eman ta zabal zazu

Universidad del País Vasco

Euskal Herriko Unibertsitatea

Traumatic Brain Injury-induced alterations in Adult Hippocampal Neurogenesis

Doctoral thesis opting to the PhD degree, presented by

Irene Durá Esteve

2022

Supervisors:

Dr. Juan Manuel Encinas Pérez

Dr. José Ramón Pineda Martí

(cc)2023 IRENE DURA ESTEVE (cc by-nc-nd 4.0)

This doctoral thesis has been performed thanks to the usage of Predoctoral Researcher Formation fellowship from the Spanish Government, Ministry of Science and Innovation between 2017-2021 (http://ciencia.gob.es) which also provided funds for the experimental work (SAF2015-70866-R)

ACKNOWLEDGEMENTS

Hice un par de entrevistas para puestos de doctorado antes de que llegara ésta. Probablemente ya no te acuerdes **Juanma**, pero la tuya duró cinco minutos. Me preguntaste si tenía experiencia usando un confocal y si estaba dispuesta a venirme a Bilbao. Y yo dije que sí a las dos. Y aquí estamos. Me parece que es muy representativo de todo lo que eres como PI. Gracias por ser el anti-jefe. Por ser paciente y comprensivo siempre. Por enseñarme a vivir la ciencia a tu propia manera, más divertida que la de los demás. Por dejarme el espacio necesario para crecer y confiar ciegamente en quien quiera que sea el misfit que se te ponga por delante. Por restarle importancia siempre a los problemas e intentar ver lo bueno en todas las situaciones. Gracias por darme la oportunidad de descubrir algo que me gusta tanto.

El que segur no m'esperava quan vaig arribar a Bilbao va ser que les correccions de la tesi acabarien sent en valencià. Gràcies **José**, per fer-me sentir com a casa estant tan lluny. Es molt més fàcil emprendre feines complicades tenint a algú com a tu al costat. Gràcies per la disponibilitat, per saber apreciar l'esforç i per sempre tenir una bona paraula, un consell, una idea que a mi mai se m'haguera ocorregut. Quin gust és treballar amb gent que disfruta el que fa. Gràcies per fer-ho tot fàcil.

Gracias también a todo el laboratorio Encinas por acompañarme a lo largo de estos años y hacer el camino más fácil a base de risas, alubias, y muchos, muchos ratos de ofi. A **Soraya, Teresa, Ane, Rodrigo, Leire y Lorena**. El futuro del laboratorio está en vuestras manos. Vamos a intentar que no salga ardiendo.

I'll always be thankful to **Emilie**, for being kind enough to welcome me, even in the midst of a pandemic. After all those months waiting and changing plans, it was all worth it. Thanks so much for the time and trust you put on me. You're the example that the effort always pays off. Thanks also to **Nora**, for giving me a space in your laboratory even in such a complicated situation and to **Muriel** for the time and patience with all of the behaviour experiments. And of course, thanks to **Pierre**, to **Nico** and to **Lyès**, for keeping me company during those three months in Bordeaux. If only I had one more month to beat you all in basketball! Merci beaucoup!

Achucarro es un lugar curioso, donde se ha juntado una peña extraña. Me resulta imposible imaginar cómo habrían sido estos años si no hubiese estado acompañada por todos vosotros.

A la persona que me abrió la puerta del laboratorio cuando llegué, aunque al principio me quisiera mandar a vivir a Zamudio. Gracias **Rober** por hacerme sentir una más desde el primer

momento. Gracias por ser la persona generosa que eres, siempre dispuesto a echar un cable, a resolver una duda, a dar una buena turra.

Thanks to **Maria**, my very first student, for always being the most motivated person in the room. I'm not sure whether you learned a lot of science with us, but I'm certain you got the most useful Spanish. Girl, we miss you.

Gracias a **Laura Escobar**. En serio, gracias. Contigo al lado casi parece que sé lo que hago en el micro. La ciencia sería mucho más fácil si hubiese más gente como tú.

Ale, gracias por los consejos, por la calma, por ser un apoyo inestimable siempre que se te necesita. Por gente como tú es que todavía conservo una poquita de salud mental.

A **Paula**, por vivir permanentemente con una sonrisa puesta. Gracias por hacer como que ser madre, doctoranda y buena persona es posible y fácil. Qué envidia, nena.

El doctorado es duro, pero lo es un poco menos si sabes que al otro lado del pasillo hay gente esperándote para echarse unas risas, pintar un bigote en un cristal o preguntarte si quieres un café. Gracias al laboratorio Sierra. A **Jorge**, por la paciencia inacabable para explicar lo que sea: estadística, macros o las reglas de un juego de mesa. A **Sol**, por ser cómplice de todas las risas. A Pili y Mili. A Pena y Pánico. A las dos tontas muy tontas. A Virelia. Os va a doler, pero os voy a separar:

No me extrañaría nada si se me pasase una factura por horas de terapia. **Vir**, reina, tú antes que bioquímica fuiste psicóloga. Con pocas personas se puede contar para un ron-cola igual que para que te aguanten los dramas. Gracias por ser siempre la más amable, la más humilde, la más atenta.

A mi crush. Aunque no quiera nada conmigo. **Noelia**, tía, si Vir fue psicóloga en otra vida, tú fuiste un señor de 80 años. Por la sabiduría, lo digo ¿eh? Gracias por ser compañera de aventuras y por la complicidad. Por ser una persona con la que se puede contar siempre sin siquiera pedirlo. Por las berenjenas, los audios de ocho minutos y por saber escuchar tan bien.

Y por supuesto, a la persona que me llevaría a una guerra, porque sé que me defendería machete en boca. **Ene**, no creo que pueda describir aquí en un par de líneas lo generosa que puedes llegar a ser. La manera que tienes de sacrificar tu tiempo sin pensártelo dos veces solo porque te lo pide un amigo. El apoyo incondicional, la complicidad. Eres la persona que se mete en el charco para desembarrar a los demás, y no hay muchas de esas. Si he sobrevivido a estos años ha sido en parte porque tú me has cogido la manita y me has dado palmaditas en el hombro. Joe, y si

Eminem puede hacer diez años sobrio, nosotras podemos hacer esto. No puedo decirlo lo suficiente, gracias.

Esta tesis ha llegado hasta aquí en gran parte gracias a la gente que me ha rodeado durante estos años. Tú imagínate el día que yo abrí la puerta de cirugía y me encontré a tres personas peleándose por ver quién inyectaba mejor.

A **Fer**, mil gracias. Por la mentorización y el pragmatismo. Gracias por ser un ejemplo de todo lo que se puede conseguir con esfuerzo y con ganas. Creo que he aprendido muchísimo simplemente estando cerca de ti. Tu motivación, tus ganas de transmitir y compartir con los demás siempre de forma desinteresada. Y hasta tus chistes, que en el fondo me hacen gracia.

Si este proyecto tiene el título que tiene, es por una casualidad del destino que me hizo coincidir con alguien muy especial. **Tati**, gracias por acogerme nada más llegar y verme por lo que podría llegar a ser más que por lo que era. Gracias por ser un poco mi hermana y mi madre a la vez. Por ser la tía más valiente que conozco. Por servir de modelo. Por saber ser terapeuta, mentora, compañera o amiga cuando toca.

A la persona que me ha acompañado durante estos años, codo con codo, compartiendo vivencias y aportando mucho más de lo que me resta, a pesar de lo que pueda parecer. Gracias **Oier**, a día de hoy no podría concebir un compañero de batalla mejor. Gracias por ayudarme a tirar del carro cuando se me hacía cuesta arriba y por disfrutarlo conmigo cuando el camino lo pone más fácil. Ha sido una suerte encontrarte en esta etapa y saber que seguirás estando en las que vengan.

Estos años fuera de casa me han hecho valorar más que nunca la suerte que he tenido de encontrarme con gente que ha sido mi familia en Bilbao. Todas las horas que he estado fuera del laboratorio, las he compartido con ellos. Y he aprendido lo mismo que dentro, o más.

Gracias a **Boris**, por ser una persona extraña, pero encantadora. Por dejarme ser extraña a tu lado. Gracias por hacerlo todo tan fácil.

A **Aaron**, por llegar cuando decidí que ya no quería hacer más amigos en Bilbao, y obligarme a tener otro. Por ser una de las personas que mejor escucha del mundo. Gracias por las listas de reproducción, los conciertos y los pimientos. Qué buena persona eres, qué asco me das.

Gracias a **Ana** y **Ainhoa**, que formaron parte de la kalea primigenia. Ese primer año de doctorado es algo a lo que me agarraré siempre que esté triste.

A **Carlos**, por aceptar que iba a ser la hija adoptiva en tu relación y hacerme sentir como tal. Gracias por traer un poco de calma a Barrenkale cada vez que venías, por aguantar lo pesadas que podemos llegar a ser y por comprarme cereales ricos. No está pagau.

Esta tesis también es un poco PrionLab. Gracias a **Hasi**, por servirnos de ejemplo a todos para ver que se puede uno mantener cuerdo en este mundo. A **Cris**, por ser de las personas más generosas que me llevo de aquí. Gracias por compartir siempre tu alegría y tu buen rollo. A **Jorge**, por sacrificar horas de niño-rata por venir a beber cerveza con nosotros. No seas sinvergüenza, que luego eres el que mejor se lo pasa. Y a **Carlos**, por demostrarnos que se puede ser un raro de la vida y majísimo a la vez. Estás fatal y soy bastante fan tuya por eso.

Y por supuesto gracias a las dos personas que salieron de ese laboratorio y han marcado mi paso por Bilbao. A **Ezequiel**. Siempre que piense en esta etapa sonreiré y tú has sido fundamental para eso. Amigo, solo espero que algún día podamos hacer un pódcast de anécdotas para que el público disfrute igual que nosotros. Gracias por acompañarme estos años.

A **Migue**. Cuando pensaba que no podía haber nadie más tonto que yo. Resulta que sí lo hay. No conozco a mucha gente que sea capaz de transmitir el buen rollo y la alegría que tienes tú. Que hayas decidido compartir eso conmigo me hace muy feliz. Estoy muy orgullosa de ser tu amiga, y, ante todo, tu público. No puedo enfatizar lo suficiente lo afortunada que me siento de haber estado rodeada de esta gente.

Bilbao me ha regalado muchas cosas bonitas. Entre ellas, a dos personas que me llevo para toda la vida. A dos hermanas. Lo siento pero ya no os vais a librar de mí.

A mi Ceuta, a mi cucharita, a mi **Marta** de orígenes combinados. La que llegó como una incorporación de última hora a suplir una baja. Y resulta que arrasó. Amiga, eres lo más parecido a una hermana pequeña que he tenido. Ya sé que tú muchas veces dices que somos iguales. Pero las dos sabemos que eso no es verdad. Tú siempre vas a ir un pasito por delante. Gracias por enseñarme tantísimas cosas sin darte cuenta siquiera. Gracias por querer tanto, tantísimo, a la gente de tu alrededor y no tener miedo a demostrarlo. Por preocuparte por todo, por todos, todo el rato. Por sentir tanto. La vida ha querido que compartamos unos años complicados y verte crecer y convertirte en la persona maravillosa, altruista, madura que eres, ha sido un regalo. Eres mi mejor público igual que yo soy el tuyo, probablemente porque somos igual de tontas. Y eso no nos lo va a quitar nadie nunca.

Y como no podía ser de otra manera, gracias a la persona que ha sido, a efectos, mi compañera de vida estos años, la versión inicial de **Marta**. Amiga, llegaste a mi vida con una confianza que

hacía imposible no dejarte entrar. Todo lo que hemos vivido estos años, bueno y malo, nos ha ido uniendo cada vez más y a día de hoy no me puedo imaginar la vida sin ti al lado. No me malinterpretes, no me preocupa en absoluto. Estoy convencida de que no existe tal cosa como una despedida entre nosotras. Tendría que escribir otras 250 páginas para darte las gracias por todo lo que has hecho por mí estos años. Creo que la manera más adecuada de expresarlo, sería decirte que gracias por ser la amiga perfecta para mí. Gracias por tener un consejo cuando estaba perdida, un chiste cuando necesitaba que me sacaran una sonrisa, un cinnamon roll cuando estaba triste. Gracias por empatizar mejor que nadie, por ser cómplice de todo. Gracias por saber cuándo era cada una de esas ocasiones sin que yo tuviese que explicar nada. Gracias por entender la importancia que tiene estar lejos de los tuyos y saber construir una familia con la gente a tu alrededor. Ese regalo nos lo hiciste tú y no creo que te lo podamos pagar nunca. Habrá más rimas y más risas y más picoteos y más vinos árabes y más de todo. La gente aún no está lo suficientemente cansada de nosotras. Aún nos queda.

No sabía yo lo que me esperaba cuando decidí dedicarme a la ciencia. No sabía la que se me venía encima. Si llegué a Bilbao, fue porque sobreviví a cuatro años en Barcelona. Y si eso sucedió, fue porque conocí a un grupo de personas maravillosas con las que aprendí mucho de la vida, y un poco de Biotecnología también.

Gracias a las personas que me hacen llorar de la risa cada vez, con cada anécdota. Caguendeu, la ciencia no sabe lo que ha ganado con cada uno de vosotros. A **Ana García**, porque no se puede ser una persona tan bonita y encima cantar así. A **David**, por ser un poco mi gemelier y entenderme tan bien. Sin ti y sin mí ellos estarían muertos, lo sabes. A **Carlos**, por ser la persona más lista y más tonta que conozco a la vez. Ah y por los apuntes jeje. A **Nando**, porque eres el sinvergüenza más grande que conozco, pero hay que ver qué risa me haces, asqueroso. A **Ana Dorrego**, por contar los peores chistes del mundo, pero persistir. Lo tuyo sí que es resiliencia, tía. A **Marina**, por ser capaz de mantenerte cuerda y mantenernos cuerdos a los demás. No es tarea fácil, la verdad. A **Iris**, por estar tan loca, porque si todos estuviésemos cuerdos, vaya gracia. Y a **Cris**, por ser capaz de aguantar a todos los anteriores sin tirarte por la ventana. Anda que vaya crew.

Gracias a mis coleguis del máster en el Instituto de Neurociencias. A **Vero**, a **Marta** y a **Rocío**, por tener la paciencia de recibirme como bebé-científica y luego lanzarme al vacío un poco menos bebé. Cuatro años he pasado sin hacer ni un contrapeso para la centrífuga. Pa que lo sepáis.

A **Vanesa**, mi primerísima compi de laboratorio. La persona con la que escribí por primera vez un informe, fregué muchos vasos de precipitados y decidí que esto era lo mío. Amiga, qué orgullosa estoy de ti, de nosotras. Suspendíamos Química pero mira a dónde hemos llegado.

