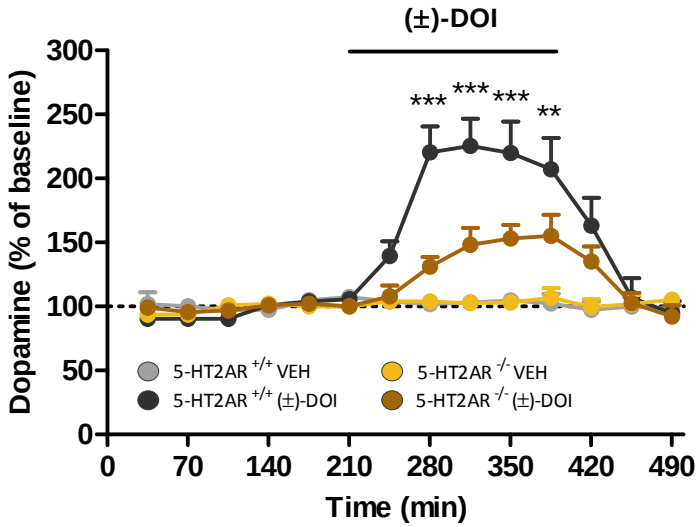


Fig. 50- Effect of cortical administration of VEH or (±)-DOI (300 µM) on extracellular DA concentrations in the FC of 5-HT2AR$^{+/+}$ (● *n*=8, ● *n*=6) and 5-HT2AR$^{-/-}$ mice (● *n*=5, ● *n*=9). Data are means ± SEM of DA expressed as a percentage of basal values (100%). The line represents drug infusion time. ***p<0.01, ***p<0.001 versus* 5-HT2AR$^{-/-}$ (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Looking at the extent of the effect, AUC values show that 300 µM (±)-DOI triggered a DA release in the FC in both genotype groups. However, in 5-HT2AR$^{+/+}$ that response was substantially higher than in 5-HT2AR$^{-/-}$ animals, unmasking a significant interaction between genotypes. Therefore, there was a significant interaction genotype x treatment ($F_i[1, 24]=8.41$; $p<0.01$) (Figure 51).

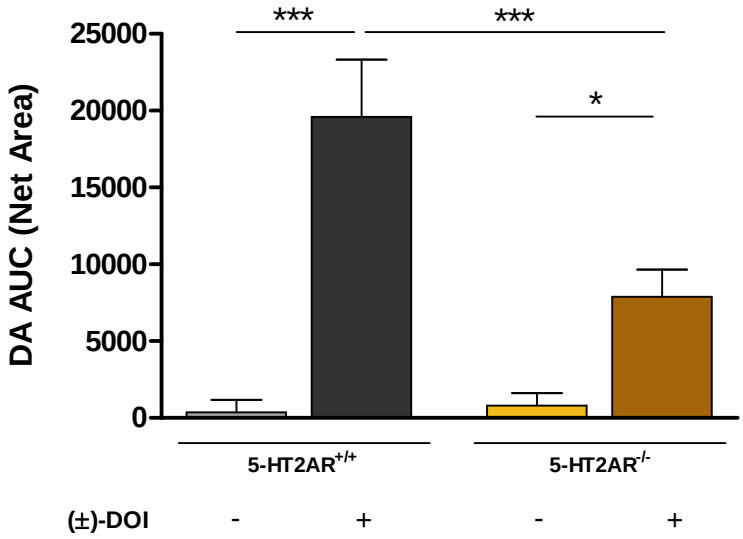


Fig. 51- Cortical DA AUC values 210 minutes after the beginning of the procedure to the end. Effect of intra-cortical infusion of vehicle or (±)-DOI (300 µM) in 5-HT2AR$^{+/+}$ (● *n*=8, ● *n*=6) and 5-HT2AR$^{-/-}$ mice (● *n*=5, ● *n*=9). All results are expressed as means ± SEM. **p<0.05, ***p<0.001* (two-way ANOVA followed by LSD's post hoc test).

Norepinephrine

NE extracellular basal values, without considering probe recovery, did not differ between 5-HT2AR$^{+/+}$ and 5-HT2AR$^{-/-}$ mice: 1.454 ± 0.101 nM (*n*=31) *versus* 1.263 ± 0.122 nM (*n*=28), respectively (*t=1.214; p=0.230*).

Local administration of 300 µM (±)-DOI remarkably increased extracellular NE outflow in the FC of 5-HT2AR$^{+/+}$ (E_{max}= 171 ± 16%) ($F_i[13, 195]=9.83$; $p<0.0001$) (Figure 52). On the contrary, a small but significant stimulatory effect was elicited in 5-HT2AR$^{-/-}$ mice (E_{max}= 119 ± 7%) ($F_i[13, 143]=2.344$; $p<0.01$) (Figure 52). Two-way repeated measures ANOVA revealed a significant interaction between (±)-DOI response over time, depending on genotype ($F_i[13, 182]=6.159$; $p<0.0001$).

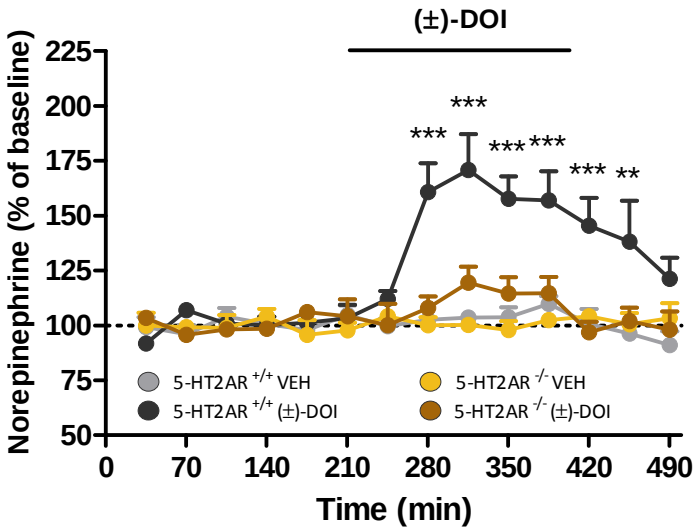


Fig. 52- Effect of cortical administration of VEH or (±)-DOI (300 µM) on extracellular NE concentrations in the FC of 5-HT2AR $^{+/+}$ (● *n*=8, ● *n*=9) and 5-HT2AR$^{-/-}$ mice (● *n*=6, ● *n*=7). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The line represents drug infusion time. ***p<0.01, ***p<0.001 versus* 5-HT2AR$^{-/-}$ (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Two-way ANOVA showed a statistically significant difference between AUC values derived from (±)-DOI-treatment and genotype, leading to a significant interaction among these two factors (F_i*[1, 26]=15.48; p<0.001*) (Figure 53).

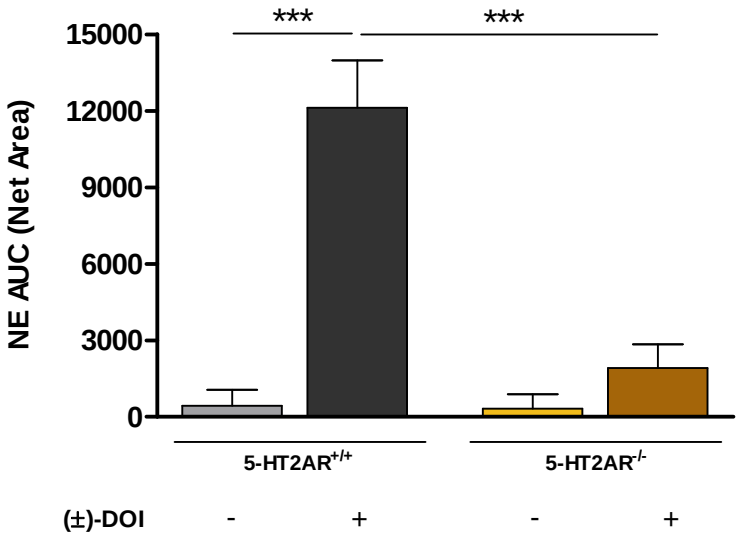


Fig. 53- Cortical NE AUC values 210 minutes after the beginning of the procedure to the end. Effect of intra-cortical infusion of vehicle or (±)-DOI (300 µM) in 5-HT2AR$^{+/+}$ (● *n*=8, ● *n*=9) and 5-HT2AR$^{-/-}$ mice (● *n*=6, ● *n*=7). All results are expressed as means ± SEM. ****p<0.001* (two-way ANOVA followed by LSD's post hoc test).

4.1.1.4. Effect of cortical infusion of (±)-DOI on DA and NE release in the frontal cortex of mGluR3$^{+/+}$ and mGluR3$^{-/-}$ mice

It has been demonstrated that 5-HT2AR forms a heterocomplex with mGluR2, and not with the closely structurally related mGluR3 (González-Maeso *et al*., 2008; Moreno *et al*., 2012). In order to further elucidate the functional implication of mGluR2 and not mGluR3 in the (±)-DOI-induced response, mGluR3 knock-out mice were used.

Note that vehicle and (±)-DOI-treated wild-type groups (mGluR3$^{+/+}$) comprised the same animals used as wild-type 5-HT2A$^{+/+}$ mice, for both dopaminergic and noradrenergic assessments, as they share the same genetic background (C57BL/6).

Dopamine

No differences in extracellular DA basal concentrations were found between wild-type (1.499 ± 0.179 nM, *n*=30) and mGluR3$^{-/-}$ mice (2.134 ± 0.27, *n*=10) (*t=1.823; p=0.076*).

Intra-cortical perfusion of (±)-DOI (300 µM) increased extracellular concentrations of DA in control animals when compared to VEH (as seen in section 4.1.1.3) (E_{max}= 225 ± 21%) (F_i*[13, 156]=25.38; p<0.0001*) (Figure 54). Similar response was yielded by their mGluR3$^{-/-}$ littermates (E_{max}= 219 ± 19%) (F_i*[13, 104]=19.90; p<0.0001*) (Figure 54). Two-way repeated measures ANOVA disclosed no differences between genotypes on extracellular DA outflow after treatment with intra-cortical (±)-DOI (300 µM) (F_i*[13, 117]=0.84; p=0.62*).

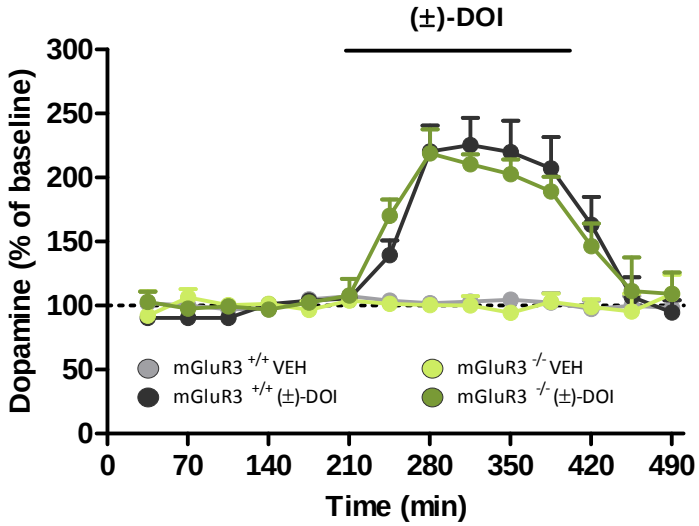


Fig. 54- Effect of cortical administration of VEH or (±)-DOI (300 µM) on extracellular DA concentrations in the FC of mGluR3 $^{+/+}$ (● *n*=8, ● *n*=6) and mGluR3$^{-/-}$ mice (● *n*=5, ● *n*=5). Data are means ± SEM of DA expressed as a percentage of basal values (100%). The line represents drug infusion time.

Two-way ANOVA of AUC values revealed a significant response to (±)-DOI administration ($F_{treatment}[1, 20]=69.49; p<0.0001$) independently of the presence or absence of mGlu3 receptor ($F_{genotype}[1, 20]=0.17; p=0.687$) in these mice (Figure 55).

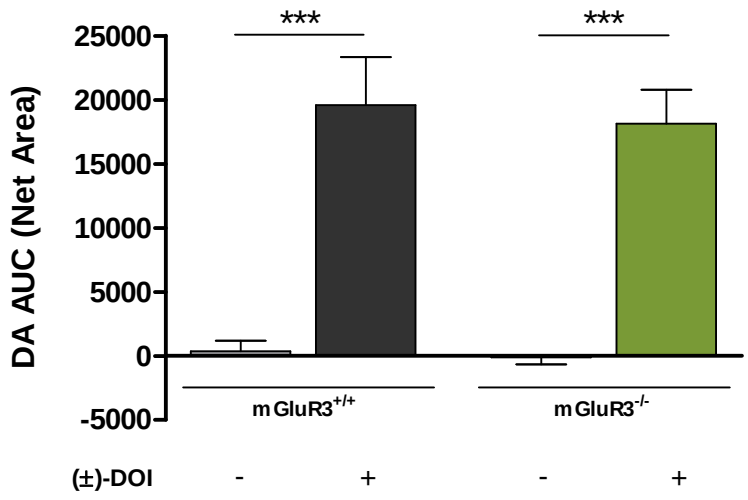


Fig. 55- Cortical DA AUC values 210 minutes after the beginning of the procedure to the end. Effect of intra-cortical infusion of vehicle or (±)-DOI (300 µM) in the FC of mGluR3$^{+/+}$ (● *n*=8, ● *n*=6) and mGluR3$^{-/-}$ mice (● *n*=5, ● *n*=5). All results are expressed as means ± SEM. ****p<0.001* (two-way ANOVA followed by LSD's post hoc test).

Norepinephrine

No differences in extracellular NE basal concentrations were found between mGluR3$^{+/+}$ (1.454 ± 0.101 nM, *n*=31) and mGluR3$^{-/-}$ mice (1.499 ± 0.15 nM, *n*=11) (*t=0.233; p=0.817*).

In accordance with the effect evoked by the 5-HT2A/2C receptors agonist (±)-DOI on DA release, intra-cortical perfusion of (±)-DOI (300 µM) significantly increased extracellular concentrations of NE in control animals, compared to VEH group (as shown in section 4.1.1.3) (E_{max}= 171 ± 16%) ($F_i[13, 195]=9.83; p<0.0001$) (Figure 56). A similar effect was observed in animals lacking mGluR3 receptors (E_{max}= 219 ± 19%) ($F_i[13, 117]=2.840; p<0.01$) (Figure 56). Two-way repeated measures ANOVA revealed no differences between genotypes on extracellular DA outflow after intra-cortical treatment with (±)-DOI (300 µM) ($F_i[13, 169]=0.499; p=0.923$).

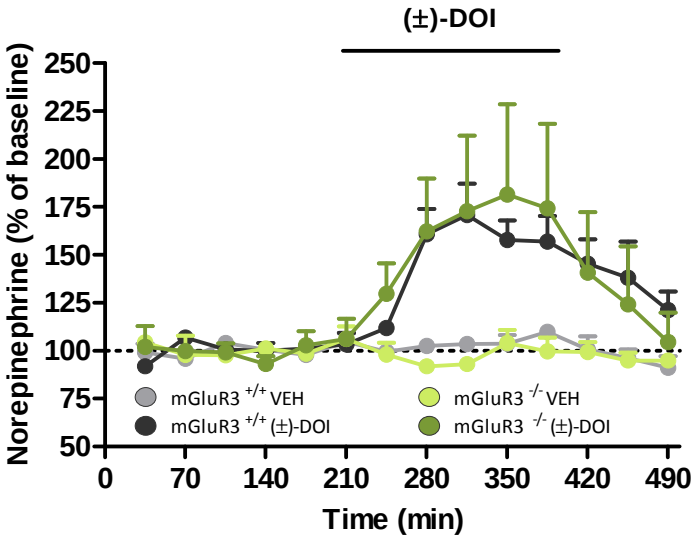


Fig. 56- Effect of cortical administration of VEH or (±)-DOI (300 µM) on extracellular NE levels in the FC of mGluR3 $^{+/+}$ (● *n*=8, ● *n*=9) and mGluR3$^{-/-}$ mice (● *n*=5, ● *n*=5). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The line represents drug infusion time.

Two-way ANOVA of AUC values found no statistically significant differences between the responses to (±)-DOI-treatment carried out by the above different genotypes. This statement indicates that (±)-DOI induced a similar noradrenergic stimulation in both genotypes and the only significant difference was reached in terms of treatment ($F_{treatment}[1, 24]=21.65$; $p<0.001$) (Figure 57).

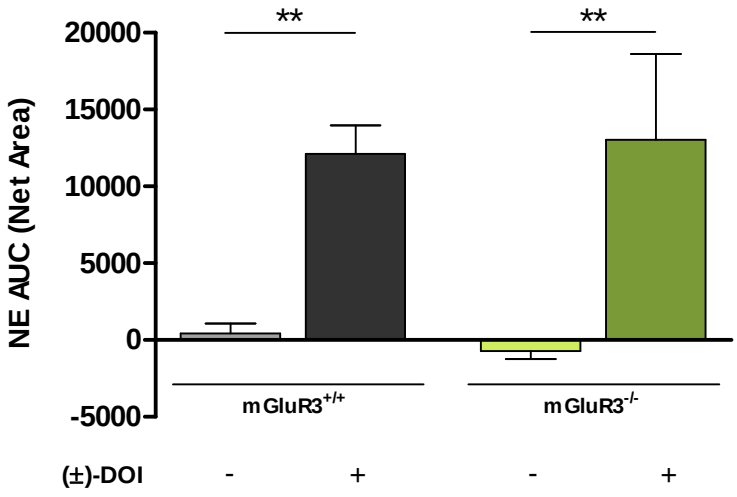


Fig. 57- Cortical NE AUC values 210 minutes after the beginning of the procedure to the end. Effect of intra-cortical infusion of vehicle or (±)-DOI (300 µM) in the FC of mGluR3$^{+/+}$ (● *n*=8, ● *n*=9) and mGluR3$^{-/-}$ mice (● *n*=5, ● *n*=5). All results are expressed as means ± SEM. ***p<0.01* (two-way ANOVA followed by LSD's post hoc test).

4.1.1.5. Effect of cortical infusion of (±)-DOI on DA and NE release in the frontal cortex, following virus-mediated overexpression of mGluR2 in mGluR2$^{-/-}$ mice

The previous results suggest that the lack of the mGluR2 leads to an impairment of catecholamine release in FC evoked by cortical administration of the hallucinogen 5-HT2A/2C receptors agonist (±)-DOI. This is potentially due to the absence of 5-HT2AR/mGluR2 heterocomplex. To fully establish the role of mGluR2 in (±)-DOI-mediated response, mGluR2 was overexpressed in the FC of mGluR2$^{-/-}$ mice to assess if the rescue of this receptor could result in similar catecholamine release as produced by their wild-type counterparts (see in 4.1.1.2 section). Mice received an intra-cortical injection of bicistronic *herpes simplex* 2 viral particles (HSV), expressing either green fluorescent protein (GFP) and mGluR2 (HSV-mGluR2) or GFP alone (HSV-GFP).

4.1.1.5.1. Immunofluorescence assay to determine virus expression location

Immunohistochemistry combines anatomical, immunological and biochemical techniques to identify tissue components by the interaction of target antigens with specific antibodies tagged with a visible label. This technique makes it possible to visualize the distribution and location of specific cellular components within cells and the proper tissue context.

It has been previously reported that mGluR2 can be rescued through HSV-mediated transgene expression in mGluR2-KO mouse FC (Moreno *et al*., 2012). In order to rescue the mGluR2, it was firstly confirmed that the virus expressed GFP protein (assuming that mGluR2 would be also expressed) in the same location as the probe would be later introduced, in the dialysis surrounding area. Four days after viral injection, when the maximum viral expression is reached, immunofluorescence assays were performed using 40-micron thick brain slices. Selected slices were those located in the surroundings of the viral injection area.

As shown in Figure 58, the maximal fluorescence intensity and subsequently the maximal viral expression, was obtained around the injection area.

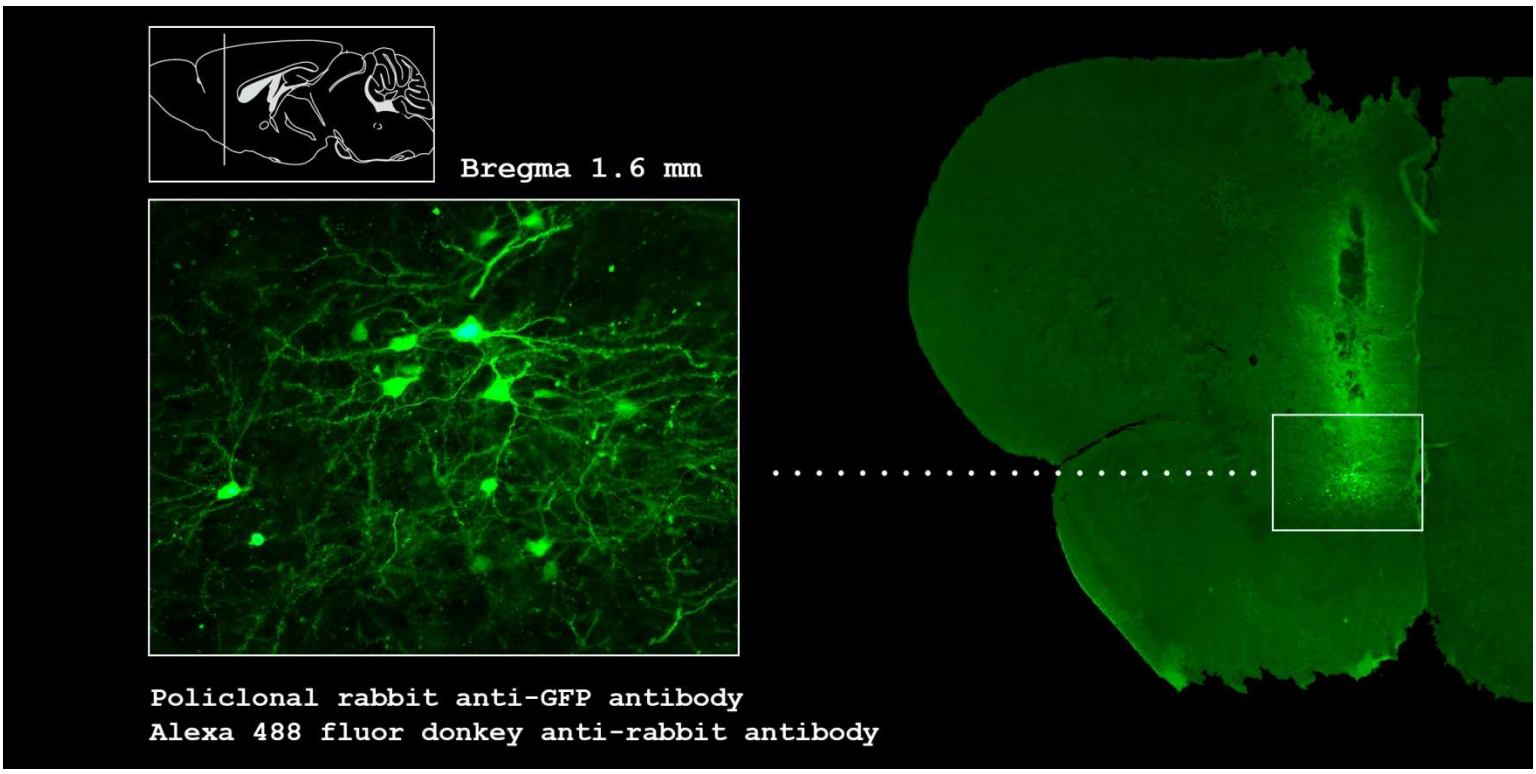


Fig. 58- Representative image of HSV-mediated transgene expression in the frontal cortex. HSV-GFP and HSV-mGluR2, which also expresses GFP, were injected into frontal cortex and GFP expression was revealed by immunofluorescence. Left upper panel: representative image showing the viral injection location, where slices were cut. Right panel: brain slice of the surrounding viral injection area observed with a fluorescence microscope: 4x magnification. Left lower panel: cortical neurons observed with a fluorescence microscope: 10x magnification.

4.1.1.5.2. Effect of cortical administration of (±)-DOI on DA and NE release in the frontal cortex of mGluR2$^{-/-}$ mice after rescuing mGluR2 in the FC

Afterwards, the neurochemical effects induced by the hallucinogenic drug (±)-DOI in HSV-mGluR2 and their HSV-GFP counterparts were then investigated. Immediately after the viral injection, a cannula was implanted and fixed in the same location until the end of the dialysis procedures. Three days after viral injection, a dialysis probe was implanted through the cannula, and microdialysis assays were performed in the following two days.

Dopamine

There were no differences in extracellular DA concentrations between mGluR2-KO mice pre-injected with HSV-GFP (2.126 ± 0.234 nM, *n*=18) or HSV-mGluR2 (1.918 ± 0.220 nM, *n*=20) (*t=0.648; p=0.521*).

The stimulatory response induced by the hallucinogenic 5-HT2A/2C receptors agonist (±)-DOI experienced in wild-type animals (E_{max}=163 ± 16%) and impaired in mGluR2 knock-out mice (E_{max}=134 ± 11%) (see 4.1.1.1. section), was rescued in mGluR2$^{-/-}$ mice overexpressing mGluR2 in the FC (E_{max}=172 ± 18%) ($F_i[13, 234]=7.322$; $p<0.0001$), but not in those animals expressing only GFP (E_{max}=137 ± 10%) ($F_i[13, 208]=4.166$; $p<0.0001$) (Figure 59). Two-way repeated measures ANOVA revealed a statistically significant difference between HSV-GFP and HSV-mGluR2 (±)-DOI-treated groups ($F_i[13, 260]=3.223$; $p<0.001$).

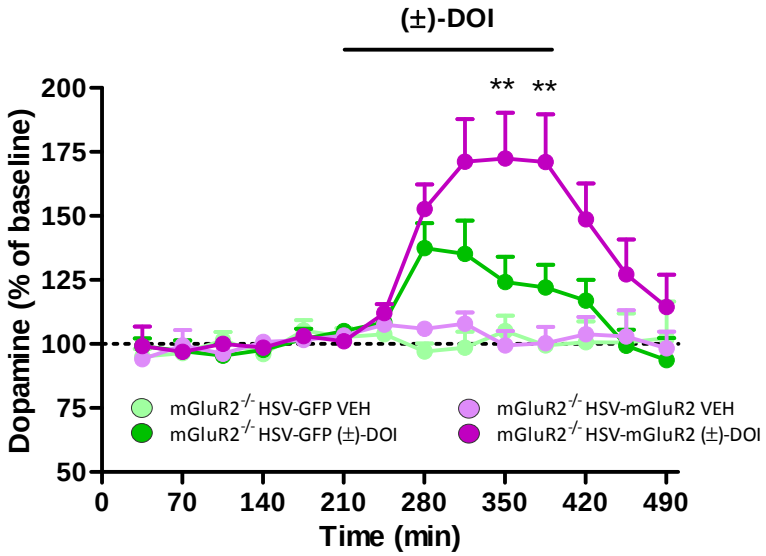


Fig. 59- Effect of cortical administration of VEH or (±)-DOI (300 µM) on extracellular DA concentrations in the FC of mGluR2$^{-/-}$ mice overexpressing GFP (● *n*=8, ● *n*=10) and mGluR2 (● *n*=8, ● *n*=12). Data are means ± SEM of DA expressed as a percentage of basal values (100%). The line represents drug infusion time**.** ***p<0.01 versus* HSV-GFP (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Two-way ANOVA analysis of AUC values within groups pre-injected with the viral vector revealed that, despite (±)-DOI was able to induce DA release in GFP- and mGluR2-HSV pre-injected mGluR2$^{-/-}$ mice ($F_{treatment}$ *[1, 34]=15.03; p<0.001*), that response had a higher extent in animals were mGluR2 was rescued ($F_{genotype}$*[1, 34]=5.03; p<0.05)* (Figure 60).

Figure 60 represents AUC values of vehicle-treated or (±)-DOI-treated wild-type and mGluR2 knock-out mice (see 4.1.1.1. section), pre and post virus-mediated GFP or mGluR2 overexpression. The hallucinogenic 5-HT2A/2C receptors agonist (±)-DOI was able to evoke a rise in extracellular DA outflow in mGluR2$^{+/+}$ mice (AUC mean=9272 ± 2132). That stimulation appeared to be impaired in mGluR2$^{-/-}$ animals (AUC mean=4573 ± 1414). (±)-DOI induced a

similar slight stimulatory effect in those mGluR2$^{-/-}$ animals pre-injected with HSV-GFP (AUC mean=4764 ± 1557), compared to mGluR2$^{-/-}$ without any previous virus transduction. Interestingly, after rescuing in the FC the expression of mGluR2 in mGluR2$^{-/-}$ mice (HSV-mGluR2), the hallucinogenic drug induced a higher extracellular DA outflow (AUC mean=12474 ± 2880), similar to the one previously displayed in mGluR2$^{+/+}$ mice.

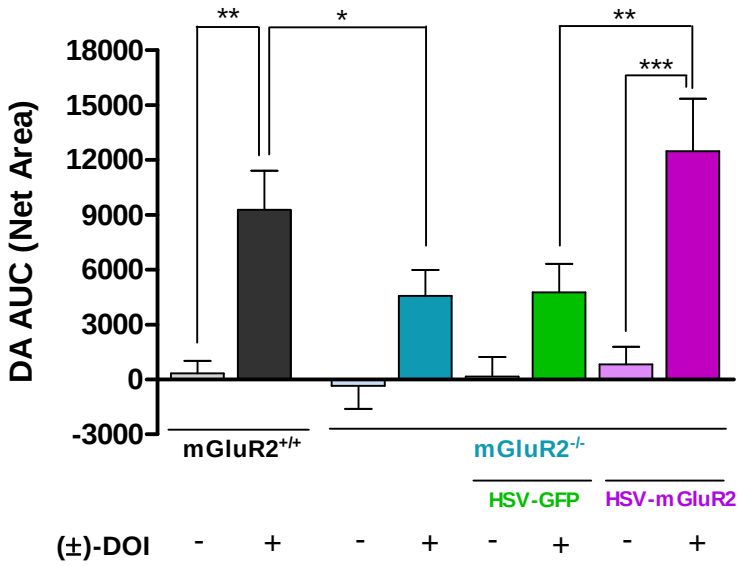


Fig. 60- Cortical DA AUC values 210 minutes after the beginning of the procedure to the end. Effect of intra-cortical infusion of vehicle or (±)-DOI (300 µM) in the FC of mGluR2$^{+/+}$ (● *n*=6, ● *n*=13), mGluR2$^{-/-}$ (● *n*=6, ● *n*=14), mGluR2$^{-/-}$ expressing GFP (HSV-GFP) (● *n*=8, ● *n*=10) and mGluR2$^{-/-}$ expressing mGluR2 (HSV-mGluR2 (● *n*=8, ● *n*=12). All results are expressed as means ± SEM. **p<0.05, **p<0.01, ***p<0.001* (two-way ANOVA followed by LSD's post hoc test).

Norepinephrine

Absolute basal NE concentrations, without considering probe recovery, did not differ between mGluR2-KO mice pre-injected with HSV-GFP (1.619 ± 0.147 nM, *n*=17) or HSV-mGluR2 (1.487 ± 0.113 nM, *n*=21) (*t= 0.726; p=0.472*).

As previously shown on dopaminergic modulation, the impaired noradrenergic stimulatory response in mGluR2 knock-out mice elicited after intra-cortical administration of (±)-DOI, was rescued in mGluR2$^{-/-}$ mice overexpressing mGluR2 in the FC (E_{max}=148 ± 9%) (F_i*[13, 247]=17.95; p<0.0001*), compared to those overexpressing only GFP (E_{max}=122 ± 6%) (F_i*[13, 195]=6.665; p<0.0001*) (Figure 61). Two-way repeated measures ANOVA revealed statistically significant

differences between HSV-GFP and HSV-mGluR2 (±)-DOI treated groups ($F_i[13, 260]=6.209$; $p<0.0001$).

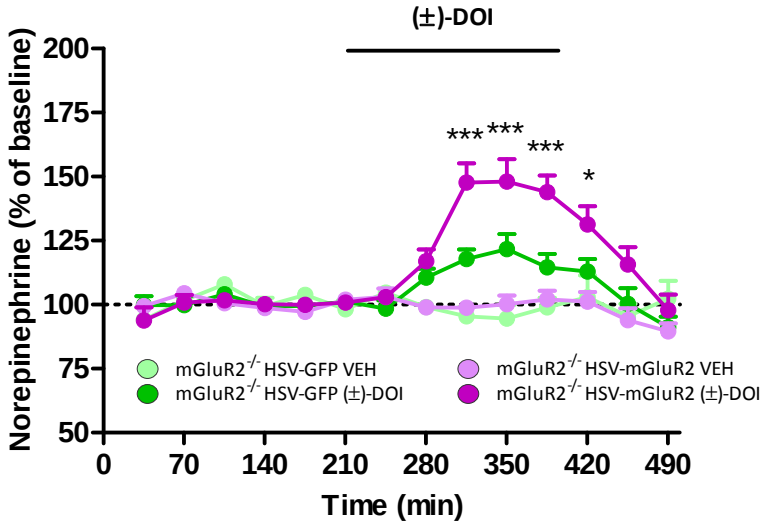


Fig. 61- Effect of cortical administration of VEH or (±)-DOI (300 µM) on extracellular NE concentrations in the FC of mGluR2$^{-/-}$ mice overexpressing GFP (● *n*=7, ● *n*=10) and mGluR2 (● *n*=9, ● *n*=12). The line represents drug infusion time. Data are means ± SEM of NE expressed as a percentage of basal values (100%). **p<0.05, ***p<0.001 versus* HSV-GFP (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Two-way ANOVA analysis of AUC values within groups pre-injected with the viral vector revealed a statistically significant difference in the (±)-DOI response between knock-out animals where mGluR2 was rescued in the FC (HSV-mGluR2) and those in which mGluR2 was not rescued (HSV-GFP) ($F_i[1, 34]=5.63$; $p<0.05$) (Figure 62).

In line with the previous studies shown above (section 4.1.1.1.), intra-cortical (±)-DOI (300 µM) enhanced NE release in the FC of mGluR2$^{+/+}$ mice (AUC mean=7980 ± 1391). That stimulation was much lower in mGluR2$^{-/-}$ mice (AUC mean=3608 ± 632). According to these results, that stimulation was likely 5-HT2AR-dependent (4.1.1.2 section), suggesting that the absence of mGluR2 forming a heterocomplex with 5-HT2AR could be responsible for the impairment of the (±)-DOI-induced effect displayed in mGluR2$^{-/-}$ mice. (±)-DOI induced a similar small increase in NE dialysate concentrations in mGluR2$^{-/-}$ animals overexpressing GFP (HSV-GFP) (AUC mean=2540 ± 894), as compared to mGluR2$^{-/-}$ without viral manipulation. Remarkably, in those mGluR2$^{-/-}$ animals where mGluR2 was rescued after viral transduction (HSV-mGluR2), the

(±)-DOI-induced extracellular NE outflow (AUC mean=7142 ± 1159) was similar to the effect seen in mGluR2$^{+/+}$ mice (Figure 62).

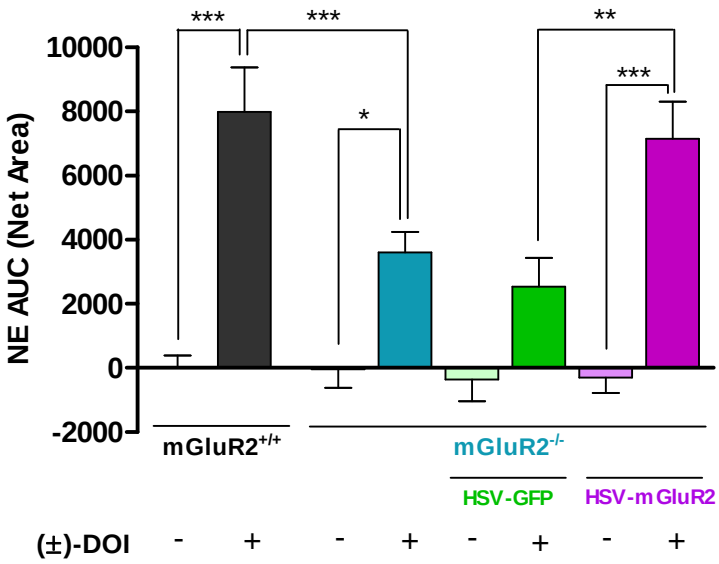


Fig. 62- Cortical NE AUC values 210 minutes after the beginning of the procedure to the end. Effect of intra-cortical infusion of vehicle or (±)-DOI (300 µM) in the FC of mGluR2$^{+/+}$ (● *n*=6, ● *n*=16), mGluR2$^{-/-}$ (● *n*=7, ● n=13), mGluR2$^{-/-}$ expressing GFP (HSV-GFP) (● *n*=7, ● *n*=10) and mGluR2$^{-/-}$ expressing mGluR2 (HSV-mGluR2) (● *n*=9, ● *n*=12). All results are expressed as means ± SEM. **p<0.05, **p<0.01, ***p<0.001* (two-way ANOVA followed by LSD's post hoc test).

4.1.2. Modulation of DA and NE release by systemic administration of the hallucinogenic 5-HT2A/2C receptor agonist (±)-DOI in mice

4.1.2.1. Effect of systemic administration of (±)-DOI on DA and NE release in the frontal cortex of wild-type C57BL/6 strain mice

Various studies have suggested that acute systemic administration of the hallucinogenic 5-HT2AR/2CR agonist drug (±)-DOI increases dialysate DA concentrations in rat FC, as well as in C57BL/6 mice (Gobert & Millan, 1999; Pehek *et al*., 2001; Bortolozzi *et al.*, 2005), apparently through the activation of 5-HT2A receptors (Huang *et al.*, 2012). By contrast, the dose of 1mg/kg (±)-DOI is able to reduce extracellular 5-HT output in rat FC (Martín-Ruiz *et al*., 2001). However, little is known about the noradrenergic modulation triggered by this hallucinogenic drug. Firstly, we determined the dopaminergic and noradrenergic modulation elicited by (±)-DOI after acute intraperitoneal injection.

Dopamine

Vehicle treatment failed to modify extracellular DA concentrations. As shown in Figure 63, systemic administration of (±)-DOI (1 mg/kg i.p.) induced a significant increase in the cortical DA efflux in wild-type animals (E_{max}=150 ± 6%). Two-way repeated-measures ANOVA displayed a statistically significant difference in the dopaminergic response elicited by (±)-DOI, compared to vehicle treatment ($F_i[11, 154]=5.543$; $p<0.0001$).

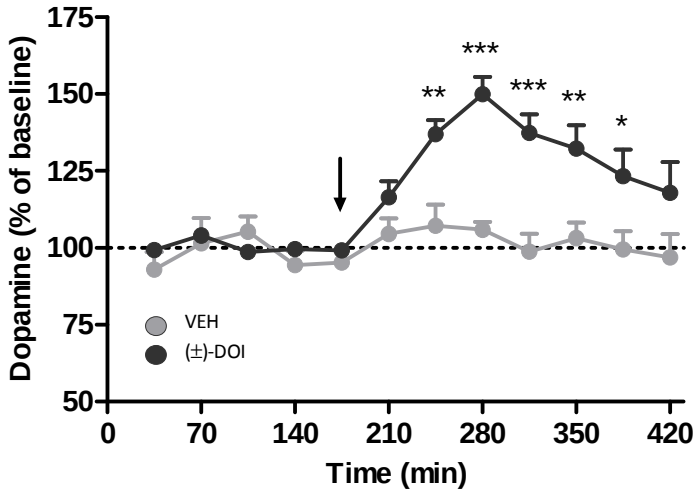


Fig. 63- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on cortical extracellular concentrations of DA in C57BL/6 mice (● *n*=6, ● *n*=10). The arrow indicates the time of drug injection. Data are means ± SEM of DA expressed as a percentage of basal values (100%). **p<0.05, **p<0.01, ***p<0.001 versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Norepinephrine

Acute administration of (±)-DOI (1 mg/kg i.p.) did not alter extracellular NE efflux in the FC of wild-type C57BL/6 mice (E_{max}=119 ± 5%). Two-way repeated measures ANOVA revealed a non-significant effect induced by (±)-DOI, compared to vehicle-treated group (*F_i[11, 132]=0.989; p=0.460*) (Figure 64).

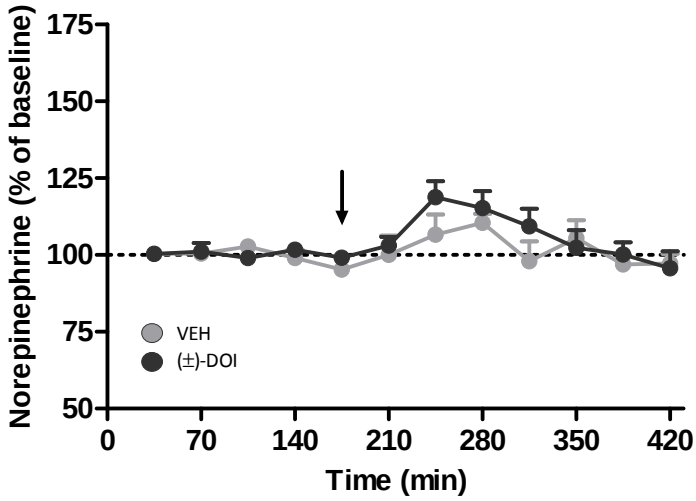


Fig. 64- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on cortical extracellular NE concentrations in C57BL/6 mice (● *n*=5, ● *n*=9). The arrow indicates the time of drug injection. Data are means ± SEM of NE expressed as a percentage of basal values (100%).

4.1.2.2. Role of the presence of the 5-HT2A receptor in (±)-DOI-induced responses

In order to evaluate the involvement of the 5-HT2A receptor on the dopaminergic stimulatory effect carried out by the systemic injection of (±)-DOI (1 mg/kg i.p.), we decided to repeat the same procedure using 5-HT2AR knock-out animals.

Dopamine

As previously mentioned in 4.1.1.3 section, absolute baseline DA concentrations, without considering probe recovery, were significantly lower in 5-HT2AR$^{+/+}$ than in 5-HT2AR$^{-/-}$ mice: 1.499 ± 0.179 nM (*n*=30) in 5-HT2AR$^{+/+}$ mice; 2.022 ± 0.160 nM (*n*=28) in 5-HT2AR$^{-/-}$ mice (*t=2.167; p<0.05*).

Acute administration of (±)-DOI induced greater DA release in 5-HT2AR wild-type mice (E_{max}=150 ± 6%), compared to the 5-HT2AR knock-out group, who apparently did not respond to (±)-DOI-derived dopaminergic modulations, when compared to saline administration (E_{max}=124 ± 4%) (*F_i[11, 132]=0.556; p=0.861)*. Statistical analysis with two-way repeated measures ANOVA showed a significant difference in the response to (±)-DOI depending on genotype (*F_i[11, 176]=3.990; p<0.0001*), suggesting that 5-HT2ARs are necessary to increase cortical extracellular DA outflow after the injection of 1 mg/kg (±)-DOI (Figure 65).

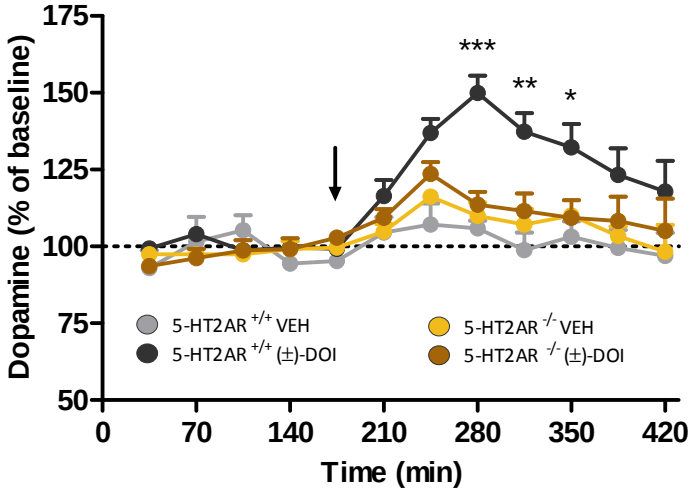


Fig. 65- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on cortical extracellular DA concentrations in 5-HT2AR$^{+/+}$ mice (● *n*=6, ● *n*=10) and in 5-HT2AR$^{-/-}$ mice (● *n*=6, ● *n*=8). The arrow indicates the time of drug injection. Data are means ± SEM of DA expressed as a percentage of basal values (100%). **p<0.05, **p<0.01, ***p<0.001 versus* 5-HT2AR$^{-/-}$ (±)-DOI-treated group (two-way repeated measures Bonferroni's post hoc test).

In terms of magnitude of the effects, regarding AUC values after the injection of saline or (±)-DOI, saline injection did not alter extracellular DA release. In contrast, acute (±)-DOI injection was able to increase extracellular DA efflux in 5-HT2AR$^{+/+}$ mice (AUC mean=6893 ± 986), but no increase was detected in 5-HT2AR$^{-/-}$ mice (AUC mean=2573 ± 846) (Figure 66). Consequently, two-way ANOVA displayed a statistically significant interaction between genotype and treatment (*F_i[1, 26]=9.64; p<0.01*).

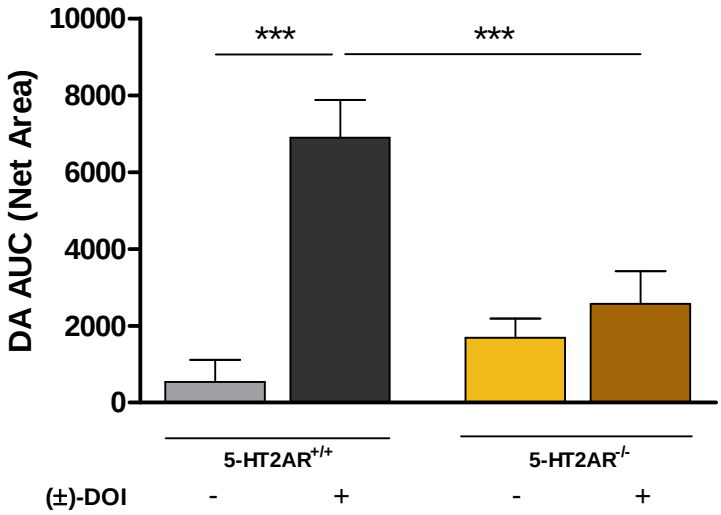


Fig. 66- Cortical DA AUC values after drug injection. Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on cortical dopaminergic modulation in 5-HT2AR$^{+/+}$ (● *n*=6, ● *n*=10) and 5-HT2AR$^{-/-}$ mice (● *n*=6, ● *n*=8). All results are expressed as means ± SEM. ****p<0.001* (two-way ANOVA followed by LSD's post hoc test).

Norepinephrine

As stated above, the pre-drug extracellular basal concentrations of NE in the FC were not different between 5-HT2AR$^{+/+}$ (1.454 ± 0.101 nM, *n*=31) and in 5-HT2AR$^{-/-}$ mice (1.263 ± 0.122 nM, *n*=28) (*t=1.214; p=0.230*) (see section 4.1.1.3).

Acute injection of (±)-DOI (1 mg/kg i.p.) did not modify extracellular concentrations of NE neither in 5-HT2AR$^{+/+}$ (E_{max}=119 ± 5%) (F_i*[11, 132]=0.989; p=0.460*) nor in 5-HT2AR$^{-/-}$ groups (E_{max}=116 ± 3%) (F_i*[11, 143]=0.528; p=0.881*) when compared to saline injection (Figure 67). Two-way repeated measures ANOVA revealed that there was no statistically significant difference attributable to genotypes on (±)-DOI-induced responses (F_i*[11, 176]=1.671; p=0.083*).

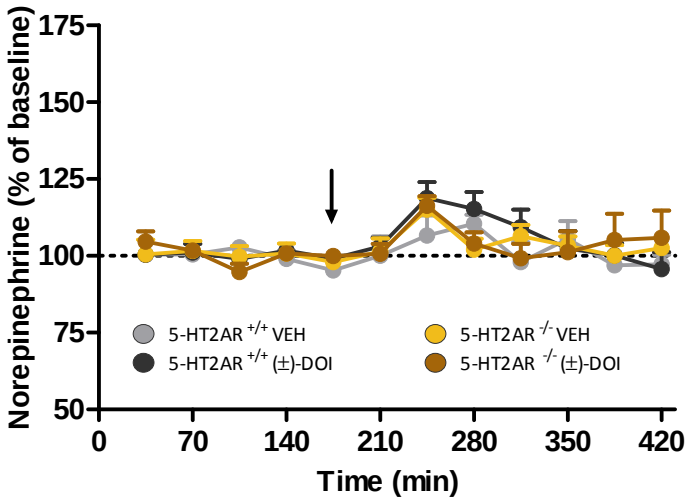


Fig. 67- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on cortical extracellular NE concentrations in 5-HT2AR$^{+/+}$ mice (● *n*=5, ● *n*=9) and in 5-HT2AR$^{-/-}$ mice (● *n*=6, ● *n*=9). The arrow indicates the time of drug injection. Data are means ± SEM of DA expressed as a percentage of basal values (100%).

As observed in Figure 68, AUC values did not differ significantly among treatments ($F_{treatment}$ *[1, 25]=0.30; p=0.588*) or genotypes ($F_{genotype}$ *[1, 25]=0.04; p=0.839*).