A ellas, a las de siempre. A **Eva**, **Maje**, **Ángela**, **Paloma** y **Fabi**. Gracias por demostrarme que pase lo que pase, vosotras siempre vais a estar. Por hacerme sentir como en casa cada vez que vuelvo. No puedo explicar lo orgullosa que estoy de vosotras, de que pasen los años y sigamos siendo las mismas, que nos hagamos mayores juntas, aunque estemos separadas.

A **Javi**, por ser la persona que me cogió de la mano al principio de esta aventura y por no soltármela hasta el final. Gracias por tener esa confianza ciega en mí, incluso cuando yo no la tenía. Gracias por ser una de las personas que mejor me ha entendido, incluso cuando yo no me entendía.

Y por supuesto, a mi familia. A toda, que no es poca. Porque cada uno de ellos ha contribuido a su manera a que yo me encuentre hoy aquí. A mis abuelos, **Armando** y **Francisco**. A mis abuelas, **Estrella** y **Ángeles**. Mi familia está llena de mujeres fuertes como ellas que me han traído hasta aquí y no puedo estarles más agradecida.

El que no sepa trabajar en equipo es porque no ha crecido en una familia numerosa. A mis hermanos, por pasarse la vida demonstrándome que puedo hacer lo que me dé la gana y va a salir bien.

A la hermana que no sabía que necesitaba. A **Rocío**. Amiga, no te imaginas lo feliz que estoy de que llegaras a la familia. Gracias por venir a iluminar todo lo que antes estaba oscuro. No puedo más que envidiar a mi hermano y a mi sobrino por compartir su vida con alguien como tú. Gracias por el apoyo incondicional, la sororidad y la comprensión.

A **Jorge**, por servir de ejemplo a todos desde bien pequeñito. Por allanar el camino y por darme las herramientas para ser independiente, por no dejarme pensar ni por un momento que merecía nada menos que cualquier otro. Por esforzarte tanto y a la vez hacer que parezca todo tan fácil.

A **Luis**, por la confianza ciega en mí. Por ser mi fan número uno desde el primer momento. Por ser la persona altruista, desinteresada y generosa con la que todo el mundo quiere estar. Por enseñarme tantas cosas sin ni siquiera ser consciente de ello. Porque aunque no lo diga muy a menudo, me siento muy afortunada de ser tu hermana.

Y por supuesto a mis padres. A las personas que me han puesto aquí, porque esta tesis es tan mérito mío como suyo:

A ti **papá**, por no permitirme dudar ni un momento de que podía hacer lo que me propusiera. Por ser un ejemplo de sacrificio, de esfuerzo, de perseverancia. Por enseñarnos desde pequeños el valor del trabajo. Si yo me atreví con esto fue porque sabía que si me caía, iba a tener una red que me recogiese. Y eso te lo debo a ti.

Y por supuesto, al espejo en el que me miro cada día. A ti, **mamá**, porque me has hecho tú. Porque todo lo bueno que tengo, te lo debo a ti. Gracias, por cuidarnos y motivarnos, por apoyarnos y por hacerlo todo sin sudar ni una gota, o al menos sin que lo notásemos nosotros. No podría haber salido de otra manera. Era imposible después de haberte visto a ti crecer y conseguir todo lo que te proponías. Eres un ejemplo, para mí, para tus alumnas, para toda persona que te rodea. Envidio tu pragmatismo, tus valores, tu capacidad de sacrificio y tu sentido del humor. Si algún día llego a ser la mitad de fuerte que tú, lo consideraré un éxito. *Yo te llevo dentro, hasta la raíz, y por más que crezca, vas a estar ahí. Aunque yo me oculte tras la montaña y encuentre un campo lleno de caña, no habrá manera, mi rayo de luna, que tú te vayas.*

Se va enredando, enredando
como en el muro la hiedra
y va brotando, brotando
como musguito en la piedra

Violeta Parra

Que no tot en la vida es faena
Que morir és no viure lluitant

ZOO

TABLE OF CONTENTS

TABLE OF CONTENTS.

LIST OF ABBREVIATIONS

1. LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
AHN	Adult Hippocampal Neurogenesis
AIS	Abbreviated Injury Scale
AMP	Adenosine Monophosphate
AMPA	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid
ANP	Amplifying Neural Progenitor
AP	Antero-Posterior
ATP	Adenosine -5'-Triphosphate
BAX	BCL2 Associated X
BBB	Blood Brain Barrier
BDNF	Brain-Derived Neurotrophic Factor
BLBP	Brain Lipid-Binding Protein
BMP	Bone Morphogenetic Protein
BSA	Bovine Serum Albumin
C57BL/6	C57 Black 6
CA	Closed Arms
CA	Cornu Ammonis
CaMK	Calmoduline-Dependent Protein Kinase
CCI	Controlled-Cortical Impact
CNS	Central Nervous System
CREB	c-AMP Response Element-Binding Protein
CS	Constant Start
CSF	Cerebrospinal Fluid
CTE	Chronic Traumatic Encephalopaty
DAI	Diffuse Axonal Injury
DAPI	4',6-diamidino-2-phenylindole
DB	Dorsal Blade
DCX	Doublecortin
DETA	Diethylenetriamine
DG	Dentate Gyrus
DNA	Deoxyribonucleic Acid
DP	Dementia Pugilistica

DV	Dorso-Ventral
EA	Epileptiform Activity
EC	Entorhinal Cortex
EEG	Electroencephalogram
EGFP	Enhanced Green Fluorescent Protein
EHU	Euskal Herriko Unibersitatea
EPM	Elevated Plus Maze
EPSP	Excitatory Post-Synaptic Potential
ERK	Extracellular Signal Regulated Kinase
ET	Emergence Test
EU	European Union
FGF	Fibroblast Growth Factor
FPI	Fluid Percussion Injury
FST	Forced Swim Test
GABA	γ-Aminobutyric Acid
GAP	GTPase-Activating Protein
GC	Granule Cell
GCL	Granule Cell Layer
GCS	Glasgow Coma Scale
GDNF	Glial Cell Derived Neurotrophic Factor
GDP	Guanosine Diphosphate
GEF	Guanine Nucleotide Exchange Factor
GFAP	Glial Fibrillary Acidic Protein
GFP	Green Fluorescent Protein
GTP	Guanine Triphosphate
HCl	Chlorhydric Acid
HPA	Hypothalamic Pituitary Adrenal Axis
HSV-TK	Herpes Simplex Virus Thymidine Kinase
IF	Immunofluorescence
IFG	Insuline-like Growth Factor
IN	Immature Neuron
IP	Intraperitoneal
KA	Kainate
KCC	Potassium-Chloride Transporter

LC	Locus Ceruleus
LD	Light/Dark
LFPI	Lateral Fluid Percussion Injury
LL	Latero-Lateral
LPAR1	Lysophosphatidic Acid Receptor 1
LPP	Lateral Perforant Path
LSD	Least Significant Difference
MKI67	Marker of Prolieration Ki67
ML	Molecular Layer
MPP	Medial Perforant Path
MRI	Magnetic Resonance Image
MTLE	Mesial Temporal Lobe Epilepsy
MWM	Morris Water Maze
NaOH	Sodium Hydroxyde
NB	Neuroblast
NCAM	Neural Cell Adhesion Protein
NE	North East
NG	Neurogenin
NIH	National Institute of Health
NKCC	Sodium-Potassium-Chloride Cotransporter
NMDA	N-Methyl D-Aspartate
NO	Nitric Oxide
NS	Novel Start
NSC	Neural Stem Cell
NT	Neurotransmiter
NW	North-West
OA	Open Arms
OF	Open Field
PBS	Phosphate Buffered Saline
PCGW	Penetrating Craniocerebral Gunshot Wounds
PCM	Pericentriolar Material 1
PCS	Post-Concussion Syndrome
PET	Positron Emission Tomography
PFA	Paraformaldehyde

PKA	Protein Kinase A
PKC	Protein Kinase C
PMD	Post-Mortem Delay
PSA-NCAM	Polysialylated Neural-Cell-Adhesion Molecule
PSD95	Post-Synaptic Density Protein 95
PTA	Post-Traumatic Amnesia
PTSD	Post-Traumatic Stress Disorder
PVC	Polyvinyl Chloride
RGC	Radial Glial Cell
RMS	Rostral Migratory Stream
RNA	Ribonucleic Acid
ROI	Region Of Interest
RV	Retrovirus
SAL	Saline
SE	South-East
SEM	Standard Error of Mean
SFFV	Spleen Focus-Forming Virus
SGZ	Subgranular Zone
SN	Substantia Nigra
ST	Sucrose Test
SVZ	Subventricular Zone
SW	South West
TAI	Traumatic Axonal Injury
TBI	Traumatic Brain Injury
TDP-43	TAR DNA-Binding Protein 43
TK	Thymidine Kinase
TNF	Tumor Necrosis Factor
TU	Transducing Units
VB	Ventral Blade
VEGF	Vascular Endothelial Growth Factor
VEGFR	VEGF-Receptor
VGCC	Voltage-Gated Calcium Channel
VS	Variable Start
WT	Wild Type

RESUMEN / SUMMARY

2. RESUMEN / SUMMARY.

2.1. RESUMEN.

La zona subgranular (SGZ del inglés "*Subgranular Zone"*) es una región del giro dentado (DG del inglés "*Dentate Gyrus"*) en la que reside una población de células madre neurales adultas (NSCs del inglés "*Neural Stem Cells"*). Estas NSCs generan nuevas células que acaban integrándose localmente como células granulares maduras en el circuito hipocampal. Las NSCs se pueden definir por su posición, morfología y expresión de varios marcadores celulares. Se dividen de manera asimétrica en su mayoría para mantener una copia de sí mismas y generar progenitores neurales (ANPs del inglés "*Amplifying Neural Progenitors"*) que tras rápidos ciclos de división celular generarán neuronas inmaduras o neuroblastos. Por último, los neuroblastos migrarán y sufrirán cambios funcionales más importantes, dado que la población evoluciona tanto morfológica electrofisiológicamente, requiriendo grandes cambios de expresión génica.

A lo largo del proceso de neurogénesis y migración neuronal, especialmente durante el desarrollo y la maduración de los neuroblastos, tiene lugar una remodelación extensa y dinámica del citoesqueleto celular. Sin embargo, todavía no está claro cómo se regulan muchos de estos procesos a nivel de señalización intracelular.

La familia de pequeñas GTPasas Rho (*Ras homologous*) está formada por reguladores clave del citoesqueleto que actúan en varios tipos celulares. Es interesante que, de los muchos miembros pertenecientes a esta familia, solo Rnd2 está selectivamente enriquecido en la SGZ adulta y por lo tanto, podría ser un buen candidato que jugara un papel importante en los cambios morfológicos celulares y de migración característicos de las neuronas inmaduras que se generan en la neurogénesis adulta hipocampal (AHN del inglés "*Adult Hippocampal Neurogenesis"*). Estudios previos han demostrado que Rnd2 es necesaria de manera intrínseca en las neuronas nacidas en el adulto para su supervivencia y maduración y también es crítica para el control de la ansiedad a nivel comportamental. Las funciones dependientes del hipocampo incluyen tanto la cognición (aprendizaje espacial y memoria contextual) como el control de las emociones (ansiedad y estrés).

La neurogénesis adulta está altamente unida al nivel de actividad del circuito hipocampal. Como consecuencia, las enfermedades que modifican dicho nivel de actividad, podrían alterarla. Investigar la neurogénesis adulta en neuropatologías que conllevan un aumento en la actividad neuronal y eventualmente el desarrollo de convulsiones, como la epilepsia, los accidentes

cerebrovasculares, el síndrome de Dravet o el daño cerebral por contusiones (TBI del inglés "*Traumatic Brain Injury"*) podría aportar información básica muy relevante sobre cómo la hiperexcitación neuronal modifica la AHN y permitir el descubrimiento de nuevas dianas con potencial terapéutico.

El TBI se define como una disrupción estructural y/o fisiológica de la función cerebral como resultado de la aplicación de una fuerza externa extrema. Así, es considerado una causa importante de muerte e incapacidad a nivel mundial. El daño cerebral producido afecta también a la neurogénesis, sin embargo, queda mucho por caracterizar sobre los efectos específicos que en ella produce. Muy pocos son los estudios que se centra en caracterizar y establecer los efectos de la integración funcional de las nuevas neuronas o incluso su resultado final en funciones dependientes del hipocampo en este tipo de alteraciones. Esto dificulta en sobremanera el poder alcanzar una conclusión clara sobre el papel de la AHN tras el TBI. Por todo ello, es necesaria una caracterización más profunda y exhaustiva para determinar el papel que ejercen las nuevas neuronas generadas tras el TBI.

En la primera parte de esta tesis, tratamos de caracterizar las alteraciones causadas por el TBI en las NSCs y su progenie. La patofisiología del TBI evoluciona desde una fase inflamatoria aguda hasta el daño secundario que genera una fase crónica. Diseñamos un paradigma experimental exhaustivo que comprende cuatro puntos temporales que cubren ambas fases para analizar la respuesta neurogénica. Tanto las NSCs como las nuevas neuronas generadas se analizaron a 3 días, 15 días, 2 meses y 4 meses post-TBI. Utilizamos un modelo de ratón transgénico Nestina-GFP (GFP del inglés "*Green Fluorescent Protein"*) que permite visualizar las NSCs fácilmente. Inyectamos intrahipocampalmente vectores retrovirales para marcar de manera permanente las células que se encuentran en división durante el evento de TBI y analizar el estado de maduración de su progenie tres semanas más tarde (*3 weeks post-TBI, 3wpTBI*).

Combinando estas estrategias, pudimos describir un aumento tanto en la proliferación como en la reactividad de las NSCs durante la fase aguda del daño. Este estímulo en la proliferación está restringido únicamente a los primeros días tras el TBI y de hecho, esta tendencia se invierte durante la fase crónica. A los 4 meses post-TBI, la proliferación es más baja cuando comparamos con controles de la misma edad. Es interesante mencionar que este primer aumento que luego se convierte en disminución no correlaciona con una disminución más rápida de la población de NSCs. Los números de éstas, evolucionan de una manera similar a los de los animales control. Respecto al estado de reactividad, concluimos que se mantiene al menos hasta 2 meses tras el daño, afectando a la morfología de las NSCs durante un largo período de tiempo.

Por otro lado, correlacionando con este aumento en la proliferación de NSCs, la población de nuevas neuronas crece 15dpTBI y se mantiene alta todavía incluso a los 2mpTBI. Sin embargo, a los 4mpTBI, la densidad celular de dichas neuronas inmaduras es menor tras el TBI que en animales control, coincidiendo con el menor índice proliferativo de las NSCs y sugiriendo así un cambio en la neurogénesis durante la fase crónica del daño.

A continuación, decidimos estudiar diferentes propiedades de maduración de las neuronas generadas durante el impacto. Usando un vector retroviral para marcarlas de manera permanente, caracterizamos un aumento en el tamaño del cuerpo celular y un patrón de migración aberrante en estas células. Sin embargo, otros parámetros como la densidad de sus espinas dendríticas permanecen invariables en la ventana de tiempo establecida.

Tras esta caracterización, en la segunda parte de esta tesis presentamos la proteína Rnd2 como una potencial candidata para revertir los cambios producidos a nivel de maduración en las nuevas neuronas tras el TBI. La falta de Rnd2 específicamente en este tipo celular en condiciones fisiológicas causa un aumento en la migración y el tamaño del soma, junto con alteraciones en la arborización dendrítica. La similitud entre este fenotipo y el generado tras el TBI nos hizo plantear la hipótesis de si un aumento de su expresión en neuronas generadas durante el TBI podría ser suficiente para revertir las alteraciones observadas. Mediante inyección intrahipocampal de un vector retroviral codificante para la secuencia de Rnd2 previo a la lesión de TBI, planteamos infectar y sobreexpresar con Rnd2 las células en división en el momento de la lesión, cuya gran parte acabarían generando neuronas. Este método ha podido corregir parcialmente las alteraciones al revertir la migración aberrante de neuronas nacidas tras el TBI sin conseguir reproducir las alteraciones en el tamaño del soma.

En la última parte de esta tesis, intentamos desvelar el papel de Rnd2 en la conducta de los animales dependiente del hipocampo. Estudios previos han demostrado que su ausencia en el hipocampo adulto en condiciones fisiológicas produce alteraciones en la supervivencia y maduración de dichas neuronas, repercutiendo conductualmente en un aumento de la ansiedad del animal. Mediante nuestra estrategia de inyección intrahipocampal bilateral de retrovirus codificantes para Rnd2 logramos incrementar la presencia de la proteína en neuronas nacidas en el adulto y caracterizamos una disminución de la ansiedad y una discreta alteración de la memoria espacial relacional. Por lo tanto, concluimos que un aumento de Rnd2 en las neuronas generadas en el adulto actúa de manera opuesta en el control de las emociones si lo comparamos con su disminución y además perjudica ligeramente la memoria espacial

relacional. Su presencia en estas células, por lo tanto, es crucial en la cantidad precisa para controlar varias funciones que dependen directamente del hipocampo.

2.2. SUMMARY.

The Subgranular Zone (SGZ) is a region of the Dentate Gyrus (DG) where a resident population of adult Neural Stem Cells (NSCs) gives rise to newborn cells that ultimately will be locally integrated as mature granule cells in the hippocampal circuitry. NSCs can be defined by their position, morphology and the expression of several cell markers. These NSCs divide mostly asymmetrically to generate copies of themselves and also Amplifying Neural Progenitors (ANPs) which act as intermediate progenitors. After these ANPs divide again, they start to undergo further differentiation to become neuroblasts that develop into maturating neurons. During this switch is when the most important functional changes happen, since the population evolves in a morphologic but also an electrophysiologic and genetic expression aspect.

An extensive and dynamic remodelling of the cell cytoskeleton is promoted during neurogenesis and neuronal migration, specifically during the development and maturation of neuroblasts. However, it is still unclear how many of these changes are regulated at the molecular level. The Rho (Ras homologous) family of small GTPases are key regulators of the cytoskeleton in various cell types. Interestingly, out of the many members in the Rho GTPase family, only Rnd2 is selectively enriched in the adult SGZ and therefore it seems to be a good candidate to play an important role in Adult Hippocampal Neurogenesis (AHN). This protein is cell-intrinsically required for the survival and maturation of adult-born neurons and it is also critical for the control of anxiety-like behaviour. Hippocampus' dependent tasks involve both cognition (spatial memory and contextual memory encoding) and mood regulation (anxiety and stress).

AHN is tightly linked to the level of hippocampal circuitry activity. As a consequence, pathologies interceding in the level of this activity may end up impairing neurogenesis. Investigating adult neurogenesis in neuropathologies which involve the increase in neuronal activity, and eventually the development of seizures, like epilepsy, stroke, Dravet syndrome or Traumatic Brain Injury (TBI) could provide very useful information on how neuronal hyperexcitation alters AHN.

TBI is defined as a structural and/or physiological disruption of the brain function as a result of an external force and it is considered a worldwide leading cause of death and disability. AHN is affected by TBI, however, the specific effect that brain injury produces over NSCs and neurogenesis is still unclear. Studies focused in the functional integration of new neurons or even their final outcome in hippocampal-related tasks are uncommon. This makes it difficult to reach a clear conclusion about the role of AHN after TBI. A more exhaustive characterization of neurogenesis post-TBI is necessary in order to clarify whether it can be targeted as a potential source of new neurons compensating for the neuronal loss following TBI.

In the first part of this thesis, we aimed to characterize the alterations caused by TBI in NSCs and their progeny. The pathophysiology of TBI starts with an acute inflammatory phase that ends up generating secondary damage into the chronic phase. In order to address the possible alterations in neurogenesis at any point, we designed an exhaustive experimental paradigm which involved four different timepoints to cover this acute-to-chronic timeline. Both NSCs and new neurons were analysed at 3 days post-TBI (3dpTBI), 15 days post-TBI (15dpTBI), 2 months post-TBI (2mpTBI) and 4 months post-TBI (4mpTBI). Furthermore, for the study of the morphology and proliferation of the NSC population, we used a Nestin-Green fluorescent protein (GFP) transgenic mice line in which NSCs are readily visualized. We also used retroviral infections containing another fluorescent protein (Venus) to permanently label dividing cells during TBI and analysed their progeny three weeks later (3wpTBI) to evaluate the fitness of newborn neurons maturation.

By combining these strategies, we were able to describe an increase in both the proliferation and reactivity of NSCs during the acute phase after the injury. The boost proliferation is restricted to the first days after the insult and actually this tendency is inverted during the chronic phase. By 4mpTBI, the proliferation in NSCs is lower than in age-matching control mice. Interestingly, this increase and later decrease in the proliferation does not correlate to a sharper decrease in the NSC pool. The population evolves in a similar manner as control animals. Regarding the reactive state of this population, it is maintained for up to two months after the injury, impairing the morphology of NSCs for a long time.

On the other hand, correlating with the increase in NSC proliferation, the population of newborn neurons grows 15dpTBI and still remains increased even 2mpTBI. However, by four months, the cellular density of immature neurons is lower after TBI than in control mice, coinciding in this case, with the lower proliferative index of NSCs, suggesting a change in neurogenesis during the chronic phase of the injury.

We next decided to further study several maturating properties of neurons that were generated during the impact. Using a retroviral vector we were able to characterize an increase in their cell body size and an aberrant pattern of migration. Other parameters like their dendritic spine density, however, remained the same.

Following this characterization, in the second part of this thesis we present the protein Rnd2 as a good candidate to revert the changes produced at the maturational level of new neurons after TBI. Lack of Rnd2 in this specific cell type has already been described to cause increased migration and soma size, together with alterations in the dendritic arborization. The similarity

between this phenotype and the one generated after TBI made us wonder whether increasing the expression of Rnd2 in neurons generated during TBI could be enough to reduce the soma size and the migration back to normal levels. Using a similar strategy to the one previously described, we injected a retroviral vector containing the Rnd2 sequence in the hippocampus previous to the TBI surgery to infect newly-generated neurons. By using this method, we were able to correct the aberrant migration of new neurons, nevertheless, the cell body size alteration could not be reproduced. Therefore, Rnd2 expression is sufficient to correct the aberrant migration of neurons born after TBI.

In the last part of this thesis, we aimed to unravel the role of Rnd2 in hippocampal-related tasks. The lack of this protein in adult-generated neurons in physiological conditions is known to produce alterations in neuron survival apart from the maturating alterations previously mentioned. This, eventually, leads to an increase in anxiety-like behaviour. By injecting a retroviral vector containing the Rnd2 sequence bilaterally into both hippocampus we were able to increment the presence of the protein in adult-born neurons and we characterized a decrease in anxiety-like behaviour and a slight impairment in spatial relational memory in these conditions. Therefore, we conclude that an increase of Rnd2 in adult-generated neurons acts in an opposite manner in mood-control when compared to its decrease and it also mildly impairs spatial relational memory. Its presence in these cells, therefore, is crucial in the proper amount to control several hippocampal-dependent tasks.

INTRODUCTION

3. INTRODUCTION.

3.1. ADULT HIPPOCAMPAL NEUROGENESIS.

3.1.1. History of the discovery.

"En las instalaciones para adultos (áreas del cerebro), los nervios son algo fijo e inmutable: todas las partes pueden morir, nada puede regenerarse. Corresponde a la ciencia del future cambiar, si es posible, este duro decreto"

"In adult centres the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree".

Santiago Ramón y Cajal, 1913-1914

When making this statement, Ramón y Cajal was just representing the mainstream understanding of his time. This "central dogma" was commonly accepted in the neuroscientific community between the late 1800s and along 1900s and it maintained that the after early postnatal period, no new neurons were generated in the mammalian brain. However, around the decade of the 1950s several authors started to publish new discoveries regarding this issue and this idea started to change.

The bases for the possibility of the existence of neurogenesis in the adult brain were set already by 1958 when Messier and colleagues reported clusters of dividing cells underlying the ependyma of the lateral ventricle in rats (Messier et al., 1958). The mere presence of cell division raised a question regarding the fate of the progeny of the newly born cells. However, no generation of new neurons was identified, instead, the cell division was suggested to represent a continuous source of glia.

Then, in 1962 Joseph Altman completed and published a manuscript that is considered ground-breaking in the field of neurogenesis and neuroscience as it shattered the former immutable dogma. He studied brain lesions in adult rats taking advantage of tritium, a radioactive isotope of hydrogen, to label thymidine ([^{3}H]-TdR). Tritiated thymidine is incorporated into dividing cells and the level of its incorporation is proportional to the amount of cell proliferation. By using this complex, he was able to mark dividing cells and subsequently identify radiolabelled neuronal cells in the cerebral cortex and the hippocampus (Altman, 1962a, 1962b). A few years later,

Michael Kaplan replicated the use of this technique to again report recently generated neurons not only in rats but also in monkeys (Kaplan, 1985). Still, these findings were largely challenged by the scientific community. For example, in a study in 1988, Pasko Rakic reported that no new neurons were generated in the brain of higher vertebrates as they got old because only a few neuronal newborn cells were spotted in the dentate gyrus (DG) right after birth (Eckenhoff & Rakic, 1988; Rakic, 1985). Also, the interpretation of [^{3}H]-TdR labelling cells being neurons was considered doubtful (Korr, 1980). The identification of newly generated cells as neurons relied only in their morphological characteristics and brain localisation, which was not considered enough evidence for some. This denial of Altman's findings by the scientific community lasted around two decades.

The discovery of more specific markers to differentiate between neurons and glia in combination with the use of monoclonal antibodies directed against 5-bromo-2'-deoxyuridine (BrdU), a synthetic nucleoside analogue of thymidine, allowed the first *in vivo* evidence for mammalian adult neurogenesis. Immunocytochemical staining is easily combinable with neuronal and glial markers using double-labelled sections to confirm the phenotype of dividing cells (Cameron et al., 1993; Kuhn et al., 1996). All these new generated data from the research community decanted the balance towards the fully support of Altman initial discoveries. Over the years, BrdU was successfully used to demonstrate the existence of adult neurogenesis in rodents in at least two areas, the so-called "canonical regions": the subventricular zone (SVZ) of the lateral ventricle, where new neurons are generated and migrate anteriorly via the rostral migratory stream (RMS) to end up differentiating into interneurons in the olfactory bulbs (Alvarez-Buylla & García-Verdugo, 2002; Corotto et al., 1993) and the subgranular zone (SGZ) of the dentate gyrus where newborn neurons are generated and integrated into the pre-existing hippocampal circuitry **(Fig. I1)** (Cameron et al., 1993; H. Kuhn et al., 1996; Seki & Arai, 1993). Furthermore, several non-canonical regions have also been proposed to contain neuronal progenitor cells like the neocortex, striatum and hypothalamus (Feliciano et al., 2015; Inta et al., 2015; K. Jin et al., 2006). Among them, hypothalamic neurogenesis has been thoroughly demonstrated by the study of tanycytes, the resident progenitor cells of this area. Tanycytes respond to nutritional signals by differentiating to orexigenic or anorexigenic neurons, playing an important role in the control of feeding behaviour (Cheng, 2013; Evans et al., 2002). Adult hippocampal neurogenesis (AHN) has also been reported to be regulated by external stimuli such as stress, learning, experience and exercise (Cameron & Glover, 2015; Gross, 2000). The end of the millenium represented, therefore, a transformative paradigm shift in the AHN field.

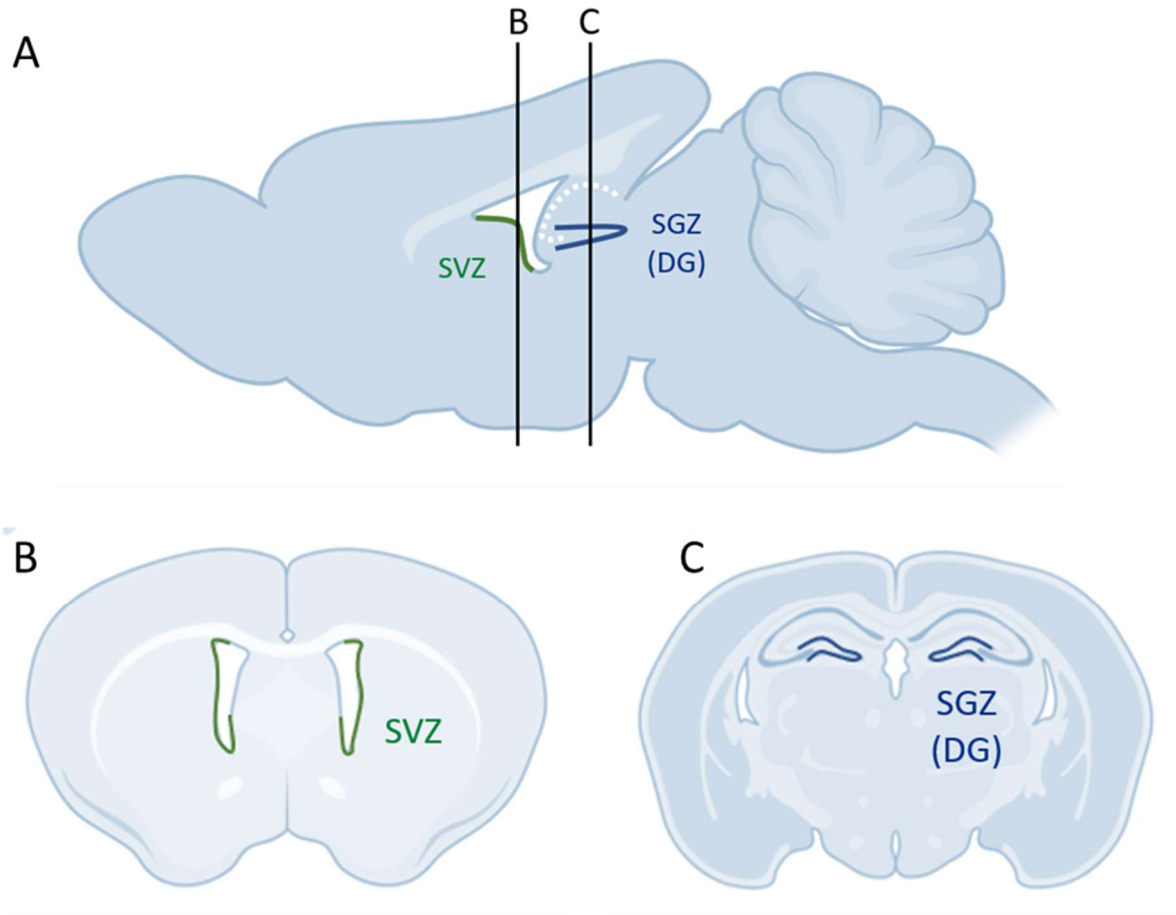


Figure I1. A. Sagittal view of the adult brain canonical neurogenic niches: SVZ (in green) and SGZ of the DG (in blue). **B.** Coronal plane of the SVZ. **C.** Coronal plane of the SGZ of the DG. SVZ: Subventricular Zone; SGZ: Subgranular Zone; DG: Dentate Gyrus.