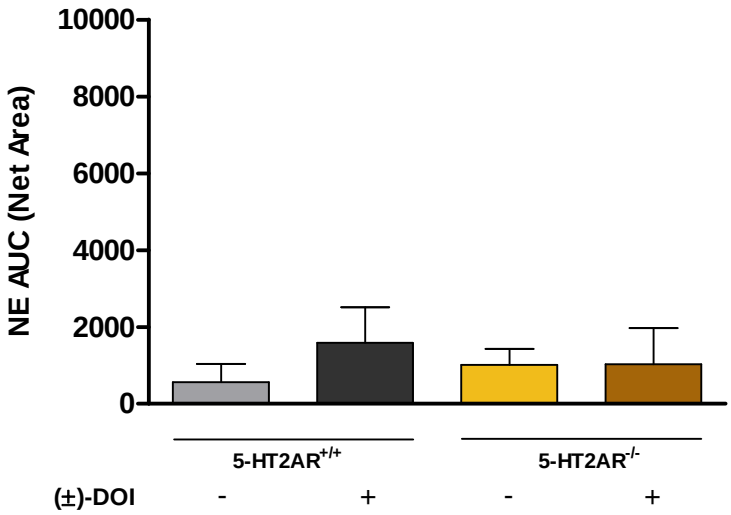


Fig. 68- NE AUC values after drug injection. Effect of systemic administration of vehicle or (±)-DOI (1 mg/kg i.p.) on cortical dopaminergic modulation in 5-HT2AR$^{+/+}$ (● *n*=6, ● *n*=9) and 5-HT2AR$^{-/-}$ mice (● *n*=6, ● *n*=9). All results are expressed as means ± SEM.

4.1.2.3. Effects of systemic (±)-DOI administration on DA and NE release in the frontal cortex of 129/Sv strain mice

In an attempt to replicate the (±)-DOI-induced dopaminergic and noradrenergic responses seen in C57BL/6 mice, the same procedure using wild-type 129/Sv mice was undertaken. At a later time, the intention was to perform the experiments in mGluR2$^{-/-}$ mice (genetically modified in a 129/Sv strain background), in order to compare systemic and local (see 4.1.1.1. section) administration of (±)-DOI.

Dopamine

The dose of 1 mg/kg i.p. of (±)-DOI unexpectedly evoked a slight, but significant, inhibitory effect on cortical extracellular DA release over time (E_{max}=-26 ± 3%) (Figure 69). Two-way repeated measures ANOVA revealed a statistically significant inhibitory effect when compared to saline-treated group ($F_i[11, 165]=4.753$; $p<0.0001$).

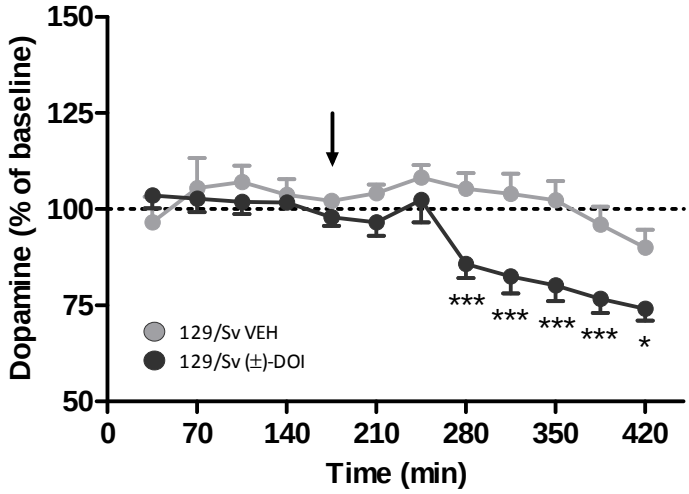


Fig. 69- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on cortical extracellular DA concentrations in 129/Sv mice (● *n*=7, ● *n*=10). The arrow indicates the time of drug injection. Data are means ± SEM of DA expressed as a percentage of basal values (100%). **p<0.05, ***p<0.001 versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Norepinephrine

Acute administration of (±)-DOI (1mg/kg i.p.) yielded an inhibitory effect, reducing extracellular NE concentrations slightly but significantly (E_{max}=-26 ± 4%) (Figure 70). Two-way repeated

measures ANOVA highlighted statistically significant differences in the (±)-DOI-induced response comparing to the one exerted by saline injection (F_i*[11, 231]=4.466; p<0.0001*).

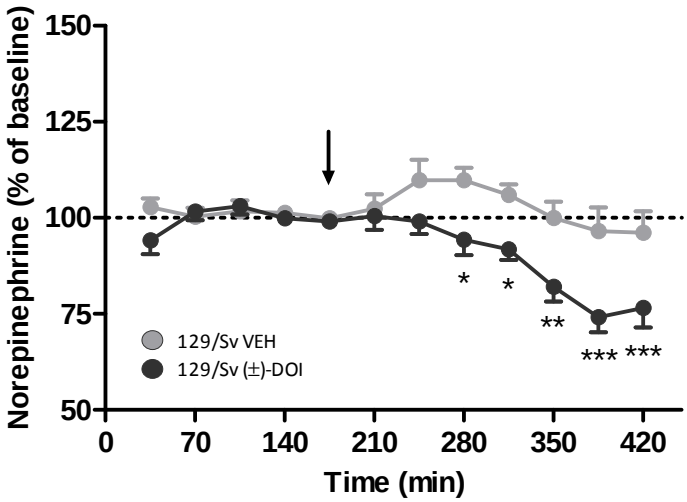


Fig. 70- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on extracellular NE concentrations in the FC of 129/Sv mice (● *n*=10, ● *n*=13). The arrow indicates the time of drug injection. Data are means ± SEM of DA expressed as a percentage of basal values (100%). **p<0.05, **p<0.01, ***p<0.001 versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

4.1.2.4. Strain differences in dopaminergic and noradrenergic responses after acute injection of the hallucinogenic 5-HT2A/2C receptors agonist (±)-DOI

There is plenty of evidence suggesting that mice can display distinct behavioral, signaling and physiological effects depending on their background strain (Weizman *et al*., 1999; Ventura *et al*., 2001; Bouwknecht & Paylor, 2002; Dimou *et al*., 2006; Tsuda *et al*., 2006; Banks *et al*., 2015; Moloney *et al*., 2015; Campus *et al*., 2016; Schwartzer *et al*., 2016). Calagno & Invernizzi (2010) also reported neurochemical strain-dependent differences in 5-HT2CR sensitivity by microdialysis experiments.

Dopamine

When represented the previous findings simultaneously, opposite strain-dependent effects induced by (±)-DOI were found. C57BL/6 experienced a strong stimulatory effect on DA release in the FC, whereas in 129/Sv mice (±)-DOI not only did not elicit a stimulatory effect, but induced an inhibitory effect (Figure 71). Repeated measures two-way ANOVA showed significant opposite responses elicited by (±)-DOI between both mouse strains (F_i*[11,198]=24.29; p<0.0001*).

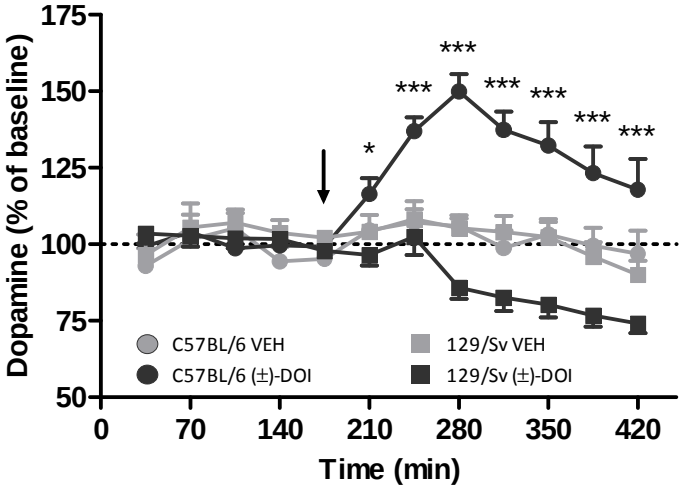


Fig. 71- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on extracellular concentrations of DA in the FC of C57BL/6 mice (● *n*=7, ● *n*=10) and 129/Sv mice (■ *n*=9, ■ *n*=10). The arrow indicates the time of drug injection. Data are means ± SEM of DA expressed as a percentage of basal values (100%). **p<0.05, ***p<0.001 versus* 129/Sv (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

As shown in Figure 72, CSF did not alter DA concentrations neither in C57BL/6, nor in 129/Sv mice (AUC mean=535 ± 575, AUC=447 ± 603; respectively). The acute systemic administration of the 5-HT2A/2C receptor agonist (±)-DOI (1 mg/kg i.p.) was able to significantly increase AUC values (AUC mean= 6893 ± 986), compared to the corresponding vehicle-treated group of C57BL/6 mice. The same dose of (±)-DOI induced a significant inhibitory effect on DA concentrations in the FC of 129/Sv mice (AUC mean=-3043 ± 642). Two-way ANOVA revealed a statistically significant difference among strains in response to treatment (F_i*[1, 29]=36.82; p<0.0001*).

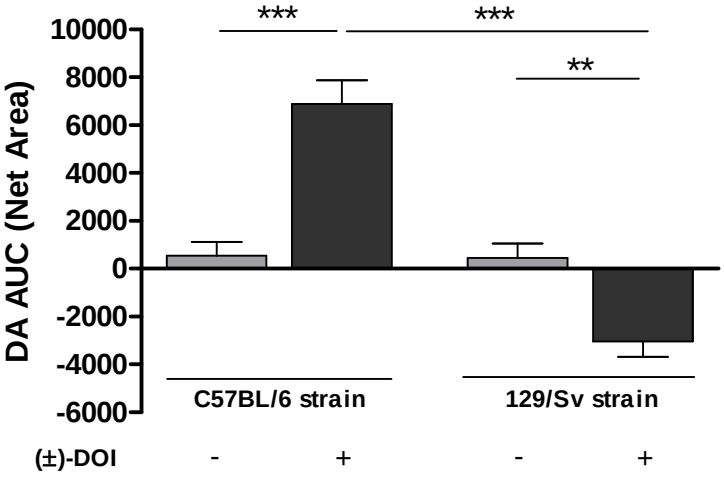


Fig. 72- DA AUC values after drug injection. Effect of systemic administration of vehicle or (±)-DOI (1 mg/kg i.p.) on cortical dopaminergic modulation in C57BL/6 mice (● *n*=7, ● *n*=10) and in 129/Sv mice (■ *n*=9, ■ *n*=10). All results are expressed as means ± SEM. ***p<0.01, ***p<0.001* (two-way ANOVA followed by LSD's post hoc test).

Norepinephrine

NE modulation seemed to follow a similar pattern as DA. Although systemic (±)-DOI failed to alter extracellular NE efflux in the FC of C57BL/6 mice (E_{max}=119 ± 5%), an inhibitory effect over time was displayed in 129/Sv mice (E_{max}=-26 ± 4%). Two-way repeated measures ANOVA displayed statistically significant differences between strains as a result of (±)-DOI injection ($F_i[11, 176]=2.731; p<0.01$) (Figure 73).

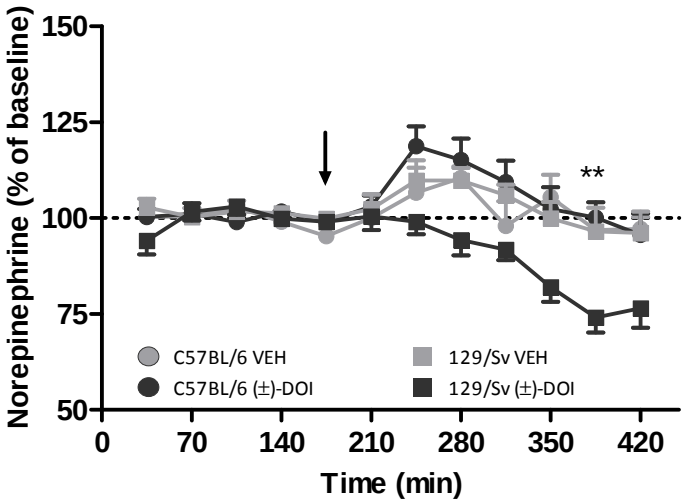


Fig. 73- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on extracellular NE concentrations in C57BL/6 mice (● *n*=7, ● *n*=10) and 129/Sv mice (■ *n*=9, ■ *n*=10). The arrow indicates the time of drug injection. Data are means ± SEM of DA expressed as a percentage of basal values (100%). ***p<0.01 versus* 129/Sv (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

AUC values analysis showed negligible effects after vehicle injection in both C57BL/6 and 129/Sv mice (AUC mean=560 ± 477, AUC mean=746 ± 574; respectively). Non-significant effect, compared to its saline-treated group, was exerted after systemic (±)-DOI administration in C57BL/6 mice (AUC mean=1584 ± 932). In contrast, a decrease in extracellular NE efflux was promoted as a result of the hallucinogenic drug administration in 129/Sv mice (AUC mean=-2458 ± 638). Hence, there was a significant interaction between strains and treatment ($F_i[1, 33]=7.54; p<0.01$) (Figure 74).

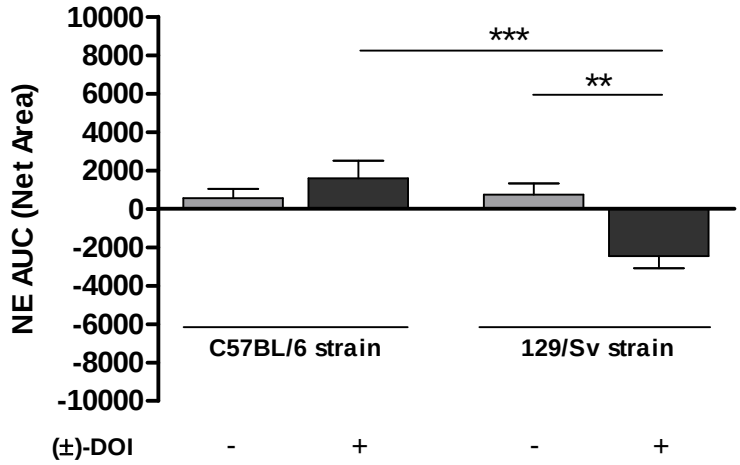


Fig. 74- Cortical NE AUC values after drug injection. Effect of systemic administration of vehicle or (±)-DOI (1 mg/kg i.p.) on cortical noradrenergic modulation in C57BL/6 mice (● *n*=7, ● =10) and 129/Sv mice (■ *n*=9, ■ *n*=10). All results are expressed as means ± SEM. ***p<0.01, ***p<0.001* (two-way ANOVA followed by LSD's post hoc test).

4.1.2.5. Dose-dependent effects of (±)-DOI on extracellular DA and NE output in 129/Sv mice

Microdialysis experiments were performed using five increasing doses of systemic (±)-DOI (0.1-5 mg/kg i.p.) in order to evaluate whether these 129/Sv mice could mimic, at a lower or higher dose, the stimulatory effect observed in C57BL/6 mice. Differences in sensitivity to specific receptors have been reported between mouse strains (Calcagno & Invernizzi, 2010). Only one dose was administered per mouse in each experiment.

The effects of the intraperitoneal administration of increasing doses of (±)-DOI (0.1-5 mg/kg) on DA and NE extracellular release is shown in Figures 75 and 76. (±)-DOI elicited a significant and dose-dependent decrease in DA efflux in the FC (*F[5, 42]=9.836; p<0.0001*, one-way ANOVA), reaching a maximal inhibitory effect at 2.5 mg/kg i.p (Figure 75). NE modulation experienced a significant inhibitory response (*F[5, 54]=3.683; p<0.01*, one-way ANOVA), with a maximal inhibitory effect at a dose of 0.5 mg/kg (Figure 76).

Dunnett's post-hoc test revealed that the unique dose which triggered a statistically significant inhibitory effect in both catecholamines was 1 mg/kg. For that reason, we decided to complete this study using this dose.

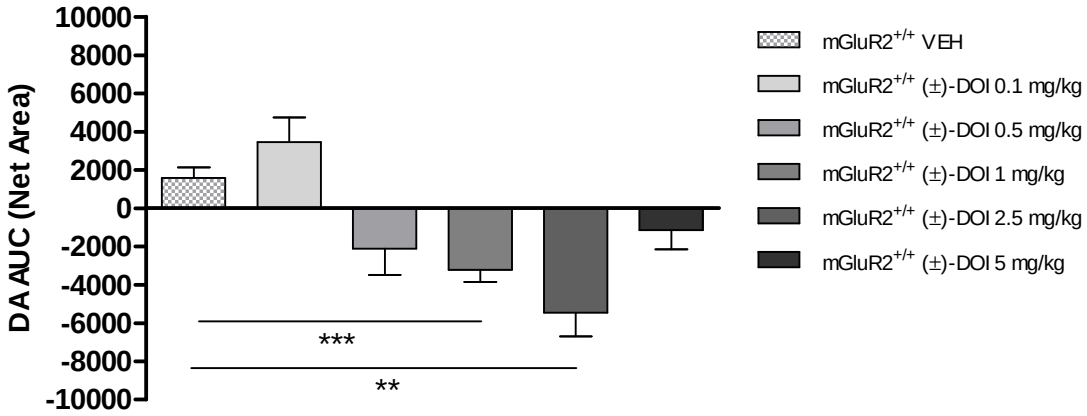


Fig. 75- Cortical DA AUC values after drug injection. Effect of intraperitoneal administration of vehicle (*n*=14), or (±)-DOI at doses of 0.1 mg/kg (*n*=9), 0.5 mg/kg (*n*=3), 1 mg/kg (*n*=10), 2.5 mg/kg (*n*=2), 5 mg/kg (*n*=5) on cortical dopaminergic modulation in 129/Sv mice. All results are expressed as means ± SEM. ***p<0.01, ***p<0.001* (one-way ANOVA followed by Dunnett's post hoc test).

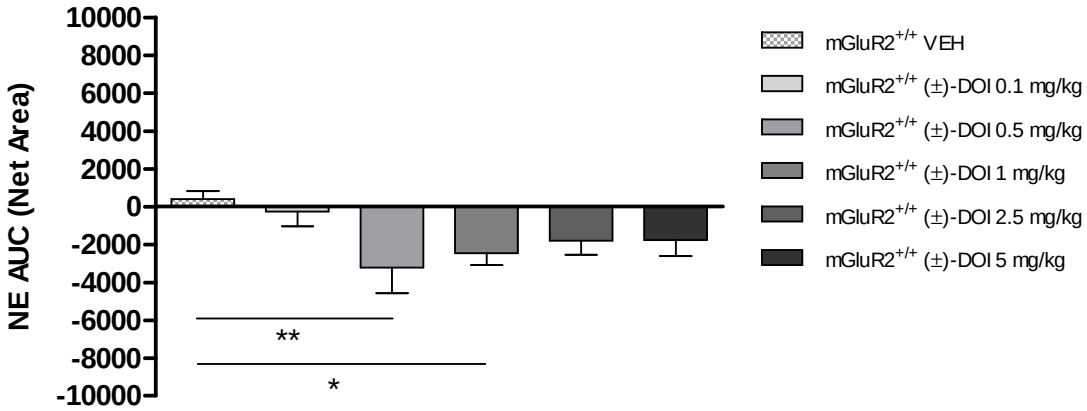


Fig. 76- Cortical NE AUC values after drug injection. Effect of intraperitoneal administration of vehicle (*n*=17), or (±)-DOI at doses of 0.1 mg/kg (*n*=9), 0.5 mg/kg (*n*=8), 1 mg/kg (*n*=13), 2.5 mg/kg (*n*=2), 5 mg/kg (*n*=6) on cortical noradrenergic modulation in 129/Sv mice. All results are expressed as means ± SEM. **p<0.05, **p<0.01* (one-way ANOVA followed by Dunnett's post hoc test).

4.1.2.6 Effect of systemic administration of M100907 on (±)-DOI-induced attenuation of extracellular DA and NE release in frontal cortex of 129/Sv mice

In order to discern whether the 5-HT2A or 5-HT2C receptors were implicated in the catecholaminergic inhibitory actions exerted by the systemic administration of the agonist 5-HT2A/2C receptors (±)-DOI, the 5-HT2AR antagonist M100907 was used. Thirty-five minutes

prior to the acute administration of (±)-DOI (1 mg/kg i.p.) a single dose of M100907 (0.5 mg/kg i.p.) was administered.

This dose has already been described to be sufficient to antagonize systemic (±)-DOI-evoked extracellular serotonergic alterations in FC (Martín-Ruiz *et al*., 2001).

Dopamine

The decrease in the extracellular DA concentration in the FC induced by (±)-DOI was reverted by the administration of the antagonist. M100907 not only was fully effective in blocking the inhibitory effect of (±)-DOI, but also increased DA output (E_{max}=131 ± 10%) (Figure 77). In these experiments - with double injection, the first one administering saline - the inhibitory effect triggered by systemic (±)-DOI was lower (E_{max}=-13 ± 8%), compared to the groups where only one injection of (±)-DOI was carried out (E_{max}=-26 ± 3%) (Figure 69). This lack of inhibitory effect could be due to a rise in DA output induced by the stress caused by subjecting the animal to a double injection.

Statistically significant differences were revealed by two-way repeated measures ANOVA analysis between saline + (±)-DOI, and M100907 + (±)-DOI groups ($F_i[12, 168]=3.68; p<0.0001$).

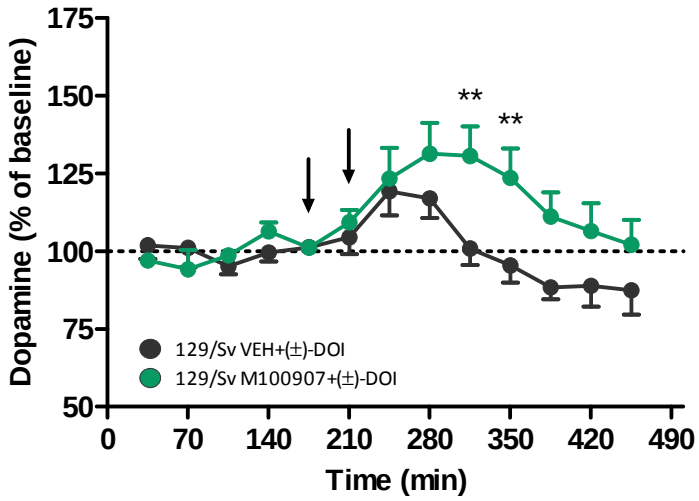


Fig. 77- Effect of systemic administration of (±)-DOI (1 mg/kg i.p.) alone (● *n*=9) or in presence of M100907 (0.5 mg/kg i.p.) (● *n*=7) on cortical extracellular DA concentrations in 129/Sv mice. The arrows indicate the time of drug injections: the first arrow represents VEH or M100907 administration; the second arrow represents (±)-DOI administration. Data are means ± SEM of DA expressed as a percentage of basal values (100%). ***p<0.01 versus* VEH + (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Norepinephrine

The previous presence of M100907 (0.5 mg/kg) abolished the (±)-DOI-induced inhibitory noradrenergic effect in the FC ($F_i[12, 216]=3.041$; $p<0.001$) (Figure 78). Once again, the inhibitory effect triggered by systemic (±)-DOI was lower (E_{max}=-8 ± 5%), as compared to the groups where only one injection - of (±)-DOI - was administered (E_{max}=-26 ± 4%) (Figure 70). These findings suggest that the lack of inhibitory effect could be a stress-induced response to the exposure to two consecutive injections.

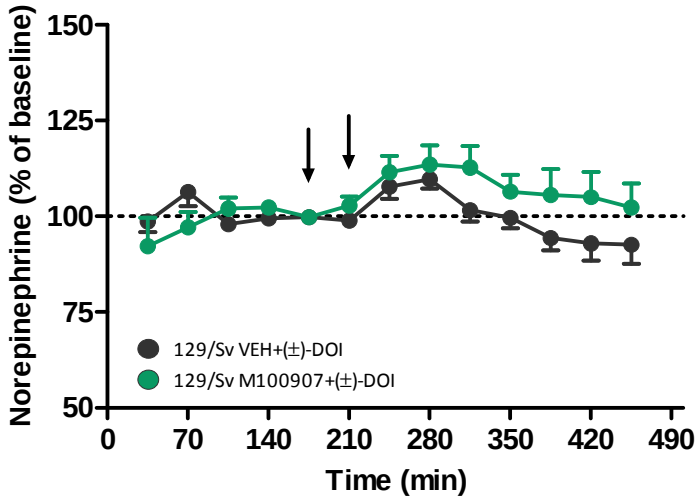


Fig. 78- Effect of systemic administration of (±)-DOI alone (1 mg/kg i.p.) (● *n*=11) or in presence of M100907 (0.5 mg/kg i.p) (● *n*=9) on cortical extracellular NE concentrations in 129/Sv mice. The arrows indicate the time of drug injections: the first arrow represents VEH or M100907 administration; the second arrow represents (±)-DOI administration. Data are means ± SEM of NE expressed as a percentage of basal values (100%) (two-way repeated measures ANOVA).

4.1.2.7. Effect of the 5-HT2CR antagonist SB242084 on (±)-DOI-induced attenuation of extracellular DA and NE release in frontal cortex of 129/Sv mice

SB242084 is primarily a 5-HT2CR antagonist, displaying 100- and 158-fold selectivity over the closely related 5-HT2B and 5-HT2A receptors respectively (Bromidge *et al*., 1997; Kennett *et al*., 1997; Gobert *et al*., 2000; Knight *et al*., 2004). A dose of 1 mg/kg was selected to be injected into the mice according to previous microdialysis studies (Calcagno *et al*., 2009, Calcagno & Invernizzi, 2010).

Dopamine

Acute injection of SB242084 (1 mg/kg i.p.) 35 minutes before the systemic administration of (±)-DOI (1 mg/kg i.p.) fully blocked the inhibitory effect induced by the hallucinogen alone. The 5-HT2CR antagonist not only significantly blocked the effect, but also rendered a stimulatory effect on DA release (E_{max}=148 ± 11%). Two-way repeated measures ANOVA revealed a statistically significant difference between saline + (±)-DOI, and SB242084 + (±)-DOI groups over time ($F_i[12, 144]=2.270; p<0.05$) (Figure 79). Taken together, these data and those under the presence of M100907, it is suggested that both 5-HT2AR and 5-HT2CR could be involved in the (±)-DOI-induced inhibitory effect.

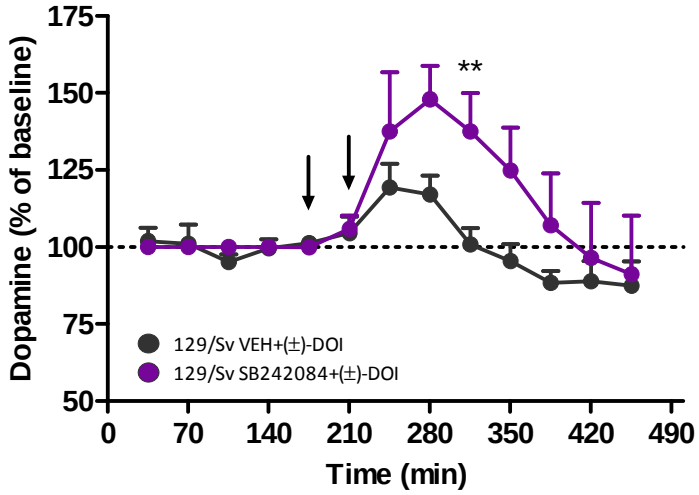


Fig. 79- Effect of systemic administration of (±)-DOI (1 mg/kg i.p.) alone (● *n*=9) or in presence of SB242084 (1 mg/kg i.p.) (● *n*=5) on cortical extracellular DA concentrations in 129/Sv mice. Arrows indicate the time of drug injections: the first arrow represents VEH or SB242084 administration; the second arrow represents (±)-DOI administration. Data are means ± SEM of DA levels expressed as a percentage of basal values (100%). ***p<0.01 versus* VEH + (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Norepinephrine

The presence of the 5-HT2CR antagonist SB242084 (1 mg/kg i.p.) did not block the slight noradrenergic response exerted by (±)-DOI (1 mg/kg i.p.) in wild-type 129/Sv strain mice. Two-way repeated measures ANOVA failed to reach significance in the interaction between treatments over time ($F_i[12, 204]=1.331; p=0.203$) (Figure 80).

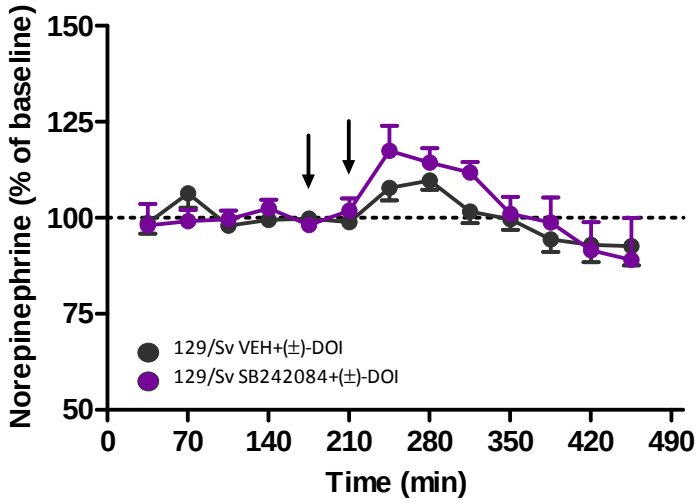


Fig. 80- Effect of systemic administration of (±)-DOI (1 mg/kg i.p.) alone (● *n*=11) or under presence of SB242084 (1 mg/kg i.p.) (● *n*=8) on cortical extracellular NE concentrations in 129/Sv mice. Arrows indicate the time of drug injections: the first arrow represents saline or SB242084 administration; the second arrow represents (±)-DOI administration. Data are means ± SEM of NE expressed as a percentage of basal values (100%).

4.1.2.8. Effect of systemic administration of the preferential 5-HT2C receptor agonist Ro 60-0175 on extracellular DA and NE release in frontal cortex of 129/Sv mice

To further discern whether the inhibitory effect on dopaminergic and noradrenergic activities was the result of 5-HT2AR or 5-HT2CR activation, experiments using a preferential 5-HT2CR agonist were performed. Ro 60-0175 has been described as an agonist at the 5-HT2CR, with roughly 10-fold higher affinity at the 5-HT2C receptor compared to the 5-HT2A subtype, and equivalent affinity for the 5-HT2B receptor (Porter *et al*., 1999; Higgins *et al*., 2001; Hemrick-Luecke & Evans, 2002; Knight *et al*., 2004). *In vitro* studies have shown reasonable affinity and efficacy for human 5-HT2AR and particularly human - and rat - 5-HT2B receptors (Porter *et al*, 1999). Nonetheless, reported findings suggest an apparent *in vivo* selectivity of this drug, because its properties as 5-HT2CR agonist inhibit the expression of 5-HT2A receptor-mediated behaviours, most notably wet-dog shakes, back muscle contractions and hyperactivity (Higgings *et al*., 2001). Dose of 3 mg/kg is commonly used to elicit 5-HT2CR related behaviors (Martin *et al*., 1998; Millan *et al*., 1998; Kennett *et al*., 2000), consistent with the relative potencies of Ro 60-0175 at human 5-HT2AR and 5-HT2CR (Porter *et al*., 1999). In addition, effects indicative of 5-HT2CR activation induced by Ro 60-0175 - e.g. hypolocomotion and penile grooming - are blocked by antagonists at the 5-HT2CR (Millan *et al*., 1998; Kennett *et al*., 2000). SB242084 (1 mg/kg) is reported to be able to antagonize dopaminergic inhibitory

effects elicited by Ro 60-0175 (1-3 mg/kg) in the NAcc (Di Matteo *et al*., 2000; De Deurwaerdère *et al*., 2004).

Dopamine

Intraperitoneal administration of 3 mg/kg of Ro 60-0175 elicited a significant decrease in DA efflux in the FC (E_{max}=-21 ± 4%). Significant differences between treatments were found after analyzing with two-way repeated measures ANOVA, compared to vehicle-treated group (*F_i[11, 132]=5.098; p<0.0001*) (Figure 81).

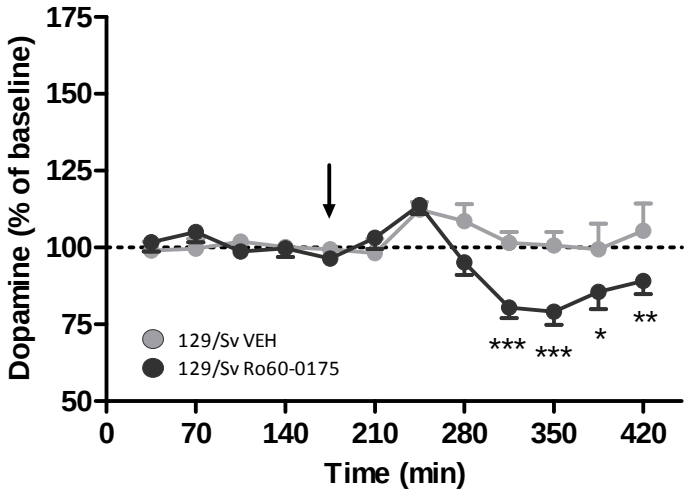


Fig. 81- Effect of systemic administration of VEH or Ro 60-0175 (3 mg/kg i.p.) on cortical extracellular DA concentrations in 129/Sv mice (● n=6, ● n=8). The arrow indicates the time of drug injection. Data are means ± SEM of DA levels expressed as a percentage of basal values (100%). **p<0.05*, ***p<0.01*, ****p<0.001 versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

Norepinephrine

Acute intra-peritoneal administration of Ro 60-0175 (3 mg/kg) significantly evoked an inhibitory effect on cortical NE output (E_{max}=-21 ± 3%) (Figure 82). Two-way repeated measures ANOVA revealed a statistically significant effect on noradrenergic modulation depending on treatments over time (*F_i[11, 143]=4.670; p<0.0001*).

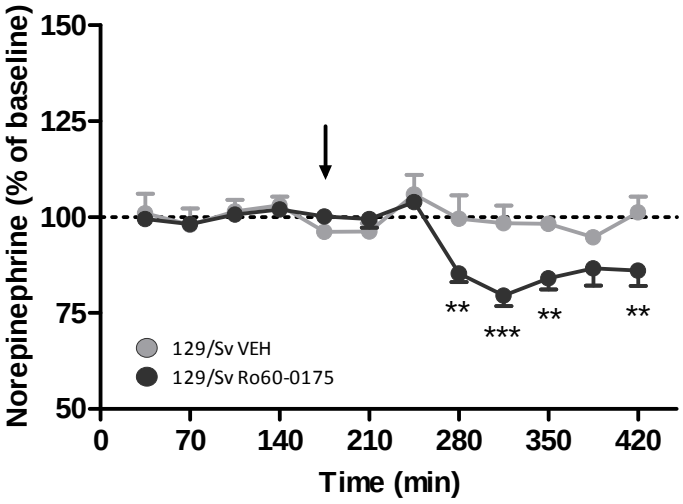


Fig. 82- Effect of systemic administration of VEH or Ro 60-0175 (3 mg/kg i.p.) on cortical extracellular NE concentrations in 129/Sv mice (● *n*=5, ● *n*=10). The arrow indicates the time of drug injection. Data are means ± SEM of NE levels expressed as a percentage of basal values (100%). ***p<0.01, ***p<0.001 versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

4.1.3. Influence of the 5-HT2AR/mGluR2 heterocomplex in the responses triggered by systemic administration of the hallucinogenic 5-HT2A/2C receptor agonist (±)-DOI

The potential functional existence of the heterocomplex in mouse frontal cortex has been previously addressed (section 4.1.1.; see introduction for further details). An impairment in the cortical stimulatory response of DA and NE to local administration of (±)-DOI was elicited in mGluR2$^{-/-}$, as compared to its wild-type counterparts (Figures 38 and 40). Overexpression of mGluR2, mediated by viral transduction, was able to restore the (±)-DOI-induced stimulatory response toward similar values to those observed in mGluR2$^{+/+}$ mice (Figures 60 and 62).

In order to establish potential differences between genotypes in the effect induced by intraperitoneal administration of (±)-DOI, the response in mGluR2$^{-/-}$ mice and wild-type mGluR2$^{+/+}$ mice was evaluated.

4.1.3.1. Dose-dependent effects of systemic administration of (±)-DOI on extracellular DA and NE release in the frontal cortex of mGluR2$^{-/-}$ 129/Sv mice

Modulation of extracellular DA and NE concentrations after the administration of three doses of (±)-DOI (0.1, 1, and 5 mg/kg i.p.) were studied. Surprisingly, the lowest dose (0.1 mg/kg) was

able to significantly rise extracellular DA (AUC mean=6962 ± 1131) and NE concentrations (AUC mean=3172 ± 1120) in the FC. However, (±)-DOI induced an overall inhibitory effect of cortical DA (*F[3, 46]=14.11; p<0.0001*, one-way ANOVA), reaching a maximal inhibitory effect at 1 mg/kg i.p. (AUC mean=-1173 ± 952) (Figure 83). Doses of 1 mg/kg and 5 mg/kg also induced a decrease in cortical NE efflux (*F[3, 50]=7.701; p<0.001*, one-way ANOVA), with a maximal inhibitory response at a dose of 1 mg/kg (AUC mean=-2132 ± 757) (Figure 84).

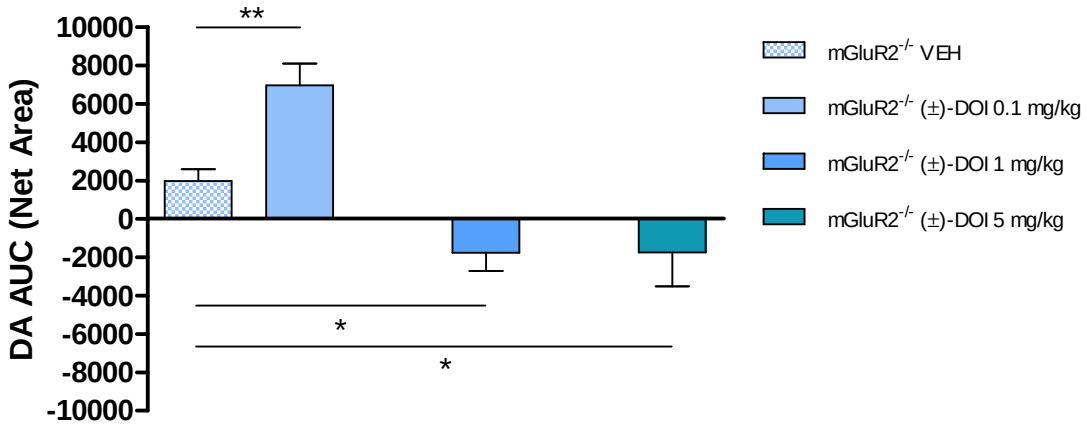


Fig. 83- Cortical DA AUC values after drug injection. Effect of intraperitoneal administration of VEH (*n*=19), or (±)-DOI at doses of 0.1 mg/kg (*n*=10), 1 mg/kg (*n*=10) and 5 mg/kg (*n*=8) on cortical dopaminergic modulation in mGluR2$^{-/-}$ 129/Sv mice. All results are expressed as means ± SEM. **p<0.05, **p<0.01* (one-way ANOVA followed by Dunnett's post hoc test).

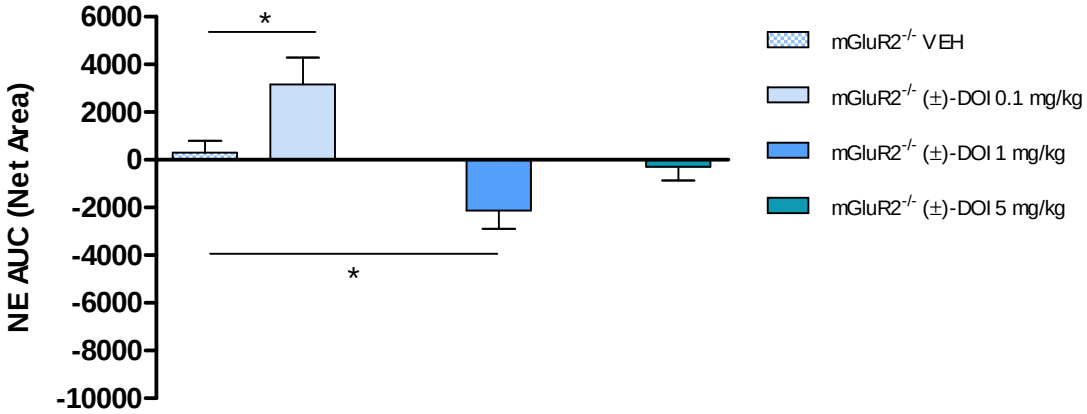


Fig. 84- Cortical NE AUC values after drug injection. Effect of intraperitoneal administration of VEH (*n*=21), or (±)-DOI at doses of 0.1 mg/kg (*n*=9), 1 mg/kg (*n*=14) and 5 mg/kg (*n*=7) on cortical noradrenergic modulation in mGluR2$^{-/-}$ 129/Sv mice. All results are expressed as means ± SEM. **p<0.05* (one-way ANOVA followed by Dunnett's post hoc test).

4.1.3.2. Effect of systemic administration of (±)-DOI (1 mg/kg) on extracellular DA and NE release in the frontal cortex in mGluR2$^{-/-}$ and mGluR2$^{+/+}$ 129/Sv strain mice

Dopamine

As mentioned above (see 4.1.1.1. section), basal dialysate concentrations of DA, by pooling from different experiments, collected from five-six consecutive samples from the FC, did not differ across genotype: 1.637 ± 0.115 nM (*n*=99) in mGluR2$^{+/+}$ mice and 1.799 ± 0.204 nM (*n*=71) in mGluR2$^{-/-}$ mice (*t=0.738; p=0.461*).

As previously stated, (±)-DOI (1 mg/kg i.p.) was able to reduce extracellular DA output in the FC of 129/Sv wild-type mice (E_{max}=-26 ± 3%) (Figure 69). Their mGluR2 knock-out counterparts seemed to display the same inhibitory pattern, exerting a significant inhibitory response when compared to saline administration (F_i*[11, 157]=2.881; p<0.01*) (E_{max}=-21 ± 7%). Two-way repeated measures ANOVA failed to reach significant interaction in the response to (±)-DOI between genotypes over time (F_i*[11, 198)=0.725; p=0.714*) (Figure 85).

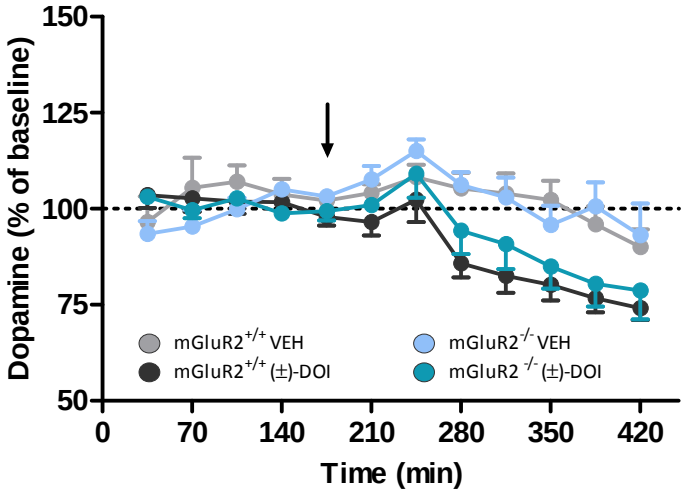


Fig. 85- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on cortical extracellular DA concentrations in mGluR2$^{+/+}$ (● *n*=7, ● *n*=10) and in mGluR2$^{-/-}$ (● *n*=9, ● *n*=10) 129/Sv mice. The arrow indicates the time of drug injection. Data are means ± SEM of DA expressed as a percentage of basal values (100%).

When analyzed the magnitude of the effects induced by systemic administration of (±)-DOI, a decrease in DA outflow in the FC in both genotypes, compared to the respective vehicle-treated groups, was observed (AUC mean=-3043 ± 642 in (±)-DOI-treated mGluR2$^{+/+}$; AUC mean=-1773 ± 952 in (±)-DOI-treated mGluR2$^{-/-}$). Two-way ANOVA only showed that (±)-DOI

was exerting an inhibitory effect, compared to vehicle ($F_{treatment}[1, 32]=14.19; p<0.001$). However, the effect was similar between genotypes ($F_i[1, 32]=0.38; p=0.540$) (Figure 86).

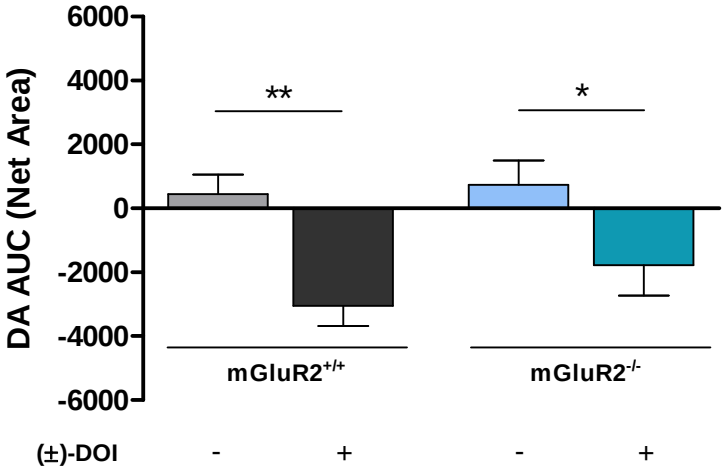


Fig. 86- Cortical DA AUC values after drug injection. Effect of intraperitoneal administration of saline or (±)-DOI (1 mg/kg i.p.) on cortical dopaminergic modulation in mGluR2$^{+/+}$ (● *n*=7, ● *n*=10) and in mGluR2$^{-/-}$ mice (● *n*=9, ● *n*=10). All results are expressed as means ± SEM. **p<0.05, **p<0.01* (two-way ANOVA followed by LSD's post-hoc test).

Norepinephrine

As mentioned above (see 4.1.1.1. section), pre-drug basal concentrations of extracellular NE in the FC under CSF infusion, by pooling dialysates from various experiments, were significantly higher in mGluR2$^{-/-}$ mice when compared to mGluR2$^{+/+}$ group: 1.420 ± 0.065 nM (*n*=125) *versus* 1.690 ± 0.106 nM (*n*=77) (*t=2.302; p<0.05*). Intra-cortical CSF infusion did not alter cortical extracellular NE concentrations in either genotype.

As previously shown, acute administration of (±)-DOI (1 mg/kg i.p.) triggered an inhibitory effect, reducing the extracellular NE release noticeably in wild-type mice (E_{max}=-26 ± 4%) (Figure 70) ($F_i[11, 231]=4.466; p<0.0001$).

Acute intraperitoneal dose of (±)-DOI (1 mg/kg), dissolved in saline as vehicle, also evoked a statistically significant inhibition of extracellular NE output in the FC of mGluR2$^{-/-}$ mice (E_{max}=-21 ± 6%) ($F_i[11, 242]=2.125; p<0.05$) (Figure 87). There were no differences between genotypes in the effect of (±)-DOI administration, as assessed by ANOVA analysis ($F_i[11, 275]=1.176; p=0.304$).

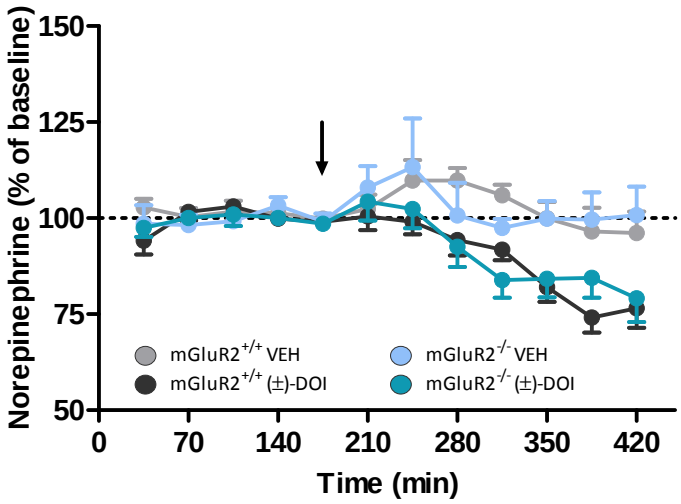


Fig. 87- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on cortical extracellular NE concentrations in mGluR2$^{+/+}$ (● *n*=10, ● *n*=13) and in mGluR2$^{-/-}$ (● *n*=10, ● *n*=14) 129/Sv mice. The arrow indicates the time of drug injection. Data are means ± SEM of NE expressed as a percentage of basal values (100%).

Regarding the magnitude of the effects displayed after saline or (±)-DOI injection, AUC values showed that there was an inhibitory effect in NE release subsequently to (±)-DOI administration ($F_{treatment}$*[1, 43]=14.78; p<0.001*), but the magnitude was not genotype-dependent (AUC mean=-2458 ± 638 in mGluR2$^{+/+}$, AUC mean=-2132 ± 757 in mGluR2$^{-/-}$) (F_i*[1, 43]=0.12; p=0.728*) (Figure 88).

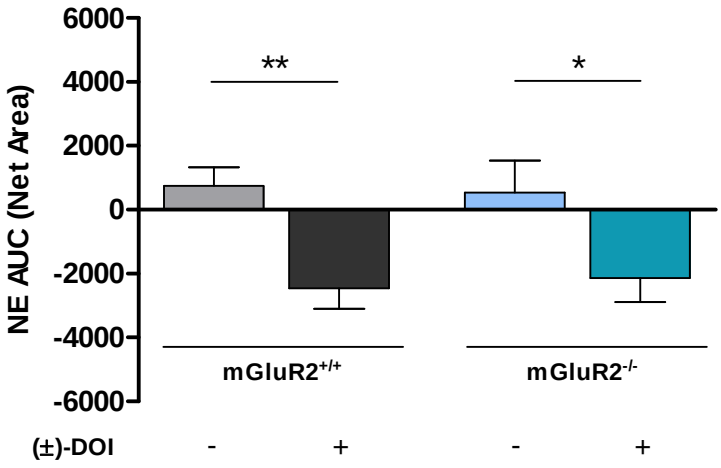


Fig. 88- Cortical NE AUC values after drug injection. Effect of intraperitoneal administration of saline or (±)-DOI (1 mg/kg i.p.) on cortical noradrenergic modulation in mGluR2*+/+* (● *n*=10, ● *n*=13) and in mGluR2$^{-/-}$ mice (● *n*=10, ● *n*=14). All results are expressed as means ± SEM. **p<0.05, **p<0.01* (two-way ANOVA followed by LSD's post hoc test).