3.1.2. Neurogenic cascade.

Once adult neurogenesis began to be accepted in the scientific field, new questions were brought to try to understand how the production of new neurons takes place and how is it regulated. Importantly, the presence of undifferentiated, multipotent stem cells potentially producing not only neurons but also several types of glial cells highlighted the possibility of regeneration in the adult brain throughout the lifespan of an animal (McKay, 1997; Seaberg & van der Kooy, 2003; Watt & Hogan, 2000; S. Weiss et al., 1996).

In the hippocampus, the SGZ is a region of the DG located between the hilus and the granule cell layer (GCL) where a resident population of adult neural stem cells (NSCs, also known as Type-1 cells or radial astrocytes) gives rise to newborn cells that ultimately will be locally integrated as mature granule cells in the DG (Obernier & Alvarez-Buylla, 2019). This neurogenic process comprises different steps in which neural stem cells proliferate and generate a progeny which

differentiates and migrates until its eventually integrated into the existing circuitry and gradually acquires physiological neuronal properties **(Fig. I2)** (Kempermann et al., 2004).

Initially, neurogenesis begins with the activation of NSCs which are normally maintained in a quiescent state with a low rate of division (2-5%) (Encinas et al., 2011a). After their activation, NSCs start mitosis which can take place in two different ways: as a symmetric division producing two new NSCs (enabling this population to self-renew by producing two daughter NSCs); or as an asymmetric division generating two daughter cells committed to different fates: one copy of themselves that will end up differentiating into an astrocyte after several rounds of division and the other giving rise to an intermediate neural progenitor which is usually known as amplifying neural progenitor (ANP) or type-2 cell (Encinas et al., 2011a; Kempermann et al., 2004). These ANPs will divide several times before they exit cell cycle and start to express doublecortin (DCX) and polysialic acid neural cell adhesion molecule (PSA-NCAM), characteristics of type-3 neuroblasts (NBs). These NBs will end up going through a stage of immature neuron until they differentiate into mature granule neurons (Encinas & Sierra, 2012). While in this neuroblasts state, these cells already start to express markers that will retain as mature neurons such as Prox1 and NeuN (Filippov et al., 2003; Kempermann et al., 2004). During the differentiation process a high percentage of neuroblasts undergo apoptosis, mostly during the first days after division being efficiently phagocytosed by microglia **(Fig. I2)** (Sierra et al., 2010). Nevertheless, the way of division, the progeny and the percentage of surviving neuroblast can change under pathological conditions (Sierra et al., 2015).

The fact that most NSCs go through asymmetric division and end up differentiating into astrocytes leads to the depletion of the NSC pool in time and the concomitant loss of their neurogenic capabilities (Encinas et al., 2011a). Nevertheless, NSC depletion may also happen by neurogenic symmetric division and ultimate neurogenic differentiation (Pilz et al., 2018). Regardless of the mechanism leading to NSC depletion, the outcome is the same in all cases: NSCs disappear over time and therefore the neurogenic capability of the hippocampus diminishes and finally gets almost completely lost (Kalamakis et al., 2019). Nevertheless, it should be noted that during animal ageing, the dynamics between quiescence and activation also change increasing the percentage of quiescence and therefore favouring the preservation of a very small population -in terms of total numbers- of NSCs and therefore a remaining neurogenic capability (Harris et al., 2021; Martín-Suárez et al., 2019; Martín-Suárez & Encinas, 2021). Different pathological conditions may also modulate the level of NSC activation differently producing an important repercussion in the numbers of newly born neurons (Sierra et al., 2015).

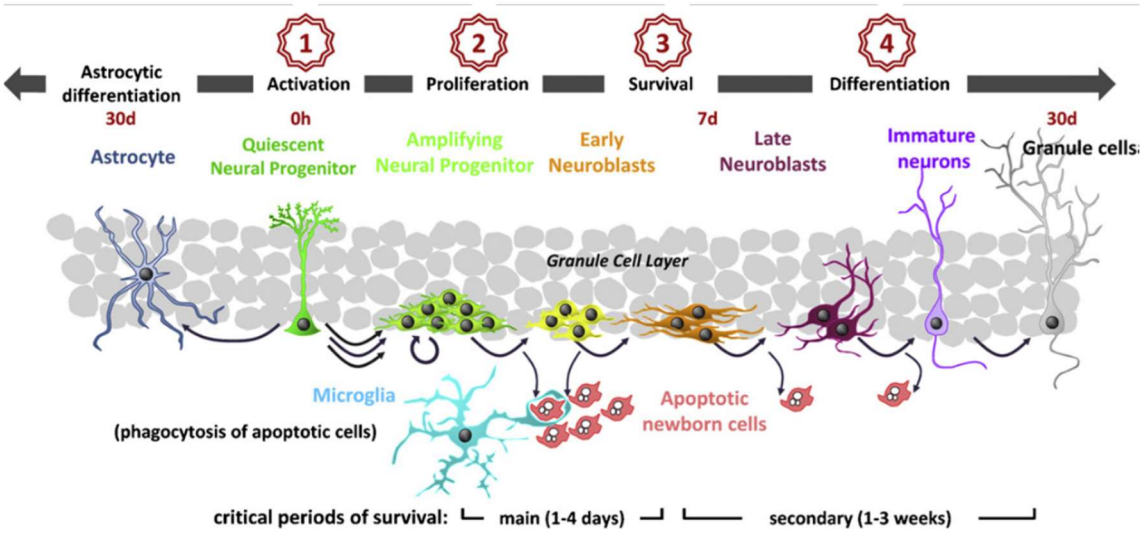


Figure I2. Adult hippocampal neurogenic cascade. NSCs (quiescent neural progenitor) with radial morphology remain inactive in the SGZ. Once they get activated, they divide several times consecutively in an asymmetric manner giving rise to ANPs that either die through apoptosis and are properly digested by microglia or slowly differentiate into mature granule cells. NSCs may also differentiate to astrocytes without generating ANPs. Modified from (Encinas & Sierra, 2012). NSCs: Neural Stem Cells; SGZ: Subgranular Zone; ANPs: Ampligying Neural Progenitors; d: days.

3.1.2.1. Adult neural stem cells.

The identification of adult NSCs (aNSCs) is crucial for the analysis of the neurogenic cascade. Years of study have been necessary to properly describe these cells as radial glia cells (RGCs) which express the glial fibrillary acid protein (GFAP) (Kosaka & Hama, 1986). The neurogenic capability of this GFAP-expressing population was later functionally demonstrated also by Seri and colleagues (Seri et al., 2001).

Nowadays, we possess more information which allows us to define aNSCs by their position, morphology and the expression -or lack of it- of several cell markers. Genetic engineering using transgenic mice opened a new era for the labelling of aNSCs through their markers. One of the most common ones is the neuroectodermal stem cell intermediate marker (Nestin) (Lendahl et al., 1990). By using these transgenic mouse lines a deeper characterization of the morphology of the aNSCs was provided (Filippov et al., 2003; Kronenberg et al., 2003). For example, Nestin-GFP cells were identified with their soma located in the subgranular zone (SGZ) and extending a process towards the molecular layer (ML) that branches once it reaches the outer region of the GCL, creating a broccoli-like shape (Filippov et al., 2003; Mignone et al., 2004). This morphology can be observed not only with Nestin, but using other markers such as Lysophosphatidic Acid Receptor 1 (LPAR1) (Walker et al., 2016). It should be considered that both these markers label

different subpopulations included in the general pool of hippocampal NSCs, pointing to a high heterogeneity of these cells (Harris et al., 2021; Walker et al., 2016). Other useful markers are the *Sex determining region Y box 2* (also known as Sox2), the intermediate filament Vimentin (Morrow et al., 2020) the Brain Lipid-Binding Protein (BLBP) (Steiner et al., 2006), or Lunatic Fringe, a key modifier of the Notch receptor (Semerci et al., 2017) which is selectively expressed in NSCs. Although many of these markers have helped during the characterization of aNSCs, their cellular specificity has always been a main issue, since they are commonly expressed in other cell types different from aNSCs.

On the other hand, aNSCs are also characterized by the lack of expression of S100B, a marker that is commonly found in mature hippocampal astrocytes (Encinas & Enikolopov, 2008; Filippov et al., 2003; Gould et al., 1992; Yamaguchi et al., 2000).

The combination of several makers combined with functional studies and single-cell sequencing analyses of these cells has revealed a high level of NSCs heterogeneity in the hippocampus (Bonaguidi et al., 2011; Filippov et al., 2003; Kronenberg et al., 2003). NSCs are connected to their environment by both receiving surrounding signals and responding to them. For example, the transition from quiescence to an active state has been largely studied in these cells (see review Urbán & Cheung, 2021). Bone morphogenetic proteins (BMPs) represent an example of NSCs interaction and allowing the regulation of quiescence, proliferation and the rate of maturation (Bond et al., 2014; Gonçalves et al., 2016; H. Mira et al., 2010) together with many others like Notch signalling and parvalbumin (Ehm et al., 2010).

3.1.2.2. Amplifying neural progenitors.

The intermediate progenitors in the neurogenic cascade are known as Amplifying Neural Progenitors (ANPs) or Type-2 cells. These are rounded cells created after one asymmetric division coming from NSCs (Encinas et al., 2006; Kempermann et al., 2004; Kronenberg et al., 2003). ANPs are is actually a quite heterogeneous population as it includes a cell type that is evolving over time, having initially more glia-like characteristics and higher proliferating rate and ending towards a more neuronal profile with less mitotic activity. For this reason, some studies have even subdivided these cells into two categories (Type-2a and Type-2b). However, due to the continuity in their changes and overlapping of some characteristics, it is hard to set a specific boundary to tell them apart (Kempermann et al., 2015).

Morphologically, ANPs extend a few, short, stubby processes oriented tangentially to the length of the SGZ and their nuclei often have an irregular shape (Llorens-Martín et al., 2016). They maintain the ability to divide at the beginning of their development but then they start to lose it as they maturate concomitantly to the expression genes associated with fate specification such as doublecortin (DCX), polysialylated neural-cell-adhesion molecule (PSA-NCAM), or Prox1 (Kempermann et al., 2015; Rao & Shetty, 2004; Seki & Arai, 1993; Steiner et al., 2006). At the end of this phase these cells start expressing doublecortin (DCX), a microtubule-associated protein related to neuronal differentiation and migration (Sánchez-Huerta et al., 2016). ANP's proliferation is influenced by different signalling pathways as it happens with NSCs, including molecules like Shh (Gonçalves et al., 2016; G. Li et al., 2013), insulin-like growth factor-1 (IFG-1), fibroblast growth factor-2 (FGF-2) (H. G. Kuhn et al., 1997; Kuwabara et al., 2009) and or vascular endothelial growth factor (VEGF) (Fabel et al., 2003).

Around 60% of these cells undergo cell death within the first 24-48 hours after cell division (Kempermann et al., 2003; Sierra et al., 2010). The mechanism for this programmed cell death, which seems to be mediated by the transcription factor BAX, is still unknown and it represents an important point of regulation which leaves ample room for increasing neurogenesis by just elevating the survival rate (Sahay et al., 2011).

3.1.2.3. Neuroblasts.

Once newborn DCX+ cells survive this crucial period, they become stable members of the DG circuitry (Kempermann et al., 2003). First, they start to undergo further differentiation to become neuroblasts also known as Type-3 cells. During this switch is when the most important functional changes happen, since the population evolves genetically and morphologically and it also changes its electrophysiological capabilities. During the first week, neuroblasts show processes parallel to the GCL and display small spikes but lack synaptic afferents. One week later, they exhibit spineless dendrites that reach the inner ML and receive functional GABAergic contacts. Finally, they get established in their final position and show dendritic spines reaching the outer ML (Esposito, 2005). However, at this stage, another wave of cell death happens, although smaller in proportion, (approximately 12-16 days counting from the birth of the cells), coinciding with the beginning of the selection that is the synaptic integration. It is up to their success in integrating in the hippocampal network whether they survive or not: newborn neurons must receive NMDA receptor-dependent input or they will be removed from the circuit (Tashiro et al., 2006).

The fact that new cells are being incorporated into the DG throughout the lifespan of an animal predicts either that the DG should grow in size together with the animals age or that previously generated dentate granule neurons should die generating space for the new ones. Both of these events do take place in the adult hippocampus: the DG volume increases in rodents until approximately 6 months of age (Bayer et al., 1982; Kempermann, 2012; Ngwenya et al., 2015), and furthermore, a percentage of early postnatally generated and adult generated granule cells have been shown to die in adult animals (Cahill et al., 2017).

Neuroblasts are already considered as post-mitotic since they have no longer the ability to proliferate. They shift their orientation from tangential to the length of the SGZ to a more polarised morphology to assume a vertical orientation with dendrites that project from the soma into the GCL and axons projecting into the hilus and eventually to the CA3 pyramidal cell layer (Danzer et al., 2008; Toni et al., 2008; C. Zhao, 2006). The dendritic tree will be acquiring complexity along with this maturation process and it is then when dendritic spines begin to be generated (C. Zhao, 2006). At about 3 weeks into maturation, excitatory synapses first appear at these newly formed dendritic spines (K. Jin et al., 2006). Axons will also keep growing during these first weeks of development gradually increasing in length and extending towards the CA3 and CA2 areas (R. L. Faulkner et al., 2008; Kohara et al., 2014). During this period, neuroblasts receive largely extra-synaptic and synaptic GABAergic (GABA, γ-aminobutyric acid) and glutamatergic input (Ge et al., 2006; Laplagne et al., 2006; Markwardt et al., 2009). This input comes initially from local neurons (largely interneurons) before they receive synaptic inputs from long-range projection neurons (Bergami et al., 2015; Deshpande et al., 2013; Vivar et al., 2012). When the first synapses are formed onto newborn neurons in the molecular layer, these neurons also form axonal-synaptic connections with hilar and pyramidal cells in the hilus and CA3 areas (R. L. Faulkner et al., 2008; Toni et al., 2008). Additionally, they will be directly connected monosynaptically with other mature granule cells to interact with the DG network activity (Luna et al., 2019).

During the period of synaptic reorganization and maturation, newborn neurons also dramatically change their intrinsic properties. Young granule cells are characterized by inverted chloride potential due to high expression of the Na^+-K^+-Cl^- cotransporter (NKCC1), causing GABA to have initially a depolarizing effect on newborn neurons' membrane potential. Eventually, they switch towards mature chloride gradients by expression of the potassium-chloride transporter (KCC2) and subsequently lose their high input resistance (Ge et al., 2006, 2007; Markwardt et al., 2009; Schmidt-Hieber et al., 2004; Wang et al., 2000).

Together with the morphological changes and as a part of their maturation journey, neuroblasts also migrate through a relocalization process. At the beginning they are located in the SGZ where the bulk of progenitors is. Then they initiate a tangential migration away from their mother progenitor cells, guided at least partly by DG vasculature, before they stop tangential migration and move radially into the GCL (Sun et al., 2015; Zhao, 2006). What causes the switch from tangential to radial migration is still unknown. They will dive themselves into first third of the GCL and remain there for the rest of their lives.

Interestingly, a number of genes (Notch, GABA, NeuroD1, Prox1…), related to developmental processes or neuronal cell fate (Gonçalves et al., 2016) are expressed in neuroblasts are also expressed in ANPs or NSCs. In particular, the small Rho GTPases family has several members crucial for early migration, neurite extension and spine formation (Beckervordersandforth et al., 2017). On the other hand, additional genes will be newly expressed and added to this panel of cell fate determining genes. For example, Reelin influences neuronal fate and migration, the formation of dendritic spines and final integration of granule neurons into the circuitry (Ampuero et al., 2017; Caruncho et al., 2016). Surrounding cAMP induces gene transcription by activating cAMP-dependent protein kinase (PKA), which phosphorylates cAMP response element-binding protein (CREB) and facilitates transcription of other molecules such as c-fos, jun-B, Bcl-2, glial-derived neurotrophic factor (GDNF) and various neurotrophins such as brain derived neurotrophic factor (BDNF) that regulate neuronal survival and regeneration (Zhang et al., 2016).

3.1.2.3.1. Role of Rnd2 in neuroblasts.

An extensive and dynamic remodelling of the cell cytoskeleton is promoted during neurogenesis and neuronal migration, specifically during the development and maturation of neuroblasts. The translocation of neuronal nucleus after neurogenesis requires dynamic changes of the cell cytoskeleton (Taverna & Huttner, 2010).

The Rho (Ras homologous) family of small GTPases are key regulators of cell cytoskeleton in various cell types **(Fig I3)** (Ridley, 2001a). It includes the classical members which cycle between an active GTP-bound state and an inactive GDP-bound state, but also some atypical members, like the case of the Rnd subfamily. This subfamily represents a distinct branch of the Rho family and consists of three different members: Rnd1/Rho6, Rnd2/Rho7 and Rnd3/Rho8/RhoE (Chardin, 2006; Riou et al., 2010). They possess low or no intrinsic GTPase activity and are therefore considered to be constitutively active (Chardin, 2006; Nobes et al., 1998; Riou et al.,

2010). Other mechanisms such as gene expression, post-transcriptional protein modifications or subcellular localization control Rnd activity.

Rnd1 and Rnd3 first functions were described by over-expressing them in cultured fibroblasts: cell retraction from the substrate and cell rounding was observed, which provided their collective name round (Rnd). However, expression of Rnd2 in the same conditions did not modulate cytoskeletal reorganization, suggesting it might act via different mechanisms in these cells (Nobes et al., 1998).

Of these three members, only Rnd2 and Rnd3 show strong expression in the developing cerebral cortex (Heng et al., 2008). *In vivo* knock down of these two in the embryonic cortex produces migratory defects characterized by distinct morphological abnormalities (Azzarelli et al., 2014; Heng et al., 2008; Nakamura et al., 2006; Pacary et al., 2011). Furthermore, Rnd2 knock down increases the fraction of neurons with a multipolar shape suggesting that Rnd2 may regulate multipolar to bipolar transition (Heng et al., 2008). Despite having similar functions, Rnd2 and Rnd3 cannot substitute for one another during cortical neuron migration. This has been attributed at least in part to their different specialized locations: Rnd3 is preferentially associated to the plasma membrane, whereas Rnd2 is cytoplasmic or associated to endomembranes (Pacary et al., 2011; Roberts et al., 2008).

A mechanism which is commonly used by Rnd proteins to control cytoskeletal dynamics is the inhibition of RhoA signalling **(Fig. I3)** (Nobes et al., 1998; Riou et al., 2010; Wennerberg et al., 2003). However, it has been proven that Rnd2 acts only partially through the suppression of RhoA activity in migrating neurons. Rnd2 and Rnd3 knock down produce an accumulation of F-actin in neuronal processes as well as in the cell body in the case of Rnd2. The activation of RhoA ultimately phosphorylates and inactivates cofilin which is an actin-disassembling factor, thus resulting in local increase of F-actin (Pacary et al., 2013). Moreover, the effect of Rnd2 over migration and cytoskeletal organization work in an independent manner. It is possible that the accumulation of F-actin and aberrant cytoskeletal organization upon Rnd2 knock down might be secondary to other events that impede migration. Rnd2 has been shown to be expressed in endosomes and to interact with molecules involved in the formation and trafficking of endocytic vesicles (Fujita et al., 2002; Tanaka et al., 2002; Wakita et al., 2011), raising the possibility that Rnd2 pro-migratory activity may involve the regulation of endocytosis. Further studies will be required to test this hypothesis.

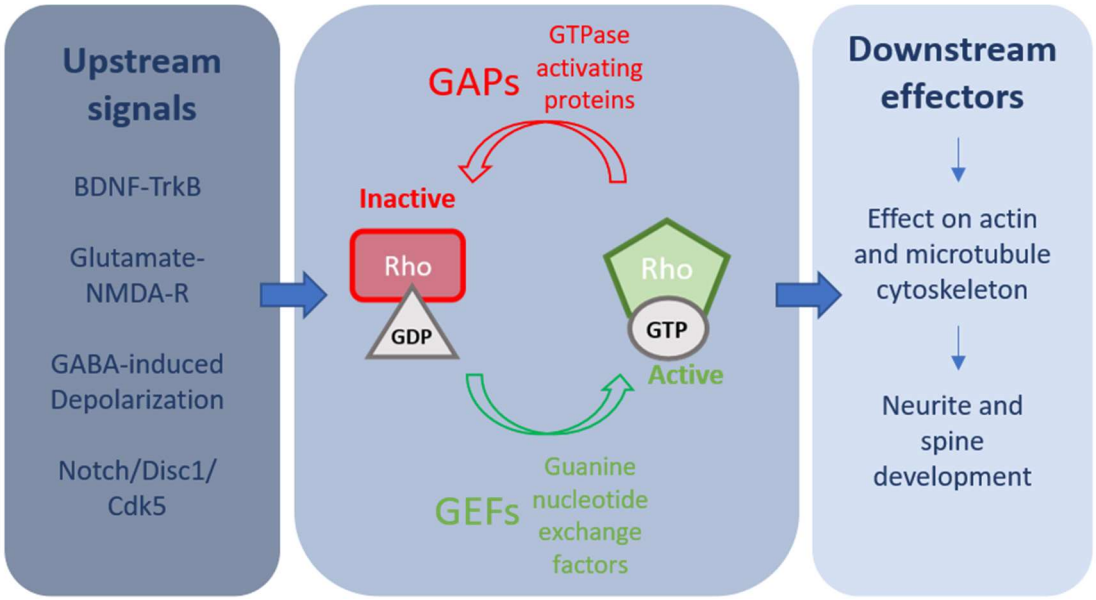


Figure I3. Small Rho GTPase signalling in neurite and spine maturation. Upstream signalling modulates GEFs and GAPs thereby regulating the activity of Rho GTPases like Rac1, Cdc42, and RhoA. GEFs promote activation (GTP-bound conformation) and GAPs promote inactivation (GDP-bound conformation). In the active state, Rho GTPases bind to several downstream effectors exerting influence on local actin and microtubule networks, thereby influencing neurite and spine growth. Modified from (Vadodaria & Jessberger, 2013). GDP: Guanine Diphosphate; GTP: Guanine Triphosphate; GEF: Guanine Nucleotide Exchange Factor; GAP: GTPase-activating protein.

The role of Rho GTPases during adult neurogenesis is mainly unknown (Vadodaria & Jessberger, 2013): Cdc42, for example, is involved in NSC proliferation, dendritic development and spine maturation while Rac1 is important in the late steps of dendritic growth and spine maturation in AHN (Vadodaria et al., 2013). Interestingly, out of the many members in the Rho GTPase family, only Rnd2 is selectively enriched in the adult SGZ **(Fig. I4)** (Miller et al., 2013) and therefore it seems to be a good candidate play an important role in AHN. In fact, Rnd2 is cell-intrinsically required for the survival and maturation of adult-born neurons and it is also critical for the control of anxiety behaviour (Kerloch et al., 2021). The maturation-related functions that are attributed to Rnd2 in AHN are similar to those that had already been described in cerebral cortex development (Heng et al., 2008; Pacary et al., 2011) including migration and neurite outgrowth. In contrast, its role in newborn neuron' survival seems to be specific to adulthood. These roles are controlled both independently and through different cellular mechanisms (Kerloch et al., 2021). The signalling pathways mediating Rnd2 action in general are poorly understood (Azzarelli et al., 2015). It has been mentioned before how Rnd2 promotes migration partially through the inhibition of RhoA but the inhibition of RhoA does not seem to be involved in Rnd2-mediated neuronal survival (Kerloch et al., 2021; Pacary et al., 2011).

As it was expected, the alteration of Rnd2 expression in newborn neurons comes with functional implications in hippocampal-related tasks. The deletion of Rnd2 in adult newborn neurons impacts anxiety-like behaviour while depression-like behaviour is not affected (Kerloch et al., 2021). Moreover, reference memory and behavioural pattern separation are not altered either, however, this result may be argued depending on the used strategy and other factors (Kerloch et al., 2021). For example, in the study cited here, the deletion is acquired through a retroviral-infection strategy, which only targets a population of newborn neurons born at the specific time of the injection (Kerloch et al., 2021). Consequently, anxiety-like behaviour could be more sensitive to the loss of new neurons compared to other memory processes. It still remains to be unravelled whether the key reason for the behaviour abnormalities is the death of newborn neurons, their mispositioning, their abnormal morphology or the result of all of these traits combined.

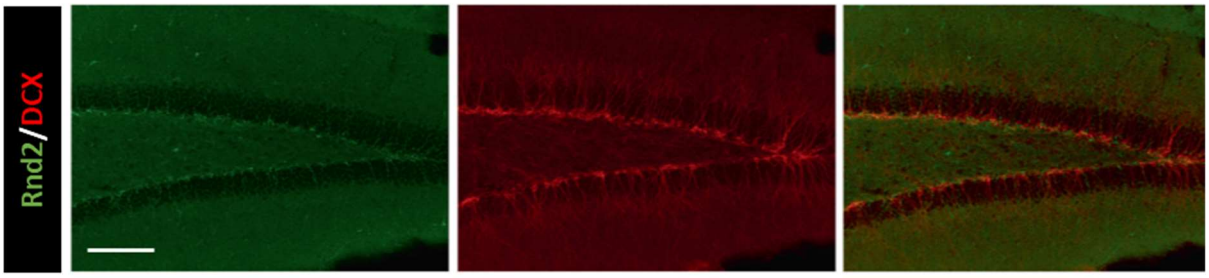


Figure I4. Rnd2 is the only Rho GTPase expressed in the adult dentate gyrus, colocalizing in the neurogenic area with cells expressing DCX. Scale bar is 100µm. (Data and images source: Kerloch et al., 2021).

3.1.3. Function of adult hippocampal neurogenesis.

Hippocampal-dependent tasks are both cognition and mood-related. It has been thoroughly studied how hippocampal function plays an important role in spatial learning, contextual memory encoding (Kheirbek et al., 2013) but also emotional behaviours such as anxiety and stress resilience (Hughes, 1965; E. Moser et al., 1993; Stevens & Cowey, 1973). AHN involvement in the functional role of the hippocampus has been largely studied. Unfortunately, the methods traditionally used to evaluate this role, have been shown to produce non-specific effects when either ablating or potentiating neurogenesis. Strategies that disrupt neurogenesis may also reduce proliferation in other cell types outside the neurogenic niche or potentiate the presence of inflammation or dying cells producing off-target effects and therefore, providing results which cause may be confusing (Dupret et al., 2008; Gu et al., 2012; Jessberger et al., 2009; Saxe et al., 2006; Shors et al., 2001). For example, using approaches based on pharmacology, transgenesis

or irradiation may not only impair NSCs function but also niche environment (Devarakonda et al., 2021; Saxe et al., 2006).

More specific tools to study AHN have been generated and allowed to prove the functional role of adult-generated neurons. By specifically blocking neurogenesis in the DG, the relevance of new neurons in many tasks becomes evident. AHN is crucial for spatial recognition and pattern separation tasks (Jessberger et al., 2009; Nakashiba et al., 2012; Sahay et al., 2011) along with the formation of new memories (Farioli-Vecchioli et al., 2008), learning (Deng et al., 2009; Zhang et al., 2008), stress-response, anxiety and fear (Aimone et al., 2011; Bergami et al., 2008; Santarelli et al., 2003; Saxe et al., 2006; Snyder et al., 2011; Zhang et al., 2008).

Furthermore, AHN is also modulated by different external factors. There is an extensive list of extrinsic stimuli that can also in return modulate neurogenesis. Some of them include living in an enriched environment (Kempermann et al., 1998; Nilsson et al., 1999), dietary restrictions (Lee et al., 2000), physical exercise (Hodge et al., 2008; Kronenberg et al., 2003; van Praag et al., 1999). All of these produce an increase in cell proliferation, which is generally related to the ANPs, therefore keeping the quiescence in NSCs. One the other hand, there are external stimuli that can regulate negatively the production of new neurons like stressful experiences (Gould et al., 1992; Tanapat et al., 2001) or ageing (Altman & Das, 1965; Barker et al., 2004; Beccari et al., 2017; Encinas et al., 2011b).

In conclusion, AHN contributes to a plethora of behavioural tasks that directly rely on the hippocampus. In return, environmental factors are also able to modulate the neurogenic niche. This bidireccional regulation of the neurogenic process turns AHN into a process of exceptional plasticity.