4.2. FUNCTIONAL REGULATION OF CORTICAL NE RELEASE BY 5-HT2A/2C RECEPTORS. NEUROCHEMICAL PATHWAYS EVALUATION BY *IN VIVO* MICRODIALYSIS IN AWAKE, FREELY MOVING RATS

Subsequent microdialysis studies were carried out in order to elucidate the pathways involved in the noradrenergic modulation exerted by (±)-DOI. As shown above, (±)-DOI evoked opposite noradrenergic responses in mouse frontal cortex after intra-cortical or systemic administration. A noradrenergic stimulation can be seen after intra-cortical administration of (±)-DOI, whereas no effect or an inhibitory response can be noted after its systemic administration in C57BL/6 or 129/Sv strain mice, respectively.

The LC is the major source of NE in the CNS and is widely known that there are projections arising from LC to the FC. In this regard, the implantation of two probes - one in the LC and the other one in the FC - allowed us to evaluate NE modulation in somatodedritic and terminal noradrenergic areas. LC in mouse brain is too tiny to ensure the dialysis in that specific area, and the overall brain size is not big enough to lodge two probes at the same time. Therefore, we decided to perform the study in rats.

Dual-probe microdialysis experiments were undertook without the presence of desipramine dissolved in the CSF. Basal extracellular NE concentrations are higher in rats than in mice and enough to detect them by HPLC. Moreover, these dialysates were measured using a very sensitive Ultra-High Performance Liquid Chromatography (UHPLC) equipment.

4.2.1. Effect of cortical infusion of the 5-HT2A/2C receptor agonist (±)-DOI on extracellular NE output in frontal cortex and locus coeruleus

Basal extracellular NE concentrations, obtained by pooling the basal values of the different experimental groups, were substantially higher in the LC (0.299 ± 0.04 nM, n=30) than in the FC (0.140 ± 0.02 nM, n=30) ($t=3.554$; $p<0.001$).

We characterized the cortical noradrenergic effect after intra-cortical (±)-DOI administration (300 µM) in rats. CSF infused as vehicle did not exert any effect. As previously shown in mice (Figures 40 and 41), the cortical infusion of the 5-HT2A/2C receptor agonist increased

extracellular NE release in the FC (E_{max}=376 ± 39%) (Figure 89). Two-way repeated measures ANOVA revealed a statistically significant stimulatory effect over time when compared to vehicle group (*F_i[10, 40]=14.07; p<0.001*).

Conversely, no modulation in NE efflux was experience in the LC after intra-cortical infusion of (±)-DOI (300 µM). Repeated measures two-way ANOVA failed to reach significance over time, when compared to vehicle-treated group (*F_i[10, 130]=0.492; p=0.892*) (Figure 90).

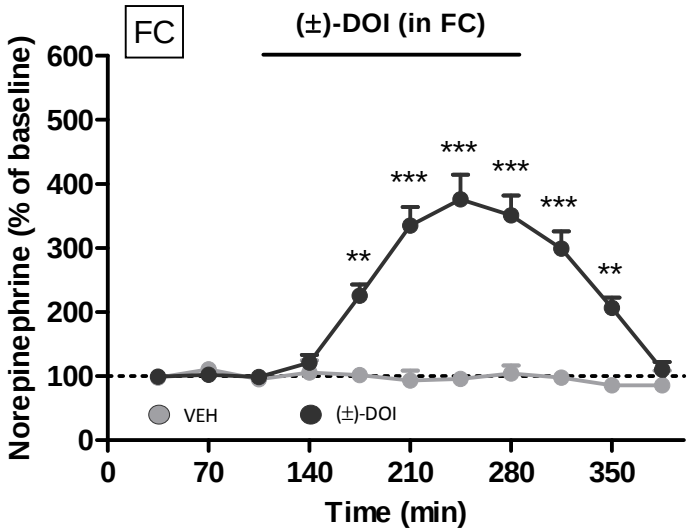


Fig. 89- Effect of cortical administration of VEH or (±)-DOI (300 µM) on extracellular concentrations of NE in rat FC (● *n*=4, ● *n*=12). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The line shows the period of drug application. ***p<0.01, ***p<0.001 versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

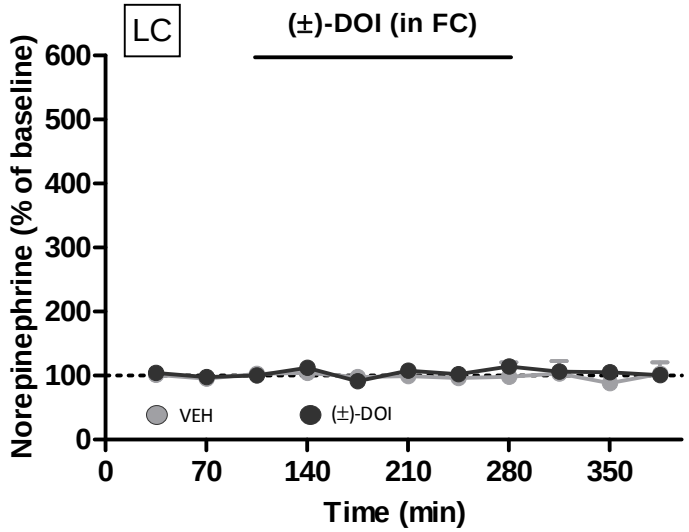


Fig. 90- Effect of cortical administration of VEH or (±)-DOI (300 µM) on extracellular NE concentrations in rat LC (● *n*=4, ● *n*=11). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The line shows the period of drug application.

4.2.2. Effect of systemic administration of the 5-HT2A/2C receptor agonist (±)-DOI on extracellular NE output in frontal cortex and locus coeruleus

Saline injection did not modify extracellular NE concentrations neither in the FC, nor in the LC. Acute administration of (±)-DOI (1 mg/kg i.p.) elicited a significant decrease of NE release in the FC (E_{max}=-28 ± 7%), reaching the maximal inhibition value 175 minutes after injection. Extracellular NE concentrations returned to basal values at the end of the procedure (Figure 91). Repeated measures two-way ANOVA showed a significant interaction in the noradrenergic modulation between vehicle-treated group and (±)-DOI-treated group over time (F_i*[9, 117]=4.283; p<0.001*).

On the contrary, right after (±)-DOI injection, a high noradrenergic stimulation was elicited in the LC (E_{max}=234 ± 24%) (Figure 92). Statistically significant differences were found by two-way repeated measures ANOVA analysis compared to the saline group (F_i*[9, 126]=9.539; p<0.0001*).

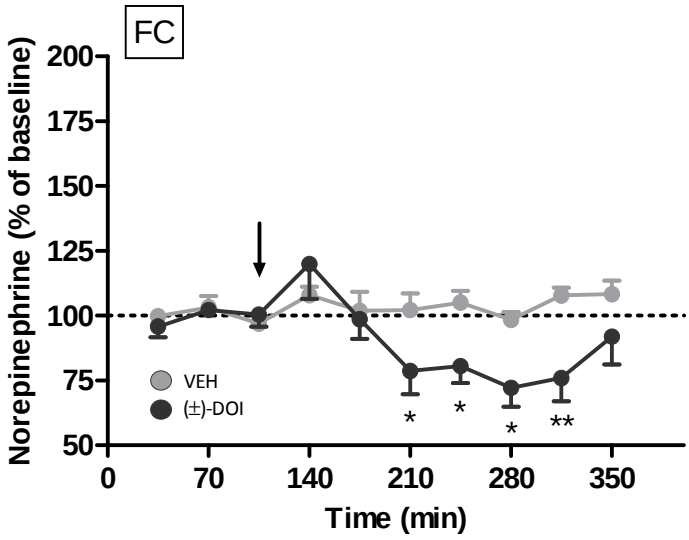


Fig. 91- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on cortical extracellular NE concentrations in rat (● *n*=7, ● *n*=8). The arrow indicates the time of drug injection. Data are means ± SEM of NE expressed as a percentage of basal values (100%). **p<0.05, **p<0.01*, *versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

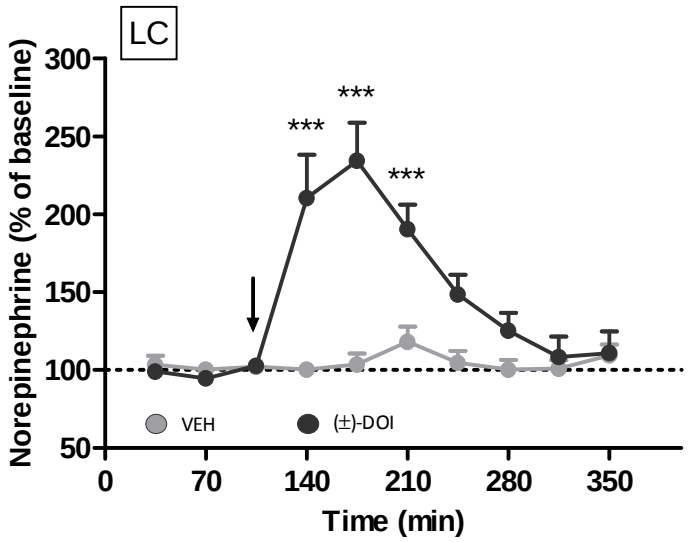


Fig. 92- Effect of systemic administration of VEH or (±)-DOI (1 mg/kg i.p.) on extracellular NE concentrations in the rat LC (● *n*=7, ● *n*=9). The arrow indicates the time of drug injection. Data are means ± SEM of NE expressed as a percentage of basal values (100%). ****p<0.001*, *versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

4.2.3. Effect of intra-locus coeruleus infusion of the 5-HT2A/2C receptor agonist (±)-DOI on extracellular NE output in locus coeruleus and frontal cortex

As a decrease in extracellular NE efflux in the FC, and an increase in the LC were observed after a systemic administration of (±)-DOI, the stimulation occurring in the LC could be responsible for the decrease in the FC. To address this hypothesis, two increasing doses were locally perfused in the LC, and extracellular NE modulations were simultaneously analyzed in both areas.

At the lowest dose (100 µM), local administration of (±)-DOI displayed a trend to increase extracellular NE output in the LC (E_{max}=192 ± 21%). This stimulation did not reach statistical significance. On the other hand, extracellular NE concentrations were gradually decreasing in the FC *versus* basal values, reaching statistical significance 210 minutes after the (±)-DOI infusion (E_{max}=-56 ± 3%) (*p<0.01*) (Figure 93 and 94). The highest dose (300 µM) of (±)-DOI evoked a greater extracellular NE release in the LC (E_{max}=278 ± 58%), following by a dramatic fall in NE efflux in the FC (E_{max}=-89 ± 3%) (Figure 93 and 94). Statistically significant effects were shown by two-way repeated measures ANOVA analysis in both areas when compared to vehicle perfusion (F_i*[13, 78]=7.486; p<0.0001*, in LC; F_i*[13, 65]=14.77; p<0.001*, in FC).

Additionally, the existence of a linear correlation between extracellular NE concentrations in the LC and the FC of the same rats was also evaluated (Figure 95). A significant negative correlation was observed between both areas ($p<0.05$, r=-0.508, n=21).

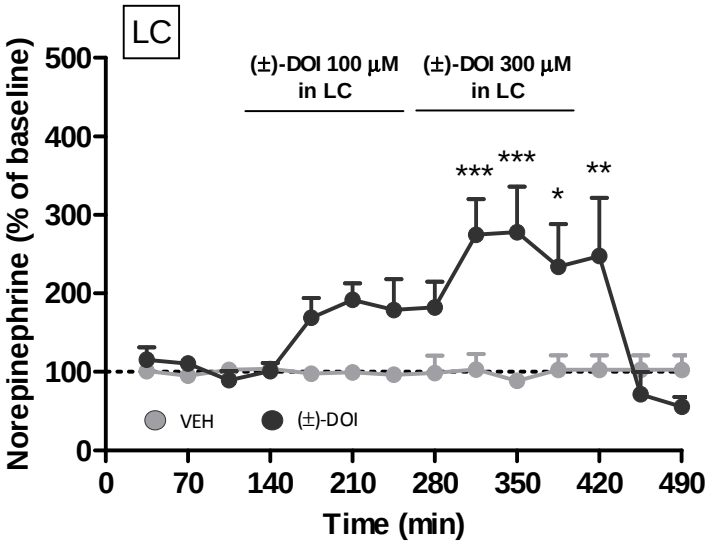


Fig. 93- Effect of intra-LC administration of VEH or (±)-DOI (100 and 300 µM) on extracellular concentrations of NE in rat LC (● n=4, ● n=4). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The lines show the period of drug application. **p<0.05, **p<0.01, ***p<0.001 versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

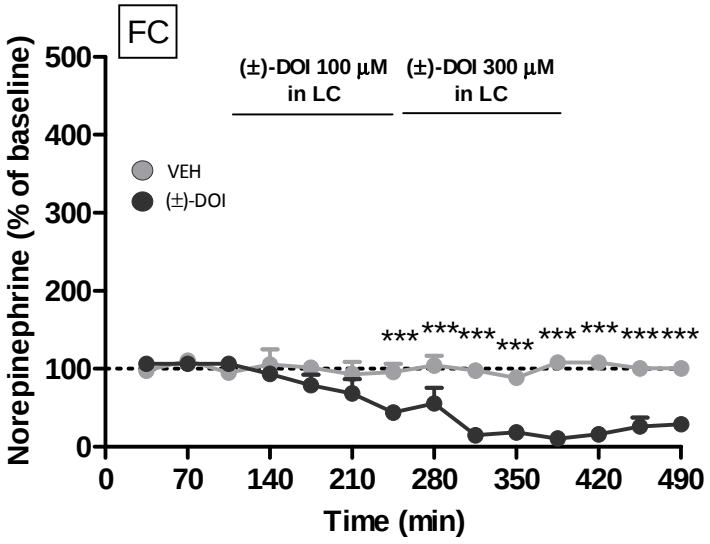


Fig. 94- Effect of intra-LC administration of VEH or (±)-DOI (100 and 300 µM) on extracellular concentrations of NE in rat FC (● n=4, ● n=3). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The lines show the period of drug application. ****p<0.001 versus* vehicle-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

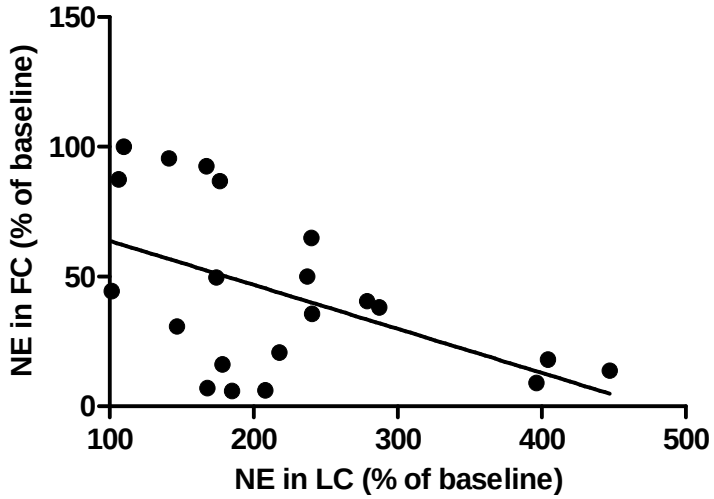


Fig. 95- Correlation between extracellular NE concentrations in rat LC and FC, following the local administration of (±)-DOI (100-300 µM) into the LC. Each point represents the percentage of NE baseline values obtained in the same animal, from samples collected every 35 minutes (*n*=3). The regression equation was *y=80.74-0.17x*.

4.2.4. α_2-Adrenoceptors in the modulation of NE by (±)-DOI

As stated above, FC is an area innervated by noradrenergic projections arising from the LC. Presynaptic α_2-adrenoceptors are located on noradrenergic nerve terminals as well as on cell bodies and dendrites of the LC neurons. It has been previously demonstrated that somatodendritic α_2-adrenoceptors in the LC exert an inhibitory tonic modulation on NE release in noradrenergic terminal areas (Fernández-Pastor & Meana, 2002) and on the firing activity of noradrenergic neurons in the LC (Mateo *et al*., 1998). The presence of the α_2-adrenoceptor antagonist RX821002 (1 µM) into the LC, despite not altering extracellular NE concentrations, is able to abolish the inhibition of NE release in FC subsequent to the increased NE release occurred in LC (Mateo *et al*., 1998).

In this regard, we sought to elucidate the influence of somatodendritic α_2-adrenoceptors located in the LC in the cortical inhibition of NE release induced by systemic administration of (±)-DOI (Figure 91 and 92).

Vehicle-treated group displayed absolute NE basal values of 0.121 ± 0.030 nM (*n*=5) in FC and 0.277 ± 0.091 nM (*n*=8) in LC. In the presence of RX821002 (1 µM) locally into the LC, basal

values were 0.450 ± 0.065 nM (*n*=5) in FC and 0.355 ± 0.041 nM in LC (*n*=9). There were no significant differences between groups.

As shown in Figure 96, the presence of RX821002 (1 µM) in the LC blocked the (±)-DOI-induced NE decrease in the FC. Indeed, a stimulatory effect in that area was found (E_{max}=156 ± 19%). Two-way repeated measures ANOVA comparing (±)-DOI *versus* RX821002+(±)-DOI groups over time, revealed statistically significant differences ($F_i[9, 126]=5.437$; $p<0.0001$).

In LC, (±)-DOI injection significantly induced a rise in NE efflux in the group with RX821002 (1 µM) (E_{max}=210 ± 26%). However the maximal value, magnitude and duration of the effect were lower compared to the group where only CSF was perfused. Two-way repeated measures ANOVA revealed a significant interaction between drug group - (±)-DOI *versus* RX821002+(±)-DOI - over time ($F_i[9, 144]=3.270$; $p<0.01$) (Figure 97).

Figure 98 and 99 represents AUC values in FC and LC triggered by the administration of saline, (±)-DOI (1 mg/kg i.p.) alone, or (±)-DOI (1 mg/kg i.p.) with previous and continuous infusion of RX821002 (1 µM) into the LC. There were statistically significant differences in the responses exerted by treatments in both areas ($F_i[2, 20]=44.61$; $p<0.0001$ in FC; $F_i[2, 22]=8.544$; $p<0.01$ in LC; one-way ANOVA).

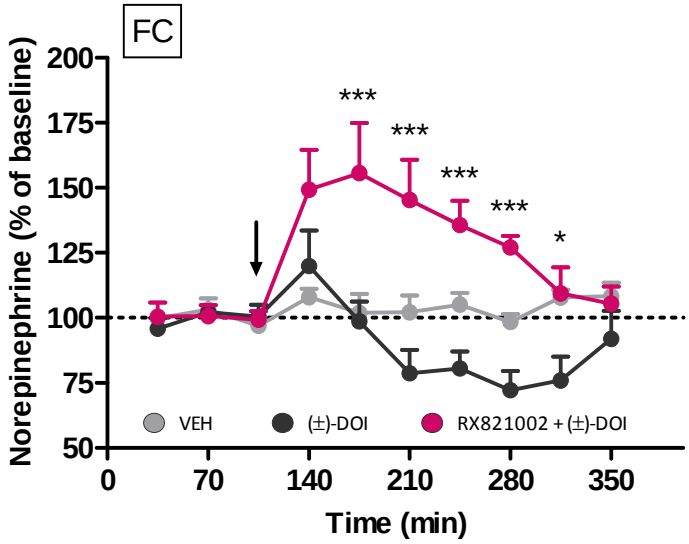


Fig. 96- Effect of the perfusion of RX821002 (1 µM) into the LC, on the (±)-DOI-induced extracellular NE variations in rat FC (● *n*=7, ● *n*=8, ● *n*=8). The arrow indicates the time of drug injection. Data are means ± SEM of NE expressed as a percentage of basal values (100%). **p<0.05, ***p<0.001 versus* (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

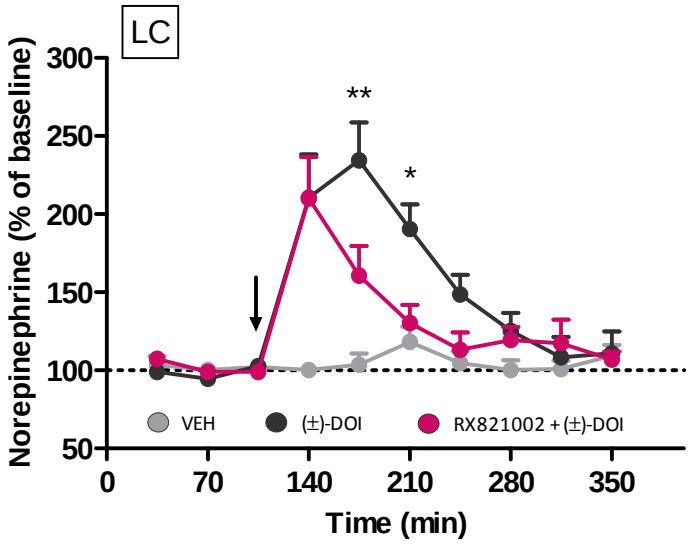


Fig. 97- Effect of the perfusion of RX821002 (1 µM) into the LC, on the (±)-DOI-induced extracellular NE variations in rat LC (● *n*=7, ● *n*=9, ● *n*=9). The arrow indicates the time of drug injection. Data are means ± SEM of NE expressed as a percentage of basal values (100%). **p<0.05, **p<0.01 versus* (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

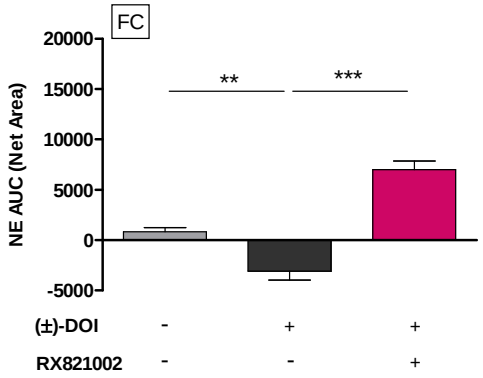


Fig. 98- NE AUC values after drug injection in rat FC. Effect of intraperitoneal administration of saline, (±)-DOI (1 mg/kg i.p.) alone or in continuous presence of RX821002 (1 µM) in the LC (● *n*=7, ● *n*=8, ● *n*=8). All results are expressed as means ± SEM. ***p<0.01, ***p<0.001* (one-way ANOVA followed by Bonferroni's post hoc test).

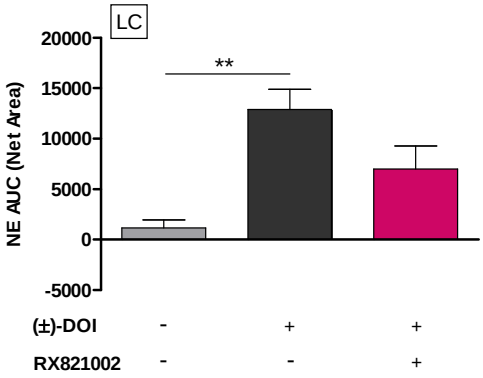


Fig. 99- NE AUC values after drug injection in rat LC. Effect of intraperitoneal administration of saline, (±)-DOI (1 mg/kg i.p.) alone or in continuous presence of RX821002 (1 µM) in the LC (● *n*=7, ● *n*=9, ● *n*=9). All results are expressed as means ± SEM. ***p<0.01* (one-way ANOVA followed by Bonferroni's post hoc test).

4.2.5. Effect of intra-locus coeruleus infusion of the glutamate receptor antagonist kynurenic acid on NE release response to systemic administration of (±)-DOI in locus coeruleus and frontal cortex

The receptors implicated in the (±)-DOI-induced increase in NE output in the LC after acute administration remained to be clarified. In an effort to elucidate the role of glutamartergic excitatory inputs arising from other areas to the LC, kynurenic acid (KYN) was perfused into the LC. KYN is a metabolite physiologically present in the CSF at nanomolar concentrations. It displays a broad spectrum of action as a glutamatergic antagonist, being able to block the glutamate receptors NMDA and AMPA, among others (Moroni *et al*., 2012).

In this section, kynurenic acid (1 mM) was perfused into the LC from the beginning of the experiment. Following the collection of 3 basal samples, (±)-DOI was intraperitoneally injected (1 mg/kg).

The administration of kynurenic acid (1 mM) into the LC did not modify basal NE concentrations in the FC (0.217 ± 0.06 nM, *n*=4 in CSF group; 0.134 ± 0.02 nM, *n*=9 in KYN group; *t=1.526; p=0.155*). Interestingly, KYN induced a markedly decrease of extracellular NE concentrations in the LC (0.599 ± 0.11 nM, *n*=5 in CSF group; 0.048 ± 0.01 nM *n*=10 in KYN group; *t=7.382; p<0.001*).

Systemic administration of (±)-DOI in the continuous presence of KYN in the LC evoked a stimulatory effect of NE release in FC 35 minutes after (±)-DOI administration (likely due to handling stress), followed by a gradual decrease in NE release (E_{max}=66 ± 14%) (Figure 100). Two-way repeated measures ANOVA indicated a significant interaction (±)-DOI *versus* KYN + (±)-DOI over time (F_i*[9, 99]=2.351; p<0.05*).

As Figure 101 shows, KYN did not blunt the stimulatory effect of (±)-DOI seen in LC, exerting again a rise in NE efflux in LC (E_{max}=251 ± 25%). No statistical differences between (±)-DOI and KYN + (±)-DOI responses over time were found by analysis with two-way repeated measures ANOVA (F_i*[9, 126]=1.634; p=0.11*2).

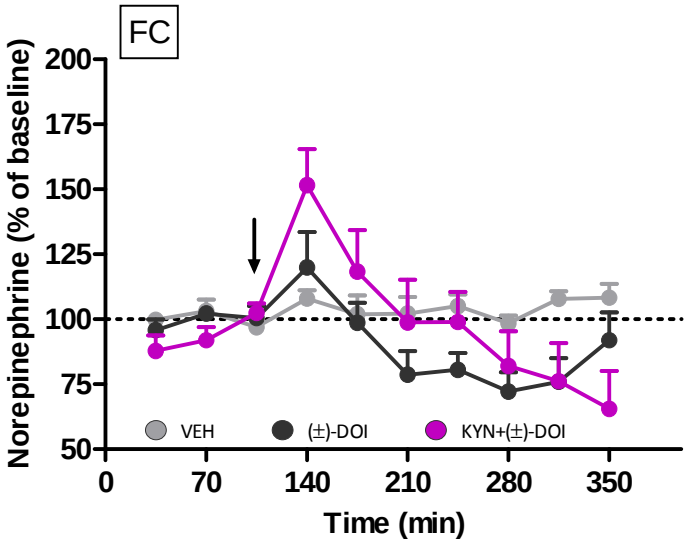


Fig. 100- Effect of the perfusion of kynurenic acid (KYN, 1 mM) into the LC, on the systemic (±)-DOI-induced extracellular NE variations in rat FC (● *n*=7, ● *n*=8, ● *n*=5). The arrow indicates the time of drug injection. Data are means ± SEM of NE expressed as a percentage of basal values (100%).

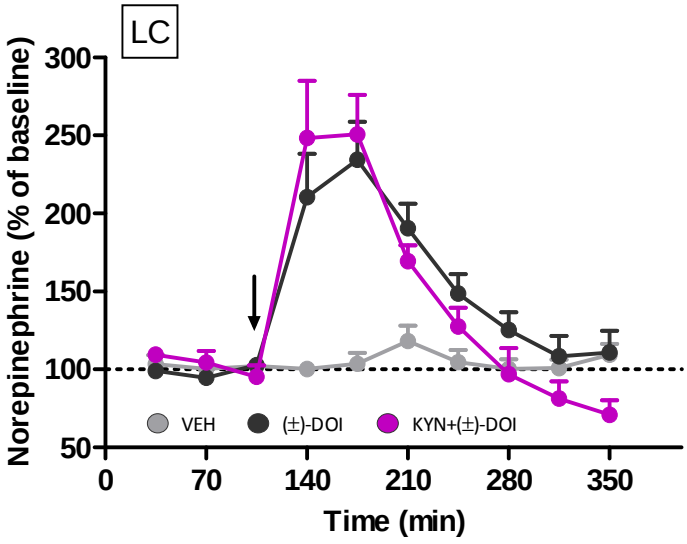


Fig. 101- Effect of the perfusion of kynurenic acid (KYN, 1 mM) into the LC, on the systemic (±)-DOI-induced extracellular NE variations in rat LC (● *n*=7, ● *n*=9, ● *n*=7). The arrow indicates the time of drug injection. Data are means ± SEM of NE expressed as a percentage of basal values (100%).

4.2.6. Effect of intra-locus coeruleus infusion of the glutamate receptor antagonist kynurenic acid on the response of NE release exerted by local administration of (±)-DOI into the frontal cortex

In order to assess the existence on an indirect loop (FC → LC → FC) responsible for the increase of extracellular NE release in the FC after the intra-cortical administration of (±)-DOI, the glutamatergic antagonist was perfused into the LC.

As mentioned in 4.2.5. section, KYN in LC (1 mM) did not modify basal NE concentrations in the FC (0.217 ± 0.06 nM, *n*=4 in CSF group *versus* 0.134 ± 0.02 nM, *n*=9 in KYN group; *t=1.526; p=0.155*). In contrast, KYN induced a markedly decrease of extracellular NE concentrations in the LC (0.599 ± 0.11 nM, *n*=5 in CSF group *versus* 0.048 ± 0.01 nM *n*=10 in KYN group; *t=7.382; p<0.001*).

The continuous perfusion of KYN (1 mM) in LC not only failed to antagonize the cortical stimulation mediated by (±)-DOI (300 μM in FC), but also enhanced that stimulation (E_{max}=500 ± 70%) (Figure 102). Two-way repeated measures ANOVA showed a statistically significant interaction between treatments - (±)-DOI alone, or KYN + (±)-DOI - over time (F_i*[10, 140]=3.295; p<0.001*).

The intra-LC infusion of KYN was not able to alter the response of extracellular NE concentrations in LC by (±)-DOI administration in the FC (Figure 103). Hence, no significant interactions were found regarding treatment and time (F_i*[10, 120]=0.410; p=0.940*).

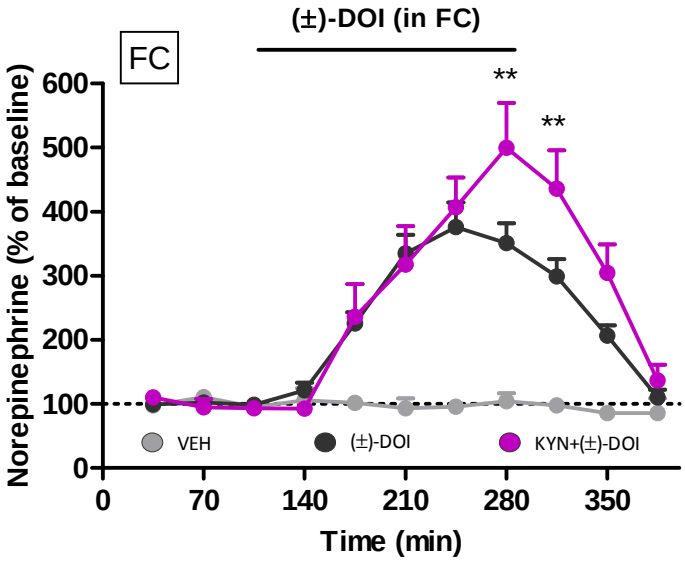


Fig. 102- Effect of the perfusion of kynurenic acid (KYN, 1 mM) into the LC on the intra-cortical (±)-DOI-induced extracellular NE variations in rat FC (● *n*=4, ● *n*=12, ● *n*=4). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The line shows the period of drug application. ***p<0.01 versus* (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

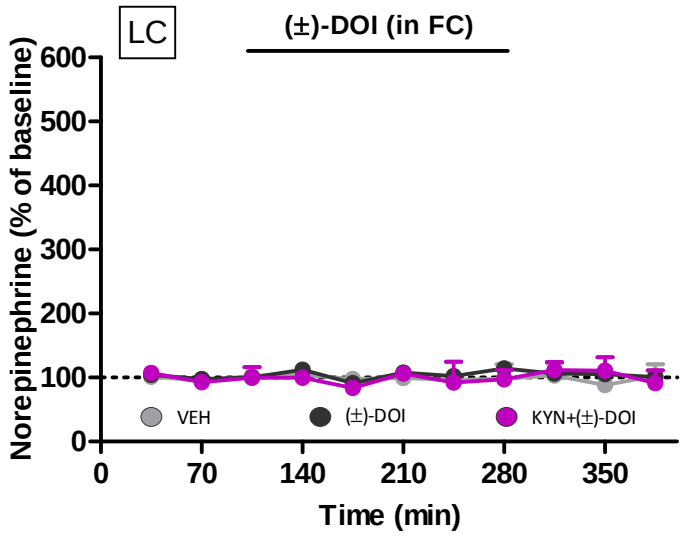


Fig. 103- Effect of the perfusion of kynurenic acid (KYN, 1 mM) into the LC on the intra-cortical (±)-DOI-induced extracellular NE variations in rat LC (● *n*=4, ● *n*=11, ● *n*=3). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The line shows the period of drug application.

4.2.7. Effect of cortical infusion of the glutamate receptor antagonist kynurenic acid on the response of NE release exerted by local administration of (±)-DOI into the frontal cortex

Martín-Ruiz and co-workers (2001) raised the hypothesis that local administration of (±)-DOI could increase the 5-HT release in FC indirectly, through the stimulation of glutamate release and subsequent activation of AMPA-KA receptors. In order to asses if in the case of NE regulation, (±)-DOI proceeds the same way as for 5-HT, the non-selective glutamatergic antagonist KYN (1 mM) was locally administered into the FC from the beginning of the experiment.

KYN perfused into FC did not alter basal NE concentrations in FC (0.217 ± 0.06 nM, *n*=4 in CSF group *versus* 0.160 ± 0.05 nM, *n*=6 in KYN group; *t=0.712; p=0.496*). Likewise, in LC no changes in baseline NE values derived from to KYN infusion were observed (0.599 ± 0.11 nM, *n*=5 in CSF group *versus* 0.678 ± 0.12 nM, *n*=5 in KYN group; *t=0.489; p=0.638*).

Intra-cortical infusion of KYN impaired the NE stimulation evoked by local administration of (±)-DOI (300 μM) in the FC, shown in Figure 89, to a maximal effect of 257 ± 51% (Figure 104). Two-way repeated measures ANOVA highlighted a statistically significant interaction treatment x time between (±)-DOI and KYN + (±)-DOI groups (F_i*[10, 170]=4.036; p<0.0001*), and between CSF and KYN + (±)-DOI groups (F_i*[10, 90]=4.310; p<0.0001*).

Conversely, no modifications in NE efflux were observed in the LC (Figure 105). Two-way repeated measures ANOVA revealed non-significant differences between treatments over time (F_i*[10, 150]=0.866; p=0.567*).

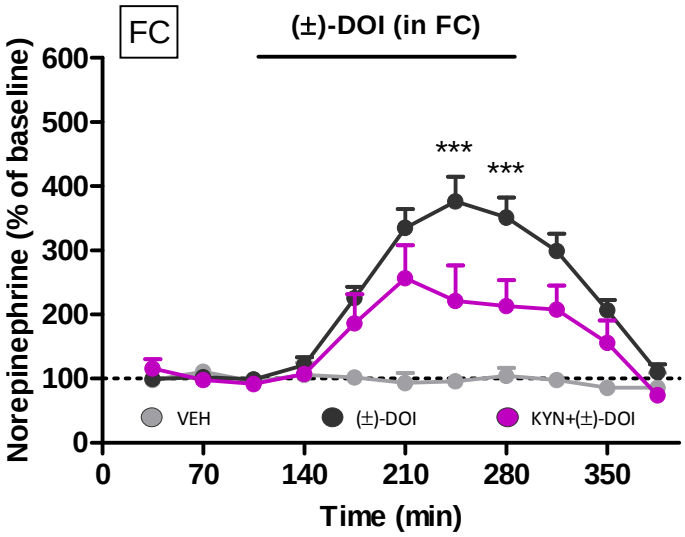


Fig. 104- Effect of the perfusion of kynurenic acid (KYN, 1 mM) into the FC on the intra-cortical (±)-DOI-induced extracellular NE variations in rat FC (● *n*=4, ● *n*=12, ● *n*=4). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The line shows the period of drug application. ****p<0.001 versus* (±)-DOI-treated group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

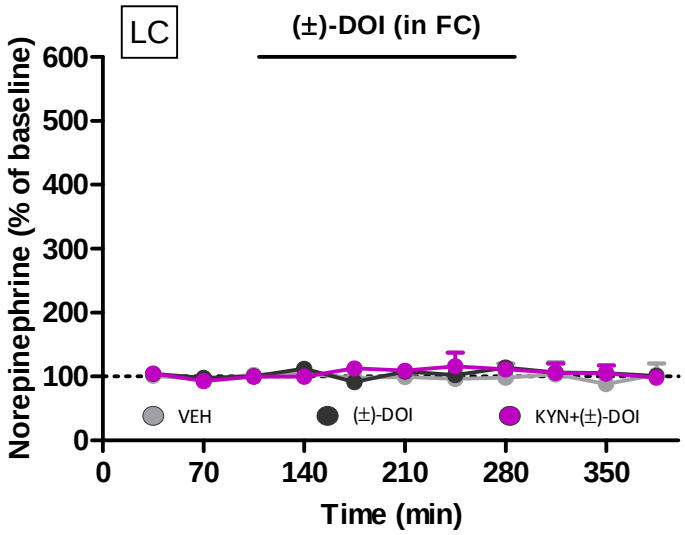


Fig. 105- Effect of the perfusion of kynurenic acid (KYN, 1 mM) into the FC on the intra-cortical (±)-DOI-induced extracellular NE variations in rat LC (● *n*=4, ● *n*=11, ● *n*=3). Data are means ± SEM of NE expressed as a percentage of basal values (100%). The line shows the period of drug application.

4.3. DETERMINATION OF THE TYPE OF G-PROTEIN SUBTYPE INVOLVED IN THE HALLUCINOGENIC-LIKE BEHAVIOR AND TEMPERATURE VARIATION INDUCED BY (±)-DOI IN MICE

4.3.1. Evaluation of the optimal dose of (±)-DOI to induce a head-twitch response in 129/Sv mice

In order to determine the efficacy of (±)-DOI (i.p.) to induce a head-twitch response, as well as to establish the optimal dose to trigger the maximal number of head-shakes, a dose-effect trial was carried out. 129/Sv mice were subjected to intraperitoneal (±)-DOI injections at doses of 0.5 mg/kg and 1 mg/kg. As illustrated in Figure 106, treatment with saline did not exert any response. In contrast, the maximal effect was achieved at a dose of 0.5 mg/kg ($p<0.001$). One-way ANOVA showed a statistically significant effect of treatment ($F_i[2, 15]=25.47; p<0.0001$).

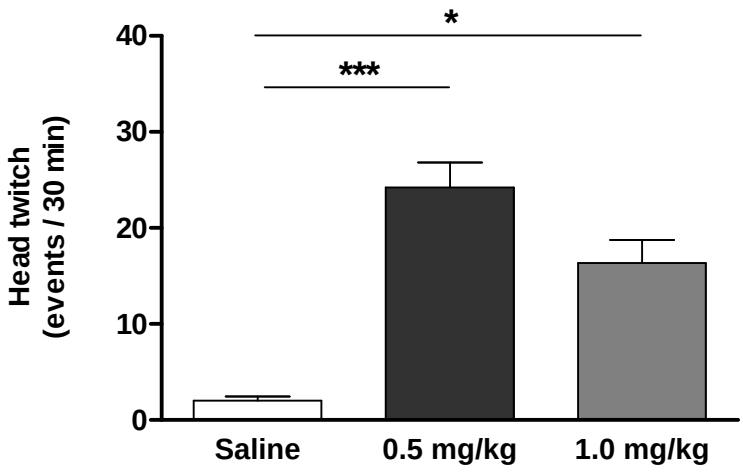


Fig. 106- Head twitch response to (±)-DOI (0.5 mg/kg or 1 mg/kg i.p.) in 129/Sv mice (○ *n*=6, ● *n*=9, ● *n*=3). Data are means ± SEM. **p<0.05, ***p<0.001 versus* saline group (one-way ANOVA followed by Bonferroni's post hoc test).

4.3.2. Role of 5-HT2A receptor in the head twitch response induced by (±)-DOI in C57B L/6 mice

To confirm that (±)-DOI elicited head twitch response by acting through 5-HT2ARs, both C57BL/6 wild type and 5-HT2AR knock-out mice were intraperitoneally single-treated with 0.5

mg/kg of (±)-DOI. As Figure 107 shows, (±)-DOI was only able to induce a significant head twitch response in wild-type mice, while this response was absent in 5-HT2AR knock-out mice (*t=11.28; p<0.0001*).

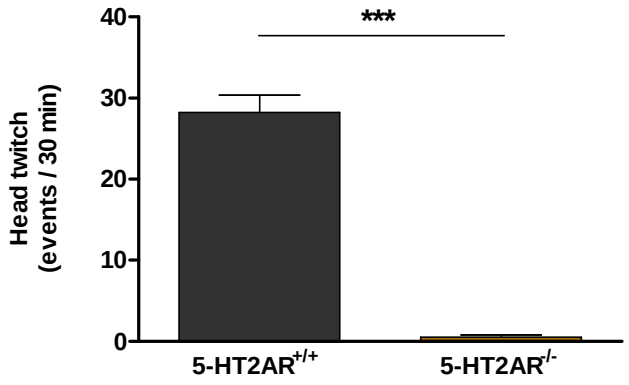


Fig. 107- Head twitch response induced by (±)-DOI (0.5 mg/kg i.p.) in 5-HT2AR$^{+/+}$ (● *n*=5) and 5-HT2AR$^{-/-}$ mice (● *n*=4). Data are means ± SEM. ****p<0.001* (Student's *t*-test).

4.3.3. Role of mGluR2 in the head twitch response induced by (±)-DOI in 129/Sv mice

As mentioned in the introduction, mGluR2 forms a functional heterocomplex with 5-HT2AR in human and mouse brain (González-Maeso *et al*., 2008). It has been demonstrated that such heterocomplex is necessary for the hallucinogenic properties derived from hallucinogenic 5-HT2AR agonist drugs. The absence of mGluR2 has been reported to abolish the hallucinogenic-like response exerted by the 5-HT2A/2C receptors agonist (±)-DOI in mice (Moreno *et al*., 2011a; Moreno *et al*., 2012).

To assess the involvement of mGluR2 in the (±)-DOI-induced hallucinogenic-like responses, HTR was scored in mGluR2$^{+/+}$ and mGluR2$^{-/-}$ mice. Mice were intraperitoneally single-treated with saline or 0.5 mg/kg of (±)-DOI. As Figure 108 shows, (±)-DOI induced a significant head twitch response in wild-type animals, whereas the absence of mGluR2 blunted that response, as seen in mGluR2$^{-/-}$ mice. Two-way ANOVA revealed significant interaction genotype x treatment (F_i*[1, 21]=28.62; p<0.0001*).

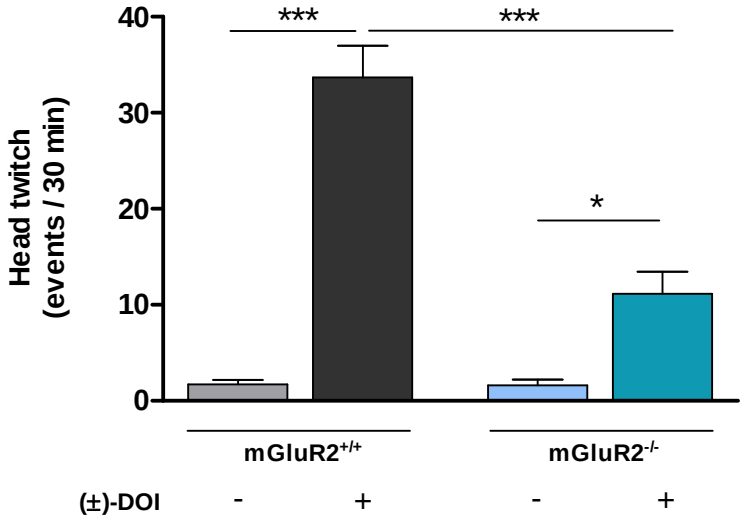


Fig. 108- Head twitch response induced by saline or (±)-DOI (0.5 mg/kg i.p.) in mGluR2$^{+/+}$ (● *n*=7, ● *n*=6) and mGluR2 $^{-/-}$ mice (● *n*=5, ● *n*=4). Data are means ± SEM. **p<0.05*, ****p<0.001* (two-way ANOVA followed by Bonferroni's post hoc test).

4.3.4. Effect of i.c.v.-administered PTX on 129/Sv mice weight variation

PTX induces several systemic effects that include a constitutive stimulation of lipid degradation in adipose tissue. This biological response is frequently used to assess that treatment with this toxin is effectively working. Therefore, after the i.c.v. administration, weight was measured daily, and its variation was calculated using the following equation:

$$\Delta\text{Weight} = \text{Weight}_{\text{day n}} - \text{Weight}_{\text{day 0}}$$

PTX pre-treated animals displayed a marked weight loss the first days after the injection of the toxin (0.4 µg), particularly at the second day after PTX administration (Figure 109). Afterwards, animals gained weight gradually, although they never recovered the initial weight. Meanwhile, animals pre-treated with vehicle did not show that drastic weight reduction and displayed a more progressive and slighter weight loss curve that was not different from baseline values (Figure 109). Two-way repeated measures ANOVA revealed a significant difference in weight loss between animals pre-treated with toxin and vehicle ($F_i[8, 184]=12.22; p<0.0001$).

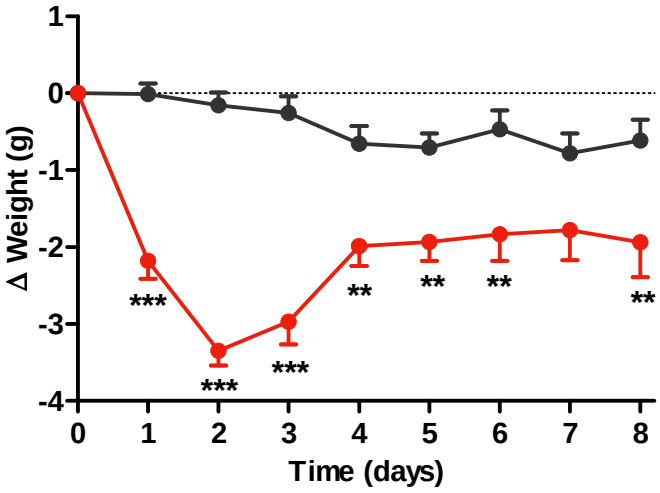


Fig. 109- Daily weight variation compared to day 0, when vehicle (● *n*=12) or PTX (● *n*=13) were intracerebroventricularly injected. ***p<0.01, ***p<0.001 versus* vehicle group (two-way repeated measures ANOVA followed by Bonferroni's post hoc test).

4.3.5. Effect of i.c.v.-administered pertussis toxin on (±)-DOI-induced head twitch response in 129/Sv mice

Based on weight loss, evaluation of HTR was decided to be performed two days after PTX administration. To evaluate whether pertussis toxin was capable of inhibiting the HTR in 129/Sv mice, animals were intracerebroventricularly injected with 0.4 µg of the neurotoxin or its vehicle. Two days after PTX administration, 0.5 mg/kg of (±)-DOI was intraperitoneally administered and the HTR was scored. As Figure 110 shows, (±)-DOI evoked a significant increase in the number of head shakes in animals pre-treated with vehicle. Lower (roughly 50% of the response displayed in vehicle-pretreated group) but still significant increase HTR was displayed in those animals pre-treated with PTX. Therefore, two-way ANOVA showed that, despite (±)-DOI somehow elicited HTR in both pre-treated groups, there was a significant inhibition of head twitch behavior in those mice which were PTX administered (F_i*[1, 20]=10.38; p<0.01*).

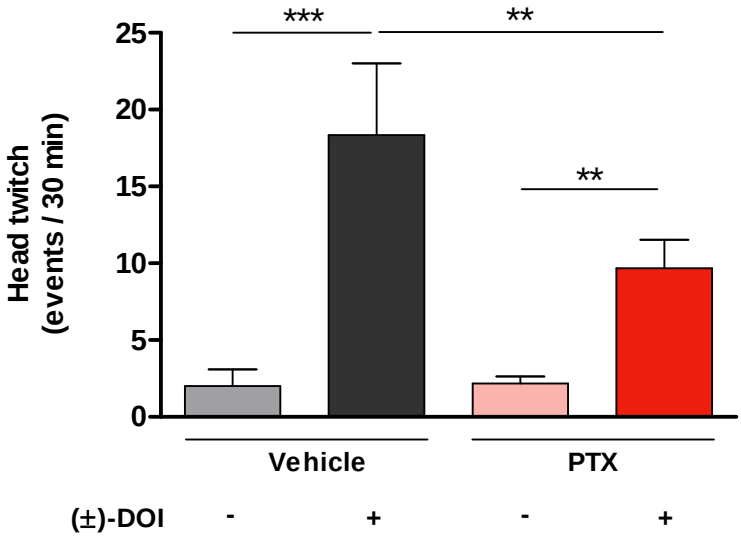


Fig. 110- Head twitch response induced by saline or (±)-DOI (0.5 mg/kg i.p.) two days after pre-treatment with vehicle (● *n*=6, ● *n*=6) or with 0.4 µg of PTX (● *n*=6, ● *n*=6). Data are means ± SEM. ***p<0.01, ***p<0.001* (two-way ANOVA followed by Bonferroni's post hoc test).

In an attempt to assess the time span of PTX inhibition over the HTR induced by (±)-DOI, a similar protocol was also followed at fourth, sixth, and eleventh day post-i.c.v. injection, with both PTX- and vehicle-pre-treated 129/Sv mice. Note that distinct animals were evaluated at these time points. Results of these experiments are represented in Figure 111.