3.1.4. AHN under pathological conditions.

GABA neurotransmitter plays an essential role into translating neuronal activity into NSCs activation. NSCs respond directly to GABA via $GABA_A$ and $GABA_B$ receptors (Giachino et al., 2014; Song et al., 2012). GABA released by parvalbumin-expressing interneurons acts tonically on NSCs maintaining them in quiescence. Blocking the action of GABA in NSCs not only increases activation but also promotes symmetrical cell division (Song et al., 2012).

AHN is tightly linked to the level of activity of the hippocampal circuitry. As a consequence, pathologies affecting to neuronal activity may end up disrupting neurogenesis. Increasing neuronal activity to a level of hyperexcitation is a hallmark in several pathologic conditions

(Huttmann et al., 2003; Segi-Nishida et al., 2008). However, hyperexcitation is not always uniform. On the contrary, several factors like the areas involved in the disturbance or the strength of the stimuli producing it, may alter the level of the hyperexcitation itself and therefore produce a different outcome in terms of the neurogenic disturbance (Martín-Suárez et al., 2020; Sierra et al., 2015).

A wide variety of disorders are characterized by neural hyperexcitation showing a plethora of different effects over neurogenesis. A very clear way to show this effect is comparing two animal models of epilepsy that only differ from one another in the severity of the hyperexcitation that characterizes each of them such as Mesial Temporal Lobe Epilepsy (MTLE) and epileptiform activity (EA)(Sierra et al., 2015). MTLE recapitulates a chronic condition in which a third of patients do not respond to medical treatment (Gibbons et al., 2013) and that commonly associates with hippocampal sclerosis (reactive gliosis, neuronal death and granule cell dispersion). On the other hand, EA consists in neuronal hyperexcitation discharges in the form of waves and spikes that do not trigger seizures, but are detectable by electroencephalographic (EEG) recordings in epilepsy patients (Bouilleret et al., 1999). Both conditions can be modelled in mice by performing intrahippocampal injections of higher and lower dose of kainite (KA) respectively (Bouilleret et al., 1999). In the case of MTLE, seizures cause hippocampal NSCs to become reactive: they develop a hypertrophic and multibranched phenotype **(Fig. I5 C)** and get activated to enter the cell cycle in large numbers. These reactive NSCs switch to a symmetric division cell mode to generate reactive astrocytes as daughter cells, while in parallel, they directly transform into reactive astrocytes as well **(Fig. I6, right panel)**. As a result, the neurogenic lineage becomes almost completely abolished (Sierra et al., 2015). However, neuronal hyperactivity in the form of EA induces more NSCs to become activated without developing a reactive phenotype **(Fig. I5 A)** or switching to symmetric division, and neurogenesis and astrogliogenesis are transiently enhanced. Nevertheless, EA accelerates the decline of the NSC population leading to decreased neurogenesis in the long term **(Fig. I6, left panel)** (Sierra et al., 2015). It seems that the change produced by EA is only at a quantitative level over the neurogenic cascade.

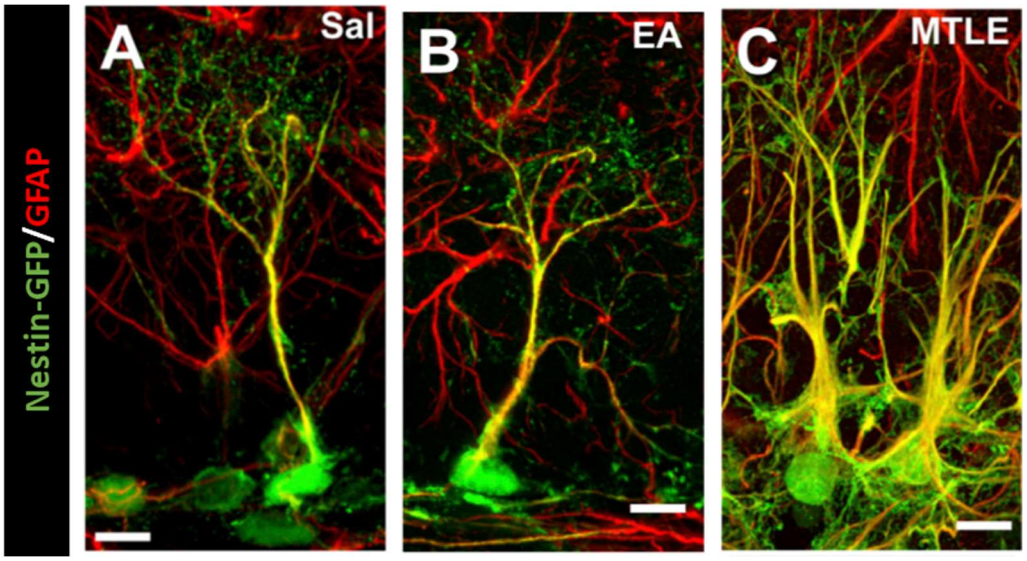


Figure I5. Nestin-GFP (green) and GFAP (red) expressing NSCs under different levels of neural hyperexcitation: **A.** SAL (saline injection, left); **B.** EA, (middle) and **C.** MTLE (right) showing their changing morphology and marker expression. (Data and images from Sierra et al., 2015). Scale bar is 10µm in A, B and 20µm in C. NSCs: Neural Stem Cells; SAL: Saline; EA: Epileptiform Activity; MTLE: Mesial Temporal Lobe Epilepsy.

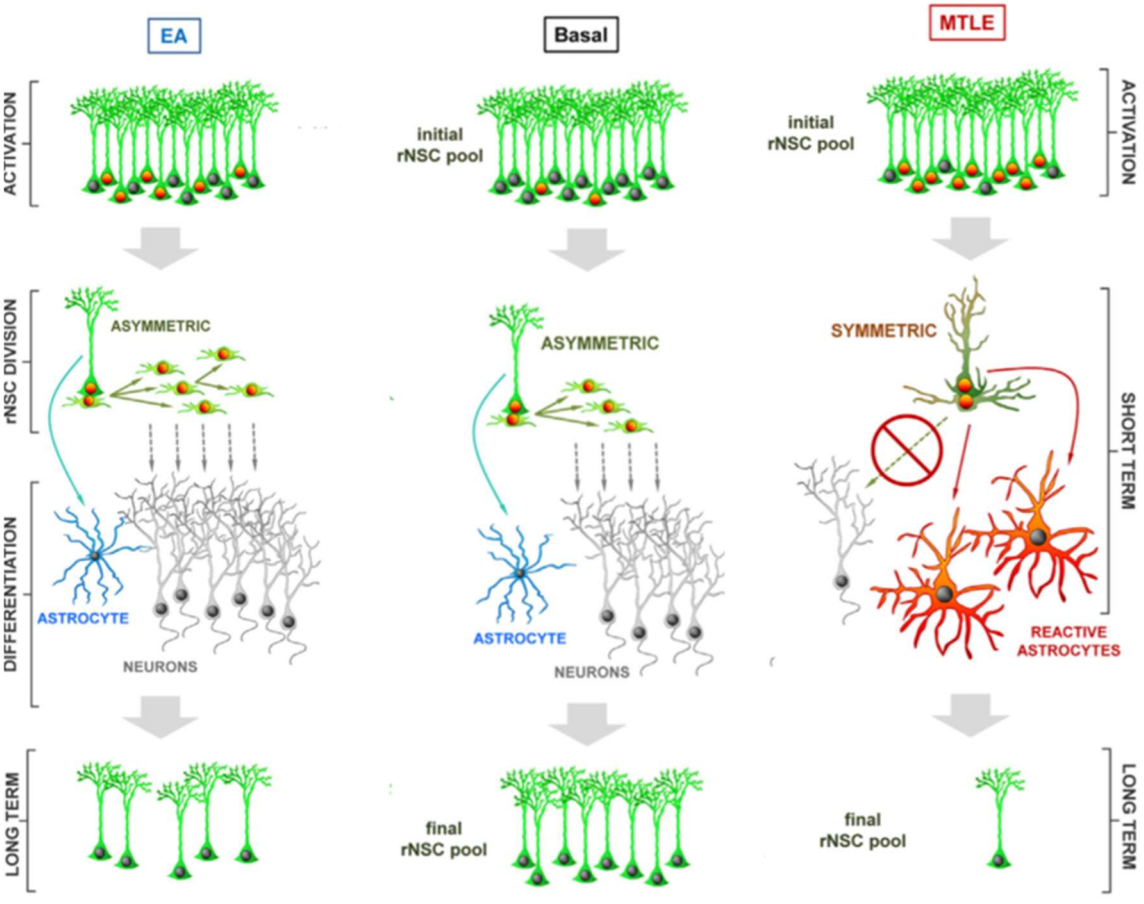


Figure I6. Schematic representation of the neurogenic cascade under different pathological conditions: Basal (middle column), EA (left column) and MTLE (right column). The different outcomes like aberrant neurogenesis or depletion of the NSCs pool are presented in the central and inferior row respectively.

(Modified from Sierra et al., 2015). EA: Epileptiform Activity; MTLE: Mesial Temporal Lobe Epilepsy; NSCs: Neural Stem Cells.

The characterization and study of the direct effects of neuronal hyperexcitation in models with a higher complexity is extremely difficult because it involves interrelated factors. Moreover, it should be taken into account that the original cause for the neurogenic disturbances cannot be clearly attributed to neuronal hyperexcitation. Nevertheless, using and integrating this knowledge in other neuropathologies involving the development of seizures like stroke, Dravet syndrome or Traumatic Brain Injury (TBI) could provide very useful information on how neuronal hyperexcitation modifies AHN (Bielefeld et al., 2019; Martín-Suárez et al., 2020; Woitke et al., 2017).

3.1.5. AHN in humans.

Ever since AHN was described in several species of mammals, it raised the question on whether there could be a neurogenic niche also in humans. For a few decades, 5-bromo-2'-deoxyuridine (BrdU), a thymidine analogue incorporated into DNA, was used as a diagnostic tool in different pathologies to measure DNA synthesis (Dolbeare, 1995). In 1998, Peter Eriksson was able to analyse human post-mortem hippocampal samples from patients suffering cancer that were intravenously treated with BrdU. This allowed Eriksson and colleagues to identify for the first time co-stained cells for both this BrdU and neuronal markers in the GCL of the DG. Therefore, they claimed that new neurons were generated in the human brain throughout life (Eriksson et al., 1998). This finding was also supported by *in vitro* data obtained later, using samples from different surgical procedures in which neural progenitors extracted from the hippocampus were able to form neurospheres and differentiate into neuronal cells (Coras et al., 2010; Hermann et al., 2006; Moe et al., 2005; T. D. Palmer et al., 2001). Nevertheless, there were still doubts regarding Eriksson's data, since other researchers were not able to detect expression of cell division markers like MKI67 (encoding for the protein Ki67) along the GCL of human patients of epilepsy (Del Bigio, 1999). Apart from division markers, specific proteins involved in neuronal maturation have also been searched in human tissue with conflicting results (Knoth et al., 2010; Mathews et al., 2017). This has fuelled much speculation regarding the accuracy of using certain markers and the conclusions that can be drawn. Interestingly, one of the studies that generated more discussion on this subject introduced a completely new technique to elucidate the issue. During this period, carbon 14 isotope (^{14}C) was released to the atmosphere after the cold-war rising nuclear-bomb tests. Taking advantage of ^{14}C integration into genomic DNA, Kirsty Spalding

et al. had the idea of using it as a tool to assess the generation of new neurons (Spalding et al., 2013). Their model suggested a neuronal turn-over in which about one-third of the total neuronal population of the hippocampus is subjected to exchange, with a modest decline during aging.

None of the studies published so far where free of criticism among the scientific community. For example, regarding Eriksson's and Spalding's work, both BrdU and ^{14}C incorporation into DNA could be theoretically caused by DNA repair or methylation (Breunig et al., 2007; Duque & Spector, 2019). Nevertheless, later studies showed how neither BrdU nor ^{14}C are taken up by dying neurons or during DNA repair, at least not in the scale that they were detected in those studies (Bauer & Patterson, 2005; Huttner et al., 2014). Spalding's work, which showed a modest decline of hAHN with aging, was also reviewed a few years later in a comparative study done by Lipp and Bonfanti. Here, they put together the different approaches used in a variety of publications and include large inter-individual and inter-species variations describing a sharper decline in humans that paralleled better the observed data found in rodents (Lipp & Bonfanti, 2016), correcting the proposed model by Spalding. On the other hand, other studies published at the time paid more attention to the conclusions that seemed to be common among what was known and try to set the ground for future work to add new ways to study the generation of new neurons in adult humans (Kempermann et al., 2018).

Recently, two studies published in a few weeks margin showed completely opposite conclusions. The first one was done by Sorrells et al., and characterized the rareness or even absence of neurogenesis in adult humans in base of the lack of morphological features and the negative detection of DCX and PSA-NCAM. He focused in fetal and perinatal ages comparing them to adult tissue. They found a huge decline of DCX-positive cells from fetal development until the first years of life, concluding with negligible amounts of DCX cells could remain in adults (Sorrells et al., 2018). The second study, done by Boldrini et al. detected a large number of DCX or PSA-NCAM positive cells interpreting that as evidence of neurogenesis. In this case, they showed the presence of dividing progenitor cells using Ki67 together with progenitor cell markers like Nestin and Sox2 together with immature neuron markers (DCX and PSA-NCAM) (Boldrini et al., 2018). Ever since then, the debate has been open and multiple causes for these conflicting results have been proposed. Some of them include the post-mortem delay (PMD), meaning the time between the death of a person and fixation of the brain; the differences in fixation methods or the clinical history of the obtained tissue (i.e. from epilepsy surgery as control) (Kempermann et al., 2018; H. G. Kuhn et al., 2018; Tartt et al., 2018). All this variability should be taken into consideration for a full and robust evaluation of the evidence.

It should be noticed how previous studies (Eriksson et al., 1998; Spalding et al., 2013) focused in the detection of precursor dividing cells and analysing their progeny using mature neuronal markers, therefore identifying integrated new neurons. In contrast, both Sorrells and Boldrini base their conclusions on histological analysis of markers for precursor cells as well as immature neurons. In this sense, the use of DCX and PSA-NCAM expression as sole indicators of neurogenesis in humans could be problematic since we could find a decoupling of the precursor cell proliferation -which would be considered a first step to neurogenesis- from the actual differentiation and integration of new neurons into the hippocampal circuitry.