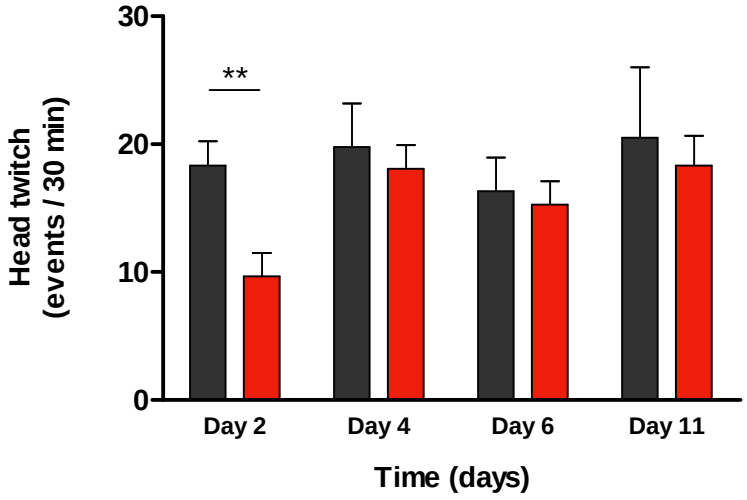


Fig. 111- Head twitch response after (±)-DOI administration (0.5 mg/kg i.p.) 2, 4, 6 or 11 days after i.c.v. pre-treatment with vehicle (●: *n*=6, *n*=10, *n*=6, *n*=2; respectively) or pertussis toxin (●: *n*=6, *n*=11, *n*=7, *n*=3; respectively). ***p<0.01* (Student's *t*-test *versus* vehicle).

4.3.6. Role of 5-HT2A receptor in the hyperthermia induced by (±)-DOI in C57BL/6 mice

Basal temperature value was 37.00 ± 0.09°C (*n*=18) in 5-HT2A$^{+/+}$ mice and 37.28 ± 0.08°C (*n*=16) in 5-HT2A$^{-/-}$ mice. Knock-out mice presented slightly higher basal body-core temperature, compared to its wild-type littermates (*t=2.350; p<0.05*).

Saline injections did not exert any change in body temperature. In contrast, 0.5 mg/kg of (±)-DOI induced a marked hyperthermia in wild-type mice (Δ_{max}=0.68 ± 0.11°C) that was not observed in 5-HT2AR$^{-/-}$ mice (Figure 112). Three-way repeated measures ANOVA revealed a statistically significant interaction between genotype x treatment x time (*F_i[6, 180]=7.78; p<0.001*) (Table 3).

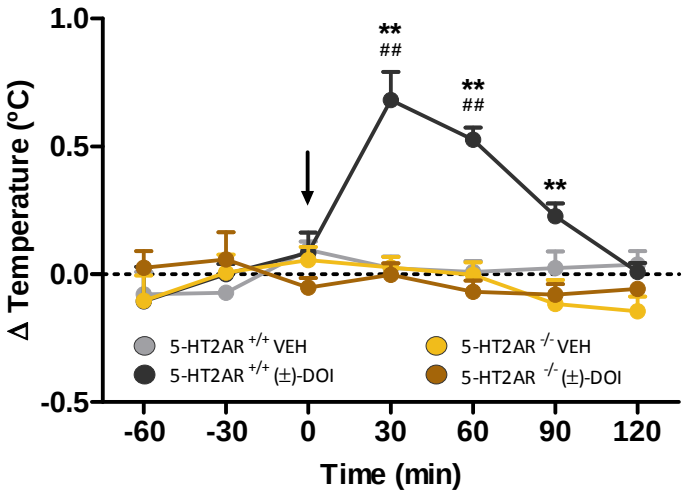


Fig. 112- Time-course for the effects of saline or (±)-DOI (0.5 mg/kg i.p.) on rectal temperature in 5-HT2AR$^{+/+}$ (● *n*=7, ● *n*=11) or 5-HT2AR$^{-/-}$ (● *n*=7, ● *n*=9) C57BL/6 mice. Values are expressed as mean temperature variation ± SEM of the mean of three basal temperatures. ***p<0.01 versus* 5-HT2AR$^{+/+}$ saline-treated group; *##p<0.01 versus* 5-HT2AR$^{-/-}$ (±)-DOI-treated group (three-way repeated measures ANOVA followed by Bonferrroni's post hoc test).

	Num. df	F-value	p-value
GENOTYPE	1	57.95	<0.0010
TREATMENT	1	26.86	<0.0010
TIME	6	12.9	<0.0010
GENOTYPE:TREATMENT	1	20.15	<0.0010
GENOTYPE:TIME	6	10.11	<0.0010
TREATMENT:TIME	6	4.54	0.0003
GENOTYPE:TREATMENT:TIME	6	7.78	<0.0010
Residuals	180		

Table 3- Statistical values derived from the three-way repeated measures ANOVA of the VEH or (±)-DOI-induced temperature variation in 5-HT2AR$^{+/+}$ and 5-HT2AR$^{-/-}$ mice.

4.3.7. Effect of i.c.v. administration of PTX on the hyperthermia induced by (±)-DOI in C57BL/6 mice

Saline injections did not alter body-core temperature. Intraperitoneal injection of the hallucinogen 5-HT2A/2C receptors agonist (±)-DOI (0.5 mg/kg i.p.) induced a substantial and similar increase in body temperature in both vehicle- or PTX-pre-treated mice (Δ_{max}=0.61 ± 0.07°C, 0.56 ± 0.06°C, respectively) ($F_{treatment}[1, 48]=35.89; p<0.001$) (Figure 113). Three-way repeated measures ANOVA showed non-significant differences between PTX or VEH pre-treatment, (±)-DOI or saline treatment, and time ($F_i[6, 288]=0.34; p=0.914$) (Table 4).

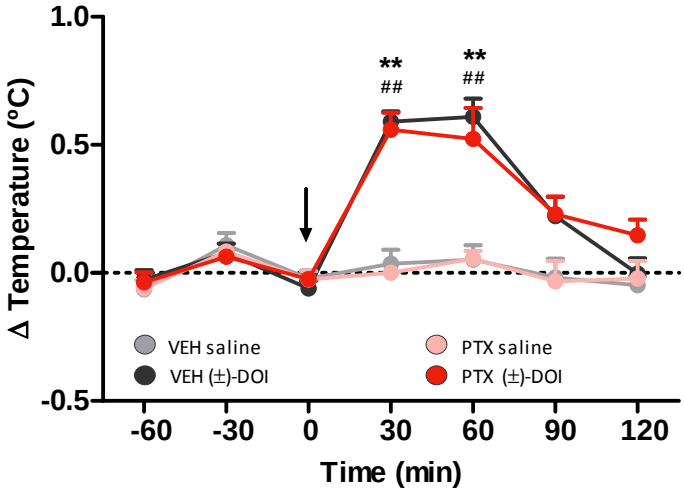


Fig. 113- Time-course for the effects of saline or (±)-DOI (0.5 mg/kg i.p.) on rectal temperature in vehicle-pre-treated (● *n*=10, ● *n*=16) or PTX-pre-treated (● *n*=9, ● *n*=17) C57BL/6 mice. Values are expressed as mean temperature variation ± SEM of the mean of three basal temperatures. ***p<0.01* vehicle-pre-treated (±)-DOI *versus* saline; *##p<0.01* PTX-pre-treated (±)-DOI *versus* saline (three-way repeated measures ANOVA followed by Bonferrroni's post hoc test).

	Num. df	F-value	p-value
GENOTYPE	1	0.10	0.7592
TREATMENT	1	35.89	<0.0010
TIME	6	45.61	<0.0010
GENOTYPE:TREATMENT	1	0.04	0.8439
GENOTYPE:TIME	6	0.81	0.5667
TREATMENT:TIME	6	20.42	<0.0010
GENOTYPE:TREATMENT:TIME	6	0.34	0.9140
Residuals	288		

Table 4- Statistical values derived from the three-way repeated measures ANOVA of the VEH or (±)-DOI-induced temperature variation in vehicle-pretreated and PTX-pretreated mice.

4.3.8. Implication of the mGluR2/5-HT2AR heterocomplex on (±)-DOI-induced hyperthermia

mGluR2 is necessary for the behavioral effects induced by hallucinogenic 5-HT2A receptor agonists, accordingly to the activation of G_i subtype proteins (Moreno *et al.*, 2011a). Data from 4.3.7. section, seem to suggest that (±)-DOI-induced hyperthermia is not elicited through G_i proteins. In order to double check whether G_i proteins were involved in this response, wild-type 129/Sv and mGluR2 knock-out mice were injected (±)-DOI (0.5 mg/kg i.p.) and rectal temperature was monitored.

No differences in basal body temperature were found between genotypes (37.80 ± 0.22°C in mGluR2$^{+/+}$ mice, 37.66 ± 0.09°C in mGluR2$^{-/-}$ mice; *t=0.562; p=0.58*). Intraperitoneal injection of (±)-DOI induced marked hyperthermia in both mGluR2$^{+/+}$ (Δ_{max}=0.850 ± 0.32°C) and mGluR2$^{-/-}$ mice (Δ_{max}=0.856 ± 0.15°C) ($F_{treatment}[1, 147]=21.83$; $p<0.0001$) (Figure 114). Three-way repeated measures ANOVA revealed no statistically significant differences between genotype x treatment x time ($F_i[6, 147]=0.09$; $p=0.997$) (Table 5).

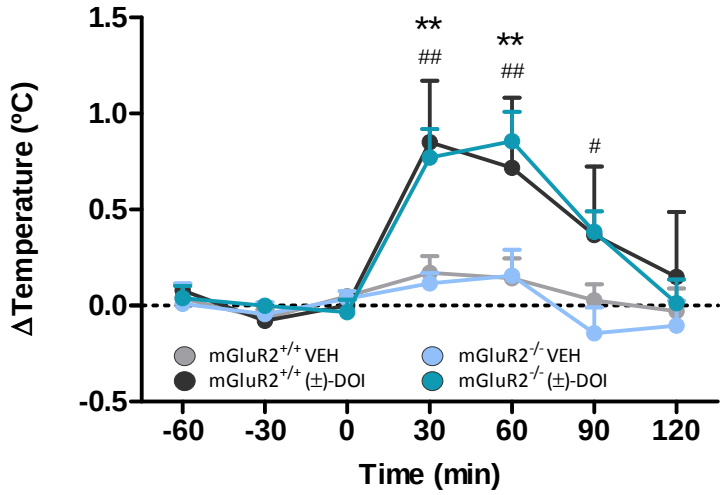
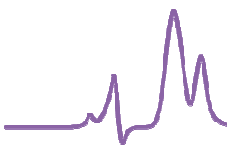


Fig. 114- Time-course for the effects of saline or (±)-DOI (0.5 mg/kg i.p.) on rectal temperature in mGluR2$^{+/+}$ (● *n*=7, ● *n*=6) or mGluR2$^{-/-}$ (● *n*=5, ● *n*=7) 129/Sv mice. Values are expressed as mean temperature variation ± SEM of the mean of three basal temperatures. ***p<0.01* mGluR2$^{+/+}$ (±)-DOI *versus* saline; *##p<0.01* mGluR2$^{-/-}$ (±)-DOI *versus* saline (three-way repeated measures ANOVA followed by Bonferrroni's post hoc test).

	Num. df	F-value	p-value
GENOTYPE	1	0.18	0.6684
TREATMENT	1	21.83	<0.0010
TIME	6	9.29	<0.0010
GENOTYPE:TREATMENT	1	0.11	0.7444
GENOTYPE:TIME	6	0.20	0.9753
TREATMENT:TIME	6	4.06	0.0008
GENOTYPE:TREATMENT:TIME	6	0.09	0.9970
Residuals	147		

Table 5- Statistical values derived from the three-way repeated measures ANOVA of the VEH or (±)-DOI-induced temperature variation in mGluR2$^{+/+}$ and mGluR2$^{-/-}$ mice.



5. DISCUSSION

Despite much research has been undertaken to develop new antipsychotic drugs for treating effectively the intrinsic positive, negative and cognitive impairments characteristic of schizophrenia, none of the medications currently available on the market has stood out for its long-lasting marked efficacy without detrimental side effects. This is partially due to the poorly understood neurobiology of schizophrenia.

Animal models have become important tools for investigating the aetiology and pathophysiology of schizophrenia, and enable progress towards the development of effective therapeutic targets for its treatment. The main obstacle in modeling psychiatric disorders has been the limited information about their origin and underlying neural mechanisms. Recently, due to the rapid growing knowledge about the neurobiology and genetics of these disorders, animal models of these diseases are gaining popularity in psychiatric research. New models of schizophrenia mimic biological phenomena associated with the clinical condition, particularly developmental changes in the cortex, abnormalities of catecholamines neurotransmission, and genetic characteristics of selected behavioral traits. The FC plays a crucial role in working memory, attention, decision making, sensorimotor gating, and impulse control, all of them features impaired in schizophrenia. Those higher-order brain functions performed by the FC are supposed to rely on rhythmic electrical activity generated by neural network oscillations determined by an excitation and inhibition balance resulting from the recurrent excitation, and feedback and feedforward inhibition. The altered cortical functions seen in schizophrenics are in part due to neurotransmission pathways disruption. Among the neurotransmitter pathways, monoamine systems represent precise modulators of cortical activity, and dysfunctions of these systems have been associated with the pathophysiology of schizophrenia. DA, NE and 5-HT release in the FC are dependent on firing activity of brainstem nuclei but also are under local control of axon terminal activities. Previous literature suggests that 5-HT2ARs are able to modulate monoamine release in FC (Gobert & Millan, 1999; Pehek *et al*., 2001; Bortolozzi *et al*., 2003; Bortolozzi *et al*., 2005; Pehek *et al*., 2006). This fact makes the use of changes in extracellular monoamine concentrations after 5-HT2AR stimulation or inhibition as a functional indicator of this receptor activity.

5-HT2ARs are key players in hallucinations and represent the main target of atypical antipsychotic drugs. On the other hand, activation of mGluR2 evokes antipsychotic-related phenotypes in preclinical models of schizophrenia (Moghaddam & Adams, 1998; Patil *et al*., 2007; Lavreysen *et al*., 2015). It has been convincingly demonstrated that 5-HT2AR is

assembled with mGluR2 in humans and rodents FC, forming a heteromer. Neuroanatomical, biophysical, genetic, epigenetic and cellular trafficking assays have demonstrated that both receptors co-localize and crosstalk in layer V mouse cortical pyramidal neurons, specifically at a postsynaptic level (González-Maeso *et al*., 2008; Fribourg *et al*., 2011; Kurita *et al*., 2012; Moreno *et al*., 2012; Moreno *et al*., 2016). Cellular trafficking assays have confirmed the crosstalk between both receptors, and the activation of 5-HT2AR signaling pathways distinct from those induced by the monomeric structure, potentially implicated in hallucinogenic effects (González-Maeso *et al*., 2008; Fribourg et al., 2011; Moreno *et al*., 2011a; Moreno *et al*., 2016). Moreover, behavioral studies also confirm that the presence of mGluR2 in the FC and the subsequent crosstalk with 5-HT2AR seems to be necessary for the hallucinogenic responses evoked by psychedelic 5-HT2AR agonist drugs in mice (Moreno *et al*., 2011a; Moreno *et al*., 2012).

Although such crosstalk has been extensively studied over the past few years from different biological levels, the existence of *in vivo* functional information about the effect of this heterocomplex on cortical catecholamine release regulation by the heterocomplex is still unknown. Therefore, the hallucinogenic 5-HT2AR agonist drug (±)-DOI was administered to rodents in order to elicit hallucinogenic states that resemble symptoms seen in schizophrenia, as well as to gain insight into the neurochemical effects triggered by this drug in the FC and LC. The involvement of the functional role of 5-HT2AR/mGluR2 heterocomplex in such responses was further evaluated using mGluR2- and 5-HT2AR-null mice. DA and NE release was quantified in the FC, which have been reported to be altered in schizophrenia and hallucinations. The NE pathways underlying (±)-DOI responses were further studied.

Within this context, the results compiled in the present work could provide new insights for the better understanding of pharmacological mechanisms, neurotransmission alterations, as well as circuits involved in hallucinations, and point to new targets for future antipsychotic treatments.

5.1. EFFECT OF THE CORTICAL ADMINISTRATION OF (±)-DOI ON THE CATECHOLAMINE RELEASE IN THE FRONTAL CORTEX

5.1.1. Cortical (±)-DOI-induced DA release is mediated by 5-HT2ARs activation

The present work found that intra-cortical infusion of the hallucinogenic 5-HT2A/2C receptors agonist (±)-DOI increased DA output in the FC (Figure 38 and 39, grayscale lines and bars), in good agreement with previous reports (Pehek *et al*., 2001; Bortolozzi *et al*., 2005). (±)-DOI acts preferentially as an agonist of 5-HT2AR, but also displays close affinity to 5-HT2CR (May *et al*., 2003; Pigott *et al*., 2012). The (±)-DOI-induced DA output enhancement was apparently, at least in part, evoked via an activation of 5-HT2ARs in cortical areas, inasmuch as the co-perfusion with the selective 5-HT2AR antagonist M100907 throughout the entire experiment was able to significantly attenuate such increase (Figure 43 and 45). However, the response was not fully abolished, but a remaining effect was still visible. To further ascertain the involvement of 5-HT2ARs in the (±)-DOI-induced DA release, the same procedure was repeated in 5-HT2AR$^{-/-}$ mice and their corresponding wild-type control littermates. 5-HT2AR$^{+/+}$ animals displayed a substantially higher stimulatory effect, when compared to 5-HT2AR$^{-/-}$ mice (Figure 65 and 66), suggesting that the majority of the stimulation elicited by (±)-DOI is mediated by 5-HT2AR activation.

It should be noted that in the latter two cases - wild-type animals in continuous presence of M100907, and 5-HT2AR$^{-/-}$ mice -, there was some remaining dopaminergic stimulatory effect, presumably 5-HT2AR-independent. It is unlikely that this remaining effect is a result of a hyperactivation of 5-HT2CRs in animals lacking 5-HT2ARs, since there are not altered compensatory changes in the 5-HT2CR mRNA or protein expression (Popa *et al*., 2005; Bortolozzi *et al*., 2010). The concentration of (±)-DOI used in the present work was chosen on the basis of a dose-effect study carried out by Bortolozzi and co-workers (2003), where the optimal concentration of (±)-DOI to elicit the greatest release of 5-HT in the FC was 300 µM. They also reported that co-administration with 300 µM M100907 fully blocked such stimulation. However, the assumption of an overestimated concentration of (±)-DOI used in this study, lacking to some extent selectivity for 5-HT2ARs, and therefore responsible for the remaining effect, cannot be ruled out. Indeed, a recent report has shown that (±)-DOI displays some affinity for certain additional receptors and transporters (Ray, 2010), which could be responsible for such remaining effect.

It has been described that the expression and activation of 5-HT2AR in the cortex is enough to induce hallucinogenic-specific behavioral and signaling effects (Willins & Meltzer, 1997; González-Maeso *et al*., 2007). As pointed out in the introduction, the two major cell types in the FC are glutamate-containing pyramidal neurons and GABAergic interneurons. Pyramidal neurons represent approximately 70-80% of the neurons in the cortex, while the remaining 20-30% are mostly inhibitory interneurons (Markram *et al*., 2004; Espuny-Camacho *et al*., 2013). 5-HT2AR and 5-HT2CR can downstream stimulate PLC, via $G_{q/11}$ subtype protein activation (Garcia *et al*., 2007). Nonetheless, both receptors display opposite behavioral and neurochemical effects within the medial FC (Alex & Pehek, 2007). In the FC, 5-HT2ARs are primarily located on apical dendrites of glutamate-containing pyramidal neurons (López-Giménez *et al*., 1997; Fiorica-Howells *et al*., 2002; Miner *et al*., 2003; Alex & Pehek, 2007), but can also be found on GABAergic interneurons, as well as the closely related 5-HT2CR (Willins *et al*., 1997; Jakab & Goldman-Rakic, 2000; Carr *et al*., 2002; Santana *et al*., 2004; Alex & Pehek, 2007; Liu *et al*., 2007; Puig & Gulledge, 2011; Santana & Artigas, 2016). A small percentage of 5-HT2CRs also co-localize with 5-HT2AR on pyramidal neurons (Nocjar *et al*., 2015). Thus, the activation of cortical 5-HT2AR/2C receptors leads to the pyramidal and GABAergic neurons excitation and *c-fos* expression in the FC (Zhai *et al*., 2003; Zhang *et al*., 2010), and subsequently increases glutamate and GABA output (Araneda & Andrade, 1991; Aghajanian & Marek, 1997; Abi-Saab *et al*., 1999; Aghajanian & Marek, 2000; Scruggs *et al*., 2003). Glutamatergic neurons can modulate DA release (Lodge, 2011) likely through serotonergic regulation (Puig & Gulledge, 2011; Meunier *et al*., 2017). GABA interneurons may regulate prefrontal DA release by the interaction with $GABA_A$ and $GABA_B$ receptors (Santiago *et al*., 1993). It is unclear if the effect of (±)-DOI on GABAergic interneurons is direct or mediated by activation of pyramidal cell collaterals. Electrophysiological studies in the neocortex suggest that the major effect of serotonin is on pyramidal cells, via 5-HT2AR agonism (Aghajanian & Marek, 1997). However, in the piriform cortex, (±)-DOI directly depolarizes interneurons predominantly via 5-HT2AR (Marek & Aghajanian 1994).

Taken together, as the FC is a region denser populated by excitatory pyramidal neurons than inhibitory GABAergic interneurons (Markram *et al*., 2004; Espuny-Camacho *et al*., 2013), and 5-HT2ARs are present in both types of neurons but mostly on the former ones (Nocjar *et al*., 2015), it is plausible that the overall effect evoked by (±)-DOI in the FC is an enhancement of DA release mediated by the activation of 5-HT2 receptors expressed on pyramidal neurons.

On the other hand, the merely intra-cortical perfusion of 300 µM M100907 dramatically decreased extracellular DA concentrations up to 65% (Figure 42, grayscale lines), reflecting a presumably tonic stimulatory 5-HT2AR-dependent effect of 5-HT on pyramidal neurons (Pehek *et al*., 2001).

5.1.1.1. DA basal dialysate concentrations

As described in the previous paragraph, DA baseline concentrations were reduced up to 57% with the cortical perfusion of M100907 in wild-type animals. Accordingly, 5-HT2AR$^{-/-}$ animals showed lower DA basal values than their wild-type counterparts. Previous research has also found lower baseline DA concentrations in 5-HT2AR$^{-/-}$ mice compared to their wild-type counterparts, although the difference did not reach significance (Bortolozzi *et al*., 2010).

Since the administration of 5-HT2AR agonists enhances DA release in the FC, it would be conceivable that its blockade triggers the decrease of such release. These observations could indicate that 5-HT2AR may tonically activate DA release in the FC or VTA.

5.1.1.2. Assessing the possible pathways involved in the (±)-DOI-induced effects

The (±)-DOI-induced DA release in the FC after cortical perfusion may be explained by the activation of different long-loop or direct pathways:

Indirect FC → VTA → FC loop: The activation of cortical 5-HT2ARs triggers the excitation of pyramidal cells and GABAergic interneurons in the FC (Araneda & Andrade, 1991; Aghajanian & Marek, 1997). Moreover, the activation of 5-HT2ARs on pyramidal neurons increases the firing rate of descending fibers projecting to the VTA, that may result in enhanced glutamate efflux in the latter area, which subsequently activates glutamate receptors on VTA DA mesocortical neurons projecting back to the FC (Pehek & Hernan, 2015), increases DA neural activity and induces DA release in VTA and FC (Murase *et al*., 1993; Overton & Clark, 1997; Bortolozzi *et al*., 2005; Alex & Pehek, 2007). In support of this interpretation, the blockade of NMDA and AMPA receptors in the VTA blocks the (±)-DOI-induced DA efflux in the FC (Alex & Pehek, 2007). Electrical stimulation mimics such increase of DA release in FC, whilst the 5-HT2AR antagonist M100907 blocks these stimulatory effects (Bortolozzi *et al*., 2005). These findings reinforce the

existence of anatomical connections between the FC and VTA (Carr & Sesack, 2000; Sesack *et al*., 2003) that can be excited after the activation of 5-HT2ARs on cortical pyramidal cells (Puig *et al*., 2003), as well as the presence of DA fibers from the VTA projecting back to the FC (Sesack *et al*., 2003).

Indirect FC → PPT → VTA → FC loop: Glutamate innervations of the VTA derive from two major inputs, the FC and the laterodorsal/pedunculopontine tegmentum (LDT/PPT). Both sources activate bursts in DA cells of the SN and VTA. The SN receives an additional glutamate input from the subthalamic nucleus, but whether there is an excitatory source for VTA is so far unknown (Sesack *et al*., 2003). Hence, more complex indirect pathways to explain the raise in cortical DA output cannot be excluded; for instance: FC → PPT → VTA → FC, or FC → NAcc → ventral pallidum → VTA → FC (Bortolozzi *et al*., 2005). Both pathways could be involved inasmuch as they have been proven to modulate DA neuron activity, via phasic and tonic inputs, respectively (Floresco *et al*., 2003).

Direct FC pathway: Another plausible explanation restricted to a merely local stimulation would involve the presence of presynaptic 5-HT2ARs on cortical dopaminergic axon terminals, which would facilitate the release of DA from the storage vesicles. Miner and co-workers (2003) showed the presence of a small percentage of 5-HT2ARs on axon terminals of cortical monoaminergic inputs, and those appear not to be neither serotonergic nor noradrenergic, positing dopaminergic fibers as the main candidates. Furthermore, moderate mRNA expression for the 5-HT2AR has been shown in dopaminergic-rich areas of the rat midbrain (Pompeiano *et al*., 1994) including cells projecting to the FC (Berger *et al*., 1976).

Alternatively, regulation by iGluRs in FC may be implicated, extending the outcomes observed in striatum. Presynaptic iGluRs are present on striatal dopaminergic nerve terminals. Activation of NMDA, kainate and AMPA receptors in synaptosomes by NMDA or non-NMDA agonists, evoke an increased release of DA. The endogenous transmitter glutamate could activate both NMDA and non-NMDA receptors, because its effect is partially blocked by both the NMDA and the AMPA/kainate receptors antagonists MK-801 or CNQX, respectively. NMDA or kainate receptor activation could increase Ca^{2+} influx and lead to transmitter release (Wang, 1991).

5.1.2. Cortical (±)-DOI-induced NE release is mediated by 5-HT2ARs activation

Intra-cortical perfusion of (±)-DOI enhanced local NE release likely through a 5-HT2AR-dependent mechanism (Figure 40 and 41, grayscale lines), since such effect was completely blunted in the local presence of M100907 (Figure 47 and 49). Supporting evidence was found when cortical perfusion of (±)-DOI in 5-HT2AR$^{-/-}$ mice failed to modulate NE output, while in wild-type animals a substantial NE release was yielded (Figure 52 and 53).

Once again, intra-cortical perfusion of M100907 (300 µM) led to a slight inhibition of NE release (E_{max}=-13 %) in the FC (Figure 46, grayscale), potentially reflecting a tonic stimulatory effect of 5-HT, mediated by 5-HT2ARs on pyramidal neurons.

5.1.2.1. NE basal dialysate concentrations

In the same way as DA, the presence of M100907 (300 µM) slightly decreased extracellular basal NE concentrations, approximately 11% from baseline. On the other hand, NE baseline concentrations in 5-HT2AR$^{-/-}$ animals did not differ from those seen in 5-HT2AR$^{+/+}$ control mice. That absence of changes might suggest that circuits regulating NE and DA release in the FC are distinct between them in response to M100907 administration.

5.1.2.2. Assessing the possible pathways involved in the (±)-DOI-induced effects

As there is little (if any) research into the cortical 5-HT2AR-induced NE output after (±)-DOI perfusion into the FC, we sought to investigate the noradrenergic pathways that could underlie such effects. The LC contains the majority of NE neurons in the brain. These neurons possess ramified axons that project to a broad array of areas, including the FC (Foote *et al*., 1983). In a bidirectional way, cortical neurons provide inputs toward the peri-LC that can modulate LC neurons activity (Cedarbaum & Aghajanian, 1978; Luppi *et al*., 1995). It was of great interest to gain insight into the potential implication of both areas - FC and LC - to explain the cortical (±)-DOI-induced NE release.

Indirect FC → LC → FC loop: In order to assess the existence of an indirect loop (FC → LC → FC) responsible for the increase of extracellular NE release in the FC after the intra-cortical administration of (±)-DOI, dual-probe microdialysis experiments were performed. One of the

limitations of the animal models in which the previous findings were discovered is that mouse brain is too tiny to accomplish dual-probe microdialysis assays. Thus, the use of rats for conducting the following experiments was required. Probes were inserted into the FC and LC, and extracellular NE concentrations were simultaneously measured in both areas.

There is some controversy regarding the influence of FC activation on the activity of NE LC neurons. While Sara & Hervé-Minvielle (1995) support an inhibitory effect of cortical neurons, other researchers stand up for an excitatory influence (Jodo *et al*., 1998). Both assays were performed in anesthetized rats.

The following goal of the present work was to evaluate the implication of excitatory inputs, involving EAA in the LC, in the (±)-DOI-induced increase of NE release in the FC. The potential glutamatergic influence in the LC was pharmacologically studied by administration of the non-selective iGluRs antagonist drug KYN (1 mM). NMDA and non-NMDA (AMPA/kainate) excitatory amino acid receptors are localized on somatodendritic sites of LC NE neurons (Olpe *et al*., 1989; Wisden & Seeburg, 1993; Luque *et al*., 1995; Van Gaalen *et al*., 1997; Kawahara *et al*., 1999; Samuels & Szabadi, 2008). Simultaneous perfusion of KYN into the LC and (±)-DOI into the FC enhanced somewhat the (±)-DOI-induced NE release in the FC (Figure 102). Similar or even higher effects compared to the sole administration of (±)-DOI were found in the FC (Figure 102), without altering NE concentrations in the LC (Figure 103).

KYN administration (1 mM) into the LC evoked a non-significant reduction of NE basal concentrations in the FC (38%), and a dramatic reduction in the LC (92%). These results might dismiss the assumption of a loop involving cortical glutamatergic pyramidal neurons activation projecting to the LC that via iGluRs activates noradrenergic projections toward the FC. On the other hand, these data suggest that the LC somatodendritic release as well as LC neural firing is under control of EAA, although this excitatory input does not seem to be regulated by cortical 5-HT2AR.

Direct cortical pathway: The feasibility of a direct cortical effect mediating the (±)-DOI-induced NE release in the FC is conceivable, inasmuch as subcortical pathways seem not to be involved in that effect, regarding the findings explained above. In fact, co-perfusion of KYN and (±)-DOI both into the FC markedly attenuated the intra-cortical (±)-DOI-induced NE release in the FC (Figure 104) without affecting NE levels in the LC (Figure 105). iGluRs have been reported to be

presynaptically located on NE nerve terminals in cortical areas and hippocampus (Pittaluga *et al*., 2005; Luccini *et al*., 2007; Nomura *et al*., 2014). Most NMDAR functions have been ascribed to postsynaptic sites of action. Moreover, it is currently known that NMDARs can be presynaptically located and modulate neurotransmitter release in many brain regions, including the neocortex (Kunz *et al*., 2013), albeit the molecular mechanisms by which these receptors can affect such release are not well-established. Stimulatory presynaptic NMDA and AMPA receptors on noradrenergic axon terminals in the spinal cord have also been found to evoke NE release (Sundström *et al*., 1998). It is probable that the activation of 5-HT2ARs on cortical layer V pyramidal neurons could lead to an enhancement of extracellular glutamate concentrations which act at iGluRs on pyramidal and non-pyramidal neurons (Martin *et al*., 1993), including NE axons in the FC, which eventually could facilitate the release of NE from storage vesicles. Therefore, the (±)-DOI-induced NE release could be restricted merely and primarily to local cortical pathways and mediated via 5-HT2ARs activation through the action of NMDA/AMPA/kainate receptors (Figure 115). In good agreement with this scenario, Fink and co-workers (1990) demonstrated that presynaptic NMDA receptors are able to facilitate the NE release in slices of rat cortex and synaptosomes preparations from hippocampus, olfactory bulb and cerebral cortex (Wang *et al*., 1992).

The local presence of KYN in the FC did not significantly modify NE basal concentrations. This suggests that the tonic control exerted by iGluRs over NE only happened after stimulation of glutamate-containing pyramidal neurons. This assumption would be in good agreement with the low inhibitory effect of M100907 on NE release in the FC (Figure 46).

Finally, it is unlikely that 5-HT2ARs presynaptically located on noradrenergic terminals in the FC mediate the observed NE release, since the LC is devoid of 5-HT2AR mRNA expression (Pompeiano *et al*., 1994; López-Giménez *et al*., 2001a; Miner *et al*., 2003). However, the existence of presynaptic 5-HT2CRs on NE axon terminals cannot be discarded, since LC express mRNA for 5-HT2CR (Pompeiano *et al*., 1994) and could travel along the axon up to terminal areas.

5.1.3. Influence of the presence of mGluR2 on the cortical (±)-DOI-induced catecholamine release. Does a functional neurochemical crosstalk between 5-HT2AR/mGluR2 in the FC exist?

The existence of a crosstalk between 5-HT2AR and mGluR2 has been broadly discussed throughout the present work (see introduction). Compelling evidence at multiple neurobiological levels has demonstrated the existence of a heteromer formation between the $G_{q/11}$-coupled 5-HT2AR and the $G_{i/o}$-coupled mGluR2 in cortical neurons of human and mouse brain (González-Maeso *et al*., 2008):

- Biophysical approaches such as BRET, microscope-based FRET, fluorescent *in situ* hybridization (FISH) or immunohistochemistry assays, among others, have determined that 5-HT2AR and mGluR2 co-localize in layer V mouse cortical pyramidal neurons (González-Maeso *et al*., 2008) and suggest the existence of a crosstalk mechanism between these two receptors that requires their physical association. Electron microscopy approaches have enabled to determine the subcellular proximity of these receptors, particularly at or near synaptic junctions (Moreno *et al*., 2012). Three residues at transmembrane domain 4 of mGluR2 were identified as necessary for the heteromerization (Moreno *et al*., 2012). Recent studies suggest that 5-HT2AR and mGluR2 could form a part of a protein complex at the postsynaptic density in mouse FC (Moreno *et al*., 2016; Gaitonde & González-Maeso, 2017).

- Biochemical studies have revealed that the $G_{q/11}$-coupled 5-HT2AR and the $G_{i/o}$-coupled mGluR2 form a heterocomplex through which ligands that bind to the 5-HT2AR modulate $G_{i/o}$-dependent signaling, whereas ligands that bind to the mGluR2 modulate $G_{q/11}$-dependent signaling (González-Maeso *et al*., 2008; Fribourg *et al*., 2011).

Under basal conditions, hallucinogenic LSD-like 5-HT2AR agonists such as (±)-DOI are able to activate $G_{q/11}$ and $G_{i/o}$ proteins through the 5-HT2AR/mGluR2 heteromer (González-Maeso *et al*., 2007; Fribourg *et al*., 2011). Thus, the presence of mGluR2 interacting with 5-HT2AR confers to the latter receptor the ability to signal via $G_{i/o}$ proteins and not only through the canonical $G_{q/11}$ proteins as a result of the 5-HT2AR activation by hallucinogenic, but not by non-hallucinogenic, 5-HT2AR agonist drugs. In fact, [^{35}S]GTPγS SPA binding assays revealed that mGluR2 is necessary and regulate (±)-DOI 5-HT2AR-mediated signaling through $G\alpha_{i1}$, $G\alpha_{i3}$, and $G_{\alpha z}$ subunits (Miranda-Azpiazu, Doctoral Thesis 2014). These findings spawn the necessity of

mGluR2 so that 5-HT2AR can mediate hallucinogenic effects via $G_{i/o}$ proteins. Supporting evidence demonstrates that *postmortem* FC of schizophrenic patients has alterations in the allosteric crosstalk between 5-HT2AR and mGluR2 as a receptor heterocomplex (Moreno *et al*., 2012).

As previously mentioned, the assembly of mGluR2 and 5-HT2AR into a functional heterocomplex allows hallucinogenic drugs to cross-signal to G_i-dependent proteins (González-Maeso *et al*., 2008). A meticulous study reported that the heterocomplex functions to establish a G_i-G_q balance for the response to glutamate and 5-HT, the endogenous ligands. Psychedelic drugs, acting through the heterocomplex, decreases G_i-dependent signaling while increases G_q-dependent signaling, tipping the G_i-$G_q$balance in favor of G_q signaling (propsychotic) (Fribourg *et al*., 2011).

- Genetic and epigenetic studies also reveal the crosstalk between 5-HT2AR and mGluR2. Schizophrenia patients who are homozygous (T/T) at single nucleotide polymorphism rs7330461 in the *Htr2a* gene showed better treatment response to pomaglumetad (LY2140023, prodrug of the mGluR2/3 agonist LY404039) compared to A/A homozygous patients (Liu *et al*., 2012; Nisenbaum *et al*., 2016). Among the epigenetic changes, it has been observed that chronic treatment with atypical antipsychotics induces repressive covalent histone modifications at the promoter region of *Grm2* gene in mice FC (Kurita *et al*., 2012). These epigenetic changes induced at the *Grm2* promoter requires 5-HT2AR signaling, as they were absent in 5-HT2AR knock-out mice (Kurita *et al*., 2012).

- Behavioral studies have also revealed that the crosstalk between 5-HT2AR and mGluR2 in mice FC is necessary to display behavioral effects derived from hallucinogenic 5-HT2AR agonist drugs. mGluR2 knock-out mice do not exhibit HTR after systemic administration of (±)-DOI. In the same way, the virus-mediated overexpression of mGluR2 in the FC of mGluR2 knock-out mice rescues the head twitch behavioral response induced by (±)-DOI, whereas overexpression of mGluR2 with the three residues necessary for the assembly modified with the corresponding residues of mGluR3 does not (Moreno *et al*., 2012). Therefore, the absence of the three amino acidic residues at TM4 of mGluR2, which leads to the disruption of a 5-HT2AR/mGluR2 complex formation, abolish the (±)-DOI-induced hallucinogenic-likes states in rodents.

Much research has been reported about this heterocomplex, but its influence in cortical monoamine release at basal conditions and after administration of 5-HT2AR agonist psychedelic drugs was still a mystery. In order to address that issue, intra-cortical perfusion of the 5-HT2A/2C receptor agonist (±)-DOI was administered to animals lacking mGluR2 and the wild-type controls.

5.1.3.1. Tackling the involvement of the heteromer in dopaminergic (±)-DOI-induced response

There was no difference in DA basal dialysate concentrations between mGluR2$^{-/-}$ and mGluR2$^{+/+}$ animals. This finding might reflect that DA baseline levels are not under mGluR2 regulation.

mGluR2$^{-/-}$ mice displayed lower (±)-DOI-induced DA release in the FC than mGluR2$^{+/+}$ animals (Figure 38 and 39). The small stimulatory effect in mGluR2$^{-/-}$ mice was insensitive to co-perfusion with M100907 (Figure 44 and 45), suggesting that most of the (±)-DOI-induced enhancement of DA output is mediated by 5-HT2ARs, and the absence of mGluR2s hampers the DA release stimulation. Possibly, the minor M100907-insensitive remaining effect could be yielded by the action of other undetermined receptors or transporters which does not interact with mGluR2. In support of this, 5-HT2AR$^{-/-}$ mice also display a remaining effect after (±)-DOI cortical perfusion.

Increasing evidence over the last years has proven that the heterocomplex formation is made up of 5-HT2AR and mGluR2 but not the mGluR3 (González-Maeso *et al*., 2008; Moreno *et al*., 2012). In order to further elucidate the potential implication of the mGluR2 receptor and not the mGluR3, the same procedure was undergone in animals lacking mGluR3. As expected, the absence of the mGluR3 receptor did not modify the (±)-DOI-induced DA increase (Figure 54 and 55). The present work shows that the absence of mGluR2 hampers to some extent the stimulatory effects on catecholamine release induced by (±)-DOI.

Previous data from the literature mention that cortical co-perfusion of (±)-DOI with the mGluR2/3 agonist 1S,3S-ACPD was able to attenuate the 5-HT release in the rat FC mediated by (±)-DOI (Bortolozzi *et al*., 2003). In line with this finding, (±)-DOI produces cortical *c-fos* expression in a number of cortical regions, including the FC (Scruggs *et al*., 2000). c-Fos is considered a marker of neuronal activity in the brain. The mGluR2/3 agonist LY379268 is able

to attenuate the (±)-DOI-induced increase in *c-fos* mRNA and that suppression is blocked by the mGluR2/3 antagonist LY341495 (Zhai *et al*., 2003).

Within this context, an apparent paradoxical situation should be noted, since animals lacking mGluR2 and the administration of a mGluR2 agonist would both block the (±)-DOI-induced effects on catecholamine release derived from its intra-cortical administration. However, both conditions are not contradictory. On the one hand, it seems that 5-HT2AR requires the presence and crosstalk with mGluR2 for its proper functioning as pro-hallucinogenic pathway. On the other hand, a mGluR2/3 agonist could lead to conformational changes in the 5-HT2AR/mGluR2 heterocomplex that hampers some of the responses mediated by 5-HT2AR activation.

The merely perfusion of the 5-HT2AR antagonist M100907 into the FC of $mGluR2^{+/+}$ and $mGluR2^{-/-}$ displayed similar extent of inhibitory responses regarding DA concentrations (Figure 42), discarding the contribution of the heterocomplex to such effect. A conceivable explanation for this finding is that cortical 5-HT2ARs exert a tonic control on DA release, which relies on the activation of its canonical signaling pathway, $G_{q/11}$ proteins. The canonical 5-HT2AR $G_{q/11}$ proteins signaling pathway is not disrupted in the absence of the heterocomplex. Thus, M100907 could block $G_{q/11}$ proteins-dependent tonic DA release in wild-type mice, and in $mGluR2^{-/-}$ mice, displaying similar responses independently of the genotype. On the other hand, M100907 blocks the enhancement of DA release induced by (±)-DOI in the FC only in $mGluR2^{+/+}$ (Figure 43), but not in $mGluR2^{-/-}$ animals (Figure 44). This outcome indicates that the phasic control of 5-HT2AR on DA release requires the presence of mGluR2. Therefore, this phasic control could be mediated through $G_{i/o}$ proteins, and is speculated that M100907 primarily blocks the $G_{i/o}$ protein-dependent effect. Thus, the coexistence of two 5-HT2AR populations in FC, with only one contributing to the heterocomplex, is suggested.

5.1.3.2. Tackling the involvement of the heteromer in noradrenergic (±)-DOI-induced response

Looking at the NE basal values, $mGluR2^{-/-}$ mice possess higher values when compared to $mGluR2^{+/+}$ animals. As mentioned in the introduction, glutamate is the main EAA neurotransmitter in the brain and can bind both ionotropic (NMDA, AMPA and KA receptors) and metabotropic receptors (coupled to G proteins, mGluR1-mGluR8). Activation of

presynaptic group II mGluRs depresses excitatory synaptic transmission and glutamate release in the cortex (Pin & Duvoisin, 1995). It has been reported a firing rate suppression of neurons in the LC subsequently to the administration of the mGluR2/3 agonist LY354740, leading to a significant decline in NE release in terminal areas such as the FC (Vandergriff & Rasmussen, 1999). Previous findings by Dubé & Marshall (1997) also suggest the inhibitory role of group II metabotropic receptors on excitatory postsynaptic potentials arising from the LC, acting as autoreceptors to block the activation of LC neurons. Conversely, animals lacking mGluR2 could conceivably prompt the opposite effect. In this way, a hyperactivation of NE neurons due to the absence of the inhibitory $G_{i/o}$-coupled mGluR2 receptor might trigger higher extracellular NE basal values, as observed in the present work.

After cortical (±)-DOI perfusion, mGluR2$^{+/+}$ mice displayed higher NE release than their mutant counterparts (Figure 40 and 41). Furthermore, perfusion of M100907 into the FC was able to fully block the NE stimulation triggered by intra-cortical infusion of (±)-DOI in mGluR2$^{+/+}$ and mGluR2$^{-/-}$ mice (Figure 47, 48 and 49). In this case, it seems that the majority of the (±)-DOI-induced effect on NE release in the FC depends on 5-HT2AR activation, but as the response is lower in mGluR2$^{-/-}$ mice, mGluR2 seems to be also necessary for the proper release. On the other hand, as the small (±)-DOI-evoked NE release in mGluR2$^{-/-}$ mice was sensitive to the presence of M100907, it is likely that a certain population of 5-HT2ARs exists without forming a heteromer with mGluR2, and would be responsible for the lower extracellular NE enhancement triggered by (±)-DOI in mGuR2$^{-/-}$ animals.

Again, the present work shows additional evidence supporting a functional crosstalk between 5-HT2AR and mGluR2, but not mGluR3, since mGluR3$^{-/-}$ mice displayed similar NE stimulatory effects elicited by (±)-DOI than their wild-type littermates (Figure 56 and 57). These data might reflect that 5-HT2AR requires the presence of mGluR2 for the proper NE release evoked by (±)-DOI.

In analogy to the other catecholamine, the sole perfusion of M100907 into the FC elicited in both mGluR2$^{+/+}$ and mGluR2$^{-/-}$ similar extent of inhibitory responses regarding NE release (Figure 46). A conceivable explanation is that the tonic stimulatory effects of the cortical 5-HT2AR on NE release are mediated by activation of its canonical $G_{q/11}$ proteins signaling pathway. Thus, the presence of the 5-HT2AR/mGluR2 heteromer and the 5-HT2AR monomer would coexist in FC.

In a similar way, previous findings investigating the crosstalk between α_2-adrenoceptors, bradykinin (B2) and angiotensin (AT1) receptors already suggested that the activation of $G_{i/o}$ proteins could conceivably be necessary for raising the effects mediated by $G_{q/11}$ proteins on NE release (Cox *et al*., 2000).

Collecting the interpretations speculated in section 5.1.2.2. for the (±)-DOI-induced effects on cortical NE release and the results included in this section, we suggest a plausible and more complex mechanism of (±)-DOI when administered cortically. This mechanism would have its roots in the activation of 5-HT2AR/mGluR2 heteromers on pyramidal neurons that could lead to glutamate release in the FC, able to interact with iGluRs located on NE axon terminals, and thus facilitating the release of NE in that area. 5-HT2ARs and 5-HT2CRs on GABAergic interneurons would be also excited and would mediate inhibitory effects. However, since the proportion of pyramidal neurons is substantially higher in the FC, the overall effect of cortical administration of (±)-DOI would be predominantly excitatory (Figure 115).

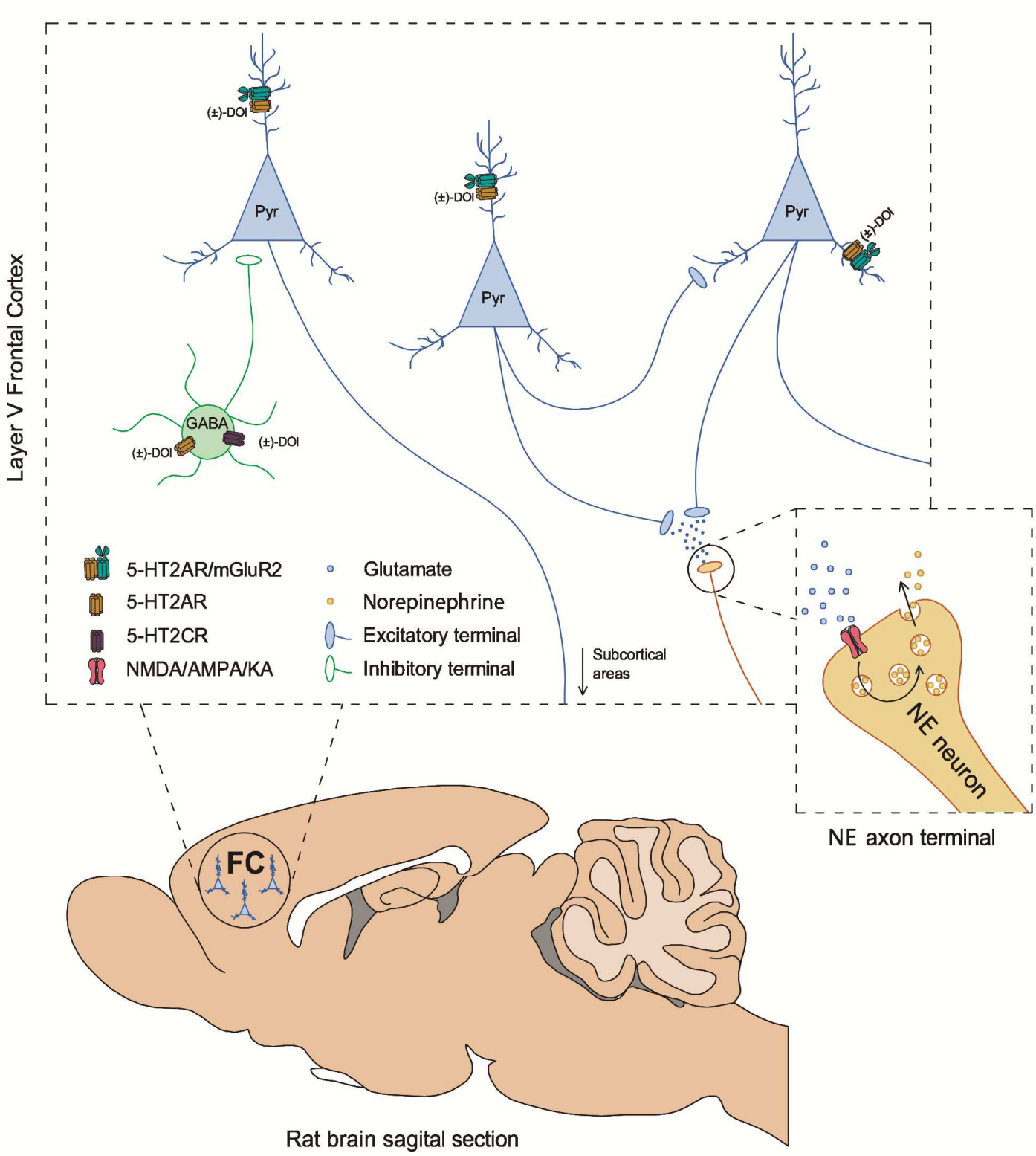


Fig. 115- Schematic picture of the plausible cortical mechanism of action of (±)-DOI on NE release in the FC. Activation of 5-HT2AR/mGluR2 heteromers on pyramidal neurons could excite glutamate neurons and promote glutamate release. This neurotransmitter could act at iGluRs (NMDA/AMPA/kainate) receptors on noradrenergic axon terminals facilitating the release of NE from the storage vesicles. 5-HT2ARs and 5-HT2CRs on GABAergic interneurons would be also excited and mediate inhibitory effects. However, since the proportion of pyramidal neurons is substantially higher in the FC, the overall effect of cortical administration of (±)-DOI would be predominantly excitatory.

5.1.3.3. Rescuing the (±)-DOI-induced catecholamine release after mGluR2 virus-mediated gene transfer in the frontal cortex of mGluR2$^{-/-}$ mice

In this work, it is demonstrated that the overexpression of mGluR2 in frontal cortical neurons of mGluR2$^{-/-}$ mice, via virus-mediated gene transfer (HSV-mGluR2), rescues the high (±)-DOI-induced extracellular DA and NE release. In contrast, the sole overexpression of the control protein (HSV-GFP) does not. These data provide supporting evidence for the necessity of the existence of mGluR2 in frontocortical neurons to induce the rise in DA and NE output in that area, subsequently to local (±)-DOI perfusion. The elegant study undertaken by Moreno and co-workers (2012) already revealed similar extendable results when head twitch behavior yielded by systemic injection of (±)-DOI was evaluated. (±)-DOI is unable to induce the psychotic-like HTR in mGluR2-null animals, leading to the assumption that mGluR2 is necessary for the behavioral effects induced by hallucinogens as (±)-DOI or LSD in mouse models (Fribourg *et al*., 2011; Moreno *et al*., 2011a).

The three amino acidic residues located in the TM4 (Ala-677$^{4.40}$, Ala-681$^{4.44}$ and Ala-685$^{4.48}$) of mGluR2 are known to be necessary for the allosteric binding mediating functional crosstalk between the components of the 5-HT2AR/mGluR2 heterocomplex (Moreno *et al*., 2012). The administration of virus-mediated plasmids substituting these three residues with the ones located at equivalent positions in the TM4 of the highly related mGluR3 leads to a disruption of the heteromeric assembly with the 5-HT2AR in living cells (Moreno *et al*., 2012).

The rescue of mGluR2 exclusively in the FC by stereotaxic injection of HSV-mGluR2 allowed mGluR2$^{-/-}$ mice to recover the high (±)-DOI-induced DA and NE release in the FC, to similar extent to those observed in wild-type animals (Figure 59 and 60 for DA; Figure 61 and 62 for NE). Such striking outcome led us to speculate that this virus-mediated gene transfer could conceivably be rescuing the functional crosstalk via the receptor heterocomplex that finally mediate the DA and NE efflux after cortical administration of (±)-DOI. Here again it should be noted that the remaining effect on DA in mGluR2$^{-/-}$ mice transfected with control virus (HSV-GFP) is visible, as expected, in this new set of experiments.

The main findings of this section can be summarized as follows: (i) the hallucinogen primarily 5-HT2AR agonist (±)-DOI, when administered locally in the FC, elicits a stimulatory effect on cortical DA and NE release in 129/Sv and C57BL/6 strain mice as well as in rats. (ii) mGluR2 seems to play a key role in facilitating such neurotransmitter release enhancement, since

mGluR2-null mice displayed stimulations of much lower degree. The previous statement was further assessed transfecting into the FC of mGluR2$^{-/-}$ mice, a virus-mediated gene to rescue the mGluR2 solely in the medial FC. After the rescue of the receptor, animals lacking mGluR2 recovered the neurochemical response with similar magnitudes to the ones observed in wild-type animals. (iii) Whereas cortical (±)-DOI-induced DA release might be a consequence of an activation of indirect loops involving the activation of cortical and subcortical areas, the cortical (±)-DOI-induced NE release might be restricted to local pathways in the FC inasmuch as the LC, the main source of NE in the brain, appears not to be implicated in the latter effect.

5.2. REGULATION OF DOPAMINERGIC AND NORADRENERGIC NEUROTRANSMISSION IN THE FC BY ACUTE SYSTEMIC ADMINISTRATION OF (±)-DOI

5.2.1. 5-HT receptors playing a role in systemic (±)-DOI-induced DA release in FC

In C57BL/6 mice, a moderate dose of (±)-DOI (1 mg/kg) intraperitoneally administered significantly increased DA release in the FC (Figure 63). It is likely that such DA increase is mediated by 5-HT2ARs, since that response was absent in 5-HT2AR knock-out animals (Figure 65). When similar experiments were carried out in rats and 129/Sv mice, it was observed that (±)-DOI mildly boosted DA release in rat FC, but in contrast, diminished DA release in the FC of 129/Sv mice.

The LSD-like hallucinogens-induced monoamine modulation has been an issue of high interest in the last two decades. There is a body of evidence reporting the ability of systemic (±)-DOI administration to enhance dialysate concentrations of DA in the FC and VTA of freely-moving rats. This is likely via activation of 5-HT2ARs inasmuch as the injection of the 5-HT2AR antagonists M100907 and MDL 11,939 blocked that effect without modifying basal levels (Gobert & Millan, 1999; Pehek *et al*., 2001; Pehek *et al*., 2006; Bortolozzi *et al*., 2005). Additionally, that stimulatory effect is increased by the co-injection of the 5-HT2B/2C receptors antagonist SB 206,553 (Gobert & Millan, 1999).

Pehek and co-workers (2001) investigated the potential presence of 5-HT2AR in the FC, and they reinforced the existence of 5-HT2ARs in cortical areas, since co-administration with M100907 (10 µM) perfused into the FC prevented the increased DA output induced by (±)-DOI (2.5 mg/kg s.c.). Cornea-Hebert and co-workers (1999) revealed by immunohistochemical assays with conventional microscopy, a weak presence of 5-HT2AR in the VTA; as earlier described by Pazos and co-workers (1985). Later, more sophisticated studies using immunohistochemistry with confocal microscopy techniques, demonstrated that 5-HT2AR are distributed on TH positive DA neurons in VTA and a sparse number on non-TH-positive cells in the VTA, probably GABAergic interneurons located in that latter area (Nocjar *et al*., 2002). In this way, the hypothesis that (±)-DOI injection may be able to enhance DA release in the FC through the activation of 5-HT2AR in VTA and in the FC seems plausible.

Herein, 5-HT2ARs in the VTA may be involved in facilitating the phasic release of frontocortical DA. Doherty & Pickel (2000) observed in C57BL/6 mice that agonism at 5-HT2ARs located on DA neurons in the VTA could stimulate DA projection fibers and finally a rise in DA output in terminal areas would be aroused. Moreover, it has been suggested that 5-HT2AR, which regulate cortical DA release, could be located in the FC. Thus, blockade of prefrontocortical 5-HT2ARs prevents the evoked release of DA from the mesocortical pathway. The application of the NMDA receptor antagonist MK-801 in the VTA suppressed the FC-evoked stimulation of DA release, supporting the involvement of NMDA-dependent inputs (Bortolozzi *et al*., 2005). NMDA and AMPA receptors within the VTA (Paquet *et al*., 1997) can excite DA neurons in this area (Rodríguez *et al*., 2000). In this sense, a rise in FC DA release following acute injection of (±)-DOI could be mediated by activation of 5-HT2AR on cortical neurons, which would induce burst firing in VTA DA neurons through a NMDA-dependent mechanism. Finally, such burst firing would be associated with a greater DA release in the FC.

However, there is some controversy regarding the effects elicited by systemic (±)-DOI in the FC. In this sense, Kuroki and co-workers (1999a) did not observe any (±)-DOI-induced (2.5 mg/kg i.p.) DA modulation in the FC of awake freely moving rats.

On the other hand, acute administration of non-selective 5-HT2AR antagonists have generally rendered increased cortical extracellular DA levels in a similar way to the one proposed by (±)-DOI. For instance, amperozide, ritanserin, risperidone, olanzapine, and clozapine have all been shown to increase cortical DA dialysate levels (Moghaddam & Bunney, 1990; Pehek *et al*., 1993; Nomikos *et al*., 1994; Pehek, 1996; Kuroki *et al*., 1999b; Kaminska *et al*., 2013; Kaminska *et al*., 2017). However, systemic administration of M100907 do not exert any modulation on DA release (Gobert *et al*., 2000). Antagonism at 5-HT2CRs slightly increases dialysate levels of DA in the FC, suggesting the existence of a tonic inhibitory regulation mediated by 5-HT2CRs (Nomikos *et al*., 1994). On the other hand, systemic administration of the preferential 5-HT2CR agonist Ro 60-0175 (3 mg/kg) decreases DA release in the FC (Ichikawa *et al*., 2001). In order to explain this paradoxical effect of antipsychotics on DA release in the FC, it has been suggested that the activation of 5-HT1AR is required (Bortolozzi *et al*., 2010). In apparent contradiction with this finding, (±)-DOI (2.5 mg/kg) but not Ro 60-0175 (3 mg/kg) when given systemically, is able to attenuate clozapine injection-induced DA release in FC, suggesting that the effect is mediated by 5-HT2ARs (Ichikawa *et al*., 2001).

In an attempt to evaluate the DA modulation in the FC underlying systemic (±)-DOI injection in 129/Sv mice, (±)-DOI (1 mg/kg i.p.) was injected in wild-type mice. Surprisingly, (±)-DOI evoked an inhibitory effect on DA release in the FC of 129/Sv mice (Figure 69), an effect antagonized by systemic administration of the 5-HT2AR antagonist M100907 (0.5 mg/kg i.p.) (Figure 77), and by the 5-HT2CR antagonist SB242084 (1 mg/kg i.p.) (Figure 79).

In view of this opposite effects mediated by 1 mg/kg of (±)-DOI in both strains, a dose-response assay was performed in 129/Sv mice, using (±)-DOI at five increasing doses (0.1, 0.5, 1, 2.5, 5 mg/kg i.p.). None of these doses was able to mimic the stimulatory effects on DA release shown in C57BL/6 mice (Figure 75). Only an upward trend was shown at the lowest dose, but it did not reach significance. Overall, there was a significant dose-dependent decrease in cortical DA efflux, reaching the maximal inhibitory values between 1 mg/kg and 2.5 mg/kg. The highest dose (5 mg/kg) did not exert that marked inhibitory effect, but only a mild inhibitory trend was evoked. The dose of 1 mg/kg was chosen to perform the following experiments in 129/Sv mice and rats.

(±)-DOI is able to induce excitatory effects on interneurons in the FC, an effect blocked with the 5-HT2A/2CRs antagonist ritanserine, and partially by SB242084, suggesting that both receptors are involved in the modulation of DA activity via excitation of interneurons in the FC (Zhang *et al*., 2010).

In order to determine if the effect observed in our assay would be mediated by 5-HT2AR and/or 5-HT2CRs activation, the preferential 5-HT2CR agonist Ro 60-0175 was systemically administered. Ro 60-0175 exerted an inhibitory effect on DA release in the FC of 129/Sv mice (Figure 81), similar to the one previously observed by (±)-DOI injection (Figure 69). This finding is in line with previous studies showing that systemic Ro 60-0175 reduces DA release in the FC (Ichikawa *et al*., 2001). Thus, it is conceivable that Ro 60-0175 decreases DA firing rate via GABAergic synapses onto DA neurons by stimulating 5-HT2CRs on GABAergic neurons. 5-HT2CRs have been localized, among others, on GABAergic neurons in the VTA (Pessia *et al*., 1994; Eberle-Wang *et al*., 1997; Theile *et al*., 2009), and its activation inhibits dopaminergic pathways (Goldstein *et al*., 1989; Prisco *et al*., 1994; Prisco & Esposito, 1995; Shi *et al*., 1995; Di Matteo *et al*., 2000) via activation of inhibitory GABAergic interneurons (Pessia *et al*., 1994). 5-HT2CRs have also been found on DA neurons as well (Bubar & Cunningham, 2007). Therefore, the inhibitory role that (±)-DOI (1 mg/kg i.p.) elicited in cortical DA release is likely

mediated by 5-HT2CRs. However, the selective 5-HT2AR antagonist M100907 also abolished the effect induced by (±)-DOI. This response seems consistent with a previous study suggesting that 5-HT2ARs directly excite dopaminergic cell bodies (Pessia *et al*., 1994). Nonetheless, the effect described in these conditions is a stimulation but not an inhibition, as the one shown in the present work.

The present findings could have the following interpretations: (i) GABAergic neurons in VTA express 5-HT2AR, since 5-HT2AR is natively resident within DA and non-DAergic (GABA- or possibly glutamate-containing) neurons in the VTA (Doherty & Pickel, 2000; Nocjar *et al*, 2002). Stimulation of 5-HT2AR on dopaminergic neurons in VTA could lead to enhanced DA release in FC - as observed in C57BL/6 mice -, whereas stimulation of 5-HT2AR or 5-HT2CR on GABAergic neurons in VTA could release GABA in this area, and synapse onto DA neurons diminishing DA release in FC - as observed in 129/Sv mice -; (ii) another explanation for the inhibitory effect observed in the FC of 129/Sv mice is that M100907 at a dose of 0.5 mg/kg could lack selectivity for 5-HT2AR (K_i=1.92 nM for 5-HT2AR; K_i=88 nM for 5-HT2CR, see on-line database: https://kidbdev.med.unc.edu/databases/kidb.php). This dose might partially block 5-HT2CRs as well.

5.2.2. 5-HT receptors playing a role in systemic (±)-DOI-induced NE release in FC

As explained above, activation of 5-HT2ARs located in the FC by intra-cortical perfusion of (±)-DOI led to a notable increase in extracellular NE output in the FC. Based on the unique published microdialysis study evaluating NE modulation in the rat FC after systemic (±)-DOI injection undertook by Gobert & Millan (1999), it was expected to observe an increase in cortical extracellular NE in the FC after administration of (±)-DOI systemically. However, doses from 0.5 to 5 mg/kg decreased NE efflux in FC, and the highest inhibitory effect was reached at 1 mg/kg (Figure 76). (±)-DOI (1 mg/kg) induced a slight but significant inhibitory effect on the FC NE release in both 129/Sv strain mice (Figure 70) and rats (Figure 91), although it did not exert any effect in C57BL/6 mice (Figure 64). Such striking outcome led to reconsider the initial scenario, suggesting that subcortical areas could conceivably be yielding the inhibitory effect on the FC.

Intriguingly, the inhibitory effect was antagonized by previous administration of the 5-HT2AR antagonist M100907 (0.5 mg/kg i.p.) (Figure 78), as well as by the selective 5-HT2CR antagonist SB242084 (1 mg/kg i.p.) (Figure 80). These data suggest that the activation of both 5-HT2AR and 5-HT2CRs could be mediating such inhibitory effect. 5-HT2ARs and 5-HT2CRs are located on GABAergic cells within the FC (Willins *et al*., 1997; Carr *et al*., 2002; Santana *et al*., 2004; Liu *et al*., 2007; Puig & Gulledge, 2011), and on GABAergic projections to LC (Pazos *et al*., 1985; Chiang & Aston-Jones, 1993; Pompeiano *et al*., 1994). Activation of these receptors in the LC could drive to an increase in extracellular GABA, which could finally diminish the firing rate of NE neurons projecting to the FC, and would decrease NE release in the FC.

In order to clarify whether 5-HT2AR or 5-HT2CR are responsible for the inhibitory effect on NE release induced by (±)-DOI, experiments were accomplished using 5-HT2AR$^{-/-}$ and 5-HT2AR$^{+/+}$ mice. These assays were performed in C57BL/6 strain mice. (±)-DOI (1 mg/kg i.p.) did not modulate NE concentrations in 5-HT2AR$^{+/+}$ mice, but neither in 5-HT2AR$^{-/-}$ mice (Figure 67).

These findings support the existence of variable effects between mouse strains (see discussion later). The involvement of 5-HT2AR and/or 5-HT2CR was further explored in 129/Sv mice by systemic administration of the preferential 5-HT2CR agonist Ro 60-0175. Ro 60-0175 (3 mg/kg i.p.) elicited a significant inhibitory response on cortical NE release (Figure 82), positing 5-HT2CRs as the main candidates involved in such inhibition. These data agree with those obtained by Gobert and co-workers (2000), showing that Ro 60-0175 (2.5 mg/kg i.p.) diminished DA and NE output in the rat FC.

Then, it was raised the question about the anatomical localization of 5-HT2AR, and/or 5-HT2CR responsible for the inhibitory effect seen in the FC. Bearing in mind the potential critical presence of 5-HT2ARs and/or 5-HT2CRs in subcortical areas underlying such inhibitory effect on FC, dual-probe microdialysis was performed in rats. Probes were inserted into the LC and the ipsilateral FC. Extracellular NE levels were simultaneously monitored in both regions. In this context, assays were carried out to assess to what extent the LC, which is major source of NE in the FC, was affected by 5-HT2AR and/or 5-HT2CR activation. Baseline NE concentration values in LC were higher than in the FC, as previously reported (Mateo *et al*., 1998; Mateo *et al*., 2000; Fernández-Pastor *et al*., 2005).

5.2.2.1. Assessing the potential role of LC NE neurons in the inhibitory effects displayed in the FC. Is the LC steering the ship?

It was previously reported that 5-HT2CRs in the LC tonically inhibit the firing activity of cortical neurons likely via activation of GABAergic interneurons in the area (Chiang & Aston-Jones, 1993). In the present study, a marked somatodendritic NE release in the LC was found after systemic administration of (±)-DOI (Figure 92). In the same animals, a simultaneous decrease of NE in the FC was yielded (Figure 91). Therefore, it can be suggested that systemic (±)-DOI could act on 5-HT2CR expressed on GABAergic neurons in the LC, and these neurons would contribute to the enhancement of NE release in the terminal area. The increase of NE output in the somatodendritic area would contribute to inhibit the tonic firing activity of LC NE neurons (Mateo *et al*., 1998). To date, there are not microdialysis data regarding the effect induced by (±)-DOI on the NE release in the LC, but electrophysiology studies have revealed that (±)-DOI attenuates the firing rate of LC NE neurons through a $GABA_A$ receptor activation, since the microiontophoretic application of a $GABA_A$ receptor antagonist on LC neurons is able to blunt that decrease (Chiang & Aston-Jones, 1993). Future microdialysis experiments using bicuculline in the LC would be necessary to evaluate the role of GABA receptors expressed on LC neurons, to elucidate whether the GABAergic system is responsible for the increments of NE release in the LC after systemic (±)-DOI administration, and its subsequent role in the regulation of the NE release in the FC.

In order to further establish the role of NE neurons in the LC in the regulation of NE release in the FC, (±)-DOI was locally perfused into the LC, and extracellular NE concentrations were measured simultaneously in LC and FC. Once again, a rapid dose-dependent stimulatory effect was found in the LC (Figure 93) and a slower but marked inhibition of NE release in the FC (Figure 94). A negative correlation between extracellular NE concentrations in LC and FC was observed (Figure 95). These findings confirm the crucial role of somatodendritic NE release in LC in the regulation of NE release in the FC. In other words, (±)-DOI could promote NE release in the LC or the peri-LC area, and such NE increase could exert a negative feedback mechanism on the firing rate of NE neurons in the LC projecting to the FC.

The ability of LC to modulate NE release in projecting noradrenergic areas such as the FC is not new. It has been previously reported that (±)-DOI suppresses the firing rate of noradrenergic neurons in the LC and decreases dialysate concentrations of NE in the hippocampus (Done & Sharp, 1992). Furthermore, systemic injection of the (±)-DOI analogues, DOM and DOB, as well

as LSD and mescaline, inhibits the firing rate of spontaneously active neurons in the LC (Rasmussen & Aghajanian, 1986; Gorea & Adrien, 1988).

α_2-Adrenoceptors present in the LC (Nicholas *et al*., 1993) have been highlighted as key players in the regulation of the firing activity of LC neurons (Svensson & Usdin, 1978; Meana *et al*., 1997; Van Gaalen *et al*., 1997; Mateo *et al*., 1998; Jedema *et al*., 2008). Agonists at α_2-adrenoceptors in the LC inhibit the firing activity and release of NE in terminal areas (Pudovkina *et al*., 2001; Jedema *et al*., 2008). The microiontophoretic administration of the α_2-adrenoceptor agonist clonidine in the LC inhibits the firing rate of these noradrenergic neurons, and also the intra-LC perfusion of this agonist commonly decreases the NE dialysate levels in the FC (Svensson *et al*., 1975; Van Gaalen *et al*., 1997; Mateo & Meana, 1999). Clonidine perfusion into the LC dramatically decreases NE in the ipsilateral FC, suggesting that the vast majority of the NE levels in the FC are derived from LC innervations, and highlighting the crucial role of α_2-adrenoceptors on noradrenergic neurons in the LC (Van Gaalen *et al*., 1997). Supporting evidence has been found inasmuch as α_2-adrenoceptor antagonists in the LC blunt both effects. The local administration of the α_2-adrenoceptor antagonist RX821002 perfused in the LC abolished the inhibition of the firing activity and the subsequent release of NE in the FC induced by the presence of an α_2-adrenoceptor agonist in the LC (Meana *et al*., 1997; Mateo *et al*., 1998; Mateo & Meana, 1999). Similar responses can be triggered by the action of the endogenous ligand in the LC through the blockade of NE reuptake in the LC (Mateo *et al*., 1998). Presynaptic α_{2A}-adrenoceptors are likely the receptor subtype involved is these inhibitory effects (Callado & Stamford, 1999; Mateo & Meana, 1999).

To assess the role of α_2-adrenoceptors on LC NE neurons in mediating the inhibition of NE release observed in the FC, systemic (±)-DOI was injected in presence of the α_2-adrenoceptor antagonist RX821002, perfused locally into the LC. RX821002 fully blocked the inhibitory effect in the cortical NE release (Figure 96), suggesting that α_2-adrenoceptors on NE neurons in the LC play a crucial role in the modulation of NE cell firing and release, as stated by Mateo and co-workers (1998). Moreover, in presence of RX821002, systemic (±)-DOI increased NE release in the FC. This stimulatory effect could probably correspond to the local effect of (±)-DOI in the FC.

According to previous findings (Mateo *et al*., 1998; Fernández-Pastor & Meana, 2002), RX821002 perfusion in the LC induced roughly a three-fold increase of basal NE concentrations

in the FC, although statistical significance was not reached, potentially due to insufficient number of animals in each group.

The effect induced by (±)-DOI (1 mg/kg i.p.) was antagonized by the preferential 5-HT2AR antagonist M100907 (0.5 mg/kg i.p.) as well as by the preferential 5-HT2CR antagonist SB242084 (1 mg/kg i.p.). We can interprete these findings regarding that both 5-HT2AR and 5-HT2CR co-localize on GABAergic cells and both could be activated by (±)-DOI. Moreover, it has been recently reported that both receptors are present alone and forming homo and heterodimers, and this heterodimerization makes 5-HT2CR blunt the 5-HT2AR-mediated signaling (Moutkine *et al*., 2017). Supporting evidence shows that the inactivation of 5-HT2CR in the LC correlates with decreased 5-HT2AR-dependent NE transmission. In this sense, inactivation of 5-HT2CR suppresses the decrease of noradenergic tone caused by activation of 5-HT2AR expressed on LC GABAergic interneurons (Moutkine *et al*., 2017). Moreover, it is difficult to determine whether this effect is mediated by the 5-HT2AR or the 5-HT2CR, since the response observed could be the combination of a 5-HT2AR- and 5-HT2CR-dependent effects, inasmuch as M100907 and (±)-DOI at the selected doses could also bind 5-HT2CRs.

Since the expression of 5-HT2CR is substantially higher in LC than of 5-HT2AR, it is more likely that this effect is mediated by 5-HT2CR (Pompeiano *et al*., 1994). However, more recent studies stand up for a preferential role of 5-HT2AR on the serotonergic modulation of NE neurons in the LC (Szabo & Blier, 2002). Underpinning that theory, Millan and co-workers (2003) observed that agomelatine (S20098), which acts as melatonin agonist and 5-HT2CR antagonist, was able to potentiate the electrical activity of noradrenergic neurons in the LC, via an antagonism of 5-HT2CRs, and also rise DA and NE concentrations in the FC. Supporting evidence to the hypothesis stated above is found by electrophysiological approaches, where it is observed that the preferential 5-HT2CRs agonist Ro 60-0175 is able to decrease the firing rate of noradrenergic neurons in the LC (Gobert *et al*., 2000). Conversely, the 5-HT2CR antagonist SB242084 enhances noradrenergic transmission (Gobert *et al*., 2000). In the present study, a similar inhibition was observed in the FC after systemic administration of Ro 60-0175 (3 mg/kg i.p.).

5.2.2.2. Considering other pathways potentially implicated in the (±)-DOI-induced inhibitory effects displayed in the FC

PGi → LC → FC: The major excitatory innervation of the LC derives from the PGi (Aston-Jones *et al*., 1986). Electrical stimulation of PGi activates most LC neurons through the EAA glutamate, since the excitatory transmission is completely abolished by the EAA antagonists KYN and γ-D-glutamylglycine (Ennis & Aston-Jones, 1988; Olpe *et al*., 1989). Kainate-type receptors seem to be the primary contributors, since the NMDA antagonist 2-amino-7-phosphonoheptanoic acid (AP7) and the preferential quisqualate receptor antagonist glutamate diethyl ester (GDEE) did not block LC responses to PGi stimulation (Ennis & Aston-Jones, 1988). Furthermore, bath application of kainate, NMDA, quisqualate and L-glutamate excited LC neurons in a concentration-dependent manner (Olpe *et al*., 1989). Indeed, the rank order for stimulation of LC neurons appears to be kainate ≈ quisqualate > NMDA > L-glutamate (Olpe *et al*., 1989). PGi is a region of high expression of 5-HT2ARs (Pazos *et al*., 1985; Pompeiano *et al*., 1994) and 5-HT2CRs (Molineaux *et al*., 1989; Pompeiano *et al*., 1994; López-Giménez *et al*., 2001a). Therefore, systemic administration of (±)-DOI could activate such excitatory nucleus projecting to the LC, and subsequently promote NE release from the LC.

Against this possibility is the fact that local administration of (±)-DOI in LC elicits similar effects to the ones observed after systemic injection.

In order to gain insight into the contribution of glutamate release from the excitatory input projections arising from PGi to evoke such (±)-DOI-induced NE release via activation of iGluRs, systemic injection of (±)-DOI was co-administrated with perfusion of KYN into the LC. No differences were found among the group treated merely with an acute injection of systemic (±)-DOI, and the group co-treated with perfusion of KYN locally into the LC, neither in the FC, nor in the LC (Figure 100 and 101). These findings also suggest that (±)-DOI-induced NE release in the LC is unlikely prompted by glutamate acting at iGluRs on LC NE neurons.

DR → LC → FC: As broadly stated in the literature, there are serotonergic inputs arising from the DR that synapse onto LC NE neurons and can modulate noradrenergic transmission (Pickel *et al*., 1977; Done & Sharp, 1994; Chiu *et al*., 1995; Haddjeri *et al*., 1997; Szabo *et al*., 1999; Szabo & Blier, 2001a). These serotonergic innervations could play a role in the increase of NE release in the LC. Biochemical and electrophysiological data implicate specific 5-HT receptor

subtypes in the inhibitory action of 5-HT on noradrenergic activity (Rasmussen & Aghajanian, 1986; Gorea & Adrien, 1988). Systemic administration of 5-HT2 receptor agonists in anesthetized rats decreases the firing rate of noradrenergic neurons in the LC (Rasmussen & Aghajanian, 1986; Gorea & Adrien, 1988).

It has been suggested that DR inervates the LC, playing a role in the modulation of NE neurons (Chiu *et al*., 1995; Szabo *et al*., 1999). The increase of 5-HT concentrations in LC is able to inhibit the electric activity of NE neurons through the boosting of somatodendritic NE release. Thus, the presence of the selective serotonin reuptake inhibitor (SSRI) citalopram locally administered, enhances the somatodendritic release of NE and reduces the firing activity of NE neurons and the subsequent release in cortical regions (Mateo *et al*., 2000; Ortega *et al*., 2010). As expected, α_2-adrenoceptors antagonists administered into the LC block this effect observed in the FC (Mateo *et al*., 2000).

On the other hand, it has been reported that the serotonergic receptor that could regulate the somatodendritic release of NE is the 5-HT3R (Ortega *et al*., 2012; Fernández-Pastor *et al*., 2013). The increase of NE output that silences the LC could be triggered by enhanced extracellular concentrations of 5-HT in LC (Chiu *et al*., 1995; Szabo *et al*., 1999). But discrepancies have been reported, since (±)-DOI inhibits the firing activity of DR neurons through the activation of 5-HT2AR on GABAergic neurons within the DR (Wright *et al*., 1990; Garratt *et al*., 1991; Boothman & Sharp, 2005), triggering an inhibitory effect on 5-HT release in terminal projection areas such as the frontal cortex (Martin-Ruiz *et al*., 2001). It has been suggested that part of the inhibitory effect of (±)-DOI on DR neurons depends directly on the LC inasmuch as the DSP4 noradrenergic toxin abolish that effect (Quesseveur *et al*., 2013).

PrH → LC → FC: Together with PGi, PrH is the other nucleus that provides LC with the major innervation inputs. Inhibitory GABAergic inputs can reduce the firing activity of LC NE neurons (Gervasoni *et al*., 1998; Szabo *et al*., 2004). Chiang & Aston-Jones (1993) support that (±)-DOI induces a decrease in spontaneous firing in LC noradrenergic neurons via activation of GABA receptors, since the GABA antagonists bicuculline or picrotoxin administered into the LC block that decrease. Hence, the influence of GABAergic pathways to explain the inhibition of the LC NE cell firing and therefore, the inhibition of the NE release in the FC cannot be ruled out. Regardless of the existence of this pathway, the local effect of (±)-DOI points out a major

influence of GABAergic neurons in the LC than more distant regions such as the PrH. Even much more complicated pathways have been postulated to mediate the decreased LC NE neurons firing rate, involving 5-HT1ARs, $GABA_A$ receptors and 5-HT2ARs (Szabo & Blier, 2001b). As previously mentioned, future experiments using the GABA receptor antagonist bicuculline or picrotoxine locally administered into the LC could be accomplished in order to better elucidate the implication of the GABAergic pathways in (±)-DOI effects on NE concentration reductions in the LC.

5.2.3. Are the strain differences accounting for the opposite effects on cortical catecholamine release induced by systemic administration of (±)-DOI?

There is growing evidence demonstrating that certain intrinsic physiological properties, conditioning factors, and pharmacological approaches can induce distinct responses depending on the background genotype of each species or strain. Thus, a broad number of articles have been published during the past decades concerning these genetic differences in drug-evoked behavioral responses among different species (Sei *et al*., 1992; Bolivar *et al*., 2001; Rikke *et al*., 2003; Nosek *et al*., 2008; Mack *et al*., 2013; Almeida Silva *et al*., 2016; Gioia *et al*., 2016; Séguret *et al*., 2016; Zimmermann *et al*., 2016; Halladay *et al*., 2017). Even quite a few articles have been published so far regarding distinct drug-induced neurochemical responses depending on the rat or mouse strain (Shoaib *et al*., 1995; He & Shippenberg, 2000; Fernandez *et al*., 2003; Calcagno *et al*., 2007). According to He & Shippenberg (2000), the response of striatal DA neurons to cocaine is enhanced in 129/Sv, as compared to C57BL/6, DBA/2, and Swiss-Webster mice. This finding is of interest as it might reflect a certain degree of hypersensitivity of 129/Sv mice at DAT blockade.

In the present work no differences were found in DA and NE basal values from 129/Sv and C57BL/6 strain mice, consistent with previous reports (He & Shippenberg, 2000). However, completely opposite effects were found following systemic (±)-DOI administration (1 mg/kg i.p.) in both strains. Whereas the drug yielded an inhibition of DA and NE release in the FC of 129/Sv mice, it elicited a stimulation of DA release, and no changes in NE output, in C57BL/6 mice. Speculating that such inhibitory effect is mediated by 5-HT2CR in subcortical areas (likely VTA for DA and LC for NE), it seems that both strains possess different sensitivity to 5-HT2CRs agonism.

Regarding NE modulation, as the outcomes obtained in rats are more similar to those observed in 129/Sv strain mice, it appears that C57BL/6 mice display some resistance to 5-HT2CR activation. In line with these findings, a very interesting study carried out in C57BL/6 and DBA/2 mice showed also some differences of sensitivity at 5-HT2CRs between both strains (Calcagno & Invernizzi, 2010). The 5-HT2CR agonist Ro 60-0175 increased extracellular GABA levels and reduced 5-HT levels in the DR of DBA/2 mice, and to a much lesser extent, in C57BL/6 mice. Indeed, C57BL/6 mice need higher doses of 5-HT2CR agonist to exert these effects and, even at higher doses, the magnitude of the effect does not reach the one showed in DBA/2 mice at lower doses. These supporting findings show a different sensitivity at 5-HT2CRs among strains, so C57BL/6 could conceivably be more resistant to 5-HT2CRs than DBA/2, and probably than 129/Sv mice.

5.2.4. Influence of the presence of mGluR2 on the systemic (±)-DOI-induced catecholamine release. Is the 5-HT2AR/mGluR2 heteromer involved in this response?

Systemic (±)-DOI injection (1 mg/kg i.p.) induced similar inhibitory effects on DA and NE release in the FC of animals lacking mGluR2, and their respective wild-type controls (Figure 85 and 86 for DA, Figure 87 and 88 for NE). Therefore, the 5-HT2AR/mGluR2 heterocomplex does not seem to be implicated in the systemic effect of (±)-DOI. Two possible alternatives could explain this finding. Firstly, since evidence of heterocomplex expression in extracortical brain areas has not been provided, the 5-HT2AR involved in the (±)-DOI effect is located in other brain regions that are not the FC, as previously discussed, where it does not form heterocomplex with the mGluR2. Secondly, the receptor implicated in the (±)-DOI effect is the 5-HT2CR, which is not assembled in heterocomplex with the mGluR2 (González-Maeso *et al*., 2008).

In conclusion, (±)-DOI induces opposite effects on DA and NE release in the FC depending on the route of administration. When (±)-DOI is administered by reversal dialysis into the FC, DA and NE concentrations increase. However, when (±)-DOI is administered systemically, distinct patterns of DA and NE release in the FC are revealed depending on mouse strains and animal species. In this sense, systemic (±)-DOI administration enhances catecholamine release in C57BL/6 mice, whereas decreases it in 129/Sv mice, and rats. 5-HT2ARs may be responsible for the stimulatory effect. By contrast, the inhibitory effect is likely mediated via 5-HT2CRs

activation. Thus, strain-dependent opposite effects could reflect a resistance toward 5-HT2CRs activation in C57BL/6 mice.

Diving deeper into the noradrenergic mechanisms involved in the opposite NE release effects induced by (±)-DOI depending on the route of administration, it is suggested that the LC is the area of greater influence on the cortical noradrenergic effects. In this regard, although intra-cortical (±)-DOI enhances NE output in the FC via 5-HT2ARs activation, the regulation exerted by the LC is more influential than the cortical one. Thus, the stimulatory effect is offset by activation of 5-HTRs in the LC, which leads to decreased NE levels in the FC after (±)-DOI injection. On the basis of these data, the inhibition of NE release in the FC after systemic (±)-DOI administration is suggested to be the result of a feedback inhibition induced by the increase of endogenous NE in the LC, which would activate α_2-adrenoceptors located at a somatodendritic level in the LC.

In line with these findings, it has been reported that schizophrenic patients have lower dopaminergic activity in the FC (Brisch *et al*., 2014). In animal models using PCP, it has been demonstrated that DA concentrations are decreased in the FC (Marcotte *et al*., 2001). Data from this study indicate that the hallucinogenic drug (±)-DOI via activation of 5-HT2AR yields similar effects in the FC, verifying that administration of (±)-DOI represents a good pharmacological model of psychosis. This deficit of DA release in the FC could produce negative and cognitive impairments. In accordance with this hypothesis, antipsychotic drugs that antagonize 5-HT2AR increase DA and NE output in the FC.

5.3. INVOLVEMENT OF mGluR2 AND $G_{i/o}$ PROTEINS IN (±)-DOI-INDUCED EFFECTS IN MICE

5.3.1. Role of 5-HT2AR in the effects induced by (±)-DOI

Schizophrenia and hallucinations occur uniquely in humans, however, the use of animal models that resembles at least some of the positive symptoms of schizophrenia is necessary to study the etiology and pathophysiology of the disease. In spite of the difficulty of modeling hallucinogen effects in nonverbal species, animal models of schizophrenia based on psychedelics have yielded important insights into the identification of receptor targets and interactions that could be exploited in the development of new therapeutic drugs.

Outcomes obtained from the present work reinforced the notion that 5-HT2AR activation is underlying (±)-DOI-induced hyperthermia, since while 5-HT2AR$^{+/+}$ mice displayed marked increase in body temperature, 5-HT2AR$^{-/-}$ mice did not (Figure 112). There is a body of evidence demonstrating that 5-HT2ARs activation leads to a rise in body temperature (Pranzatelli, 1990; Mazzola-Pomietto *et al*., 1995; Mazzola-Pomietto *et al*., 1997; Salmi & Ahlenius, 1998; Zhang & Tao, 2011). 5-HT2AR antagonists such as ritanserin, ketanserin, metergoline, mesulergine, and methysergide, while they yield no effects by their own, attenuate (±)-DOI- and DOM-induced hyperthermia, unmasking the contribution of 5-HT2AR to evoke such effect (Pranzatelli, 1990; Aulakh *et al*., 1994; Mazzola-Pomietto, 1995; Salmi & Ahlenius, 1998). 5-HT2ARs on hypothalamic neurons could presumably produce temperature variation, since it is a brain area implicated in the regulation of body temperature.

On the other hand, there is some controversy regarding the implication of 5-HT2CR activation in thermogenic effects. Thus, some researchers also suggest a role of 5-HT2CR activation in hyperthermia (Hayashi *et al*., 2004; Mazzola-Pomietto *et al*., 1997), blunted by 5-HT2CR antagonistm (Hayashi *et al*., 2004). Interestingly, 5-HT1ARs seem to exert opposite effects. In this sense, systemic administration of the 5-HT1AR agonist 8-OHDPAT induces hypothermia (Hjorth, 1985), and co-treatment with the 5-HT1AR antagonist WAY-100635 enhances (±)-DOI-induced hyperthermia (Salmi & Ahlenius, 1998).

Several lines of evidence indicate that 5-HT2AR activation induces HTR, sensoritomor gating impairments, hyperthermia, and hyperlocomotion, among others (Herin *et al*., 2005; Halberstadt *et al*., 2009). Previous findings have reported that LSD, mescaline, psylocybin and

(±)-DOI are all psychedelic drugs and potent 5-HT2A/2C receptor agonists that closely resemble positive clinical symptoms in schizophrenia, including perceptual disturbances, sensory processing deficits or changes in brain metabolism.

Systemic administration of the potent agonist 5-HT2A/2C receptors (±)-DOI strongly yielded HTR in 129/Sv mice. Two different doses were tested intraperitoneally, 0.5 mg/kg and 1 mg/kg. Both doses of (±)-DOI exerted HTR, but the lowest dose induced significantly higher number of head movements than the highest dose (Figure 106). In order to evaluate the involvement of 5-HT2ARs in (±)-DOI-induced HTR, this behavioral response was performed in 5-HT2A$^{+/+}$ and 5-HT2AR$^{-/-}$ C57BL/6 strain mice. As the provision of 5-HT2AR knockout mice on a 129/Sv strain mice was unavailable in our laboratory, experiments were carried out using C57BL/6 strain. Both mouse strains showed HTR when the 5-HT2AR agonist (±)-DOI (0.5 mg/kg i.p.) was administered, even C57BL/6 displayed higher number of head twitch events than 129/Sv mice, as previously reported (Canal & Morgan, 2012). Taking into account the bell-shaped response exerted by increasing doses of (±)-DOI, high doses could be binding to the 5-HT2CR. In this sense, dose of 1 mg/kg in 129/Sv mice could be acting at least partially through 5-HT2CR. In line with the results shown in the present work, there is evidence suggesting that this 5-HT2A/2CRs agonism seems to be responsible for the bell-shaped dose-response curves obtained at some (±)-DOI-induced HTR experiments (Pranzatelli, 1990; Willins & Meltzer, 1997). Thus, activation of 5-HT2AR appears to be responsible for the ascending portion of this bell-shaped dose-response curve, and therefore eliciting higher number of head movements. Meanwhile, 5-HT2CR activation accounts for the descending limb of the dose-effect curve of (±)-DOI indicating that, at higher doses of (±)-DOI, the hallucinogenic capability of this drug is reversed by its 5-HT2CR agonism (Fantegrossi *et al*., 2010). As mentioned above, a vast number of publications have earmarked 5-HT2AR for such HTR, an effect fully abolished by antagonists with higher affinity for 5-HT2AR such as ketanserin, ritanserin, mianserin, metergoline, methysergide and M100907, rather than for 5-HT2CR antagonists (Schreiber *et al*., 1995; Vickers *et al*., 2001). Supporting evidence to establish the no involvement of 5-HT2CR in triggering HTR can be found when the 5-HT2CR agonist Ro 60-0175 is systemically administered alone and no head twitch response is observed. However, in the presence of pretreatment with 5-HT2CRs antagonist SB 242084, there is a dose-dependent increase in the number of head twitches induced by Ro 60-0175, probably attributable to 5-HT2ARs activation, inasmuch as it is abolished by low, selective doses of 5-HT2ARs antagonists as ketanserin or M100907 (Vickers *et al*., 2001).

The absence of 5-HT2AR leads to the suppression of HTR in mice, indicating that HTR requires the activation of 5-HT2AR (Figure 107). In that sense, results shown in the present work are consistent with previous studies showing null HTR in 5-HT2AR$^{-/-}$ (González-Maeso *et al*., 2007). It seems that the ability of (±)-DOI to evoke HTR in rodents is an effect restrictively associated to the activation of 5-HT2ARs located on cortical neurons of the mFC. Thus, the direct bilateral administration of (±)-DOI into both mFCs is able to induce HTR (Willins & Meltzer, 1997), and loss of the HTR in 5-HT2AR$^{-/-}$ mice can be rescued by selective restoration of the receptor in cortical regions (González-Maeso *et al*., 2007).

5.3.2. Role of the 5-HT2AR/mGluR2 complex in the effects induced by (±)-DOI

Several findings have demonstrated the necessity of the mGluR2 existence to evoke pharmacological and behavioral effects induced by hallucinogenic 5-HT2AR agonists. The present work shows a much lower number of (±)-DOI-induced head twitch movements in mGluR2$^{-/-}$ than in mGluR2$^{+/+}$ mice, suggesting that the absence of mGluR2 is somewhat impairing the hallucinogenic 5-HT2AR-dependent effects, which is consistent with previous reports (Moreno *et al*., 2011a; Moreno *et al*., 2012). Looking deeper into the mechanisms underlying such effect, the existence of a heteromer made up between 5-HT2AR and mGluR2 receptors being able to modulate distinct G protein signaling pathways has been thoroughly demonstrated. Psychoactive drugs as LSD or mescaline stimulate specific conformations of this 5-HT2AR/mGluR2 heterodimer, whereas non-psychoactive 5-HT2AR agonists lisuride and ergotamine do not stimulate those conformations, leading to a differential signaling (González-Maeso *et al*., 2007).

Moreover, it has been demonstrated that hallucinogenic 5-HT2AR agonists induce a unique pattern of gene expression in mouse frontal cortex that predicts the behavioral effects (González-Maeso *et al*., 2003; González-Maeso *et al*., 2007). Thus, 5-HT2AR agonist hallucinogens induce *c-fos* and *egr-2* expression, whereas non-hallucinogens induce only *c-fos* expression. In mGluR2$^{-/-}$ mice, 5-HT2AR agonist hallucinogenic drugs do not induce *egr-2* expression, only *c-fos*, unraveling the necessity of both receptors to exert that pattern of gene expression (Moreno *et al*., 2011a). This can lead to the assumption that the hallucinogenic-specific signaling signature is affected in the absence of mGluR2, and suggests that the 5-HT2AR/mGluR2 heteromer is necessary for the responses induced by LSD-like drugs.

Noteworthy, the level of expression of 5-HT2AR in mGluR2$^{-/-}$ mice is unaffected in mouse FC (Moreno *et al*., 2011a).

In an attempt to investigate to what extent the 5-HT2AR/mGluR2 heteromer was involved in non-psychotic related behavior as hyperthermia, the hallucinogenic drug (±)-DOI was injected in mGluR2$^{-/-}$ mice and their respective wild-type controls. It was found that both mGluR2$^{+/+}$ and mGluR2$^{-/-}$ displayed similar magnitude of hyperthermia (Figure 114), suggesting that the 5-HT2AR/mGluR2 heterocomplex is not necessary for this effect induced by (±)-DOI. This finding reinforces the notion that (i) the heterocomplex is located only in FC and not in hypothalamus; (ii) it is more linked to psychosis-like features of hallucinogens; (iii) or both reasons.

5.3.3. Role of the $G_{i/o}$-proteins in the effects induced by (±)-DOI

In mouse cortical primary neurons it has been discovered that the signaling elicited by hallucinogen and non-hallucinogen 5-HT2AR agonists requires $G_{q/11}$-dependent signaling. However, the signaling of hallucinogenic 5-HT2AR agonists seems to be $G_{i/o}$ protein-dependent. In order to further investigate the role of $G_{i/o}$-dependent signaling in HTR induced by (±)-DOI, the G_i proteins-inhibitor PTX was intracerebroventricularly administered. One of the most extended approaches to test PTX activity when used in *in vivo* assays is weight monitoring, since the α subunit of PTX induces lipolysis in adipocytes, so weight loss monitoring can be use as a good tool to verify the $G_{i/o}$ protein signaling blockade. Mice exposed to i.c.v. injection of PTX showed a dramatic weight loss within the first two days that was not fully recovered over time (Figure 109), a hallmark that did not occur in VEH-injected mice. The largest weight loss was achieved two days after PTX injection, correlating to the highest (±)-DOI-induced HTR inhibition (Figure 110). It seems that after the second day of PTX administration, the (±)-DOI-induced HTR is recovered as weight gain is gradually increasing (Figure 111).

The explanation regarding the loss of PTX efficacy over time might be partially explained by $G_{i/o}$ protein turnover. As PTX inhibits $G_{i/o}$ proteins in an irreversible way, its effects could conceivably be dissipated as new $G_{i/o}$ proteins are synthesized, while inhibited G proteins are degraded. However, previous studies showed long-lasting suppressive effects of $G_{i/o}$ proteins,

although observations evaluated completely different effects that comprise distinct mouse brain areas (Self *et al*., 1994; Shah *et al*., 1997).

The role of $G_{i/o}$ proteins, however, seems to be only partial, since the (±)-DOI-elicited HTR was not fully abolished. The latter can leads to the assumption that $G_{i/o}$ proteins are involved in HTR but not exclusively. In this sense, the involvement of other signaling pathways cannot be ruled out, and the remaining $G_{i/o}$-insensitive effect could be yielded by activation of other pathways such as G_q proteins (Garcia *et al*., 2007). Additional reasons could be the use of insufficient dose of PTX, or a lack of dissipation of this toxin through the brain from the ventricular space.

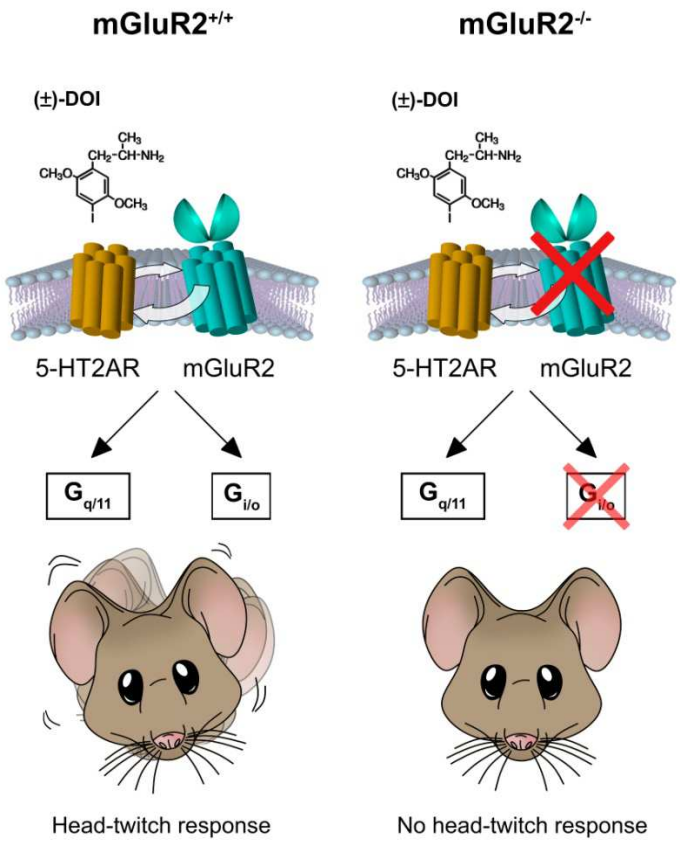


Fig. 116- Schematic picture of G-protein-dependent signaling and behavioral responses that require a GPCR heterocomplex. (±)-DOI acting at the 5-HT2AR/mGluR2 heteromer (mGluR2$^{+/+}$) activates both $G_{q/11}$- and $G_{i/o}$-dependent signaling, triggering head twitch behavior responses. Conversely, when 5-HT2AR and mGluR2 are prevented from forming a heteromer (mGluR2$^{-/-}$), activation of 5-HT2AR by (±)-DOI evokes signaling through $G_{q/11}$ protein subtypes.