The one thing that most authors agree on is the need for additional approximations to study the generation of new neurons in adult humans, for example single-cell RNA-sequencing, which is likely to provide very valuable information. However, while the application of these state-of-the-art technique arrives, other scientists focused in trying to reduce the variability of the results refining the protocols and eliminating the possible sources of controversy. For example, by minimizing the post-mortem delay, improving the method of fixation and antigen retrieval, and making an extensive study of the majority of the available antibodies for immature neuron detection, Flor-García et al. provided a very detailed precise protocol setting the ground for an improved way to analyse human brain tissue (Flor-García et al., 2020). Using this protocol they were able to identify newborn neurons in the adult brains of human up to the ninth decade of life **(Fig. I7)** and even notice a difference when comparing from tissue coming from Alzheimer's patients (Moreno-Jiménez et al., 2019). Parallelly, another report studying DCX expression in elder individuals supported these results (Tobin et al., 2019). They did not only identify developing neurons but also what they considered to be progenitors: Nestin and Sox2-expressing cells in the DG.

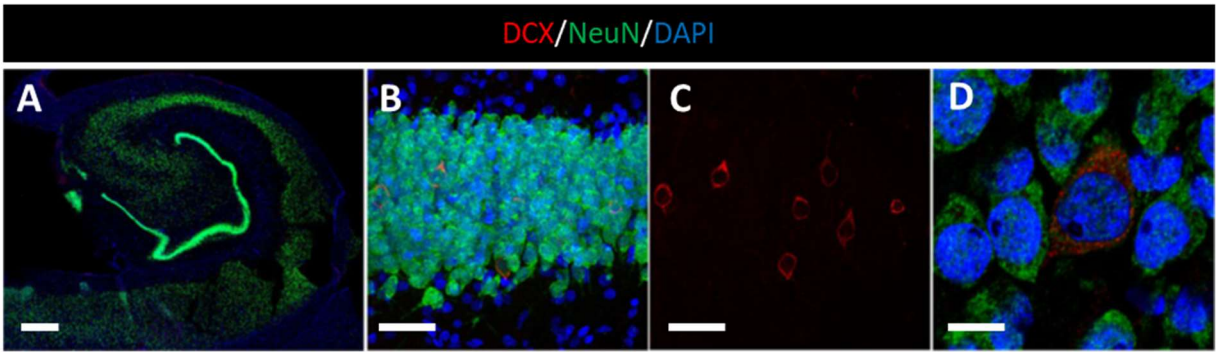


Figure I7. Whole human hippocampus **A.** together with representative images of the DG showing the presence of $DCX^{+}/NeuN^{-}$ immature neurons **B-D.** (Images and data from Moreno-Jiménez et al., 2019). Scale bar in A is 200µm; scale bar in B and C is 50µM and scale bar in D is 10µm. DG: Dentate Gyrus.

Perfecting the protocol to detect adult-generated neurons in human tissue opens the door for future research to expand our knowledge about their implication in several pathologies. Abnormal morphological development and changes in the expression of different markers of adult-born neurons has already been described in tissue from patients of amyotrophic lateral sclerosis, Huntington's disease or Parkinson's disease, for example (Terreros-Roncal et al., 2021).

All these recent works of the last triennium are the best example of how new approaches and improvements of old techniques can expand our knowledge of hAHN.

3.2. TRAUMATIC BRAIN INJURY.

3.2.1. Definition, epidemiology, causes and vulnerability.

Traumatic Brain Injury (TBI) is defined as a structural and/or physiological disruption of the brain as a result of an external force. According to the Centre for Disease Control and Prevention (National Public Health Agency of the United States), a TBI can be caused by a bump, blow or jolt to the head or a penetrating head injury (Menon et al., 2010).

Due to the high variability of lesions, cognitive problems and sequels involved in TBI, its definition, classification, diagnosis and treatment have been in constant evolution to date. It is not surprising that case definitions and characterization of the injury vary importantly when interpreting TBI patients. For this reason, the real incidence in the population is underestimated.

TBI is known to be a worldwide leading cause of death and disability. An extrapolation of internationally reported cases estimates over 50-60 million individuals are affected by TBI every year predicting that ≈50% of the world's population will sustain a TBI during their lifetime (Capizzi et al., 2020; Maas et al., 2017). The pooled international incidence of TBI is reported to be around 250 cases per 100.000 people in one year (Nguyen et al., 2016). However, most of these TBI are mild in severity **(Fig. I8 A)**. The annual incidence of mild TBI (mTBI) is 224 cases per 100.000 person-year, almost 10 times the incidence of moderate TBI and 17 times the incidence of severe TBI (Nguyen et al., 2016).

The leading cause for TBI in 2014 of youngest (0-4 years) and oldest (>75 years) age groups were unintentional falls. Meanwhile, in middle-aged groups the most common cause for TBI hospitalizations and death are motor vehicle crashes. There are a plethora of different causes and mechanisms by which a TBI could take place: intentional self-harm, participation in contact

sports like boxing, American football or skating; assaults or even being struck by or against an object (Peterson et al., 2019). Vulnerability and/or increased risk of injury towards TBI is can be related to biologic factors, as it happens with the elder population (Ramanathan et al., 2012) but it is more commonly linked with socioeconomic and environmental factors, like homelessness, workers in high-risk occupations, prison populations or women exposed to intimate partner violence (Engström et al., 2018; Mollayeva et al., 2018).

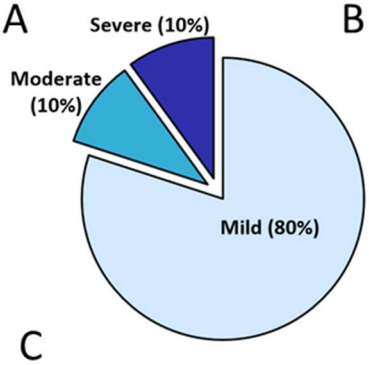
3.2.2. Severity measurement.

A correct evaluation of the brain injury severity helps to make a precise prognosis and estimate the functional recovery, anticipating thus patient's rehabilitation needs. However, the main problem identified in many studies is a lack of proper head-injury classification aiming to analyse TBI-related complications (Nguyen et al., 2016). Next, we expose the current diagnostic scales:

3.2.2.1. Diagnosis: Glasgow Coma Scale and Abbrevieated Injury Scale.

Glasgow coma scale (GCS) is the most common tool to assess the severity of TBI in human patients in emergency departments. GCS is based in three behavioural aspects: (1) motor responsiveness, (2) verbal performance and (3) eye opening. Depending on the obtained score in the GCS, patients are classified into three definitive categories as mild (GCS = 14-15), moderate (GCS = 9-13) or severe (GCS = 3-8) (Grote et al., 2011) **(Fig. I8 B-C)**. However, it is important to take into account that TBI patients often show up in the hospital while suffering from psychological stress, facial and multiple injuries or under the influence of alcohol or drugs. For this reason, the deterioration in consciousness state with low level of GCS may be related to other non-TBI injury (Kraus & McArthur, 1996).

A



B

Glasgow Coma Scale		
Behaviour	Response	
Eye opening response	1	No response
	2	To pain
	3	To speech
	4	Spontaneously
Verbal response	1	No response
	2	Incomprehensible sounds
	3	Inappropiate words
	4	Confused, but able to answuer to questions
	5	Oriented to time, person and place
Motor response	1	No response
	2	Abnormal extension, decerebrate posture
	3	Abnormal flexion, decorticate posture
	4	Flex to withdraw from pain
	5	Moves to localised pain
	6	Obeys commands

C

GCS Score	Injury Severity
3-8	Severe
9-12	Moderate
13-15	Mild

Figure I8. A. TBI incidence distributed by severity in the general population. Data from (Capizzi et al., 2020). **B.** GCS evaluating scale. **C.** GCS score resulting from the evaluation. TBI: Traumatic Brain Injury; GCS: Glasgow Coma Scale.

To solve the previously mentioned problem, physicians have been working on developing another tool for the assessment of brain injury severity: the Abbreviated Injury Scale (AIS). In contrast with GCS, AIS relies into an anatomical scoring scale, which ranks each anatomic region on a 1-6 ordinal scale using neuroradiology or operative findings (Haasper et al., 2010). To allow valid comparison between studies dealing with brain injury, a uniform terminology of TBI severity definition is needed (Savitsky et al., 2016). When dealing with animal models, both GCS and AIS scales are (obviously) useless, thus an alternative classification for the degree of injury is needed. In these cases, severity is fixed prior to the injury depending on the factors acting on it (see **3.2.5.3.2. Animal models**).

Apart from the above-mentioned executive, behavioural and functional tests there are fewer additional alternatives to diagnose TBI and assess the severity of it. From these ones, traditional neuroimaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) can be very useful for identification of life-threatening intracranial injury at the acute stage. However, they are not sensitive enough to discern between micro-damages such as diffuse axonal injury (DAI). Furthermore, these tests remain poorly correlated with functional outcome and cognitive impairment (Azouvi, 2000). However, recent advances in neuroimaging have led to a better understanding of the functional neuroanatomy of cognitive disorders associated with TBI, like positron emission tomography (PET) in patients with severe TBI, with no detectable macroscopic structural lesions (Fontaine et al., 1999). In conclusion, more

research is needed in the neuroimaging field in order to detect earlier severe damage, preventing thus the later negative outcomes that arise after rehabilitation therapies.

Historically it has been assumed that patients with a similar GCS had sustained comparable forms of injury (Grote et al., 2011). However, recent studies have illustrated the inaccuracy of such assumptions (Saatman et al., 2008). In light of this, more recent studies have focused on patient pathoanatomic features allowing more accurate comparisons. Considering the pathobiology of TBI across the spectrum of the disease -ranging from mild to severe- it is common to discuss the disease within the context of terms such as *focal* versus *diffuse* changes (McGinn et al, 2016) as we describe later; or even combination of both in the most severe and moderate forms of injury (Povlishock & Katz, 2005).

3.2.3. Pathophysiology.

TBI is a dynamic process which results in alterations in function and structure of virtually all elements of the brain that may continue up to several years after the moment when injury took place **(Fig I9)**. Depending on the distribution of the structural damage we can differentiate between focal and diffuse injury.

3.2.3.1. Focal and diffuse injury.

The brain is floating within the skull and it is surrounded by cerebral spinal fluid (CSF). *Focal brain injury* is produced by collision forces acting on the skull and resulting in brain displacement through CSF reaching the tissue underneath the skull at the site of impact (direct) or oppositely to the impact (indirect) (Pudenz & Shelden, 1946). Thus, after the initial direct impact the brain will rebound towards the opposite side of the skull, resulting in a secondary or indirect impact after the acceleration-deceleration forces. The location and severity of the impact to the skull ultimately determine the cerebral pathology and neurological deficits (Cifu & Eapen, 2019).

The term *diffuse brain injury* entails widely distributed damage to axons, diffuse vascular injury, hypoxic-ischemic injury and brain swelling (Gennarelli et al., 1982). This is driven by the fast acceleration-deceleration of the head. Brain structures are heterogeneous both in terms of degree of fixation to other parts of the brain and skull and in terms of tissue consistency. As a result, during the movement of the head, certain segments of the brain move at a slower rate than others, causing shear, tensile and compressive forces within the brain tissue (Gentry et al.,

1988; Strich, 1956). During normal head movement, strain deformation manifested among axons is not harmful: due to their viscoelastic nature axons return to their normal shape and structure (Dennerll et al., 1989). However, under more extreme circumstances the threshold of maximum elasticity is exceeded, resulting in damage to axonal integrity (Meaney et al., 1995; Meythaler et al., 2001). Both the degree of the force applied to the axons and the length of time over which the force is applied influence the magnitude of axonal damage. An essential factor in the development of shear strain is the direction of the head movement: lateral head movement is associated with more severe diffuse damage than sagittal head movement (Tang-Schomer et al., 2010). Diffuse injury is more scattered and is not linked to a specific focus of destructive tissue damage. Rather, it shows a more widespread distribution. It potentially involves diffuse neuronal damage, microvascular change and axonal perturbation and disconnection (Farkas, 2006; Farkas & Povlishock, 2007). The understanding of the pathogenesis of neuronal and microvascular change, however is limited and focuses on the potential that the mechanical forces of injury elicit various forms of membrane change leading to ionic dysregulation and/or altered permeability that in turn evokes lethal and sublethal damage. In contrast to the incomplete appreciation clinicians have of the pathophysiology of diffuse neuronal and microvascular change, understanding of scattered axonal injury is much more complete and its implications for animal and patient morbidity are better understood.

Axonal injury is the most common consequence of diffuse TBI first described in 1956 by the pathologist S. Jeannette Strich as a devastating clinicopathological syndrome with extensive damage to the white matter. Later, the term Diffuse Axonal Injury (DAI), was suggested by J. Hume Adams and colleagues, referring to prolonged coma (more than 6 hours) and widespread injuries to white matter regions (Adams et al., 1989).

Focal and diffuse injuries can and usually do co-exist within a single individual (McGinn & Povlishock, 2016). Neuronal cell death has mostly been studied in focal contusional or pericontusional regions whereas axonal injury is considered a consequence of diffuse TBI. However, the distinction between focal and diffuse injuries is artificial. A more recent MRI study in moderate and severe TBI revealed both focal lesions (contusions or haematomas) and DAI in 50% of the patients (Skandsen et al., 2010). The coexistence of multiple injury types provides a further difficulty and it has been suggested that for effective treatment multi-therapy strategies should be applied.

3.2.3.2. Pathophysiological mechanisms: Focal Injury.

3.2.3.2.1. Excitotoxicity.

In normal conditions, excitatory neurotransmitters are released by the pre-synaptic vesicles after depolarization. However, after TBI, the energy transferred towards the brain tissue triggers a massive depolarization from the nerve cells which results in their uncontrolled excessive release producing excitotoxicity. The main excitatory neurotransmitter released in brain injury is glutamate **(Fig. I10)** (Bullock et al., 1995, 1998). This rapid and transient increase has been described within minutes following the brain insult. Its magnitude and duration have been correlated with the severity of the injury (Bullock et al., 1995; Faden et al., 1989; Katayama et al., 1990; Koura et al., 1998; Palmer et al., 1993).

Apart from depolarization, the neurotransmitter also leaks through damaged neuronal membranes and the supportive astrocytes may not be able to re-uptake it through its ATP-dependent sodium-cotransport (ATP, adenosine triphosphate) system due its cellular energy depletion. Thus, high extracellular glutamate concentrations will initiate a massive influx of both Ca^{2+} and Na^{+} influx into the neurons and glial cells (Choi, 1987). Glutamate binds the N-methyl-D-aspartate aspartic acid and alfa-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors resulting in an overactivation of the ion channels responsible for Na^{+} and Ca^{2+} influxes (Faden et al., 1989). This effect provokes a vicious loop in which more Ca^{2+} is sequentially released from intracellular stores like the endoplasmatic reticulum, and therefore intracellular Ca^{2+} levels raise and provoke a depolarization of the neuronal membrane through voltage-dependent Ca^{2+} channels (VGCC) which in turn increase cellular Ca^{2+} influx.

This cascade initiated with the glutamate secretion has many consequences at different levels. The first of them is neuronal swelling as a consequence of passive water movement which comes from the Na^{+}/ Ca^{2+} influx. Mitochondrial function is also affected after this misplacement of ions and cell death happens through different mechanisms as we will discuss.