Whereas the canonical signaling pathway of 5-HT2AR is mediated by $G_{q/11}$ proteins, it has been suggested the hallucinogenic effects evoked by (±)-DOI are $G_{i/o}$-dependent (González-Maeso *et al*., 2007). It has been indicated that this hallucinogenic effect evoked by the 5-HT2AR activation requires the crosstalk with mGluR2 to activate $G_{i/o}$-dependent signaling (González-Maeso *et al*., 2008). Altogether provide additional insights into the *in vivo* 5-HT2AR/mGluR2 heteromer functionality.

Few articles have elucidated the potential signaling pathways involve in (±)-DOI-induced hyperthermia. Thus, the hallucinogen 5-HT2AR agonists (±)-DOI and LSD evoke 5-HT2AR-dependent body temperature variation, whereas the non-hallucinogen R-lisuride evoked 5-HT2AR-independent body temperature variation (González-

Maeso *et al*., 2007). Since the hallucinogenic and non-hallucinogenic 5-HT2AR agonist drugs differ in the presence or absence of $G_{i/o}$-protein coupling activation through 5-HT2AR, hyperthermia should be considered as a pharmacological response non-dependent on $G_{i/o}$ proteins.

For this purpose, PTX was i.c.v. administered and (±)-DOI-induced temperature variation was monitored. Animals pretreated with PTX showed similar magnitude of hyperthermia when administered (±)-DOI as the VEH-pretreated mice (Figure 113), suggesting that this non-psychosis-like functional response is not mediated via $G_{i/o}$-proteins.

It should be noted that warm ambient temperature strikingly influences the magnitude of the evoked hyperthermia. In this sense, (±)-DOI induces higher hyperthermia when experiments are undertaken in a thermoneutral zone or warm ambient temperatures (27-32°C) than in colder or even room temperature (12-22°C) (Zhang & Tao, 2011). Therefore, experiments in this work were carried out at a 26-27 °C ambient room temperature.

In conclusion, these findings demonstrate for the first time that activation of 5-HT2AR and subsequent signaling through $G_{i/o}$-proteins are a prerequisite for triggering hallucinogenic-like responses inasmuch as inactivation of $G_{i/o}$ proteins by PTX hampers HTR in mice. 5-HT2AR needs the functional presence of mGluR2 to evoke such HTR (Figure 116). Moreover, PTX, albeit inactivating $G_{i/o}$ proteins, does not affect (±)-DOI-induced non-hallucinogenic responses, since (±)-DOI evokes a similar degree of body core variation. This finding indirectly suggests that hyperthermia induced by (±)-DOI administration is mediated by $G_{q/11}$ proteins, the canonical pathway of 5-HT2ARs.

6. CONCLUSIONS

1. The cortical administration of the hallucinogenic 5-HT2A/2C receptor agonist (±)-DOI evokes DA and NE release in the FC through the activation of 5-HT2ARs expressed on cortical neurons. In animals lacking 5-HT2AR, as well as co-perfusion of (±)-DOI with the 5-HT2AR antagonist M100907 in the FC of control mice, the effect was null or attenuated, respectively.

2. The cortical administration of the hallucinogenic 5-HT2AR agonist (±)-DOI evokes NE release through the activation of iGluRs expressed on cortical neurons. The cortical co-perfusion of (±)-DOI with the iGluRs antagonist kynurenic acid blunted the (±)-DOI-induced effects on NE release.

3. The proper cortical DA and NE release following the perfusion of (±)-DOI into the FC requires the presence of mGluR2, but not mGluR3, in this region. Animals lacking mGluR2 displayed lower catecholamine release induced by cortical perfusion of (±)-DOI, whereas animals lacking mGluR3 displayed a similar magnitude of catecholamine release as the one observed in control mice.

4. Systemic administration of (±)-DOI enhanced DA release in the FC of C57BL/6 mice, through the activation of 5-HT2ARs. Animals lacking 5-HT2AR displayed negligible effects on DA release following systemic (±)-DOI administration. However, (±)-DOI did not exert any modulation on NE output in C57BL/6 mice.

5. Systemic administration of (±)-DOI decreased DA and NE release in the FC of 129/Sv mice. The degree of implication of 5-HT2AR and/or 5-HT2CR in triggering these (±)-DOI-induced effect remains to be further elucidated. In the same way, systemic administration of the 5-HT2CR agonist Ro 60-0175 decreases DA and NE release in the FC. Co-injection of (±)-DOI with the 5-HT2AR antagonist M100907, as well as with the 5-HT2CR antagonist SB242084, antagonizes the inhibitory effects induced by (±)-DOI in the FC.

6. The presence of mGluR2 is not required for the inhibitory effects on DA and NE release induced by systemic administration of (±)-DOI in the FC of 129/Sv mice. Animals lacking mGluR2 displayed similar magnitude of inhibition that the one observed in control mice.

7. Systemic administration of (±)-DOI enhanced NE release in rat LC, whereas decreased NE output in the FC. A negative correlation between NE concentrations in FC and LC was revealed. Subcortical pathways seem to play a role in the effects observed in the FC following (±)-DOI injection.

8. The α_2-adrenoceptors antagonism in the LC abolished the inhibitory effects on NE release in the FC of rats induced by (±)-DOI.

9. Perfusion of the iGluRs antagonist kynurenic acid into the rat LC did not blunt either the inhibitory effects on NE release in the FC induced by (±)-DOI or the stimulatory effects observed in LC. Glutamatergic afferent projections toward the LC do not seem to play a role in these effects.

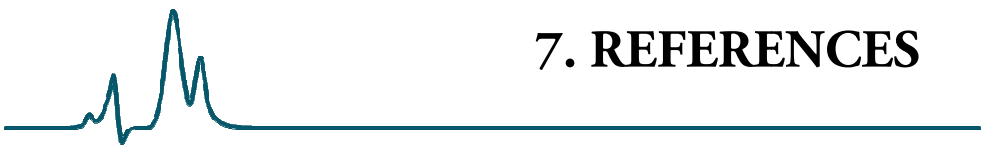
10. Systemic administration of (±)-DOI induced head twitch response through a mechanism that requires the presence of 5-HT2AR and mGluR2. Animals lacking 5-HT2AR, or mGluR2, exhibited negligible or much lower number of head movements, respectively.

11. $G_{i/o}$ proteins are involved in the head twitch response induced by (±)-DOI. The intracerebroventricular administration of the $G_{i/o}$ protein inhibitor pertussis toxin, attenuated the head-twitch response induced by systemic administration of (±)-DOIin mice. The maximal attenuation was yielded two days after the injection of pertussis toxin, which correlates with the greater weight loss.

12. Systemic administration of (±)-DOI induces hyperthermia through a mechanism that requires the presence of 5-HT2AR but not mGluR2. Animals lacking 5-HT2AR did not display any body temperature variation following (±)-DOI injection, but animals lacking mGluR2 showed increased body temperature similar to their respective controls.

13. The hyperthermia evoked by systemic administration of (±)-DOI seems not to be $G_{i/o}$-dependent, since the intracerebroventricular injection of pertussis toxin did not abolish that effect.

14. The findings shown in the present work suggest that modulation of DA and NE release in FC could represent an *in vivo* functional index of the mGluR2/5-HT2AR heterocomplex activation by hallucinogenic 5-HT2AR agonists. This activation is mediated by the non-canonical 5-HT2AR signaling pathway coupled to $G_{i/o}$ proteins. Therefore, an *in vivo* biased agonism activation of this receptor is demonstrated.



7. REFERENCES

Abbas AI, Yadav PN, Yao WD, Arbuckle MI, Grant SG, Caron MG, Roth BL (2009) PSD-95 is essential for hallucinogen and atypical antipsychotic drug actions at serotonin receptors. Journal of Neuroscience 29:7124-7136.

Abi-Saab WM, Bubser M, Roth RH, Deutch AY (1999) 5-HT2 receptor regulation of extracellular GABA levels in the prefrontal cortex. Neuropsychopharmacology 20:92-96.

Abi-Saab WM, D'Souza DC, Moghaddam B, Krystal JH (1998) The NMDA antagonist model for schizophrenia: promise and pitfalls. Pharmacopsychiatry 31:104-109.

Adell A, Artigas F (2004) The somatodendritic release of dopamine in the ventral tegmental area and its regulation by afferent transmitter systems. Neuroscience & Biobehavioral Reviews 28:415-431.

Aghajanian GK, Cedarbaum JM, Wang RY (1977) Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons. Brain Research 136:570-577.

Aghajanian GK, Marek GJ (1997) Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. Neuropharmacology 36:589-599.

Aghajanian GK, Marek GJ (1999) Serotonin and hallucinogens. Neuropsychopharmacology 21:16S-23S.

Aghajanian GK, Marek GJ (2000) Serotonin model of schizophrenia: emerging role of glutamate mechanisms. Brain Research Reviews 31:302-312.

Akhondzadeh S, Malek-Hosseini M, Ghoreishi A, Raznahan M, Rezazadeh SA (2008) Effect of ritanserin, a 5HT2A/2C antagonist, on negative symptoms of schizophrenia: a double-blind randomized placebo-controlled study. Progress in Neuro-Psychopharmacology & Biological Psychiatry 32:1879-1883.

Albanese A, Minciacchi D (1983) Organization of the ascending projections from the ventral tegmental area: a multiple fluorescent retrograde tracer study in the rat. Journal of Comparative Neurology 216:406-420.

Albizu L, Holloway T, Gonzalez-Maeso J, Sealfon SC (2011) Functional crosstalk and heteromerization of serotonin 5-HT2A and dopamine D2 receptors. Neuropharmacology 61:770-777.

Alex KD, Pehek EA (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. Pharmacology & Therapeutics 113:296-320.

Alexander SP, Davenport AP, Kelly E, Marrion N, Peters JA, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Southan C, Davies JA, Collaborators C (2015) The Concise Guide to PHARMACOLOGY 2015/16: G protein-coupled receptors. British Journal of Pharmacology 172:5744-5869.

Almeida Silva LF, Engel T, Reschke CR, Conroy RM, Langa E, Henshall DC (2016) Distinct behavioral and epileptic phenotype differences in 129/P mice compared to C57BL/6 mice subject to intraamygdala kainic acid-induced status epilepticus. Epilepsy & Behavior : E&B 64:186-194.

Alvir JM, Lieberman JA, Safferman AZ, Schwimmer JL, Schaaf JA (1993) Clozapine-induced agranulocytosis. Incidence and risk factors in the United States. The New England Journal of Medicine 329:162-167.

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. International Journal of Neuropsychopharmacology 9:565-573.

American Psychiatric Association (2010) Practice guideline for the treatment of patients with schizophrenia. 2nd edition. Arlington, VA, USA.

American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders: DSM-5. 5th edition. Washington, DC, USA.

Andreasen NC (1997) Linking mind and brain in the study of mental illnesses: a project for a scientific psychopathology. Science 275:1586-1593.

Andreasen NC, Flashman L, Flaum M, Arndt S, Swayze V, 2nd, O'Leary DS, Ehrhardt JC, Yuh WT (1994) Regional brain abnormalities in schizophrenia measured with magnetic resonance imaging. JAMA 272:1763-1769.

Andrews RC (1990) Unification of the findings in schizophrenia by reference to the effects of gestational zinc deficiency. Medical Hypotheses 31:141-153.

Angers S, Salahpour A, Bouvier M (2002) Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. Annual Review of Pharmacology and Toxicology 42:409-435.

Anneken JH, Cunningham JI, Collins SA, Yamamoto BK, Gudelsky GA (2013) MDMA increases glutamate release and reduces parvalbumin-positive GABAergic cells in the dorsal hippocampus of the rat: role of cyclooxygenase. Journal of Neuroimmune Pharmacology 8:58-65.

Appel NM, Mitchell WM, Garlick RK, Glennon RA, Teitler M, De Souza EB (1990) Autoradiographic characterization of (+-)-1-(2,5-dimethoxy-4-[125I] iodophenyl)-2-aminopropane ([125I]DOI) binding to 5-HT2 and 5-HT1c receptors in rat brain. Journal of Pharmacology and Experimental Therapeutics 255:843-857.

Araneda R, Andrade R (1991) 5-Hydroxytryptamine2 and 5-hydroxytryptamine1A receptors mediate opposing responses on membrane excitability in rat association cortex. Neuroscience 40:399-412.

Arnt J, Skarsfeldt T (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacology 18:63-101.

Arvanov VL, Liang X, Magro P, Roberts R, Wang RY (1999) A pre- and postsynaptic modulatory action of 5-HT and the 5-HT2A, 2C receptor agonist DOB on NMDA-evoked responses in the rat medial prefrontal cortex. European Journal of Neuroscience 11:2917-2934.

Ashby CR, Jr., Jiang LH, Kasser RJ, Wang RY (1990) Electrophysiological characterization of 5-hydroxytryptamine2 receptors in the rat medial prefrontal cortex. Journal of Pharmacology and Experimental Therapeutics 252:171-178.

Astman N, Gutnick MJ, Fleidervish IA (1998) Activation of protein kinase C increases neuronal excitability by regulating persistent Na+ current in mouse neocortical slices. Journal of Neurophysiology 80:1547-1551.

Aston-Jones G, Ennis M, Pieribone VA, Nickell WT, Shipley MT (1986) The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. Science 234:734-737.

Aston-Jones G, Shipley MT, Chouvet G, Ennis M, van Bockstaele E, Pieribone V, Shiekhattar R, Akaoka H, Drolet G, Astier B, et al. (1991) Afferent regulation of locus coeruleus neurons: anatomy, physiology and pharmacology. Progress in Brain Research 88:47-75.

Aulakh CS, Hill JL, Yoney HT, Murphy DL (1992) Evidence for involvement of 5-HT1C and 5-HT2 receptors in the food intake suppressant effects of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Psychopharmacology 109:444-448.

Aulakh CS, Mazzola-Pomietto P, Wozniak KM, Hill JL, Murphy DL (1994) Evidence that 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane-induced hypophagia and hyperthermia in rats is mediated by serotonin-2A receptors. Journal of Pharmacology and Experimental Therapeutics 270:127-132.

Austin JH, Takaori S (1976) Studies of connections between locus coeruleus and cerebral cortex. Japanese Journal of Pharmacology 26:145-160.

Baez M, Kursar JD, Helton LA, Wainscott DB, Nelson DL (1995) Molecular biology of serotonin receptors. Obesity Research 3 Suppl 4:441S-447S.

Ballanger B, Strafella AP, van Eimeren T, Zurowski M, Rusjan PM, Houle S, Fox SH (2010) Serotonin 2A receptors and visual hallucinations in Parkinson disease. Archives of Neurology 67:416-421.

Bangalore SS, Prasad KM, Montrose DM, Goradia DD, Diwadkar VA, Keshavan MS (2008) Cannabis use and brain structural alterations in first episode schizophrenia--a region of interest, voxel based morphometric study. Schizophrenia Research 99:1-6.

Banks G, Heise I, Starbuck B, Osborne T, Wisby L, Potter P, Jackson IJ, Foster RG, Peirson SN, Nolan PM (2015) Genetic background influences age-related decline in visual and nonvisual retinal responses, circadian rhythms, and sleep. Neurobiology of Aging 36:380-393.

Barbey AK, Koenigs M, Grafman J (2013) Dorsolateral prefrontal contributions to human working memory. Cortex 49:1195-1205.

Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38:1083-1152.

Barr CE, Mednick SA, Munk-Jorgensen P (1990) Exposure to influenza epidemics during gestation and adult schizophrenia. A 40-year study. Archives of General Psychiatry 47:869-874.

Barrot M, Olivier JD, Perrotti LI, DiLeone RJ, Berton O, Eisch AJ, Impey S, Storm DR, Neve RL, Yin JC, Zachariou V, Nestler EJ (2002) CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. Proceedings of the National Academy of Sciences of the United States of America 99:11435-11440.

Becker KP, Hannun YA (2005) Protein kinase C and phospholipase D: intimate interactions in intracellular signaling. Cellular and Molecular Life Sciences 62:1448-1461.

Bedard P, Pycock C (1977) The 'wet dog shake" behaviour in the rat and 5-hydroxytryptamine [proceedings]. British Journal of Pharmacology 59:450P-451P.

Benarroch EE (2008) Metabotropic glutamate receptors: synaptic modulators and therapeutic targets for neurologic disease. Neurology 70:964-968.

Bender L (1947) Childhood schizophrenia; clinical study on one hundred schizophrenic children. The American Journal of Orthopsychiatry 17:40-56.

Benneyworth MA, Xiang Z, Smith RL, Garcia EE, Conn PJ, Sanders-Bush E (2007) A selective positive allosteric modulator of metabotropic glutamate receptor subtype 2 blocks a hallucinogenic drug model of psychosis. Molecular Pharmacology 72:477-484.

Benveniste H, Drejer J, Schousboe A, Diemer NH (1987) Regional cerebral glucose phosphorylation and blood flow after insertion of a microdialysis fiber through the dorsal hippocampus in the rat. Journal of Neurochemistry 49:729-734.

Beraki S, Diaz-Heijtz R, Tai F, Ogren SO (2009) Effects of repeated treatment of phencyclidine on cognition and gene expression in C57BL/6 mice. International Journal of Neuropsychopharmacology 12:243-255.

Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P, Clarke WP (1998) Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. Molecular Pharmacology 54:94-104.

Berger B, Thierry AM, Tassin JP, Moyne MA (1976) Dopaminergic innervation of the rat prefrontal cortex: a fluorescence histochemical study. Brain Research 106:133-145.

Berlim MT, Mattevi BS, Belmonte-de-Abreu P, Crow TJ (2003) The etiology of schizophrenia and the origin of language: overview of a theory. Comprehensive Psychiatry 44:7-14.

Berridge CW, Waterhouse BD (2003) The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Research Reviews 42:33-84.

Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: dynamics, homeostasis and remodelling. Nature Reviews Molecular Cell Biology 4:517-529.

Berry N, Jobanputra V, Pal H (2003) Molecular genetics of schizophrenia: a critical review. Journal of Psychiatry & Neuroscience 28:415-429.

Bjorklund A, Enemar A, Falck B (1968) Monoamines in the hypothalamo-hypophyseal system of the mouse with special reference to the ontogenetic aspects. Zeitschrift für Zellforschung und Mikroskopische Anatomie 89:590-607.

Bleich A, Brown SL, Kahn R, van Praag HM (1988) The role of serotonin in schizophrenia. Schizophrenia Bulletin 14:297-315.

Bleuler M (1978) The Schizophrenic Disorders Translated by S Clemens Yale University Press. New Haven and London.

Body S, Asgari K, Cheung TH, Bezzina G, Fone KF, Glennon JC, Bradshaw CM, Szabadi E (2006) Evidence that the effect of 5-HT2 receptor stimulation on temporal differentiation is not mediated by receptors in the dorsal striatum. Behavioural Processes 71:258-267.

Bokoch MP, Zou Y, Rasmussen SG, Liu CW, Nygaard R, Rosenbaum DM, Fung JJ, Choi HJ, Thian FS, Kobilka TS, Puglisi JD, Weis WI, Pardo L, Prosser RS, Mueller L, Kobilka BK (2010) Ligand-specific regulation of the extracellular surface of a G-protein-coupled receptor. Nature 463:108-112.

Bolivar VJ, Pooler O, Flaherty L (2001) Inbred strain variation in contextual and cued fear conditioning behavior. Mammalian Genome 12:651-656.

Bongiovanni R, Kirkbride B, Walmire P, Jaskiw GE (2005) Tyrosine administration does not affect desipramine-induced dopamine levels as measured in vivo in prefrontal cortex. Brain Research 1054:203-206.

Boothman LJ, Sharp T (2005) A role for midbrain raphe gamma aminobutyric acid neurons in 5-hydroxytryptamine feedback control. Neuroreport 16:891-896.

Bortolozzi A, Amargos-Bosch M, Adell A, Diaz-Mataix L, Serrats J, Pons S, Artigas F (2003) In vivo modulation of 5-hydroxytryptamine release in mouse prefrontal cortex by local 5-HT(2A) receptors: effect of antipsychotic drugs. European Journal of Neuroscience 18:1235-1246.

Bortolozzi A, Diaz-Mataix L, Scorza MC, Celada P, Artigas F (2005) The activation of 5-HT receptors in prefrontal cortex enhances dopaminergic activity. Journal of Neurochemistry 95:1597-1607.

Bortolozzi A, Masana M, Diaz-Mataix L, Cortes R, Scorza MC, Gingrich JA, Toth M, Artigas F (2010) Dopamine release induced by atypical antipsychotics in prefrontal cortex requires 5-HT(1A) receptors but not 5-HT(2A) receptors. International Journal of Neuropsychopharmacology 13:1299-1314.

Bouwknecht JA, Paylor R (2002) Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. Behavioural Brain Research 136:489-501.

Boydell J, van Os J, McKenzie K, Allardyce J, Goel R, McCreadie RG, Murray RM (2001) Incidence of schizophrenia in ethnic minorities in London: ecological study into interactions with environment. British Medical Journal 323:1336-1338.

Bozymski KM, Lowe DK, Pasternak KM, Gatesman TL, Crouse EL (2017) Pimavanserin: A Novel Antipsychotic for Parkinson's Disease Psychosis. Annals of Pharmacotherapy 51:479-487.

Brisch R, Saniotis A, Wolf R, Bielau H, Bernstein HG, Steiner J, Bogerts B, Braun K, Jankowski Z, Kumaratilake J, Henneberg M, Gos T (2014) The role of dopamine in schizophrenia from a neurobiological and evolutionary perspective: old fashioned, but still in vogue. Frontiers in Psychiatry 5:47.

Bromidge SM, Duckworth M, Forbes IT, Ham P, King FD, Thewlis KM, Blaney FE, Naylor CB, Blackburn TP, Kennett GA, Wood MD, Clarke SE (1997) 6-Chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline (SB-242084): the first selective and brain penetrant 5-HT2C receptor antagonist. Journal of Medicinal Chemistry 40:3494-3496.

Broome MR, Woolley JB, Tabraham P, Johns LC, Bramon E, Murray GK, Pariante C, McGuire PK, Murray RM (2005) What causes the onset of psychosis? Schizophrenia Research 79:23-34.

Brown AS, Derkits EJ (2010) Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. The American Journal of Psychiatry 167:261-280.

Bruce ML, Takeuchi DT, Leaf PJ (1991) Poverty and psychiatric status. Longitudinal evidence from the New Haven Epidemiologic Catchment Area study. Archives of General Psychiatry 48:470-474.

Bubar MJ, Cunningham KA (2007) Distribution of serotonin 5-HT2C receptors in the ventral tegmental area. Neuroscience 146:286-297.

Buchanan RW, Vladar K, Barta PE, Pearlson GD (1998) Structural evaluation of the prefrontal cortex in schizophrenia. The American Journal of Psychiatry 155:1049-1055.

Buckley PF (2005) Neuroimaging of schizophrenia: structural abnormalities and pathophysiological implications. Neuropsychiatric Disease and Treatment 1:193-204.

Calcagno E, Canetta A, Guzzetti S, Cervo L, Invernizzi RW (2007) Strain differences in basal and post-citalopram extracellular 5-HT in the mouse medial prefrontal cortex and dorsal hippocampus: relation with tryptophan hydroxylase-2 activity. Journal of Neurochemistry 103:1111-1120.

Calcagno E, Guzzetti S, Canetta A, Fracasso C, Caccia S, Cervo L, Invernizzi RW (2009) Enhancement of cortical extracellular 5-HT by 5-HT1A and 5-HT2C receptor blockade restores the antidepressant-like effect of citalopram in non-responder mice. International Journal of Neuropsychopharmacology 12:793-803.

Calcagno E, Invernizzi RW (2010) Strain-dependent serotonin neuron feedback control: role of serotonin 2C receptors. Journal of Neurochemistry 114:1701-1710.

Callado LF, Stamford JA (1999) Alpha2A- but not alpha2B/C-adrenoceptors modulate noradrenaline release in rat locus coeruleus: voltammetric data. European Journal of Pharmacology 366:35-39.

Callicott JH, Bertolino A, Mattay VS, Langheim FJ, Duyn J, Coppola R, Goldberg TE, Weinberger DR (2000) Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. Cerebral Cortex 10:1078-1092.

Campus P, Accoto A, Maiolati M, Latagliata C, Orsini C (2016) Role of prefrontal 5-HT in the strain-dependent variation in sign-tracking behavior of C57BL/6 and DBA/2 mice. Psychopharmacology 233:1157-1169.

Canal CE, Booth RG, Morgan D (2013) Support for 5-HT2C receptor functional selectivity in vivo utilizing structurally diverse, selective 5-HT2C receptor ligands and the 2,5-dimethoxy-4-iodoamphetamine elicited head-twitch response model. Neuropharmacology 70:112-121.

Canal CE, Morgan D (2012) Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: a comprehensive history, a re-evaluation of mechanisms, and its utility as a model. Drug Testing and Analysis 4:556-576.

Cannon M, Jones PB, Murray RM (2002) Obstetric complications and schizophrenia: historical and meta-analytic review. The American Journal of Psychiatry 159:1080-1092.

Carbonetti NH (2010) Pertussis toxin and adenylate cyclase toxin: key virulence factors of Bordetella pertussis and cell biology tools. Future Microbiology 5:455-469.

Carboni E, Tanda GL, 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. Journal of Neurochemistry 55:1067-1070.

Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, Davies NJ, Venturi P, Jones LA, Lewis SW, Sham PC, Gottesman, II, Farmer AE, McGuffin P, Reveley AM, Murray RM (1999) Heritability estimates for psychotic disorders: the Maudsley twin psychosis series. Archives of General Psychiatry 56:162-168.

Carlsson A (1959) The occurrence, distribution and physiological role of catecholamines in the nervous system. Pharmacological Reviews 11:490-493.

Carlsson A, Carlsson ML (2006) A dopaminergic deficit hypothesis of schizophrenia: the path to discovery. Dialogues in Clinical Neuroscience 8:137-142.

Carlsson A, Lindqvist M (1963) Effect of Chlorpromazine or Haloperidol on Formation of 3methoxytyramine and Normetanephrine in Mouse Brain. Acta Pharmacologica et Toxicologica 20:140-144.

Carlsson A, Lindqvist M, Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180:1200.

Carpenter WT, Koenig JI (2008) The evolution of drug development in schizophrenia: past issues and future opportunities. Neuropsychopharmacology 33:2061-2079.

Carr DB, Cooper DC, Ulrich SL, Spruston N, Surmeier DJ (2002) Serotonin receptor activation inhibits sodium current and dendritic excitability in prefrontal cortex via a protein kinase C-dependent mechanism. Journal of Neuroscience 22:6846-6855.

Carr DB, Sesack SR (2000) Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. Journal of Neuroscience 20:3864-3873.

Carr VJ, Neil AL, Halpin SA, Holmes S, Lewin TJ (2003) Costs of schizophrenia and other psychoses in urban Australia: findings from the Low Prevalence (Psychotic) Disorders Study. The Australian & New Zealand Journal of Psychiatry 37:31-40.

Cartmell J, Monn JA, Schoepp DD (1999) The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine versus d-amphetamine motor behaviors in rats. Journal of Pharmacology and Experimental Therapeutics 291:161-170.

Cartmell J, Schoepp DD (2000) Regulation of neurotransmitter release by metabotropic glutamate receptors. Journal of Neurochemistry 75:889-907.

Castle DJ, Abel K, Takei N, Murray RM (1995) Gender differences in schizophrenia: hormonal effect or subtypes? Schizophrenia Bulletin 21:1-12.

Cedarbaum JM, Aghajanian GK (1976) Noradrenergic neurons of the locus coeruleus: inhibition by epinephrine and activation by the alpha-antagonist piperoxane. Brain Research 112:413-419.

Cedarbaum JM, Aghajanian GK (1978) Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. Journal of Comparative Neurology 178:1-16.

Celada P, Puig M, Amargos-Bosch M, Adell A, Artigas F (2004) The therapeutic role of 5-HT1A and 5-HT2A receptors in depression. Journal of Psychiatry & Neuroscience 29:252-265.

Celada P, Puig MV, Artigas F (2013) Serotonin modulation of cortical neurons and networks. Frontiers in Integrative Neuroscience 7:25.

Chadman KK, Yang M, Crawley JN (2009) Criteria for validating mouse models of psychiatric diseases. American Journal of Medical Genetics Part B, Neuropsychiatric Genetics 150B:1-11.

Chagraoui A, Thibaut F, Skiba M, Thuillez C, Bourin M (2016) 5-HT2C receptors in psychiatric disorders: A review. Progress in Neuro-Psychopharmacology & Biological Psychiatry 66:120-135.

Chefer VI, Thompson AC, Zapata A, Shippenberg TS (2009) Overview of brain microdialysis. Current Protocols in Neuroscience Chapter 7:Unit7 1.

Chen H, Lambert NA (2000) Endogenous regulators of G protein signaling proteins regulate presynaptic inhibition at rat hippocampal synapses. Proceedings of the National Academy of Sciences of the United States of America 97:12810-12815.

Cheramy A, Leviel V, Glowinski J (1981) Dendritic release of dopamine in the substantia nigra. Nature 289:537-542.

Chiang C, Aston-Jones G (1993) A 5-hydroxytryptamine2 agonist augments gamma-aminobutyric acid and excitatory amino acid inputs to noradrenergic locus coeruleus neurons. Neuroscience 54:409-420.

Chiu TH, Yang YR, Yang JJ, Chen CL (1995) Inhibition of locus coeruleus neurons by serotonin at high doses. The Chinese Journal of Physiology 38:153-157.

Cho KK, Sohal VS (2014) Optogenetic approaches for investigating neural pathways implicated in schizophrenia and related disorders. Human Molecular Genetics 23:R64-68.

Chun L, Zhang WH, Liu JF (2012) Structure and ligand recognition of class C GPCRs. Acta Pharmacologica Sinica 33:312-323.

Clark MS, Sexton TJ, McClain M, Root D, Kohen R, Neumaier JF (2002) Overexpression of 5-HT1B receptor in dorsal raphe nucleus using Herpes Simplex Virus gene transfer increases anxiety behavior after inescapable stress. Journal of Neuroscience 22:4550-4562.

Cleghorn JM, Garnett ES, Nahmias C, Firnau G, Brown GM, Kaplan R, Szechtman H, Szechtman B (1989) Increased frontal and reduced parietal glucose metabolism in acute untreated schizophrenia. Psychiatry Research 28:119-133.

Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KC (2000) Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. Neuropharmacology 39:123-132.

Conn PJ, Christopoulos A, Lindsley CW (2009) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. Nature Reviews Drug discovery 8:41-54.

Conn PJ, Jones CK (2009) Promise of mGluR2/3 activators in psychiatry. Neuropsychopharmacology 34:248-249.

Conn PJ, Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. Annual review of Pharmacology and Toxicology 37:205-237.

Conrad LC, Leonard CM, Pfaff DW (1974) Connections of the median and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study. Journal of Comparative Neurology 156:179-205.

Corbett R, Camacho F, Woods AT, Kerman LL, Fishkin RJ, Brooks K, Dunn RW (1995) Antipsychotic agents antagonize non-competitive N-methyl-D-aspartate antagonist-induced behaviors. Psychopharmacology 120:67-74.

Corlett PR, Honey GD, Fletcher PC (2007) From prediction error to psychosis: ketamine as a pharmacological model of delusions. Journal of Psychopharmacology 21:238-252.

Corlew R, Brasier DJ, Feldman DE, Philpot BD (2008) Presynaptic NMDA receptors: newly appreciated roles in cortical synaptic function and plasticity. The Neuroscientist 14:609-625.

Cornea-Hebert V, Riad M, Wu C, Singh SK, Descarries L (1999) Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. Journal of Comparative Neurology 409:187-209.

Corti C, Restituito S, Rimland JM, Brabet I, Corsi M, Pin JP, Ferraguti F (1998) Cloning and characterization of alternative mRNA forms for the rat metabotropic glutamate receptors mGluR7 and mGluR8. European Journal of Neuroscience 10:3629-3641.

Cox SL, Schelb V, Trendelenburg AU, Starke K (2000) Enhancement of noradrenaline release by angiotensin II and bradykinin in mouse atria: evidence for cross-talk between G(q/11) protein- and G(i/o) protein-coupled receptors. British Journal of Pharmacology 129:1095-1102.

Coyle JT (2006) Glutamate and schizophrenia: beyond the dopamine hypothesis. Cellular and Molecular Neurobiology 26:365-384.

Crespo-Facorro B, Kim J, Andreasen NC, O'Leary DS, Magnotta V (2000) Regional frontal abnormalities in schizophrenia: a quantitative gray matter volume and cortical surface size study. Biological Psychiatry 48:110-119.

Crow TJ (2000) Schizophrenia as the price that homo sapiens pays for language: a resolution of the central paradox in the origin of the species. Brain Research Reviews 31:118-129.

Crow TJ, Baker HF, Cross AJ, Joseph MH, Lofthouse R, Longden A, Owen F, Riley GJ, Glover V, Killpack WS (1979) Monoamine mechanisms in chronic schizophrenia: post-mortem neurochemical findings. British Journal of Psychiatry 134:249-256.

Cryan JF, Dev KK (2008) The glutamatergic system as a potential therapeutic target for the treatment of anxiety disorders. In: Handobook of anxiety and fear. Blanchard RJ, Blanchard DC, Griebel G, Nutt D (Eds.). Elsevier. Oxford, United Kingdom. Pag: 269-301.

Daly DA, Moghaddam B (1993) Actions of clozapine and haloperidol on the extracellular levels of excitatory amino acids in the prefrontal cortex and striatum of conscious rats. Neuroscience Letters 152:61-64.

Davis KL, Kahn RS, Ko G, Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. The American Journal of Psychiatry 148:1474-1486.

De Deurwaerdere P, Navailles S, Berg KA, Clarke WP, Spampinato U (2004) Constitutive activity of the serotonin2C receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. Journal of Neuroscience 24:3235-3241.

De Gregorio D, Comai S, Posa L, Gobbi G (2016) d-Lysergic Acid Diethylamide (LSD) as a Model of Psychosis: Mechanism of Action and Pharmacology. International Journal of Molecular Sciences 17.

de Lange EC, Danhof M, de Boer AG, Breimer DD (1997) Methodological considerations of intracerebral microdialysis in pharmacokinetic studies on drug transport across the blood-brain barrier. Brain Research Reviews 25:27-49.

De Paulis (2001) M-100907 (Aventis). Current Opinion in Investigational Drugs 2:123-32.

Deakin JF, Lees J, McKie S, Hallak JE, Williams SR, Dursun SM (2008) Glutamate and the neural basis of the subjective effects of ketamine: a pharmaco-magnetic resonance imaging study. Archives of General Psychiatry 65:154-164.

Dean B (2003) The cortical serotonin2A receptor and the pathology of schizophrenia: a likely accomplice. Journal of Neurochemistry 85:1-13.

Dean K, Murray RM (2005) Environmental risk factors for psychosis. Dialogues in Clinical Neuroscience 7:69-80.

Debure LI, Moyse E, Fevre-Montange M, Hardin H, Belin MF, Rousset C, Pujol JF, Weissmann D (1992) Somatotopic organization of tyrosine hydroxylase expression in the rat locus coeruleus: long term effect of RU24722. Brain Research 581:19-32.

Delille HK, Becker JM, Burkhardt S, Bleher B, Terstappen GC, Schmidt M, Meyer AH, Unger L, Marek GJ, Mezler M (2012) Heterocomplex formation of 5-HT2A-mGlu2 and its relevance for cellular signaling cascades. Neuropharmacology 62:2184-2191.

Demjaha A, Egerton A, Murray RM, Kapur S, Howes OD, Stone JM, McGuire PK (2014) Antipsychotic treatment resistance in schizophrenia associated with elevated glutamate levels but normal dopamine function. Biological Psychiatry 75:e11-13.

D'Esposito M, Cooney JW, Gazzaley A, Gibbs SE, Postle BR (2006) Is the prefrontal cortex necessary for delay task performance? Evidence from lesion and FMRI data. Journal of the International Neuropsychological Society 12:248-260.

Devoto P, Flore G, Longu G, Pira L, Gessa GL (2003a) Origin of extracellular dopamine from dopamine and noradrenaline neurons in the medial prefrontal and occipital cortex. Synapse 50:200-205.

Devoto P, Flore G, Vacca G, Pira L, Arca A, Casu MA, Pani L, Gessa GL (2003b) Co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex induced by clozapine, the prototype atypical antipsychotic. Psychopharmacology 167:79-84.

Di Forti M, Lappin JM, Murray RM (2007) Risk factors for schizophrenia--all roads lead to dopamine. European Neuropsychopharmacology 17 Suppl 2:S101-107.

Di Matteo V, Di Giovanni G, Di Mascio M, Esposito E (2000) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin(2C) receptors. Brain Research 865:85-90.

Diez-Alarcia R, Ibarra-Lecue I, Lopez-Cardona AP, Meana J, Gutierrez-Adan A, Callado LF, Agirregoitia E, Uriguen L (2016) Biased Agonism of Three Different Cannabinoid Receptor Agonists in Mouse Brain Cortex. Frontiers in Pharmacology 7:415.

Dimou L, Schnell L, Montani L, Duncan C, Simonen M, Schneider R, Liebscher T, Gullo M, Schwab ME (2006) Nogo-A-deficient mice reveal strain-dependent differences in axonal regeneration. Journal of Neuroscience 26:5591-5603.

Dinan TG, Cryan JF (2017) Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. The Journal of Physiology 595:489-503.

Diverse-Pierluissi MA, Fischer T, Jordan JD, Schiff M, Ortiz DF, Farquhar MG, De Vries L (1999) Regulators of G protein signaling proteins as determinants of the rate of desensitization of presynaptic calcium channels. Journal of Biological Chemistry 274:14490-14494.

Doherty MD, Pickel VM (2000) Ultrastructural localization of the serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. Brain Research 864:176-185.

Done CJ, Sharp T (1992) Evidence that 5-HT2 receptor activation decreases noradrenaline release in rat hippocampus in vivo. British Journal of Pharmacology 107:240-245.

Done CJ, Sharp T (1994) Biochemical evidence for the regulation of central noradrenergic activity by 5-HT1A and 5-HT2 receptors: microdialysis studies in the awake and anaesthetized rat. Neuropharmacology 33:411-421.

D'Souza DC (2007) Cannabinoids and psychosis. International Review of Neurobiology 78:289-326.

Dube GR, Marshall KC (1997) Modulation of excitatory synaptic transmission in locus coeruleus by multiple presynaptic metabotropic glutamate receptors. Neuroscience 80:511-521.

Dunlop BW, Nemeroff CB (2007) The role of dopamine in the pathophysiology of depression. Archives of General Psychiatry 64:327-337.

Dutta R, Murray RM, Hotopf M, Allardyce J, Jones PB, Boydell J (2010) Reassessing the long-term risk of suicide after a first episode of psychosis. Archives of General Psychiatry 67:1230-1237.

Eagle DM, Baunez C, Hutcheson DM, Lehmann O, Shah AP, Robbins TW (2008) Stop-signal reaction-time task performance: role of prefrontal cortex and subthalamic nucleus. Cerebral Cortex 18:178-188.

Eberle-Wang K, Mikeladze Z, Uryu K, Chesselet MF (1997) Pattern of expression of the serotonin2C receptor messenger RNA in the basal ganglia of adult rats. Journal of Comparative Neurology 384:233-247.

Egerton A, Brugger S, Raffin M, Barker GJ, Lythgoe DJ, McGuire PK, Stone JM (2012) Anterior cingulate glutamate levels related to clinical status following treatment in first-episode schizophrenia. Neuropsychopharmacology 37:2515-2521.

el Mestikawy S, Glowinski J, Hamon M (1986) Presynaptic dopamine autoreceptors control tyrosine hydroxylase activation in depolarized striatal dopaminergic terminals. Journal of Neurochemistry 46:12-22.

Ellman LM, Deicken RF, Vinogradov S, Kremen WS, Poole JH, Kern DM, Tsai WY, Schaefer CA, Brown AS (2010) Structural brain alterations in schizophrenia following fetal exposure to the inflammatory cytokine interleukin-8. Schizophrenia Research 121:46-54.

Englisch S, Jung HS, Lewien A, Becker A, Nowak U, Braun H, Thiem J, Eisenacher S, Meyer-Lindenberg A, Zink M (2016) Agomelatine for the Treatment of Major Depressive Episodes in Schizophrenia-Spectrum Disorders: An Open-Prospective Proof-of-Concept Study. Journal of Clinical Psychopharmacology 36:597-607.

Ennis M, Aston-Jones G (1986) Evidence for self- and neighbor-mediated postactivation inhibition of locus coeruleus neurons. Brain Research 374:299-305.

Ennis M, Aston-Jones G (1988) Activation of locus coeruleus from nucleus paragigantocellularis: a new excitatory amino acid pathway in brain. Journal of Neuroscience 8:3644-3657.

Ennis M, Aston-Jones G (1989) Potent inhibitory input to locus coeruleus from the nucleus prepositus hypoglossi. Brain Research Bulletin 22:793-803.

Ennis M, Behbehani M, Shipley MT, Van Bockstaele EJ, Aston-Jones G (1991) Projections from the periaqueductal gray to the rostromedial pericoerulear region and nucleus locus coeruleus: anatomic and physiologic studies. Journal of Comparative Neurology 306:480-494.

Espuny-Camacho I, Michelsen KA, Gall D, Linaro D, Hasche A, Bonnefont J, Bali C, Orduz D, Bilheu A, Herpoel A, Lambert N, Gaspard N, Peron S, Schiffmann SN, Giugliano M, Gaillard A, Vanderhaeghen P (2013) Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. Neuron 77:440-456.

Faludi G, Dome P, Lazary J (2011) Origins and perspectives of schizophrenia research. Neuropsychopharmacologia Hungarica 13:185-192.

Fantegrossi WE, Simoneau J, Cohen MS, Zimmerman SM, Henson CM, Rice KC, Woods JH (2010) Interaction of 5-HT2A and 5-HT2C receptors in R(-)-2,5-dimethoxy-4-iodoamphetamine-elicited head twitch behavior in mice. Journal of Pharmacology and Experimental Therapeutics 335:728-734.

Fay R, Kubin L (2000) Pontomedullary distribution of 5-HT2A receptor-like protein in the rat. Journal of Comparative Neurology 418:323-345.

Fernandez F, Sarre S, Launay JM, Aguerre S, Guyonnet-Duperat V, Moisan MP, Ebinger G, Michotte Y, Mormede P, Chaouloff F (2003) Rat strain differences in peripheral and central serotonin transporter protein expression and function. European Journal of Neuroscience 17:494-506.

Fernandez-Pastor B, Mateo Y, Gomez-Urquijo S, Javier Meana J (2005) Characterization of noradrenaline release in the locus coeruleus of freely moving awake rats by in vivo microdialysis. Psychopharmacology 180:570-579.

Fernandez-Pastor B, Meana JJ (2002) In vivo tonic modulation of the noradrenaline release in the rat cortex by locus coeruleus somatodendritic alpha(2)-adrenoceptors. European Journal of Pharmacology 442:225-229.

Fernandez-Pastor B, Ortega JE, Meana JJ (2013) Involvement of serotonin 5-HT3 receptors in the modulation of noradrenergic transmission by serotonin reuptake inhibitors: a microdialysis study in rat brain. Psychopharmacology 229:331-344.

Filip M, Spampinato U, McCreary AC, Przegalinski E (2012) Pharmacological and genetic interventions in serotonin (5-HT)(2C) receptors to alter drug abuse and dependence processes. Brain Research 1476:132-153.

Fink K, Bonisch H, Gothert M (1990) Presynaptic NMDA receptors stimulate noradrenaline release in the cerebral cortex. European Journal of Pharmacology 185:115-117.

Fiorica-Howells E, Hen R, Gingrich J, Li Z, Gershon MD (2002) 5-HT(2A) receptors: location and functional analysis in intestines of wild-type and 5-HT(2A) knockout mice. American Journal of Physiology. Gastrointestinal and Liver Physiology 282:G877-893.

Fischman LG (1983) Dreams, hallucinogenic drug states, and schizophrenia: a psychological and biological comparison. Schizophrenia Bulletin 9:73-94.

Fletcher PC, Honey GD (2006) Schizophrenia, ketamine and cannabis: evidence of overlapping memory deficits. Trends in Cognitive Sciences 10:167-174.

Flor PJ, Van Der Putten H, Ruegg D, Lukic S, Leonhardt T, Bence M, Sansig G, Knopfel T, Kuhn R (1997) A novel splice variant of a metabotropic glutamate receptor, human mGluR7b. Neuropharmacology 36:153-159.

Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nature Neuroscience 6:968-973.

Florin-Lechner SM, Druhan JP, Aston-Jones G, Valentino RJ (1996) Enhanced norepinephrine release in prefrontal cortex with burst stimulation of the locus coeruleus. Brain Research 742:89-97.

Foote SL, Bloom FE, Aston-Jones G (1983) Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. Physiological Reviews 63:844-914.

Franceschetti S, Taverna S, Sancini G, Panzica F, Lombardi R, Avanzini G (2000) Protein kinase C-dependent modulation of Na+ currents increases the excitability of rat neocortical pyramidal neurones. The Journal of Physiology 528 Pt 2:291-304.

Franklin KBJ, Paxinos G (2008). The Mouse brain in stereotaxic coordinates. Academic Press, Elsevier. 3rd edition. Amsterdam, The Netherlands.

Fribourg M, Moreno JL, Holloway T, Provasi D, Baki L, Mahajan R, Park G, Adney SK, Hatcher C, Eltit JM, Ruta JD, Albizu L, Li Z, Umali A, Shim J, Fabiato A, MacKerell AD, Jr., Brezina V, Sealfon SC, Filizola M, Gonzalez-Maeso J, Logothetis DE (2011) Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. Cell 147:1011-1023.

Fritts ME, Asbury ET, Horton JE, Isaac WL (1998) Medial prefrontal lesion deficits involving or sparing the prelimbic area in the rat. Physiology & Behavior 64:373-380.

Fusar-Poli P, Stone JM, Broome MR, Valli I, Mechelli A, McLean MA, Lythgoe DJ, O'Gorman RL, Barker GJ, McGuire PK (2011) Thalamic glutamate levels as a predictor of cortical response during executive functioning in subjects at high risk for psychosis. Archives of General Psychiatry 68:881-890.

Gaitonde SA, Gonzalez-Maeso J (2017) Contribution of heteromerization to G protein-coupled receptor function. Current Opinion in Pharmacology 32:23-31.

Garcia EE, Smith RL, Sanders-Bush E (2007) Role of G(q) protein in behavioral effects of the hallucinogenic drug 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane. Neuropharmacology 52:1671-1677.

Gardier AM (2014) Brain microdialysis in knockout mice: drawbacks and advantages to study the role of 5-HT1A and 5-HT1B autoreceptors in the mechanism of action of antidepressants. In: Neurobiology of Mood Disorders. Guiard BP, Dremencov E (Eds.). Bentham Science Publishers Ltd. Netherlands. Pag: 3-22.

Garratt JC, Kidd EJ, Wright IK, Marsden CA (1991) Inhibition of 5-hydroxytryptamine neuronal activity by the 5-HT agonist, DOI. European Journal of Pharmacology 199:349-355.

Geisler S, Wise RA (2008) Functional implications of glutamatergic projections to the ventral tegmental area. Reviews in the Neurosciences 19:227-244.

George SR, Fan T, Xie Z, Tse R, Tam V, Varghese G, O'Dowd BF (2000) Oligomerization of mu- and delta-opioid receptors. Generation of novel functional properties. Journal of Biological Chemistry 275:26128-26135.

Geranton SM, Heal DJ, Stanford SC (2003) Differences in the mechanisms that increase noradrenaline efflux after administration of d-amphetamine: a dual-probe microdialysis study in rat frontal cortex and hypothalamus. British Journal of Pharmacology 139:1441-1448.

Gervasoni D, Darracq L, Fort P, Souliere F, Chouvet G, Luppi PH (1998) Electrophysiological evidence that noradrenergic neurons of the rat locus coeruleus are tonically inhibited by GABA during sleep. European Journal of Neuroscience 10:964-970.

Gewirtz JC, Marek GJ (2000) Behavioral evidence for interactions between a hallucinogenic drug and group II metabotropic glutamate receptors. Neuropsychopharmacology 23:569-576.

Geyer MA, Vollenweider FX (2008) Serotonin research: contributions to understanding psychoses. Trends in Pharmacological Sciences 29:445-453.

Gilman AG (1995) Nobel Lecture. G proteins and regulation of adenylyl cyclase. Bioscience Reports 15:65-97.

Gioia DA, Alexander NJ, McCool BA (2016) Differential Expression of Munc13-2 Produces Unique Synaptic Phenotypes in the Basolateral Amygdala of C57BL/6J and DBA/2J Mice. Journal of Neuroscience 36:10964-10977.

Gobert A, Millan MJ (1999) Serotonin (5-HT)2A receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. Neuropharmacology 38:315-317.

Gobert A, Rivet JM, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas JP, Cistarelli L, Melon C, Millan MJ (2000) Serotonin(2C) receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. Synapse 36:205-221.

Goldstein JM, Litwin LC, Sutton EB, Malick JB (1989) Effects of ICI 169,369, a selective serotonin2 antagonist, in electrophysiological tests predictive of antipsychotic activity. Journal of Pharmacology and Experimental Therapeutics 249:673-680.

Gomes I, Jordan BA, Gupta A, Trapaidze N, Nagy V, Devi LA (2000) Heterodimerization of mu and delta opioid receptors: A role in opiate synergy. Journal of Neuroscience 20:RC110.

Gonzalez-Burgos G, Hashimoto T, Lewis DA (2010) Alterations of cortical GABA neurons and network oscillations in schizophrenia. Current Psychiatry Reports 12:335-344.

Gonzalez-Maeso J (2011) GPCR oligomers in pharmacology and signaling. Molecular Brain 4:20.

Gonzalez-Maeso J (2014) Family a GPCR heteromers in animal models. Frontiers in Pharmacology 5:226.

Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF, Zhou M, Okawa Y, Callado LF, Milligan G, Gingrich JA, Filizola M, Meana JJ, Sealfon SC (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 452:93-97.

Gonzalez-Maeso J, Meana JJ (2006) Heterotrimeric g proteins: insights into the neurobiology of mood disorders. Current Neuropharmacology 4:127-138.

Gonzalez-Maeso J, Sealfon SC (2009a) Agonist-trafficking and hallucinogens. Current Medicinal Chemistry 16:1017-1027.

Gonzalez-Maeso J, Sealfon SC (2009b) Psychedelics and schizophrenia. Trends in Neurosciences 32:225-232.

Gonzalez-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R, Lira A, Bradley-Moore M, Ge Y, Zhou Q, Sealfon SC, Gingrich JA (2007) Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. Neuron 53:439-452.

Gonzalez-Maeso J, Yuen T, Ebersole BJ, Wurmbach E, Lira A, Zhou M, Weisstaub N, Hen R, Gingrich JA, Sealfon SC (2003) Transcriptome fingerprints distinguish hallucinogenic and nonhallucinogenic 5-hydroxytryptamine 2A receptor agonist effects in mouse somatosensory cortex. Journal of Neuroscience 23:8836-8843.

Goodman R (1988) Are complications of pregnancy and birth causes of schizophrenia? Developmental Medicine & Child Neurology 30:391-395.

Gorea E, Adrien J (1988) Serotonergic regulation of noradrenergic coerulean neurons: electrophysiological evidence for the involvement of 5-HT2 receptors. European Journal of Pharmacology 154:285-291.

Grace AA (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41:1-24.

Grace AA (1993) Cortical regulation of subcortical dopamine systems and its possible relevance to schizophrenia. Journal of Neural Transmission General Section 91:111-134.

Grattan DR (2015) 60 Years of neuroendocrinology: The hypothalamo-prolactin axis. Journal of Endocrinology 226:T101-122.

Gray JA, Roth BL (2007) The pipeline and future of drug development in schizophrenia. Molecular Psychiatry 12:904-922.

Graybiel AM (2000) The basal ganglia. Current Biology 10:R509-511.

Greenwood BN, Strong PV, Loughridge AB, Day HE, Clark PJ, Mika A, Hellwinkel JE, Spence KG, Fleshner M (2012) 5-HT2C receptors in the basolateral amygdala and dorsal striatum are a novel target for the anxiolytic and antidepressant effects of exercise. PloS One 7:e46118.

Groves PM, Wilson CJ (1980) Fine structure of rat locus coeruleus. Journal of Comparative Neurology 193:841-852.

Grzanna R, Molliver ME, Coyle JT (1978) Visualization of central noradrenergic neurons in thick sections by the unlabeled antibody method: a transmitter-specific Golgi image. Proceedings of the National Academy of Sciences of the United States of America 75:2502-2506.

Gu G, Lorrain DS, Wei H, Cole RL, Zhang X, Daggett LP, Schaffhauser HJ, Bristow LJ, Lechner SM (2008) Distribution of metabotropic glutamate 2 and 3 receptors in the rat forebrain: Implication in emotional responses and central disinhibition. Brain Research 1197:47-62.

Gupta S, Kulhara P (2010) What is schizophrenia: A neurodevelopmental or neurodegenerative disorder or a combination of both? A critical analysis. Indian Journal of Psychiatry 52:21-27.

Gurevich EV, Gurevich VV (2006) Arrestins: ubiquitous regulators of cellular signaling pathways. Genome Biology 7:236.

Gutierrez-Galve L, Wheeler-Kingshott CA, Altmann DR, Price G, Chu EM, Leeson VC, Lobo A, Barker GJ, Barnes TR, Joyce EM, Ron MA (2010) Changes in the frontotemporal cortex and cognitive correlates in first-episode psychosis. Biological Psychiatry 68:51-60.

Guyenet PG (1991) Central noradrenergic neurons: the autonomic connection. Progress in Brain Research 88:365-380.

Haddjeri N, de Montigny C, Blier P (1997) Modulation of the firing activity of noradrenergic neurones in the rat locus coeruleus by the 5-hydroxtryptamine system. British Journal of Pharmacology 120:865-875.

Hafner H, Maurer K, Loffler W, Riecher-Rossler A (1993) The influence of age and sex on the onset and early course of schizophrenia. British Journal of Psychiatry 162:80-86.

Halberstadt AL (2015) Recent advances in the neuropsychopharmacology of serotonergic hallucinogens. Behavioural Brain Research 277:99-120.

Halberstadt AL, Geyer MA (2010) LSD but not lisuride disrupts prepulse inhibition in rats by activating the 5-HT(2A) receptor. Psychopharmacology 208:179-189.

Halberstadt AL, Geyer MA (2011) Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. Neuropharmacology 61:364-381.

Halberstadt AL, Geyer MA (2013a) Characterization of the head-twitch response induced by hallucinogens in mice: detection of the behavior based on the dynamics of head movement. Psychopharmacology 227:727-739.

Halberstadt AL, Geyer MA (2013b) Serotonergic hallucinogens as translational models relevant to schizophrenia. International Journal of Neuropsychopharmacology 16:2165-2180.

Halberstadt AL, Geyer MA (2014) Effects of the hallucinogen 2,5-dimethoxy-4-iodophenethylamine (2C-I) and superpotent N-benzyl derivatives on the head twitch response. Neuropharmacology 77:200-207.

Halberstadt AL, van der Heijden I, Ruderman MA, Risbrough VB, Gingrich JA, Geyer MA, Powell SB (2009) 5-HT(2A) and 5-HT(2C) receptors exert opposing effects on locomotor activity in mice. Neuropsychopharmacology 34:1958-1967.

Halbreich U, Kahn LS (2003) Hormonal aspects of schizophrenias: an overview. Psychoneuroendocrinology 28 Suppl 2:1-16.

Halladay LR, Kocharian A, Holmes A (2017) Mouse strain differences in punished ethanol self-administration. Alcohol 58:83-92.

Hamada S, Senzaki K, Hamaguchi-Hamada K, Tabuchi K, Yamamoto H, Yamamoto T, Yoshikawa S, Okano H, Okado N (1998) Localization of 5-HT2A receptor in rat cerebral cortex and olfactory system revealed by immunohistochemistry using two antibodies raised in rabbit and chicken. Brain Research. Molecular Brain Research 54:199-211.

Hambrecht M, Maurer K, Hafner H, Sartorius N (1992) Transnational stability of gender differences in schizophrenia? An analysis based on the WHO study on determinants of outcome of severe mental disorders. European Archives of Psychiatry and Clinical Neuroscience 242:6-12.

Hanks JB, Gonzalez-Maeso J (2013) Animal models of serotonergic psychedelics. ACS Chemical Neuroscience 4:33-42.

Hardingham GE, Bading H (2010) Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nature Reviews Neuroscience 11:682-696.

Hartig PR, Hoffman BJ, Kaufman MJ, Hirata F (1990) The 5-HT1C receptor. Annals of the New York Academy of Sciences 600:149-166; discussion 166-147.

Harvey PD, Rosenthal JB (2016) Treatment resistant schizophrenia: Course of brain structure and function. Progress in Neuro-Psychopharmacology & Biological Psychiatry 70:111-116.

Hayashi A, Suzuki M, Sasamata M, Miyata K (2004) Thermogenic effect of YM348, a novel 5-HT2C-receptor agonist, in rats. Journal of Pharmacy and Pharmacology 56:1551-1556.

He M, Shippenberg TS (2000) Strain differences in basal and cocaine-evoked dopamine dynamics in mouse striatum. Journal of Pharmacology and Experimental Therapeutics 293:121-127.

Hemrick-Luecke SK, Evans DC (2002) Comparison of the potency of MDL 100,907 and SB 242084 in blocking the serotonin (5-HT)(2) receptor agonist-induced increases in rat serum corticosterone concentrations: evidence for 5-HT(2A) receptor mediation of the HPA axis. Neuropharmacology 42:162-169.

Herdegen T, Leah JD (1998) Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Research Reviews 28:370-490.

Herin DV, Liu S, Ullrich T, Rice KC, Cunningham KA (2005) Role of the serotonin 5-HT2A receptor in the hyperlocomotive and hyperthermic effects of (+)-3,4-methylenedioxymethamphetamine. Psychopharmacology 178:505-513.

Hertzmann M, Reba RC, Kotlyarov EV (1990) Single photon emission computed tomography in phencyclidine and related drug abuse. American Journal of Psychiatry 147:255-256.

Herve D, Pickel VM, Joh TH, Beaudet A (1987) Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. Brain Research 435:71-83.

Higashino K, Ago Y, Umehara M, Kita Y, Fujita K, Takuma K, Matsuda T (2014) Effects of acute and chronic administration of venlafaxine and desipramine on extracellular monoamine levels in the mouse prefrontal cortex and striatum. European Journal of Pharmacology 729:86-93.

Higgins GA, Fletcher PJ (2003) Serotonin and drug reward: focus on 5-HT2C receptors. European Journal of Pharmacology 480:151-162.

Higgins GA, Fletcher PJ (2015) Therapeutic Potential of 5-HT2C Receptor Agonists for Addictive Disorders. ACS Chemical Neuroscience 6:1071-1088.

Higgins GA, Ouagazzal AM, Grottick AJ (2001) Influence of the 5-HT(2C) receptor antagonist SB242,084 on behaviour produced by the 5-HT(2) agonist Ro60-0175 and the indirect 5-HT agonist dexfenfluramine. British Journal of Pharmacology 133:459-466.

Higgins GA, Silenieks LB, Altherr EB, MacMillan C, Fletcher PJ, Pratt WE (2016) Lorcaserin and CP-809101 reduce motor impulsivity and reinstatement of food seeking behavior in male rats: Implications for understanding the anti-obesity property of 5-HT2C receptor agonists. Psychopharmacology 233:2841-2856.

Hilfiker S, Pieribone VA, Czernik AJ, Kao HT, Augustine GJ, Greengard P (1999) Synapsins as regulators of neurotransmitter release. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 354:269-279.

Hirvonen J, Hietala J (2011) Dysfunctional brain networks and genetic risk for schizophrenia: specific neurotransmitter systems. CNS Neuroscience & Therapeutics 17:89-96.

Hjorth S (1985) Hypothermia in the rat induced by the potent serotoninergic agent 8-OH-DPAT. Journal of Neural Transmission 61:131-135.

Hof PR YW, Bloom FE, Belichenko PV, Celio MR (2000) Comparative Cytoarchitectonic Atlas of the C57BL/6 and 129/Sv Mouse Brains. Elsevier. Amstersam, The Netherlands.

Hoftman GD, Lewis DA (2011) Postnatal developmental trajectories of neural circuits in the primate prefrontal cortex: identifying sensitive periods for vulnerability to schizophrenia. Schizophrenia Bulletin 37:493-503.

Hollerman JR, Abercrombie ED, Grace AA (1992) Electrophysiological, biochemical, and behavioral studies of acute haloperidol-induced depolarization block of nigral dopamine neurons. Neuroscience 47:589-601.

Hollerman JR, Grace AA (1990) The effects of dopamine-depleting brain lesions on the electrophysiological activity of rat substantia nigra dopamine neurons. Brain Research 533:203-212.

Holloway T, Moreno JL, Umali A, Rayannavar V, Hodes GE, Russo SJ, Gonzalez-Maeso J (2013) Prenatal stress induces schizophrenia-like alterations of serotonin 2A and metabotropic glutamate 2 receptors in the adult offspring: role of maternal immune system. Journal of Neuroscience 33:1088-1098.

Homayoun H, Moghaddam B (2007) NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. Journal of Neuroscience 27:11496-11500.

Honea R, Crow TJ, Passingham D, Mackay CE (2005) Regional deficits in brain volume in schizophrenia: a meta-analysis of voxel-based morphometry studies. The American Journal of Psychiatry 162:2233-2245.

Horacek J, Bubenikova-Valesova V, Kopecek M, Palenicek T, Dockery C, Mohr P, Hoschl C (2006) Mechanism of action of atypical antipsychotic drugs and the neurobiology of schizophrenia. CNS Drugs 20:389-409.

Hori T, Abe S, Baba A, Suzuki T, Shiraishi H (2000) Effects of repeated phencyclidine treatment on serotonin transporter in rat brain. Neuroscience Letters 280:53-56.

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophrenia Bulletin 35:549-562.

Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacology Biochemistry and Behavior 71:533-554.

https://kidbdev.med.unc.edu/databases/kidb.php

Huang HP, Zhu FP, Chen XW, Xu ZQ, Zhang CX, Zhou Z (2012) Physiology of quantal norepinephrine release from somatodendritic sites of neurons in locus coeruleus. Frontiers in Molecular Neuroscience 5:29.

Huang M, Ichiwaka J, Li Z, Dai J, Meltzer HY (2006) Augmentation by citalopram of risperidone-induced monoamine release in rat prefrontal cortex. Psychopharmacology 185:274-281.

Hyde C, Glancy G, Omerod P, Hall D, Taylor GS (1978) Abuse of indigenous psilocybin mushrooms: a new fashion and some psychiatric complications. British Journal of Psychiatry 132:602-604.

Ichikawa J, Dai J, Meltzer HY (2001) DOI, a 5-HT2A/2C receptor agonist, attenuates clozapine-induced cortical dopamine release. Brain Research 907:151-155.

Ichikawa J, Kuroki T, Dai J, Meltzer HY (1998) Effect of antipsychotic drugs on extracellular serotonin levels in rat medial prefrontal cortex and nucleus accumbens. European Journal of Pharmacology 351:163-171.

Ichikawa J, Meltzer HY (1995) DOI, a 5-HT2A/2C receptor agonist, potentiates amphetamine-induced dopamine release in rat striatum. Brain Research 698:204-208.

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. Journal of Neuroscience 4:966-977.

Insel TR (2010) Rethinking schizophrenia. Nature 468:187-193.

Iqbal N, Asnis GM, Wetzler S, Kay SR, van Praag HM (1991) The role of serotonin in schizophrenia. New findings. Schizophrenia Research 5:181-182.

Iqbal N, van Praag HM (1995) The role of serotonin in schizophrenia. European Neuropsychopharmacology 5 Suppl:11-23.

Jablensky A, Sartorius N, Ernberg G, Anker M, Korten A, Cooper JE, Day R, Bertelsen A (1992) Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization ten-country study. Psychological Medicine Monograph Supplement 20:1-97.

Jackson DM, Johansson C, Lindgren LM, Bengtsson A (1994) Dopamine receptor antagonists block amphetamine and phencyclidine-induced motor stimulation in rats. Pharmacology, Biochemistry, and Behavior 48:465-471.

Jakab RL, Goldman-Rakic PS (1998) 5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. Proceedings of the National Academy of Sciences of the United States of America 95:735-740.

Jakab RL, Goldman-Rakic PS (2000) Segregation of serotonin 5-HT2A and 5-HT3 receptors in inhibitory circuits of the primate cerebral cortex. Journal of Comparative Neurology 417:337-348.

Javitt DC (2010) Glutamatergic theories of schizophrenia. The Israel Journal of Psychiatry and Related Sciences 47:4-16.

Jedema HP, Gold SJ, Gonzalez-Burgos G, Sved AF, Tobe BJ, Wensel T, Grace AA (2008) Chronic cold exposure increases RGS7 expression and decreases alpha(2)-autoreceptor-mediated inhibition of noradrenergic locus coeruleus neurons. European Journal of Neuroscience 27:2433-2443.

Jenkins TA, Harte MK, McKibben CE, Elliott JJ, Reynolds GP (2008) Disturbances in social interaction occur along with pathophysiological deficits following sub-chronic phencyclidine administration in the rat. Behavioural Brain Research 194:230-235.

Jentsch JD, Roth RH (1999) The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20:201-225.

Jodo E, Aston-Jones G (1997) Activation of locus coeruleus by prefrontal cortex is mediated by excitatory amino acid inputs. Brain Research 768:327-332.

Jodo E, Chiang C, Aston-Jones G (1998) Potent excitatory influence of prefrontal cortex activity on noradrenergic locus coeruleus neurons. Neuroscience 83:63-79.

Johnson KA, Lovinger DM (2016) Presynaptic G Protein-Coupled Receptors: Gatekeepers of Addiction? Frontiers in Cellular Neuroscience 10:264.

Johnstone EC, Crow TJ, Frith CD, Husband J, Kreel L (1976) Cerebral ventricular size and cognitive impairment in chronic schizophrenia. Lancet 2:924-926.

Jones CA, Watson DJ, Fone KC (2011) Animal models of schizophrenia. British Journal of Pharmacology 164:1162-1194.

Jorm CM, Stamford JA (1993) Actions of the hypnotic anaesthetic, dexmedetomidine, on noradrenaline release and cell firing in rat locus coeruleus slices. British Journal of Anaesthesia 71:447-449.

Julio-Pieper M, Flor PJ, Dinan TG, Cryan JF (2011) Exciting times beyond the brain: metabotropic glutamate receptors in peripheral and non-neural tissues. Pharmacological Reviews 63:35-58.

Kaiya H (1980) Neuromelanin, neuroleptics and schizophrenia: hypothesis of an interaction between noradrenergic and dopaminergic system. Neuropsychobiology 6:241-248.

Kalivas PW, Duffy P (1991) A comparison of axonal and somatodendritic dopamine release using in vivo dialysis. Journal of Neurochemistry 56:961-967.

Kaminska K, Golembiowska K, Rogoz Z (2013) Effect of risperidone on the fluoxetine-induced changes in extracellular dopamine, serotonin and noradrenaline in the rat frontal cortex. Pharmacological Reports 65:1144-1151.

Kaminska K, Noworyta-Sokolowska K, Jurczak A, Gorska A, Rogoz Z, Golembiowska K (2017) Risperidone and escitalopram co-administration: A potential treatment of schizophrenia symptoms with less side effects. Pharmacological Reports 69:13-21.

Kapur S, Seeman P (2002) NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D(2) and serotonin 5-HT(2)receptors-implications for models of schizophrenia. Molecular Psychiatry 7:837-844.

Kawahara Y, Kawahara H, Westerink BH (1999) Tonic regulation of the activity of noradrenergic neurons in the locus coeruleus of the conscious rat studied by dual-probe microdialysis. Brain Research 823:42-48.

Kegeles LS, Abi-Dargham A, Zea-Ponce Y, Rodenhiser-Hill J, Mann JJ, Van Heertum RL, Cooper TB, Carlsson A, Laruelle M (2000) Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia. Biological Psychiatry 48:627-640.

Kehne JH, Baron BM, Carr AA, Chaney SF, Elands J, Feldman DJ, Frank RA, van Giersbergen PL, McCloskey TC, Johnson MP, McCarty DR, Poirot M, Senyah Y, Siegel BW, Widmaier C (1996) Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100,907 as a potent 5-HT2A antagonist with a favorable CNS safety profile. Journal of Pharmacology and Experimental Therapeutics 277:968-981.

Keller DL, Umbreit WW (1956) Permanent alteration of behavior in mice by chemical and psychological means. Science 124:723-724.

Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R, Ben-Jonathan N, Grandy DK, Low MJ (1997) Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. Neuron 19:103-113.

Kempton MJ, Stahl D, Williams SC, DeLisi LE (2010) Progressive lateral ventricular enlargement in schizophrenia: a meta-analysis of longitudinal MRI studies. Schizophrenia Research 120:54-62.

Kenakin T (2003) Ligand-selective receptor conformations revisited: the promise and the problem. Trends in Pharmacological Sciences 24:346-354.

Kenakin T (2007) Functional selectivity through protean and biased agonism: who steers the ship? Molecular Pharmacology 72:1393-1401.

Kennett G, Lightowler S, Trail B, Bright F, Bromidge S (2000) Effects of RO 60 0175, a 5-HT(2C) receptor agonist, in three animal models of anxiety. European Journal of Pharmacology 387:197-204.

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, Avenell KY, Stean T, Upton N, Bromidge S, Forbes IT, Brown AM, Middlemiss DN, Blackburn TP (1997) SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. Neuropharmacology 36:609-620.

Kessler RM, Ansari MS, Riccardi P, Li R, Jayathilake K, Dawant B, Meltzer HY (2005) Occupancy of striatal and extrastriatal dopamine D2/D3 receptors by olanzapine and haloperidol. Neuropsychopharmacology 30:2283-2289.

Kim Y, Simon NW, Wood J, Moghaddam B (2016) Reward Anticipation Is Encoded Differently by Adolescent Ventral Tegmental Area Neurons. Biological Psychiatry 79:878-886.

Kitaichi K, Yamada K, Hasegawa T, Furukawa H, Nabeshima T (1994) Effects of risperidone on phencyclidine-induced behaviors: comparison with haloperidol and ritanserin. Japanese Journal of Pharmacology 66:181-189.

Klosterkotter J, Hellmich M, Steinmeyer EM, Schultze-Lutter F (2001) Diagnosing schizophrenia in the initial prodromal phase. Archives of General Psychiatry 58:158-164.

Knight AR, Misra A, Quirk K, Benwell K, Revell D, Kennett G, Bickerdike M (2004) Pharmacological characterisation of the agonist radioligand binding site of 5-HT(2A), 5-HT(2B) and 5-HT(2C) receptors. Naunyn-Schmiedeberg's Archives of Pharmacology 370:114-123.

Kobilka BK, Deupi X (2007) Conformational complexity of G-protein-coupled receptors. Trends in Pharmacological Sciences 28:397-406.

Koethe D, Gerth CW, Neatby MA, Haensel A, Thies M, Schneider U, Emrich HM, Klosterkotter J, Schultze-Lutter F, Leweke FM (2006) Disturbances of visual information processing in early states of psychosis and experimental delta-9-tetrahydrocannabinol altered states of consciousness. Schizophrenia Research 88:142-150.

Koethe D, Hoyer C, Leweke FM (2009) The endocannabinoid system as a target for modelling psychosis. Psychopharmacology 206:551-561.

Koh MT, Shao Y, Sherwood A, Smith DR (2016) Impaired hippocampal-dependent memory and reduced parvalbumin-positive interneurons in a ketamine mouse model of schizophrenia. Schizophrenia Research 171:187-194.

Kolakowski LF, Jr. (1994) GCRDb: a G-protein-coupled receptor database. Receptors & Channels 2:1-7.

Kometer M, Schmidt A, Jancke L, Vollenweider FX (2013) Activation of serotonin 2A receptors underlies the psilocybin-induced effects on alpha oscillations, N170 visual-evoked potentials, and visual hallucinations. Journal of Neuroscience 33:10544-10551.

Krabbendam L, van Os J (2005) Schizophrenia and urbanicity: a major environmental influence--conditional on genetic risk. Schizophrenia Bulletin 31:795-799.

Kraepelin E (1896) Ein Lehrbuch für Studierende und Ärzte. Psychiatrie 5:789-814.

Kress GJ, Yamawaki N, Wokosin DL, Wickersham IR, Shepherd GM, Surmeier DJ (2013) Convergent cortical innervation of striatal projection neurons. Nature Neuroscience 16:665-667.

Kristiansen H, Elfving B, Plenge P, Pinborg LH, Gillings N, Knudsen GM (2005) Binding characteristics of the 5-HT2A receptor antagonists altanserin and MDL 100907. Synapse 58:249-257.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Jr., Charney DS (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Archives of General Psychiatry 51:199-214.

Krystal JH, Perry EB, Jr., Gueorguieva R, Belger A, Madonick SH, Abi-Dargham A, Cooper TB, Macdougall L, Abi-Saab W, D'Souza DC (2005) Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. Archives of General Psychiatry 62:985-994.

Kubota T, Anzawa N, Hirota K, Yoshida H, Kushikata T, Matsuki A (1999) Effects of ketamine and pentobarbital on noradrenaline release from the medial prefrontal cortex in rats. Canadian Journal of Anaesthesia 46:388-392.

Kumar JS, Mann JJ (2014) PET tracers for serotonin receptors and their applications. Central Nervous System Agents in Medicinal Chemistry 14:96-112.

Kunz PA, Roberts AC, Philpot BD (2013) Presynaptic NMDA receptor mechanisms for enhancing spontaneous neurotransmitter release. Journal of Neuroscience 33:7762-7769.

Kurita M, Holloway T, Garcia-Bea A, Kozlenkov A, Friedman AK, Moreno JL, Heshmati M, Golden SA, Kennedy PJ, Takahashi N, Dietz DM, Mocci G, Gabilondo AM, Hanks J, Umali A, Callado LF, Gallitano AL, Neve RL, Shen L, Buxbaum JD, Han MH, Nestler EJ, Meana JJ, Russo SJ, Gonzalez-Maeso J (2012) HDAC2 regulates atypical antipsychotic responses through the modulation of mGlu2 promoter activity. Nature Neuroscience 15:1245-1254.

Kuroki T, Kawahara T, Yonezawa Y, Tashiro N (1999a) Effects of the serotonin2A/2C receptor agonist and antagonist on phencyclidine-induced dopamine release in rat medial prefrontal cortex. Progress in Neuro-Psychopharmacology & Biological Psychiatry 23:1259-1275.

Kuroki T, Meltzer HY, Ichikawa J (1999b) Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. Journal of Pharmacology and Experimental Therapeutics 288:774-781.

Lahti AC, Koffel B, LaPorte D, Tamminga CA (1995) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13:9-19.

Laman-Maharg A, Trainor BC (2017) Stress, sex, and motivated behaviors. Journal of Neuroscience Research 95:83-92.

Laruelle M (2014) Schizophrenia: from dopaminergic to glutamatergic interventions. Current Opinion in Pharmacology 14:97-102.

Latek D, Modzelewska A, Trzaskowski B, Palczewski K, Filipek S (2012) G protein-coupled receptors--recent advances. Acta Biochimica Polonica 59:515-529.

Lavreysen H, Langlois X, Donck LV, Nunez JM, Pype S, Lutjens R, Megens A (2015) Preclinical evaluation of the antipsychotic potential of the mGlu2-positive allosteric modulator JNJ-40411813. Pharmacology Research & Perspectives 3:e00097.

Lechin F, van der Dijs B (2005) Noradrenergic hypothesis of schizophrenia. Progress in Neuro-Psychopharmacology & Biological Psychiatry 29:777-778.

Lee A, Rosin DL, Van Bockstaele EJ (1998) alpha2A-adrenergic receptors in the rat nucleus locus coeruleus: subcellular localization in catecholaminergic dendrites, astrocytes, and presynaptic axon terminals. Brain Research 795:157-169.

Lee KW, Woon PS, Teo YY, Sim K (2012) Genome wide association studies (GWAS) and copy number variation (CNV) studies of the major psychoses: what have we learnt? Neuroscience & Biobehavioral Reviews 36:556-571.

Legault M, Rompre PP, Wise RA (2000) Chemical stimulation of the ventral hippocampus elevates nucleus accumbens dopamine by activating dopaminergic neurons of the ventral tegmental area. Journal of Neuroscience 20:1635-1642.

Leggio GM, Cathala A, Moison D, Cunningham KA, Piazza PV, Spampinato U (2009) Serotonin2C receptors in the medial prefrontal cortex facilitate cocaine-induced dopamine release in the rat nucleus accumbens. Neuropharmacology 56:507-513.

Lewis DA (2011) The chandelier neuron in schizophrenia. Developmental Neurobiology 71:118-127.

Lewis DA, Campbell MJ, Foote SL, Goldstein M, Morrison JH (1987) The distribution of tyrosine hydroxylase-immunoreactive fibers in primate neocortex is widespread but regionally specific. Journal of Neuroscience 7:279-290.

Lewis DA, Gonzalez-Burgos G (2008) Neuroplasticity of neocortical circuits in schizophrenia. Neuropsychopharmacology 33:141-165.

Leysen JE, Gommeren W, Van Gompel P, Wynants J, Janssen PF, Laduron PM (1985) Receptor-binding properties in vitro and in vivo of ritanserin: A very potent and long acting serotonin-S2 antagonist. Molecular Pharmacology 27:600-611.

Li ML, Hu XQ, Li F, Gao WJ (2015) Perspectives on the mGluR2/3 agonists as a therapeutic target for schizophrenia: Still promising or a dead end? Progress in Neuro-Psychopharmacology & Biological Psychiatry 60:66-76.

Liao Y, Tang J, Corlett PR, Wang X, Yang M, Chen H, Liu T, Chen X, Hao W, Fletcher PC (2011) Reduced dorsal prefrontal gray matter after chronic ketamine use. Biological Psychiatry 69:42-48.

Lin HH (2013) G-protein-coupled receptors and their (Bio) chemical significance win 2012 Nobel Prize in Chemistry. Biomedical Journal 36:118-124.

Liu S, Bubar MJ, Lanfranco MF, Hillman GR, Cunningham KA (2007) Serotonin2C receptor localization in GABA neurons of the rat medial prefrontal cortex: implications for understanding the neurobiology of addiction. Neuroscience 146:1677-1688.

Liu W, Downing AC, Munsie LM, Chen P, Reed MR, Ruble CL, Landschulz KT, Kinon BJ, Nisenbaum LK (2012) Pharmacogenetic analysis of the mGlu2/3 agonist LY2140023 monohydrate in the treatment of schizophrenia. The Pharmacogenomics Journal 12:246-254.

Llorca-Torralba M, Borges G, Neto F, Mico JA, Berrocoso E (2016) Noradrenergic Locus Coeruleus pathways in pain modulation. Neuroscience 338:93-113.

Lodge DJ (2011) The medial prefrontal and orbitofrontal cortices differentially regulate dopamine system function. Neuropsychopharmacology 36:1227-1236.

Lopez-Gimenez JF, Mengod G, Palacios JM, Vilaro MT (1997) Selective visualization of rat brain 5-HT2A receptors by autoradiography with [3H]MDL 100,907. Naunyn-Schmiedeberg's Archives of Pharmacology 356:446-454.

Lopez-Gimenez JF, Mengod G, Palacios JM, Vilaro MT (2001a) Regional distribution and cellular localization of 5-HT2C receptor mRNA in monkey brain: comparison with [3H]mesulergine binding sites and choline acetyltransferase mRNA. Synapse 42:12-26.

Lopez-Gimenez JF, Villazon M, Brea J, Loza MI, Palacios JM, Mengod G, Vilaro MT (2001b) Multiple conformations of native and recombinant human 5-hydroxytryptamine(2a) receptors are labeled by agonists and discriminated by antagonists. Molecular Pharmacology 60:690-699.

Lorrain DS, Baccei CS, Bristow LJ, Anderson JJ, Varney MA (2003a) Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. Neuroscience 117:697-706.

Lorrain DS, Schaffhauser H, Campbell UC, Baccei CS, Correa LD, Rowe B, Rodriguez DE, Anderson JJ, Varney MA, Pinkerton AB, Vernier JM, Bristow LJ (2003b) Group II mGlu receptor activation suppresses norepinephrine release in the ventral hippocampus and locomotor responses to acute ketamine challenge. Neuropsychopharmacology 28:1622-1632.

Lovett Doust JW, Huszka L, Lovett Doust JN (1975) Psychotropic drugs and gender as modifiers of the role of plasma tryptophan and serotonin in schizophrenia. Comprehensive Psychiatry 16:349-355.

Lu L, Mamiya T, Koseki T, Mouri A, Nabeshima T (2011) Genetic animal models of schizophrenia related with the hypothesis of abnormal neurodevelopment. Biological & Pharmaceutical Bulletin 34:1358-1363.

Luccini E, Musante V, Neri E, Brambilla Bas M, Severi P, Raiteri M, Pittaluga A (2007) Functional interactions between presynaptic NMDA receptors and metabotropic glutamate receptors co-expressed on rat and human noradrenergic terminals. British Journal of Pharmacology 151:1087-1094.

Luppi PH, Aston-Jones G, Akaoka H, Chouvet G, Jouvet M (1995) Afferent projections to the rat locus coeruleus demonstrated by retrograde and anterograde tracing with cholera-toxin B subunit and Phaseolus vulgaris leucoagglutinin. Neuroscience 65:119-160.

Luque JM, Malherbe P, Richards JG (1995) Localization of NMDA receptor subunit mRNAs in the rat locus coeruleus. Brain Research. Molecular Brain Research 29:224-232.

MacDowell KS, Munarriz-Cuezva E, Caso JR, Madrigal JL, Zabala A, Meana JJ, Garcia-Bueno B, Leza JC (2016) 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.

Mack LA, Felver-Gant JN, Dennis RL, Cheng HW (2013) Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. Poultry Science 92:285-294.

Mackay E, Dalman C, Karlsson H, Gardner RM (2017) Association of Gestational Weight Gain and Maternal Body Mass Index in Early Pregnancy With Risk for Nonaffective Psychosis in Offspring. JAMA Psychiatry 74:339-349.

MacLeod RM, Fontham EH, Lehmeyer JE (1970) Prolactin and growth hormone production as influenced by catecholamines and agents that affect brain catecholamines. Neuroendocrinology 6:283-294.

Maeda T (2000) The locus coeruleus: history. Journal of Chemical Neuroanatomy 18:57-64.

Malherbe P, Kew JN, Richards JG, Knoflach F, Kratzeisen C, Zenner MT, Faull RL, Kemp JA, Mutel V (2002) Identification and characterization of a novel splice variant of the metabotropic glutamate receptor 5 gene in human hippocampus and cerebellum. Brain Research. Molecular Brain Research 109:168-178.

Mangmool S, Kurose H (2011) G(i/o) protein-dependent and -independent actions of Pertussis Toxin (PTX). Toxins 3:884-899.

Mansour A, Meador-Woodruff JH, Bunzow JR, Civelli O, Akil H, Watson SJ (1990) Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in the rat brain and pituitary: an in situ hybridization-receptor autoradiographic analysis. Journal of Neuroscience 10:2587-2600.

Marcelis M, Takei N, van Os J (1999) Urbanization and risk for schizophrenia: does the effect operate before or around the time of illness onset? Psychological Medicine 29:1197-1203.

Marcotte ER, Pearson DM, Srivastava LK (2001) Animal models of schizophrenia: a critical review. Journal of Psychiatry & Neuroscience 26:395-410.

Marek GJ, Aghajanian GK (1994) Excitation of interneurons in piriform cortex by 5-hydroxytryptamine: blockade by MDL 100,907, a highly selective 5-HT2A receptor antagonist. European Journal of Pharmacology 259:137-141.

Marek GJ, Aghajanian GK (1999) 5-HT2A receptor or alpha1-adrenoceptor activation induces excitatory postsynaptic currents in layer V pyramidal cells of the medial prefrontal cortex. European Journal of Pharmacology 367:197-206.

Marek GJ, Wright RA, Gewirtz JC, Schoepp DD (2001) A major role for thalamocortical afferents in serotonergic hallucinogen receptor function in the rat neocortex. Neuroscience 105:379-392.

Marek GJ, Wright RA, Schoepp DD (2006) 5-Hydroxytryptamine2A (5-HT2A) receptor regulation in rat prefrontal cortex: interaction of a phenethylamine hallucinogen and the metabotropic glutamate2/3 receptor agonist LY354740. Neuroscience Letters 403:256-260.

Marek GJ, Wright RA, Schoepp DD, Monn JA, Aghajanian GK (2000) Physiological antagonism between 5-hydroxytryptamine(2A) and group II metabotropic glutamate receptors in prefrontal cortex. Journal of Pharmacology and Experimental Therapeutics 292:76-87.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. Nature Reviews Neuroscience 5:793-807.

Marona-Lewicka D, Nichols CD, Nichols DE (2011) An animal model of schizophrenia based on chronic LSD administration: old idea, new results. Neuropharmacology 61:503-512.

Marsman A, Mandl RC, Klomp DW, Bohlken MM, Boer VO, Andreychenko A, Cahn W, Kahn RS, Luijten PR, Hulshoff Pol HE (2014) GABA and glutamate in schizophrenia: a 7 T (1)H-MRS study. NeuroImage Clinical 6:398-407.

Marsman A, van den Heuvel MP, Klomp DW, Kahn RS, Luijten PR, Hulshoff Pol HE (2013) Glutamate in schizophrenia: a focused review and meta-analysis of (1)H-MRS studies. Schizophrenia Bulletin 39:120-129.

Martin JR, Bos M, Jenck F, Moreau J, Mutel V, Sleight AJ, Wichmann J, Andrews JS, Berendsen HH, Broekkamp CL, Ruigt GS, Kohler C, Delft AM (1998) 5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential. Journal of Pharmacology and Experimental Therapeutics 286:913-924.

Martin LJ, Blackstone CD, Levey AI, Huganir RL, Price DL (1993) AMPA glutamate receptor subunits are differentially distributed in rat brain. Neuroscience 53:327-358.

Martin-Ruiz R, Puig MV, Celada P, Shapiro DA, Roth BL, Mengod G, Artigas F (2001) Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. Journal of Neuroscience 21:9856-9866.

Marti-Solano M, Iglesias A, de Fabritiis G, Sanz F, Brea J, Loza MI, Pastor M, Selent J (2015) Detection of new biased agonists for the serotonin 5-HT2A receptor: modeling and experimental validation. Molecular Pharmacology 87:740-746.

Masu M, Iwakabe H, Tagawa Y, Miyoshi T, Yamashita M, Fukuda Y, Sasaki H, Hiroi K, Nakamura Y, Shigemoto R, et al. (1995) Specific deficit of the ON response in visual transmission by targeted disruption of the mGluR6 gene. Cell 80:757-765.

Mateo Y, Meana JJ (1999) Determination of the somatodendritic alpha2-adrenoceptor subtype located in rat locus coeruleus that modulates cortical noradrenaline release in vivo. European Journal of Pharmacology 379:53-57.

Mateo Y, Pineda J, Meana JJ (1998) Somatodendritic alpha2-adrenoceptors in the locus coeruleus are involved in the in vivo modulation of cortical noradrenaline release by the antidepressant desipramine. Journal of Neurochemistry 71:790-798.

Mateo Y, Ruiz-Ortega JA, Pineda J, Ugedo L, Meana JJ (2000) Inhibition of 5-hydroxytryptamine reuptake by the antidepressant citalopram in the locus coeruleus modulates the rat brain noradrenergic transmission in vivo. Neuropharmacology 39:2036-2043.

Matsumoto T, Maeno Y, Kato H, Seko-Nakamura Y, Monma-Ohtaki J, Ishiba A, Nagao M, Aoki Y (2014) 5-hydroxytryptamine- and dopamine-releasing effects of ring-substituted amphetamines on rat brain: a comparative study using in vivo microdialysis. European Neuropsychopharmacology 24:1362-1370.

Maurel D, Comps-Agrar L, Brock C, Rives ML, Bourrier E, Ayoub MA, Bazin H, Tinel N, Durroux T, Prezeau L, Trinquet E, Pin JP (2008) Cell-surface protein-protein interaction analysis with time-resolved FRET and snap-tag technologies: application to GPCR oligomerization. Nature Methods 5:561-567.

May JA, Chen HH, Rusinko A, Lynch VM, Sharif NA, McLaughlin MA (2003) A novel and selective 5-HT2 receptor agonist with ocular hypotensive activity: (S)-(+)-1-(2-aminopropyl)-8,9-dihydropyrano[3,2-e]indole. Journal of Medicinal Chemistry 46:4188-4195.

Mazzola-Pomietto P, Aulakh CS, Tolliver T, Murphy DL (1997) Functional subsensitivity of 5-HT2A and 5-HT2C receptors mediating hyperthermia following acute and chronic treatment with 5-HT2A/2C receptor antagonists. Psychopharmacology 130:144-151.

Mazzola-Pomietto P, Aulakh CS, Wozniak KM, Hill JL, Murphy DL (1995) Evidence that 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced hyperthermia in rats is mediated by stimulation of 5-HT2A receptors. Psychopharmacology 117:193-199.

McFarland K, Price DL, Bonhaus DW (2011) Pimavanserin, a 5-HT2A inverse agonist, reverses psychosis-like behaviors in a rodent model of Parkinson's disease. Behavioural Pharmacology 22:681-692.

McGrath J, Saha S, Chant D, Welham J (2008) Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiologic Reviews 30:67-76.

McGrath J, Saha S, Welham J, El Saadi O, MacCauley C, Chant D (2004) A systematic review of the incidence of schizophrenia: the distribution of rates and the influence of sex, urbanicity, migrant status and methodology. BMC Medicine 2:13.

McGrath JJ (2005) Myths and plain truths about schizophrenia epidemiology--the NAPE lecture 2004. Acta Psychiatrica Scandinavica 111:4-11.

McGuffin P, Owen MJ, Farmer AE (1995) Genetic basis of schizophrenia. Lancet 346:678-682.

Meana JJ, Barturen F, Garcia-Sevilla JA (1989) Characterization and regional distribution of alpha 2-adrenoceptors in postmortem human brain using the full agonist [3H]UK 14304. Journal of Neurochemistry 52:1210-1217.

Meana JJ, Herrera-Marschitz M, Goiny M, Silveira R (1997) Modulation of catecholamine release by alpha 2-adrenoceptors and I1-imidazoline receptors in rat brain. Brain Research 744:216-226.

Mednick SA, Machon RA, Huttunen MO, Bonett D (1988) Adult schizophrenia following prenatal exposure to an influenza epidemic. Archives of General Psychiatry 45:189-192.

Melkersson K, Dahl ML (2004) Adverse metabolic effects associated with atypical antipsychotics: literature review and clinical implications. Drugs 64:701-723.

Meltzer HY (1989) Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. Psychopharmacology 99 Suppl:S18-27.

Meltzer HY (1999) Treatment of schizophrenia and spectrum disorders: pharmacotherapy, psychosocial treatments, and neurotransmitter interactions. Biological Psychiatry 46:1321-1327.

Meltzer HY (2001) Treatment of suicidality in schizophrenia. Annals of the New York Academy of Sciences 932:44-60.

Meltzer HY, Matsubara S, Lee JC (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values. Journal of Pharmacology and Experimental Therapeutics 251:238-246.

Mengod G (2011) Serotonin 5-HT2C Receptors: Chemical Neuroanatomy in the Mammalian Brain. In 5-HT2C Receptors in the Pathophysiology of CNS Disease: Humana Press. New York, USA.

Merritt EA, Hol WG (1995) AB5 toxins. Current Opinion in Structural Biology 5:165-171.

Messias EL, Chen CY, Eaton WW (2007) Epidemiology of schizophrenia: review of findings and myths. Psychiatric Clinics of North America 30:323-338.

Meunier CN, Chameau P, Fossier PM (2017) Modulation of Synaptic Plasticity in the Cortex Needs to Understand All the Players. Frontiers in Synaptic Neuroscience 9:2.

Millan MJ, 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 37:953-955.

Millan MJ, Gobert A, Lejeune F, Dekeyne A, Newman-Tancredi A, Pasteau V, Rivet JM, Cussac D (2003) The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine2C receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. Journal of Pharmacology and Experimental Therapeutics 306:954-964.

Millan MJ, Marin P, Bockaert J, Mannoury la Cour C (2008) Signaling at G-protein-coupled serotonin receptors: recent advances and future research directions. Trends in Pharmacological Sciences 29:454-464.

Milligan G (2007) G protein-coupled receptor dimerisation: molecular basis and relevance to function. Biochimica et Biophysica Acta 1768:825-835.

Milligan G, Kostenis E (2006) Heterotrimeric G-proteins: a short history. British Journal of Pharmacology 147 Suppl 1:S46-55.

Miner LA, Backstrom JR, Sanders-Bush E, Sesack SR (2003) Ultrastructural localization of serotonin2A receptors in the middle layers of the rat prelimbic prefrontal cortex. Neuroscience 116:107-117.

Minzenberg MJ, Yoon JH (2011) An index of relative central alpha-adrenergic receptor antagonism by antipsychotic medications. Experimental and Clinical Psychopharmacology 19:31-39.

Miranda-Azpiazu (2014). Biochemistry, function and pharmacology of serotonin 5-HT2A and group II metabotropic glutamate receptors in postmortem human brain. Interest for psychosis. Doctoral Thesis. University of the Basque Country, Spain.

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. Journal of Neuroscience 17:2921-2927.

Moghaddam B, Adams BW (1998) Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. Science 281:1349-1352.

Moghaddam B, Bunney BS (1990) Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an in vivo microdialysis study. Journal of Neurochemistry 54:1755-1760.

Molinaro G, Traficante A, Riozzi B, Di Menna L, Curto M, Pallottino S, Nicoletti F, Bruno V, Battaglia G (2009) Activation of mGlu2/3 metabotropic glutamate receptors negatively regulates the stimulation of inositol phospholipid hydrolysis mediated by 5-hydroxytryptamine2A serotonin receptors in the frontal cortex of living mice. Molecular Pharmacology 76:379-387.

Molineaux SM, Jessell TM, Axel R, Julius D (1989) 5-HT1c receptor is a prominent serotonin receptor subtype in the central nervous system. Proceedings of the National Academy of Sciences of the United States of America 86:6793-6797.

Molliver ME, Kristt DA (1975) The fine structural demonstration of monoaminergic synapses in immature rat neocortex. Neuroscience Letters 1:305-310.

Moloney RD, Dinan TG, Cryan JF (2015) Strain-dependent variations in visceral sensitivity: relationship to stress, anxiety and spinal glutamate transporter expression. Genes, Brain and Behavior 14:319-329.

Moreno JL, Holloway T, Albizu L, Sealfon SC, Gonzalez-Maeso J (2011a) Metabotropic glutamate mGlu2 receptor is necessary for the pharmacological and behavioral effects induced by hallucinogenic 5-HT2A receptor agonists. Neuroscience Letters 493:76-79.

Moreno JL, Holloway T, Gonzalez-Maeso J (2013) G protein-coupled receptor heterocomplexes in neuropsychiatric disorders. Progress in Molecular Biology and Translational Science 117:187-205.

Moreno JL, Kurita M, Holloway T, Lopez J, Cadagan R, Martinez-Sobrido L, Garcia-Sastre A, Gonzalez-Maeso J (2011b) Maternal influenza viral infection causes schizophrenia-like alterations of 5-HT(2)A and mGlu(2) receptors in the adult offspring. Journal of Neuroscience 31:1863-1872.

Moreno JL, Miranda-Azpiazu P, Garcia-Bea A, Younkin J, Cui M, Kozlenkov A, Ben-Ezra A, Voloudakis G, Fakira AK, Baki L, Ge Y, Georgakopoulos A, Moron JA, Milligan G, Lopez-Gimenez JF, Robakis NK, Logothetis DE, Meana JJ, Gonzalez-Maeso J (2016) Allosteric signaling through an mGlu2 and 5-HT2A heteromeric receptor complex and its potential contribution to schizophrenia. Science Signaling 9:ra5.

Moreno JL, Muguruza C, Umali A, Mortillo S, Holloway T, Pilar-Cuellar F, Mocci G, Seto J, Callado LF, Neve RL, Milligan G, Sealfon SC, Lopez-Gimenez JF, Meana JJ, Benson DL, Gonzalez-Maeso J (2012) Identification of three residues essential for 5-hydroxytryptamine 2A-metabotropic glutamate 2 (5-HT2A.mGlu2) receptor heteromerization and its psychoactive behavioral function. Journal of Biological Chemistry 287:44301-44319.

Morgan CJ, Mofeez A, Brandner B, Bromley L, Curran HV (2004) Acute effects of ketamine on memory systems and psychotic symptoms in healthy volunteers. Neuropsychopharmacology 29:208-218.

Moroni F, Cozzi A, Sili M, Mannaioni G (2012) Kynurenic acid: a metabolite with multiple actions and multiple targets in brain and periphery. Journal of Neural Transmission 119:133-139.

Morrison JH, Grzanna R, Molliver ME, Coyle JT (1978) The distribution and orientation of noradrenergic fibers in neocortex of the rat: an immunofluorescence study. Journal of Comparative Neurology 181:17-39.

Morrison PD, Stone JM (2011) Synthetic delta-9-tetrahydrocannabinol elicits schizophrenia-like negative symptoms which are distinct from sedation. Human Psychopharmacology 26:77-80.

Morrison PD, Zois V, McKeown DA, Lee TD, Holt DW, Powell JF, Kapur S, Murray RM (2009) The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. Psychological Medicine 39:1607-1616.

Mortensen PB, Pedersen CB, Westergaard T, Wohlfahrt J, Ewald H, Mors O, Andersen PK, Melbye M (1999) Effects of family history and place and season of birth on the risk of schizophrenia. The New England Journal of Medicine 340:603-608.

Moutkine I, Quentin E, Guiard BP, Maroteaux L, Doly S (2017) Heterodimers of serotonin receptor subtypes 2 are driven by 5-HT2C protomers. Journal of Biological Chemistry 292:6352-6368.

Mueser KT, McGurk SR (2004) Schizophrenia. Lancet 363:2063-2072.

Muguruza C, Meana JJ, Callado LF (2016) Group II Metabotropic Glutamate Receptors as Targets for Novel Antipsychotic Drugs. Frontiers in Pharmacology 7:130.

Muguruza C, Miranda-Azpiazu P, Diez-Alarcia R, Morentin B, Gonzalez-Maeso J, Callado LF, Meana JJ (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.

Muguruza C, Moreno JL, Umali A, Callado LF, Meana JJ, Gonzalez-Maeso J (2013) Dysregulated 5-HT(2A) receptor binding in postmortem frontal cortex of schizophrenic subjects. European Neuropsychopharmacology 23:852-864.

Muller N, Weidinger E, Leitner B, Schwarz MJ (2015) The role of inflammation in schizophrenia. Frontiers in Neuroscience 9:372.

Munk C, Isberg V, Mordalski S, Harpsoe K, Rataj K, Hauser AS, Kolb P, Bojarski AJ, Vriend G, Gloriam DE (2016) GPCRdb: the G protein-coupled receptor database - an introduction. British Journal of Pharmacology 173:2195-2207.

Murase S, Grenhoff J, Chouvet G, Gonon FG, Svensson TH (1993) Prefrontal cortex regulates burst firing and transmitter release in rat mesolimbic dopamine neurons studied in vivo. Neuroscience Letters 157:53-56.

Murray CJ, Lopez AD (1996) Evidence-based health policy--lessons from the Global Burden of Disease Study. Science 274:740-743.

Murray RM, Lewis SW, Reveley AM (1985) Towards an aetiological classification of schizophrenia. Lancet 1:1023-1026.

Muschamp JW, Regina MJ, Hull EM, Winter JC, Rabin RA (2004) Lysergic acid diethylamide and [-]-2,5-dimethoxy-4-methylamphetamine increase extracellular glutamate in rat prefrontal cortex. Brain Research 1023:134-140.

Narendran R, Frankle WG, Keefe R, Gil R, Martinez D, Slifstein M, Kegeles LS, Talbot PS, Huang Y, Hwang DR, Khenissi L, Cooper TB, Laruelle M, Abi-Dargham A (2005) Altered prefrontal dopaminergic function in chronic recreational ketamine users. American Journal of Psychiatry 162:2352-2359.

Narr KL, Bilder RM, Toga AW, Woods RP, Rex DE, Szeszko PR, Robinson D, Sevy S, Gunduz-Bruce H, Wang YP, DeLuca H, Thompson PM (2005) Mapping cortical thickness and gray matter concentration in first episode schizophrenia. Cerebral Cortex 15:708-719.

Nemani K, Hosseini Ghomi R, McCormick B, Fan X (2015) Schizophrenia and the gut-brain axis. Progress in Neuro-Psychopharmacology & Biological Psychiatry 56:155-160.

Nestler EJ, Duman RS (1999) Heterotrimeric G Proteins. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition. American Society for Neurochemistry. Philadelphia, USA.

Nestler EJ, Hyman SE (2010) Animal models of neuropsychiatric disorders. Nature Neuroscience 13:1161-1169.

Nicholas AP, Pieribone V, Hokfelt T (1993) Distributions of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: an in situ hybridization study. Journal of Comparative Neurology 328:575-594.

Nisenbaum LK, Downing AM, Zhao F, Millen BA, Munsie L, Kinon BJ, Adams DH, Gomez JC, Penny MA (2016) Serotonin 2A Receptor SNP rs7330461 Association with Treatment Response to Pomaglumetad Methionil in Patients with Schizophrenia. Journal of Personalized Medicine 6.

Nocjar C, Alex KD, Sonneborn A, Abbas AI, Roth BL, Pehek EA (2015) Serotonin-2C and -2a receptor co-expression on cells in the rat medial prefrontal cortex. Neuroscience 297:22-37.

Nocjar C, Roth BL, Pehek EA (2002) Localization of 5-HT(2A) receptors on dopamine cells in subnuclei of the midbrain A10 cell group. Neuroscience 111:163-176.

Nomikos GG, Iurlo M, Andersson JL, Kimura K, Svensson TH (1994) Systemic administration of amperozide, a new atypical antipsychotic drug, preferentially increases dopamine release in the rat medial prefrontal cortex. Psychopharmacology 115:147-156.

Nomura S, Bouhadana M, Morel C, Faure P, Cauli B, Lambolez B, Hepp R (2014) Noradrenalin and dopamine receptors both control cAMP-PKA signaling throughout the cerebral cortex. Frontiers in Cellular Neuroscience 8:247.

Nosek K, Dennis K, Andrus BM, Ahmadiyeh N, Baum AE, Solberg Woods LC, Redei EE (2008) Context and strain-dependent behavioral response to stress. Behavioral and Brain Functions 4:23.

Ogren SO, Goldstein M (1994) Phencyclidine- and dizocilpine-induced hyperlocomotion are differentially mediated. Neuropsychopharmacology 11:167-177.

Ohishi H, Neki A, Mizuno N (1998) Distribution of a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat and mouse: an immunohistochemical study with a monoclonal antibody. Neuroscience Research 30:65-82.

Ohuoha DC, Hyde TM, Kleinman JE (1993) The role of serotonin in schizophrenia: an overview of the nomenclature, distribution and alterations of serotonin receptors in the central nervous system. Psychopharmacology 112:S5-15.

Olabi B, Ellison-Wright I, McIntosh AM, Wood SJ, Bullmore E, Lawrie SM (2011) Are there progressive brain changes in schizophrenia? A meta-analysis of structural magnetic resonance imaging studies. Biological Psychiatry 70:88-96.

Olney JW, Farber NB (1995) NMDA antagonists as neurotherapeutic drugs, psychotogens, neurotoxins, and research tools for studying schizophrenia. Neuropsychopharmacology 13:335-345.

Olpe HR, Steinmann M (1991) Responses of locus coeruleus neurons to neuropeptides. Progress in Brain Research 88:241-248.

Olpe HR, Steinmann MW, Brugger F, Pozza MF (1989) Excitatory amino acid receptors in rat locus coeruleus. An extracellular in vitro study. Naunyn-Schmiedeberg's Archives of Pharmacology 339:312-314.

Ordway GA, Stockmeier CA, Cason GW, Klimek V (1997) Pharmacology and distribution of norepinephrine transporters in the human locus coeruleus and raphe nuclei. Journal of Neuroscience 17:1710-1719.

Orejarena MJ, Lanfumey L, Maldonado R, Robledo P (2011) Involvement of 5-HT2A receptors in MDMA reinforcement and cue-induced reinstatement of MDMA-seeking behaviour. International Journal of Neuropsychopharmacology 14:927-940.

Ortega JE, Fernandez-Pastor B, Callado LF, Meana JJ (2010) In vivo potentiation of reboxetine and citalopram effect on extracellular noradrenaline in rat brain by alpha2-adrenoceptor antagonism. European Neuropsychopharmacology 20:813-822.

Ortega JE, Mendiguren A, Pineda J, Meana JJ (2012) Regulation of central noradrenergic activity by 5-HT(3) receptors located in the locus coeruleus of the rat. Neuropharmacology 62:2472-2479.

Overton PG, Clark D (1997) Burst firing in midbrain dopaminergic neurons. Brain Research Reviews 25:312-334.

Paasonen J, Salo RA, Ihalainen J, Leikas JV, Savolainen K, Lehtonen M, Forsberg MM, Grohn O (2017) Dose-response effect of acute phencyclidine on functional connectivity and dopamine levels, and their association with schizophrenia-like symptom classes in rat. Neuropharmacology 119:15-25.

Palczewski K, Orban T (2013) From atomic structures to neuronal functions of g protein-coupled receptors. Annual Review of Neuroscience 36:139-164.

Pan WH, Yang SY, Lin SK (2004) Neurochemical interaction between dopaminergic and noradrenergic neurons in the medial prefrontal cortex. Synapse 53:44-52.

Paquet M, Tremblay M, Soghomonian JJ, Smith Y (1997) AMPA and NMDA glutamate receptor subunits in midbrain dopaminergic neurons in the squirrel monkey: an immunohistochemical and in situ hybridization study. Journal of Neuroscience 17:1377-1396.

Parellada E, Catafau AM, Bernardo M, Lomena F, Gonzalez-Monclus E, Setoain J (1994) Prefrontal dysfunction in young acute neuroleptic-naive schizophrenic patients: a resting and activation SPECT study. Psychiatry Research 55:131-139.

Pascual J, del Arco C, Gonzalez AM, Pazos A (1992) Quantitative light microscopic autoradiographic localization of alpha 2-adrenoceptors in the human brain. Brain Research 585:116-127.

Pasqualetti M, Ori M, Castagna M, Marazziti D, Cassano GB, Nardi I (1999) Distribution and cellular localization of the serotonin type 2C receptor messenger RNA in human brain. Neuroscience 92:601-611.

Pasternak O, Kubicki M, Shenton ME (2016) In vivo imaging of neuroinflammation in schizophrenia. Schizophrenia Research 173:200-212.

Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, Avedisova AS, Bardenstein LM, Gurovich IY, Morozova MA, Mosolov SN, Neznanov NG, Reznik AM, Smulevich AB, Tochilov VA, Johnson BG, Monn JA, Schoepp DD (2007) Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nature Medicine 13:1102-1107.

Paxinos G, Watson C (1986). The rat brain in stereotaxic coordinates. Academic press. 2nd edition. Orlando, USA.

Pazos A, Cortes R, Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Research 346:231-249.

Pazos A, Gonzalez AM, Pascual J, Meana JJ, Barturen F, Garcia-Sevilla JA (1988) Alpha 2-adrenoceptors in human forebrain: autoradiographic visualization and biochemical parameters using the agonist [3H]UK-14304. Brain Research 475:361-365.

Pazos A, Probst A, Palacios JM (1987) Serotonin receptors in the human brain--III. Autoradiographic mapping of serotonin-1 receptors. Neuroscience 21:97-122.

Peen J, Dekker J (1997) Admission rates for schizophrenia in The Netherlands: an urban/rural comparison. Acta Psychiatrica Scandinavica 96:301-305.

Pehek EA (1996) Local infusion of the serotonin antagonists ritanserin or ICS 205,930 increases in vivo dopamine release in the rat medial prefrontal cortex. Synapse 24:12-18.

Pehek EA, Hernan AE (2015) Stimulation of glutamate receptors in the ventral tegmental area is necessary for serotonin-2 receptor-induced increases in mesocortical dopamine release. Neuroscience 290:159-164.

Pehek EA, McFarlane HG, Maguschak K, Price B, Pluto CP (2001) M100,907, a selective 5-HT(2A) antagonist, attenuates dopamine release in the rat medial prefrontal cortex. Brain Research 888:51-59.

Pehek EA, Meltzer HY, Yamamoto BK (1993) The atypical antipsychotic drug amperozide enhances rat cortical and striatal dopamine efflux. European Journal of Pharmacology 240:107-109.

Pehek EA, Nocjar C, Roth BL, Byrd TA, Mabrouk OS (2006) Evidence for the preferential involvement of 5-HT2A serotonin receptors in stress- and drug-induced dopamine release in the rat medial prefrontal cortex. Neuropsychopharmacology 31:265-277.

Pessia M, Jiang ZG, North RA, Johnson SW (1994) Actions of 5-hydroxytryptamine on ventral tegmental area neurons of the rat in vitro. Brain Research 654:324-330.

Petralia RS, Wang YX, Niedzielski AS, Wenthold RJ (1996) The metabotropic glutamate receptors, mGluR2 and mGluR3, show unique postsynaptic, presynaptic and glial localizations. Neuroscience 71:949-976.

Phillipson OT (1979) Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: a horseradish peroxidase study in the rat. Journal of Comparative Neurology 187:117-143.

Pickel VM, Joh TH, Reis DJ (1977) A serotonergic innervation of noradrenergic neurons in nucleus locus coeruleus: demonstration by immunocytochemical localization of the transmitter specific enzymes tyrosine and tryptophan hydroxylase. Brain Research 131:197-214.

Pigott A, Frescas S, McCorvy JD, Huang XP, Roth BL, Nichols DE (2012) trans-2-(2,5-Dimethoxy-4-iodophenyl)cyclopropylamine and trans-2-(2,5-dimethoxy-4-bromophenyl)cyclopropylamine as potent agonists for the 5-HT(2) receptor family. Beilstein Journal of Organic Chemistry 8:1705-1709.

Pin JP, Duvoisin R (1995) The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34:1-26.

Pino O, Guilera G, Gomez-Benito J, Najas-Garcia A, Rufian S, Rojo E (2014) Neurodevelopment or neurodegeneration: review of theories of schizophrenia. Actas Espanolas de Psiquiatria 42:185-195.

Pistis M, Ferraro L, Pira L, Flore G, Tanganelli S, Gessa GL, Devoto P (2002) Delta(9)-tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study. Brain Research 948:155-158.

Pittaluga A, Segantini D, Feligioni M, Raiteri M (2005) Extracellular protons differentially potentiate the responses of native AMPA receptor subtypes regulating neurotransmitter release. British Journal of Pharmacology 144:293-299.

Pletscher A, Shore PA, Brodie BB (1955) Serotonin release as a possible mechanism of reserpine action. Science 122:374-375.

Pompeiano M, Palacios JM, Mengod G (1994) Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. Brain Research Molecular Brain Research 23:163-178.

Popa D, Lena C, Fabre V, Prenat C, Gingrich J, Escourrou P, Hamon M, Adrien J (2005) Contribution of 5-HT2 receptor subtypes to sleep-wakefulness and respiratory control, and functional adaptations in knock-out mice lacking 5-HT2A receptors. Journal of Neuroscience 25:11231-11238.

Porter RH, Benwell KR, Lamb H, Malcolm CS, Allen NH, Revell DF, Adams DR, Sheardown MJ (1999) Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells. British Journal of Pharmacology 128:13-20.

Powell SB, Zhou X, Geyer MA (2009) Prepulse inhibition and genetic mouse models of schizophrenia. Behavioural Brain Research 204:282-294.

Pralong E, Magistretti P, Stoop R (2002) Cellular perspectives on the glutamate-monoamine interactions in limbic lobe structures and their relevance for some psychiatric disorders. Progress in Neurobiology 67:173-202.

Pranzatelli MR (1990) Evidence for involvement of 5-HT2 and 5-HT1C receptors in the behavioral effects of the 5-HT agonist 1-(2,5-dimethoxy-4-iodophenyl aminopropane)-2 (DOI). Neuroscience Letters 115:74-80.

Prisco S, Esposito E (1995) Differential effects of acute and chronic fluoxetine administration on the spontaneous activity of dopaminergic neurones in the ventral tegmental area. British Journal of Pharmacology 116:1923-1931.

Prisco S, Pagannone S, Esposito E (1994) Serotonin-dopamine interaction in the rat ventral tegmental area: an electrophysiological study in vivo. Journal of Pharmacology and Experimental Therapeutics 271:83-90.

Pudovkina OL, Kawahara Y, de Vries J, Westerink BH (2001) The release of noradrenaline in the locus coeruleus and prefrontal cortex studied with dual-probe microdialysis. Brain Research 906:38-45.

Puig MV (2004). Control recíproco entre la corteza prefrontal y los núcleos del rafe. Papel de los receptores de serotonina. Doctoral Thesis. Universidad de Barcelona.

Puig MV (2011) Serotonergic modulation of the prefrontal cortex: from neurons to brain waves. Psychiatric Disorders. Worldwide Advances. Uehara T (Ed.), InTech.

Puig MV, Celada P, Diaz-Mataix L, Artigas F (2003) In vivo modulation of the activity of pyramidal neurons in the rat medial prefrontal cortex by 5-HT2A receptors: relationship to thalamocortical afferents. Cerebral Cortex 13:870-882.

Puig MV, Gulledge AT (2011) Serotonin and prefrontal cortex function: neurons, networks, and circuits. Molecular Neurobiology 44:449-464.

Quarta D, Large CH (2011) Effects of lamotrigine on PCP-evoked elevations in monoamine levels in the medial prefrontal cortex of freely moving rats. Journal of Psychopharmacology 25:1703-1711.

Quednow BB, Kometer M, Geyer MA, Vollenweider FX (2012) Psilocybin-induced deficits in automatic and controlled inhibition are attenuated by ketanserin in healthy human volunteers. Neuropsychopharmacology 37:630-640.

Quesseveur G, Reperant C, David DJ, Gardier AM, Sanchez C, Guiard BP (2013) 5-HT(2)A receptor inactivation potentiates the acute antidepressant-like activity of escitalopram: involvement of the noradrenergic system. Experimental Brain Research 226:285-295.

Raja (2009) Pharmacotherapy update: risperidone in the treatment os schizophrenia. Clinical Medicine: Therapeutics 1:1199-1214.

Rang HP, Dale MM, Ritter JM, Flower R (2007) Rang and Dale's Pharmacology. 6th edition. Edinburgh, United Kindgom.

Raote I, Bhattacharya A, Panicker MM (2007) Serotonin 2A (5-HT2A) Receptor Function: Ligand-Dependent Mechanisms and Pathways. In: Serotonin Receptors in Neurobiology. Amitabha Chattopadhyay. Hyderabad, India.

Rasmussen K, Aghajanian GK (1986) Effect of hallucinogens on spontaneous and sensory-evoked locus coeruleus unit activity in the rat: reversal by selective 5-HT2 antagonists. Brain Research 385:395-400.

Ray TS (2010) Psychedelics and the human receptorome. PloS One 5:e9019.

Realini N, Rubino T, Parolaro D (2009) Neurobiological alterations at adult age triggered by adolescent exposure to cannabinoids. Pharmacological Research 60:132-138.

Ribeiro FM, Vieira LB, Pires RG, Olmo RP, Ferguson SS (2017) Metabotropic glutamate receptors and neurodegenerative diseases. Pharmacological Research 115:179-191.

Rikke BA, Yerg JE, 3rd, Battaglia ME, Nagy TR, Allison DB, Johnson TE (2003) Strain variation in the response of body temperature to dietary restriction. Mechanisms of Ageing and Development 124:663-678.

Rodriguez JJ, Doherty MD, Pickel VM (2000) N-methyl-D-aspartate (NMDA) receptors in the ventral tegmental area: subcellular distribution and colocalization with 5-hydroxytryptamine(2A) receptors. Journal of Neuroscience Research 60:202-211.

Rogers GB, Keating DJ, Young RL, Wong ML, Licinio J, Wesselingh S (2016) From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. Molecular Psychiatry 21:738-748.

Rosso IM, Makris N, Thermenos HW, Hodge SM, Brown A, Kennedy D, Caviness VS, Faraone SV, Tsuang MT, Seidman LJ (2010) Regional prefrontal cortex gray matter volumes in youth at familial risk for schizophrenia from the Harvard Adolescent High Risk Study. Schizophrenia Research 123:15-21.

Rotaru DC, Lewis DA, Gonzalez-Burgos G (2012) The role of glutamatergic inputs onto parvalbumin-positive interneurons: relevance for schizophrenia. Reviews in the Neurosciences 23:97-109.

Roth BL (2011) Irving Page Lecture: 5-HT(2A) serotonin receptor biology: interacting proteins, kinases and paradoxical regulation. Neuropharmacology 61:348-354.

Rowland LM, Bustillo JR, Mullins PG, Jung RE, Lenroot R, Landgraf E, Barrow R, Yeo R, Lauriello J, Brooks WM (2005) Effects of ketamine on anterior cingulate glutamate metabolism in healthy humans: a 4-T proton MRS study. American Journal of Psychiatry 162:394-396.

Rubesa G, Gudelj L, Kubinska N (2011) Etiology of schizophrenia and therapeutic options. Psychiatria Danubina 23:308-315.

Rubino T, Vigano D, Realini N, Guidali C, Braida D, Capurro V, Castiglioni C, Cherubino F, Romualdi P, Candeletti S, Sala M, Parolaro D (2008) Chronic delta 9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. Neuropsychopharmacology 33:2760-2771.

Ruiz-Ortega JA, Ugedo L (1997) Activation of 5-HT1A receptors potentiates the clonidine inhibitory effect in the locus coeruleus. European Journal of Pharmacology 333:159-162.

Saiardi A, Bozzi Y, Baik JH, Borrelli E (1997) Antiproliferative role of dopamine: loss of D2 receptors causes hormonal dysfunction and pituitary hyperplasia. Neuron 19:115-126.

Salmi P, Ahlenius S (1998) Evidence for functional interactions between 5-HT1A and 5-HT2A receptors in rat thermoregulatory mechanisms. Pharmacology & Toxicology 82:122-127.

Salon JA, Lodowski DT, Palczewski K (2011) The significance of G protein-coupled receptor crystallography for drug discovery. Pharmacological Reviews 63:901-937.

Sams-Dodd F (1998b) Effects of continuous D-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. Neuropsychopharmacology 19:18-25.

Sams-Dodd F (1998a) Effects of dopamine agonists and antagonists on PCP-induced stereotyped behaviour and social isolation in the rat social interaction test. Psychopharmacology 135:182-193.

Sams-Dodd F (1999) Phencyclidine in the social interaction test: an animal model of schizophrenia with face and predictive validity. Reviews in the Neurosciences 10:59-90.

Samuels ER, Szabadi E (2008) Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part I: principles of functional organisation. Current Neuropharmacology 6:235-253.

Sanchez-Blazquez P, Garcia-Espana A, Garzon J (1995) In vivo injection of antisense oligodeoxynucleotides to G alpha subunits and supraspinal analgesia evoked by mu and delta opioid agonists. Journal of Pharmacology and Experimental Therapeutics 275:1590-1596.

Santana N, Artigas F (2016) Expression of Serotonin2C Receptors in Pyramidal and GABAergic Neurons of Rat Prefrontal Cortex: A Comparison with Striatum. Cerebral Cortex.

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. Cerebral Cortex 14:1100-1109.

Santiago M, Machado A, Cano J (1993) Regulation of the prefrontal cortical dopamine release by GABAA and GABAB receptor agonists and antagonists. Brain Research 630:28-31.

Santiago M, Westerink BH (1991) The regulation of dopamine release from nigrostriatal neurons in conscious rats: the role of somatodendritic autoreceptors. European Journal of Pharmacology 204:79-85.

Sara SJ (2009) The locus coeruleus and noradrenergic modulation of cognition. Nature Reviews Neuroscience 10:211-223.

Sara SJ, Herve-Minvielle A (1995) Inhibitory influence of frontal cortex on locus coeruleus neurons. Proceedings of the National Academy of Sciences of the United States of America 92:6032-6036.

Sartorius LJ, Nagappan G, Lipska BK, Lu B, Sei Y, Ren-Patterson R, Li Z, Weinberger DR, Harrison PJ (2006) Alternative splicing of human metabotropic glutamate receptor 3. Journal of Neurochemistry 96:1139-1148.

Sarva H, Henchcliffe C (2016) Evidence for the use of pimavanserin in the treatment of Parkinson's disease psychosis. Therapeutic Advances in Neurological Disorders 9:462-473.

Sayo A, Jennings RG, Van Horn JD (2012) Study factors influencing ventricular enlargement in schizophrenia: a 20 year follow-up meta-analysis. NeuroImage 59:154-167.

Schellekens H, van Oeffelen WE, Dinan TG, Cryan JF (2013) Promiscuous dimerization of the growth hormone secretagogue receptor (GHS-R1a) attenuates ghrelin-mediated signaling. Journal of Biological Chemistry 288:181-191.

Schioth HB, Fredriksson R (2005) The GRAFS classification system of G-protein coupled receptors in comparative perspective. General and Comparative Endocrinology 142:94-101.

Schmid CL, Bohn LM (2010) Serotonin, but not N-methyltryptamines, activates the serotonin 2A receptor via a β-arrestin2/Src/Akt signaling complex in vivo. Journal of Neuroscience 30:13513-13524.

Schmid CL, Raehal KM, Bohn LM (2008) Agonist-directed signaling of the serotonin 2A receptor depends on beta-arrestin-2 interactions in vivo. Proceedings of the National Academy of Sciences of the United States of America 105:1079-1084.

Schmid CL, Streicher JM, Meltzer HY, Bohn LM (2014) Clozapine acts as an agonist at serotonin 2A receptors to counter MK-801-induced behaviors through a betaarrestin2-independent activation of Akt. Neuropsychopharmacology 39:1902-1913.

Schmidt MJ, Mirnics K (2015) Neurodevelopment, GABA system dysfunction, and schizophrenia. Neuropsychopharmacology 40:190-206.

Schneider M, Koch M (2003) Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. Neuropsychopharmacology 28:1760-1769.

Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S, Millan MJ (1995) (1-(2,5-dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT) 2A receptors: modulation by novel 5-HT2A/2C antagonists, D1 antagonists and 5-HT1A agonists. Journal of Pharmacology and Experimental Therapeutics 273:101-112.

Schreiber R, Brocco M, Millan MJ (1994) Blockade of the discriminative stimulus effects of DOI by MDL 100,907 and the 'atypical' antipsychotics, clozapine and risperidone. European Journal of Pharmacology 264:99-102.

Schwartzer JJ, Careaga M, Coburn MA, Rose DR, Hughes HK, Ashwood P (2016) Behavioral impact of maternal allergic-asthma in two genetically distinct mouse strains. Brain, Behavior, and Immunity.

Scruggs JL, Patel S, Bubser M, Deutch AY (2000) DOI-Induced activation of the cortex: dependence on 5-HT2A heteroceptors on thalamocortical glutamatergic neurons. Journal of Neuroscience 20:8846-8852.

Scruggs JL, Schmidt D, Deutch AY (2003) The hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) increases cortical extracellular glutamate levels in rats. Neuroscience Letters 346:137-140.

Sealfon SC, Gonzalez-Maeso J (2008) Receptor pair for schizophrenia. Pediatric Research 64:1.

Seamans J (2008) Losing inhibition with ketamine. Nature Chemical Biology 4:91-93.

Seeman P (2014) Clozapine, a fast-off-D2 antipsychotic. ACS Chemical Neuroscience 5:24-29.

Seguret A, Collignon B, Halloy J (2016) Strain differences in the collective behaviour of zebrafish (Danio rerio) in heterogeneous environment. Royal Society Open Science 3:160451.

Sei Y, Skolnick P, Arora PK (1992) Strain variation in immune response and behavior following the death of cage cohorts. International Journal of Neuroscience 65:247-258.

Selemon LD, Zecevic N (2015) Schizophrenia: a tale of two critical periods for prefrontal cortical development. Translational Psychiatry 5:e623.

Self DW, Terwilliger RZ, Nestler EJ, Stein L (1994) Inactivation of Gi and G(o) proteins in nucleus accumbens reduces both cocaine and heroin reinforcement. Journal of Neuroscience 14:6239-6247.

Semenova S, Markou A (2010) The alpha2 adrenergic receptor antagonist idazoxan, but not the serotonin-2A receptor antagonist M100907, partially attenuated reward deficits associated with nicotine, but not amphetamine, withdrawal in rats. European Neuropsychopharmacology 20:731-746.

Sesack SR, Carr DB, Omelchenko N, Pinto A (2003) Anatomical substrates for glutamate-dopamine interactions: evidence for specificity of connections and extrasynaptic actions. Annals of the New York Academy of Sciences 1003:36-52.

Sesack SR, Snyder CL, Lewis DA (1995) Axon terminals immunolabeled for dopamine or tyrosine hydroxylase synapse on GABA-immunoreactive dendrites in rat and monkey cortex. Journal of Comparative Neurology 363:264-280.

Shah S, Breivogel C, Selly D, Munirathinam G, Childers S, Yoburn BC (1997) Time-dependent effects of in vivo pertussis toxin on morphine analgesia and G-proteins in mice. Pharmacology Biochemistry and Behavior 56:465-469.

Shepherd AM, Laurens KR, Matheson SL, Carr VJ, Green MJ (2012) Systematic meta-review and quality assessment of the structural brain alterations in schizophrenia. Neuroscience & Biobehavioral Reviews 36:1342-1356.

Shi L, Fatemi SH, Sidwell RW, Patterson PH (2003) Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. Journal of Neuroscience 23:297-302.

Shi WX, Nathaniel P, Bunney BS (1995) Ritanserin, a 5-HT2A/2C antagonist, reverses direct dopamine agonist-induced inhibition of midbrain dopamine neurons. Journal of Pharmacology and Experimental Therapeutics 274:735-740.

Shimizu N, Imamoto K (1970) Fine structure of the locus coeruleus in the rat. Archivum histologicum Japonicum = Nihon soshikigaku kiroku 31:229-246.

Shoaib M, Spanagel R, Stohr T, Shippenberg TS (1995) Strain differences in the rewarding and dopamine-releasing effects of morphine in rats. Psychopharmacology 117:240-247.

Singewald N, Philippu A (1998) Release of neurotransmitters in the locus coeruleus. Progress in Neurobiology 56:237-267.

Sipes TA, Geyer MA (1994) Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats. Neuropharmacology 33:441-448.

Slifstein M, van de Giessen E, Van Snellenberg J, Thompson JL, Narendran R, Gil R, Hackett E, Girgis R, Ojeil N, Moore H, D'Souza D, Malison RT, Huang Y, Lim K, Nabulsi N, Carson RE, Lieberman JA, Abi-Dargham A (2015) Deficits in prefrontal cortical and extrastriatal dopamine release in schizophrenia: a positron emission tomographic functional magnetic resonance imaging study. JAMA Psychiatry 72:316-324.

Snyder SH (2008) Neuroscience: a complex in psychosis. Nature 452:38-39.

Sorensen SM, Kehne JH, Fadayel GM, Humphreys TM, Ketteler HJ, Sullivan CK, Taylor VL, Schmidt CJ (1993) Characterization of the 5-HT2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. Journal of Pharmacology and Experimental Therapeutics 266:684-691.

Spauwen J, Krabbendam L, Lieb R, Wittchen HU, van Os J (2006) Evidence that the outcome of developmental expression of psychosis is worse for adolescents growing up in an urban environment. Psychological Medicine 36:407-415.

Stahl SM (2016) Mechanism of action of pimavanserin in Parkinson's disease psychosis: targeting serotonin 5HT2A and 5HT2C receptors. CNS Spectrums 21:271-275.

Stahle L (2000) On mathematical models of microdialysis: geometry, steady-state models, recovery and probe radius. Advanced Drug Delivery Reviews 45:149-167.

Steeds H, Carhart-Harris RL, Stone JM (2015) Drug models of schizophrenia. Therapeutic Advances in Psychopharmacology 5:43-58.

Steen RG, Mull C, McClure R, Hamer RM, Lieberman JA (2006) Brain volume in first-episode schizophrenia: systematic review and meta-analysis of magnetic resonance imaging studies. British Journal of Psychiatry 188:510-518.

Stein L, Wise CD (1971) Possible etiology of schizophrenia: progressive damage to the noradrenergic reward system by 6-hydroxydopamine. Science 171:1032-1036.

Stein PE, Boodhoo A, Armstrong GD, Cockle SA, Klein MH, Read RJ (1994) The crystal structure of pertussis toxin. Structure 2:45-57.

Steinpreis RE (1996) The behavioral and neurochemical effects of phencyclidine in humans and animals: some implications for modeling psychosis. Behavioural Brain Research 74:45-55.

Stone JM, Dietrich C, Edden R, Mehta MA, De Simoni S, Reed LJ, Krystal JH, Nutt D, Barker GJ (2012) Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. Molecular Psychiatry 17:664-665.

Stuber GD, Britt JP, Bonci A (2012) Optogenetic modulation of neural circuits that underlie reward seeking. Biological Psychiatry 71:1061-1067.

Stuber GD, Mason AO (2013) Integrating optogenetic and pharmacological approaches to study neural circuit function: current applications and future directions. Pharmacological Reviews 65:156-170.

Sudhof TC (2004) The synaptic vesicle cycle. Annual Review of Neuroscience 27:509-547.

Sudhof TC & Starke K (2008) Pharmacology of Neurotransmitter Release. Springer. Berlin, Germany.

Sundstrom E, Holmberg L, Souverbie F (1998) NMDA and AMPA receptors evoke transmitter release from noradrenergic axon terminals in the rat spinal cord. Neurochemical Research 23:1501-1507.

Susser E, Neugebauer R, Hoek HW, Brown AS, Lin S, Labovitz D, Gorman JM (1996) Schizophrenia after prenatal famine. Further evidence. Archives of General Psychiatry 53:25-31.

Svensson TH, Bunney BS, Aghajanian GK (1975) Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. Brain Research 92:291-306.

Svensson TH, Usdin T (1978) Feedback inhibition of brain noradrenaline neurons by tricyclic antidepressants: alpha-receptor mediation. Science 202:1089-1091.

Swanson CJ, Perry KW, Schoepp DD (2004) The mGlu2/3 receptor agonist, LY354740, blocks immobilization-induced increases in noradrenaline and dopamine release in the rat medial prefrontal cortex. Journal of Neurochemistry 88:194-202.

Swanson LW (1976) The locus coeruleus: a cytoarchitectonic, Golgi and immunohistochemical study in the albino rat. Brain Research 110:39-56.

Swartz MS, Stroup TS, McEvoy JP, Davis SM, Rosenheck RA, Keefe RS, Hsiao JK, Lieberman JA (2008) What CATIE found: results from the schizophrenia trial. Psychiatric Services 59:500-506.

Szabo B, Fritz T, Wedzony K (2001) Effects of imidazoline antihypertensive drugs on sympathetic tone and noradrenaline release in the prefrontal cortex. British Journal of Pharmacology 134:295-304.

Szabo ST, Blier P (2001a) Functional and pharmacological characterization of the modulatory role of serotonin on the firing activity of locus coeruleus norepinephrine neurons. Brain Research 922:9-20.

Szabo ST, Blier P (2001b) Serotonin (1A) receptor ligands act on norepinephrine neuron firing through excitatory amino acid and GABA(A) receptors: a microiontophoretic study in the rat locus coeruleus. Synapse 42:203-212.

Szabo ST, Blier P (2002) Effects of serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibition plus 5-HT(2A) receptor antagonism on the firing activity of norepinephrine neurons. Journal of Pharmacology and Experimental Therapeutics 302:983-991.

Szabo ST, de Montigny C, Blier P (1999) Modulation of noradrenergic neuronal firing by selective serotonin reuptake blockers. British Journal of Pharmacology 126:568-571.

Szabo ST, Gold MS, Goldberger BA, Blier P (2004) Effects of sustained gamma-hydroxybutyrate treatments on spontaneous and evoked firing activity of locus coeruleus norepinephrine neurons. Biological Psychiatry 55:934-939.

Szulc A, Konarzewska B, Galinska-Skok B, Lazarczyk J, Waszkiewicz N, Tarasow E, Milewski R, Walecki J (2013) Proton magnetic resonance spectroscopy measures related to short-term symptomatic outcome in chronic schizophrenia. Neuroscience Letters 547:37-41.

Takahashi S, Matsuura M, Tanabe E, Yara K, Nonaka K, Fukura Y, Kikuchi M, Kojima T (2000) Age at onset of schizophrenia: gender differences and influence of temporal socioeconomic change. Psychiatry and Clinical Neurosciences 54:153-156.

Talley EM, Rosin DL, Lee A, Guyenet PG, Lynch KR (1996) Distribution of alpha 2A-adrenergic receptor-like immunoreactivity in the rat central nervous system. Journal of Comparative Neurology 372:111-134.

Talpos J, Aerts N, Waddell J, Steckler T (2015) MK-801 and amphetamine result in dissociable profiles of cognitive impairment in a rodent paired associates learning task with relevance for schizophrenia. Psychopharmacology 232:3911-3920.

Tamaru Y, Nomura S, Mizuno N, Shigemoto R (2001) Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: differential location relative to pre- and postsynaptic sites. Neuroscience 106:481-503.

Taylor SR, Badurek S, Dileone RJ, Nashmi R, Minichiello L, Picciotto MR (2014) GABAergic and glutamatergic efferents of the mouse ventral tegmental area. Journal of Comparative Neurology 522:3308-3334.

Tejani-Butt SM (1992) [3H]nisoxetine: a radioligand for quantitation of norepinephrine uptake sites by autoradiography or by homogenate binding. Journal of Pharmacology and Experimental Therapeutics 260:427-436.

The European Network of Schizophrenia Netwroks for the Study of Gene-Environment Interaction (EU-GEI) (2008) Schizophrenia aetiology: Do gene-environment interactions hold the key? Schizophrenia Research 102:21-26.

Theile JW, Morikawa H, Gonzales RA, Morrisett RA (2009) Role of 5-hydroxytryptamine2C receptors in Ca2+-dependent ethanol potentiation of GABA release onto ventral tegmental area dopamine neurons. Journal of Pharmacology and Experimental Therapeutics 329:625-633.

Thomas HV, Dalman C, David AS, Gentz J, Lewis G, Allebeck P (2001) Obstetric complications and risk of schizophrenia. Effect of gender, age at diagnosis and maternal history of psychosis. The British Journal of Psychiatry 179:409-414.

Tissot R (1975) The common pathophysiology of monaminergic psychoses: a new hypothesis. Neuropsychobiology 1:243-260.

Torres US, Portela-Oliveira E, Borgwardt S, Busatto GF (2013) Structural brain changes associated with antipsychotic treatment in schizophrenia as revealed by voxel-based morphometric MRI: an activation likelihood estimation meta-analysis. BMC Psychiatry 13:342.

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R (2010) Glutamate receptor ion channels: structure, regulation, and function. Pharmacological Reviews 62:405-496.

Tritsch NX, Sabatini BL (2012) Dopaminergic modulation of synaptic transmission in cortex and striatum. Neuron 76:33-50.

Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nature Neuroscience 9:519-525.

Tseng K, Atzori M (2007) Monoaminergic Modulation of Cortical Excitability. Springer. Berlin, Germany.

Tsuda N, Tohmi M, Mizuno M, Nawa H (2006) Strain-dependent behavioral alterations induced by peripheral interleukin-1 challenge in neonatal mice. Behavioural Brain Research 166:19-31.

Tzschentke TM (2001) Pharmacology and behavioral pharmacology of the mesocortical dopamine system. Progress in Neurobiology 63:241-320.

Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, Javitch JA, Roth BL, Christopoulos A, Sexton PM, Miller KJ, Spedding M, Mailman RB (2007) Functional selectivity and classical concepts of quantitative pharmacology. Journal of Pharmacology and Experimental Therapeutics 320:1-13.

Uriguen L, Gil-Pisa I, Munarriz-Cuezva E, Berrocoso E, Pascau J, Soto-Montenegro ML, Gutierrez-Adan A, Pintado B, Madrigal JL, Castro E, Sanchez-Blazquez P, Ortega JE, Guerrero MJ, Ferrer-Alcon M, Garcia-Sevilla JA, Mico JA, Desco M, Leza JC, Pazos A, Garzon J, Meana JJ (2013) Behavioral, neurochemical and morphological changes induced by the overexpression of munc18-1a in brain of mice: relevance to schizophrenia. Translational Psychiatry 3:e221.

Usunoff KG, Itzev DE, Ovtscharoff WA, Marani E (2002) Neuromelanin in the human brain: a review and atlas of pigmented cells in the substantia nigra. Archives of Physiology and Biochemistry 110:257-369.

Valerio A, Ferraboli S, Paterlini M, Spano P, Barlati S (2001) Identification of novel alternatively-spliced mRNA isoforms of metabotropic glutamate receptor 6 gene in rat and human retina. Gene 262:99-106.

Van Gaalen M, Kawahara H, Kawahara Y, Westerink BH (1997) The locus coeruleus noradrenergic system in the rat brain studied by dual-probe microdialysis. Brain Research 763:56-62.

van Os J (2004) Does the urban environment cause psychosis? The British Journal of Psychiatry 184:287-288.

Van Sant SP, Buckley PF (2011) Pharmacotherapy for treatment-refractory schizophrenia. Expert Opinion on Pharmacotherapy 12:411-434.

Vandergriff J, Rasmussen K (1999) The selective mGlu2/3 receptor agonist LY354740 attenuates morphine-withdrawal-induced activation of locus coeruleus neurons and behavioral signs of morphine withdrawal. Neuropharmacology 38:217-222.

Vardy MM, Kay SR (1983) LSD psychosis or LSD-induced schizophrenia? A multimethod inquiry. Archives of General Psychiatry 40:877-883.

Varty GB, Bakshi VP, Geyer MA (1999) M100907, a serotonin 5-HT2A receptor antagonist and putative antipsychotic, blocks dizocilpine-induced prepulse inhibition deficits in Sprague-Dawley and Wistar rats. Neuropsychopharmacology 20:311-321.

Venkatadri PS, Lee CC (2014) Differential expression of mGluR2 in the developing cerebral cortex of the mouse. Journal of Biomedical Science and Engineering 7:1030-1037.

Venkatesh BK, Thirthalli J, Naveen MN, Kishorekumar KV, Arunachala U, Venkatasubramanian G, Subbakrishna DK, Gangadhar BN (2008) Sex difference in age of onset of schizophrenia: findings from a community-based study in India. World Psychiatry 7:173-176.

Ventura R, Cabib S, Puglisi-Allegra S (2001) Opposite genotype-dependent mesocorticolimbic dopamine response to stress. Neuroscience 104:627-631.

Vetulani J (2001) Drug addiction. Part II. Neurobiology of addiction. Polish Journal of Pharmacology 53:303-317.

Vickers SP, Easton N, Malcolm CS, Allen NH, Porter RH, Bickerdike MJ, Kennett GA (2001) Modulation of 5-HT(2A) receptor-mediated head-twitch behaviour in the rat by 5-HT(2C) receptor agonists. Pharmacology Biochemistry and Behavior 69:643-652.

Vinals X, Moreno E, Lanfumey L, Cordomi A, Pastor A, de La Torre R, Gasperini P, Navarro G, Howell LA, Pardo L, Lluis C, Canela EI, McCormick PJ, Maldonado R, Robledo P (2015) Cognitive Impairment Induced by Delta9-tetrahydrocannabinol Occurs through Heteromers between Cannabinoid CB1 and Serotonin 5-HT2A Receptors. PLoS Biology 13:e1002194.

Vollenweider FX, Csomor PA, Knappe B, Geyer MA, Quednow BB (2007) The effects of the preferential 5-HT2A agonist psilocybin on prepulse inhibition of startle in healthy human volunteers depend on interstimulus interval. Neuropsychopharmacology 32:1876-1887.

Vollenweider FX, Leenders KL, Scharfetter C, Maguire P, Stadelmann O, Angst J (1997) Positron emission tomography and fluorodeoxyglucose studies of metabolic hyperfrontality and psychopathology in the psilocybin model of psychosis. Neuropsychopharmacology 16:357-372.

Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, Vogel H, Hell D (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. Neuroreport 9:3897-3902.

Vuoristo JT, Berrettini WH, Overhauser J, Prockop DJ, Ferraro TN, Ala-Kokko L (2000) Sequence and genomic organization of the human G-protein Golfalpha gene (GNAL) on chromosome 18p11, a susceptibility region for bipolar disorder and schizophrenia. Molecular Psychiatry 5:495-501.

Vysokanov A, Flores-Hernandez J, Surmeier DJ (1998) mRNAs for clozapine-sensitive receptors co-localize in rat prefrontal cortex neurons. Neuroscience Letters 258:179-182.

Walker AG, Conn PJ (2015) Group I and group II metabotropic glutamate receptor allosteric modulators as novel potential antipsychotics. Current Opinion in Pharmacology 20:40-45.

Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H, Bader M (2003) Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science 299:76.

Wang JK (1991) Presynaptic glutamate receptors modulate dopamine release from striatal synaptosomes. Journal of Neurochemistry 57:819-822.

Wang JK, Andrews H, Thukral V (1992) Presynaptic glutamate receptors regulate noradrenaline release from isolated nerve terminals. Journal of Neurochemistry 58:204-211.

Watt NF (1978) Patterns of childhood social development in adult schizophrenics. Archives of General Psychiatry 35:160-165.

Weber ET, Andrade R (2010) Htr2a Gene and 5-HT(2A) Receptor Expression in the Cerebral Cortex Studied Using Genetically Modified Mice. Frontiers in Neuroscience 4.

Weinberger DR, Torrey EF, Neophytides AN, Wyatt RJ (1979) Lateral cerebral ventricular enlargement in chronic schizophrenia. Archives of General Psychiatry 36:735-739.

Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP, Sibille E, Underwood M, Itohara S, Dauer WT, Ansorge MS, Morelli E, Mann JJ, Toth M, Aghajanian G, Sealfon SC, Hen R, Gingrich JA (2006) Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. Science 313:536-540.

Weizman R, Paz L, Backer MM, Amiri Z, Modai I, Pick CG (1999) Mouse strains differ in their sensitivity to alprazolam effect in the staircase test. Brain Research 839:58-65.

Westerink BHC, Rea K, Oldenziel WH, Cremers TIFH (2007) Microdialysis of glutamate and GABA in the brain: analysis and interpretation. In: Handbook of Microdialysis. Westerink BHC and Cremers TIFH (Eds.) Elsevier. Amsterdam, The Netherlands. Pag: 17-31.

Westfall DP, Todorov LD, Mihaylova-Todorova ST (2002) ATP as a cotransmitter in sympathetic nerves and its inactivation by releasable enzymes. Journal of Pharmacology and Experimental Therapeutics 303:439-444.

Wilkinson ST, Radhakrishnan R, D'Souza DC (2015) Impact of Cannabis Use on the Development of Psychotic Disorders. Current Addiction Reports 1:115–128.

Willard SS, Koochekpour S (2013) Glutamate, glutamate receptors, and downstream signaling pathways. International Journal of Biological Sciences 9:948-959.

Willins DL, Berry SA, Alsayegh L, Backstrom JR, Sanders-Bush E, Friedman L, Roth BL (1999) Clozapine and other 5-hydroxytryptamine-2A receptor antagonists alter the subcellular distribution of 5-hydroxytryptamine-2A receptors in vitro and in vivo. Neuroscience 91:599-606.

Willins DL, Deutch AY, Roth BL (1997) Serotonin 5-HT2A receptors are expressed on pyramidal cells and interneurons in the rat cortex. Synapse 27:79-82.

Willins DL, Meltzer HY (1997) Direct injection of 5-HT2A receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. Journal of Pharmacology and Experimental Therapeutics 282:699-706.

Winter JC, Eckler JR, Rabin RA (2004) Serotonergic/glutamatergic interactions: the effects of mGlu2/3 receptor ligands in rats trained with LSD and PCP as discriminative stimuli. Psychopharmacology 172:233-240.

Wisden W, Seeburg PH (1993) A complex mosaic of high-affinity kainate receptors in rat brain. Journal of Neuroscience 13:3582-3598.

Woolley DW, Shaw E (1954) A Biochemical and Pharmacological Suggestion About Certain Mental Disorders. Proceedings of the National Academy of Sciences of the United States of America 40:228-231.

World Health Organization. Division of Mental Health (1992). The ICD-10 classification of mental and behavioural disorders: Clinical descriptions and diagnostic guidelines, vol. I. Geneva, Italy.

Wright IC, Rabe-Hesketh S, Woodruff PW, David AS, Murray RM, Bullmore ET (2000) Meta-analysis of regional brain volumes in schizophrenia. The American Journal of Psychiatry 157:16-25.

Wright IK, Garratt JC, Marsden CA (1990) Effects of a selective 5-HT2 agonist, DOI, on 5-HT neuronal firing in the dorsal raphe nucleus and 5-HT release and metabolism in the frontal cortex. British Journal of Pharmacology 99:221-222.

Wright RA, Arnold MB, Wheeler WJ, Ornstein PL, Schoepp DD (2001) [3H]LY341495 binding to group II metabotropic glutamate receptors in rat brain. Journal of Pharmacology and Experimental Therapeutics 298:453-460.

Xiberas X, Martinot JL, Mallet L, Artiges E, Loc HC, Maziere B, Paillere-Martinot ML (2001) Extrastriatal and striatal D(2) dopamine receptor blockade with haloperidol or new antipsychotic drugs in patients with schizophrenia. The British Journal of Psychiatry 179:503-508.

Yamaguchi T, Qi J, Wang HL, Zhang S, Morales M (2015) Glutamatergic and dopaminergic neurons in the mouse ventral tegmental area. European Journal of Neuroscience 41:760-772.

Yamamoto BK, Cooperman MA (1994) Differential effects of chronic antipsychotic drug treatment on extracellular glutamate and dopamine concentrations. Journal of Neuroscience 14:4159-4166.

Yamamoto K, Hornykiewicz O (2004) Proposal for a noradrenaline hypothesis of schizophrenia. Progress in Neuro-Psychopharmacology & Biological Psychiatry 28:913-922.

Yamamura S, Ohoyama K, Hamaguchi T, Kashimoto K, Nakagawa M, Kanehara S, Suzuki D, Matsumoto T, Motomura E, Shiroyama T, Okada M (2009) Effects of quetiapine on monoamine, GABA, and glutamate release in rat prefrontal cortex. Psychopharmacology 206:243-258.

Yang C, Li W, Weiwei W, Wu S, Lu W (2014) A Review: Detecting Alterations of Brain Connectivity in Schizophrenia based on Structural MRI. Journal of Psychiatry 17.

Yau HJ, Wang DV, Tsou JH, Chuang YF, Chen BT, Deisseroth K, Ikemoto S, Bonci A (2016) Pontomesencephalic Tegmental Afferents to VTA Non-dopamine Neurons Are Necessary for Appetitive Pavlovian Learning. Cell Reports 16:2699-2710.

Yokoi M, Kobayashi K, Manabe T, Takahashi T, Sakaguchi I, Katsuura G, Shigemoto R, Ohishi H, Nomura S, Nakamura K, Nakao K, Katsuki M, Nakanishi S (1996) Impairment of hippocampal mossy fiber LTD in mice lacking mGluR2. Science 273:645-647.

Yun HM, Rhim H (2011) The serotonin-6 receptor as a novel therapeutic target. Experimental Neurobiology 20:159-168.

Zhai Y, George CA, Zhai J, Nisenbaum ES, Johnson MP, Nisenbaum LK (2003) Group II metabotropic glutamate receptor modulation of DOI-induced c-fos mRNA and excitatory responses in the cerebral cortex. Neuropsychopharmacology 28:45-52.

Zhang G, Tao R (2011) Enhanced responsivity of 5-HT(2A) receptors at warm ambient temperatures is responsible for the augmentation of the 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced hyperthermia. Neuroscience Letters 490:68-71.

Zhang QJ, Wang S, Liu J, Ali U, Gui ZH, Wu ZH, Hui YP, Wang Y, Chen L (2010) Unilateral lesion of the nigrostriatal pathway decreases the response of interneurons in medial prefrontal cortex to 5-HT 2A/2C receptor stimulation in the rat. Brain Research 1312:127-137.

Zhu H, Ryan K, Chen S (1999) Cloning of novel splice variants of mouse mGluR1. Brain Research Molecular Brain Research 73:93-103.

Zifa E, Fillion G (1992) 5-Hydroxytryptamine receptors. Pharmacological Reviews 44:401-458.

Zimmermann KS, Hsu CC, Gourley SL (2016) Strain commonalities and differences in response-outcome decision making in mice. Neurobiology of Learning and Memory 131:101-108.

Zlamy M (2016) Rediscovering Pertussis. Frontiers in Pediatrics 4:52.