3.2.3.2.2. Mitochondrial dysfunction.

Mitochondria is the cellular organelle responsible for respiratory chain and ATP energy production. Mitochondria also serves (up to some degree) as an organelle to accumulate intracellular Ca^{2+} concentration to preserve cellular homeostasis. Thus, after the injury, the raised levels of cytoplasmic Ca^{2+} result in mitochondrial calcium sequestration. Excessive Ca^{2+} overload directly impairs on its oxidative phosphorylation processes (Xiong et al., 1997) leading

to mitochondrial membrane depolarization and the generation of transition pores which increase its permeability. Then, passive water entry into the mitochondria results in osmotic swelling (Lifshitz et al., 2003; Singh et al., 2006) and the eventual loss of mitochondrial ATP production. Since energy demands are high shortly after TBI, the decreased ATP levels are extra harmful.

The increase in glutamate concentration into the extracellular milieu following injury induces a marked increase in the use of cerebral glucose and a consequent accumulation of extracellular lactate due to the shift to anaerobic metabolism **(Fig I10)** (Kawamata et al., 1992; Scafidi et al., 2009). Following this brief period of hyperglycolysis in the acute phase (i.e., first few days postinjury in humans), a global reduction in glycolysis occurs, persisting until recovery, which in humans can range from weeks to months postinjury. The clinical course of recovery typically parallels the restoration of normal brain glucose use (Bergsneider et al., 1997, 2000, 2001; Wu et al., 2004).

3.2.3.2.3. Neuroinflammation.

The inflammatory response of the brain to traumatic insult is multifactorial, encompassing the activation of resident CNS immune cells and the infiltration of peripheral immune cells due to the break produced in the blood brain barrier (BBB). Both types of cells mediate inflammatory processes through a variety of inflammatory cytokines, chemokines, adhesion molecules, reactive oxygen and nitrogen species and complement factors, among others **(Fig. I10)**. For example, local microglia is activated and in addition to the scavenging and phagocytosis of cellular debris, these resident immune cells mediate inflammation through the production of several inflammatory cytokines, proteases and reactive free radical species **(Fig. I9)** (Benveniste, 1998; Gehrmann et al., 1995; Nakajima & Kohsaka, 2004).

Recent studies have shown that microglia can maintain a primed or proinflammatory profile for weeks to months after the acute effects of injury have disappeared **(Fig. I9)** (Witcher et al., 2015). This primed and possible hyperreactive microglial phenotype can potentially set the stage for more progressive degenerative change and chronic patient morbidity, along with an increased vulnerability to subsequent insult.

Together with the local microglial inflammatory response, the BBB acquires an increased permeability caused by mechanical disruption producing the cerebral infiltration and accumulation of peripheral immune cells via their extravasation (Holmin et al., 1998; Royo et al.,

1999). Neutrophils and machrophages/monocytes mediate early inflammatory events via the release of various inflammatory cytokines, proteases, free radicals and other inflammatory mediators in addition to phagocytosing cellular and/or axotomy-related debris (Holmin et al., 1998).

Although most cytokines have traditionally been associated with neuroinflammatory damage, more recent findings suggest that certain factors may also serve neuroprotective and neurotrophic roles in the injured brain (Morganti-Kossman et al., 1997). Depending on their concentrations and the timing/conditions of their expression following TBI, such factors as interleukin-6 and tumour necrosis factor alpha (TNFα) may also serve beneficial roles in the injured brain, possible setting the stage for and promoting regenerative and reparative processes (Maier et al., 2001).

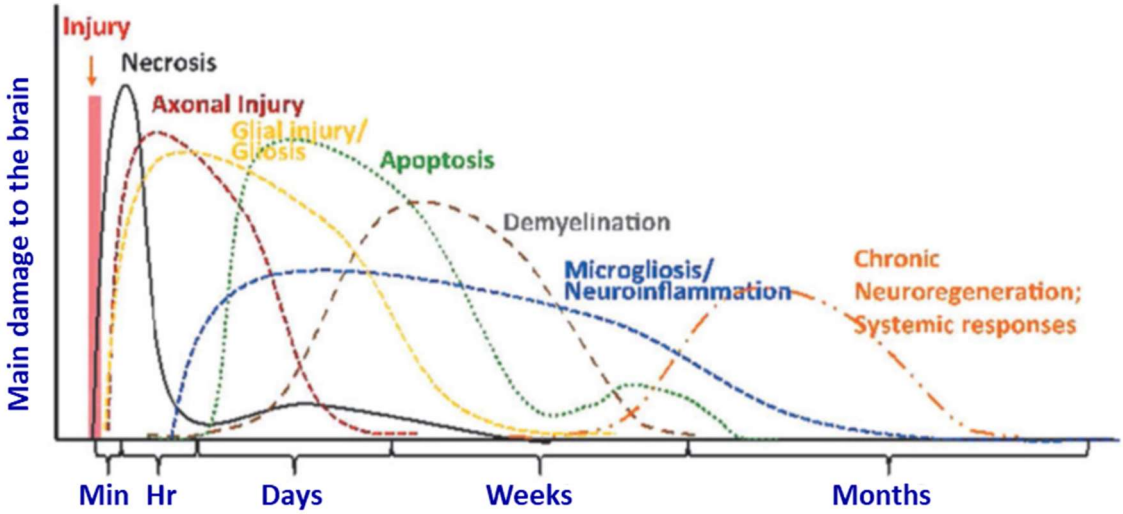


Figure I9. Progressive evolution of detrimental effects of TBI pathophysiology over time. (Modified from Pavlovic et al., 2019). TBI: Traumatic Brain Injury.

3.2.3.2.4. Cell death.

Both necrosis and apoptosis simultaneously occur in TBI. Necrosis is due to physical irrevocable cell damage, breaking of the plasmatic membrane and the consequently releasing cytoplasm from the cell. However, apoptosis (or programmed cell death) can occur through either intrinsic or extrinsic caspase activation pathways. For the intrinsic activation, following the mitochondrial membrane dysfunction, cytochrome-c is released into the cytoplasm and it binds to the apoptosis activating protein-1 (Apaf-1) subsequently activating the caspase cascade form caspase-9 to the effector caspase-3 (Clark et al., 2001; Sullivan et al., 2002). Concerning the extrinsic activation, this pathway is triggered through specific activation of death receptors like

the tumour necrosis factor alfa receptor 1 and caspase-8 present in the cell surface (Ashkenazi & Dixit, 1998).

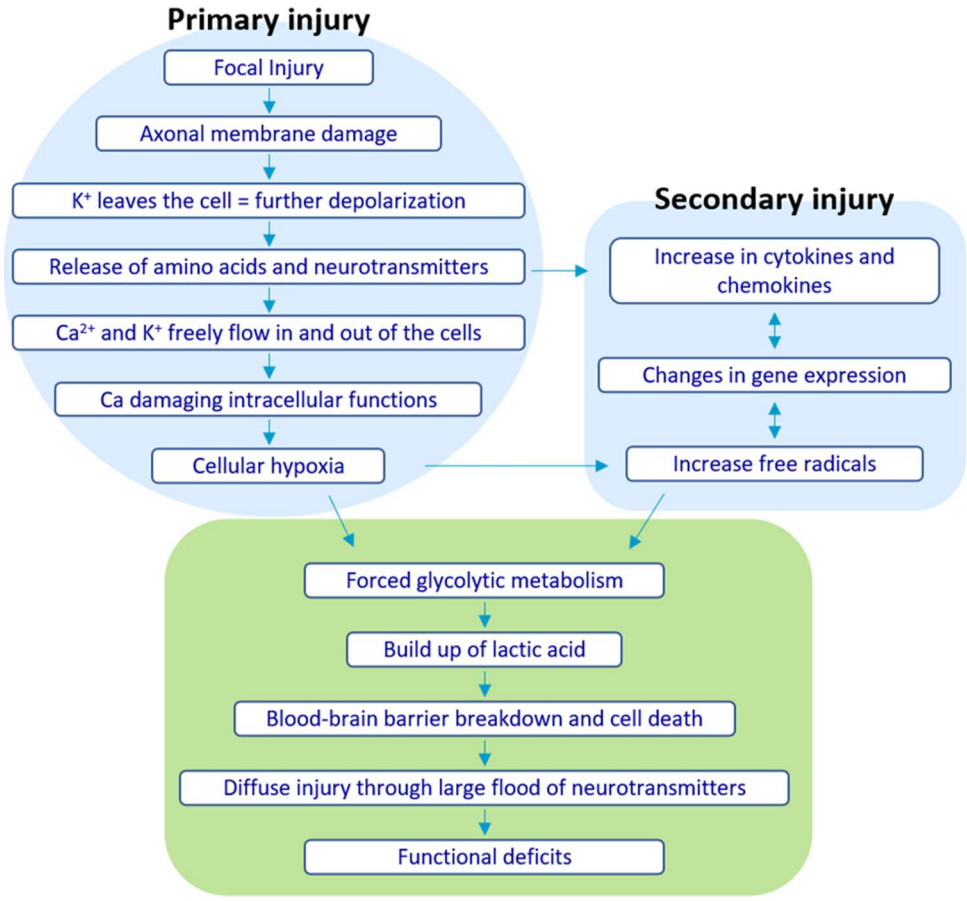


Figure I10. Pathophysiology of TBI: Main effects of the primary and secondary injury and how they interact with each other. An external force produces axonal damage, general depolarization and NTs release. The NTs induce a massive influx of Ca^{2+} which will be sequestrated in mitochondria leading to energy deficits, free radical formation like hydroxyl and peroxide and initiation of apoptosis. TBI upregulates many transcription factors, inflammatory mediators and neuroprotective genes but downregulates NT receptors. Increased expression of detrimental cytokines and chemokines induces BBB damage and cell death. The result of these complex cascades is eventually cell damage and death, which causes functional deficits. Modified from Capizzi et al., 2020; Xiong et al., 2013. TBI: Traumatic Brain Injury; NTs: Neurotransmiters; BBB: Blood Brain Barrier.

3.2.3.3. Pathophysiological mechanisms: Diffuse injury.

As mentioned above, axonal injury is the most common consequence of diffuse TBI. Axonal bulbs, -grossly swollen axons- are a pathological hallmark of DAI. After axonal disconnection, the downstream segments of the axons undergo a breakdown of the myelin sheath and the axon cylinder known as Wallerian degeneration (Gaudet et al., 2011). The time course of this process is highly variable with degeneration initiating as early as 1 to 3 hours after injury but potentially extending up to several months after the impact (Kelley et al., 2006; Maxwell et al., 2003). In

diffuse brain injury, data on the processes following the deafferentation of target sites, now failing to receive input from the detached axon is sparse. The quality of synaptic reorganization differs across the spectrum of TBI with maladaptive changes potentially consisting of inapt fibre ingrowth or abnormal alterations of the cytoarchitecture (Büki & Povlishock, 2006). Upstream axonal disconnection triggers temporary changes within the soma. Interestingly, contrary to what occurs in models of primary axotomy produced by transaction of axons, cell-soma dysfunction does not necessarily lead to neuronal cell death (Singleton et al., 2002).

3.2.4. Symptomatology.

The functional and cognitive symptoms after TBI are highly variable. These symptoms may be influenced by many factors which comprehend, between others, the area and severity of the injury, the delay of medical attention, and the response to treatment, in addition to genetic susceptibility or environmental factors affecting also to the TBI outcome.

3.2.4.1. Implications of acute and chronic TBI.

After TBI, some symptoms may resolve within two weeks (acute TBI) but some others can be persistent (chronic TBI). In acute, both focal and diffuse damage are part of the primary injuries taking place in the brain. Primary injury is the result of the immediate mechanical disruption of brain tissue that occurs at the time of exposure to the external force and includes contusion, damage to blood vessels (haemorrhage) and axonal shearing, in which the axons are stretched and torn (Cernak, 2005; Gaetz, 2004). However, in chronic phase there is also the apparition of a secondary injury due to systemic complications such as ischemia, brain swelling and other complex inflammatory mechanisms that may have severe consequences (Nortje and Menon, 2004). Secondary injury evolves over minutes to months after the primary injury and is the result of cascades of metabolic, cellular and molecular events that ultimately lead to brain cell death, tissue damage and atrophy (Bramlett & Dietrich, 2007; Marklund et al., 2006; Thompson et al., 2005). In this context, the different degree of tissue damage can contribute to the variability of both symptoms and TBI outcome, although in general terms, it can be classified as acute (short-term outcome) or chronic (long-term outcome).

3.2.4.2. TBI, long-term memory and post-traumatic amnesia.

The cognitive alteration that patients suffer after TBI, are confusion, spatial and temporal disorientation, inability to save and/or retrieve new information and alterations termed as post traumatic amnesia (PTA) (Russell & Smith, 1961). The cognitive recovery is usually gradual, however, a high percentage of patients (67%) still experiencing long-term memory failures that last up to four years after the traumatic event. Long-term memory deficits have also been found not only in the ability to recall memories, but also in the ability to mentally travel back through subjective time to re-experience the past (autonoetic consciousness) and to perceive oneself as a continuous entity across time (self-perspective) (Piolino et al., 2007).

3.2.4.3. Post-traumatic seizures.

Abnormal excessive or synchronous neuronal activity in the brain leads the apparition of seizures. Post-traumatic seizures are a common complication of TBI. Seizures following TBI are classified as immediate, early, or late. Focal seizures, formerly known as simple partial (pure motor) seizures, are the most common type to occur late in TBI patients, although others (complex partial, generalized and so forth) are documented (Berg et al., 2010). More than 80% of patients who develop seizure activity will present it in the first two years after injury (Berg et al., 2010).

3.2.4.4. Speed of processing and attention.

Speed of processing is a cognitive capacity that consists of the relationship between the response to a cognitive demand and the time invested in that operation. TBI patients still report mental slowness and concentration difficulties at a rate of 56.7% as late as four years after the injury (Jourdan et al., 2016), becoming one of the most frequent sequel in TBI survivors and complaints from their close relatives.

Attention is a multidimensional concept that can be subdivided into various modules such as phasic alertness, sustained attention, focused attention and divided attention. Several studies have addressed which one of these components could be the most affected after TBI, however, there is a lot of debate in the subject and it is still not clear (Dockree et al., 2006; Ponsford et al., 2008; Whyte et al., 1995, 2006).

3.2.4.5. Mental fatigue.

Mental fatigue is a very frequent sequel post-TBI, reported by 30-70% of patients (Belmont et al., 2006; Englander et al., 2010). Regarding the outcome in the long-term TBI, mental fatigue is reported to improve during the first year after the injury reaching the plateau during the next two years (Bushnik et al., 2008). The mechanism behind this symptom is still under debate. Some authors consider that mental fatigue could be the collateral effect of an increased effort in a scenario of cognitive deficits and slower processing conditions in an attempt to reach adequate levels of performance (Azouvi et al., 2004).

3.2.4.6. Executive function.

During brain injury, several areas of the brain are more susceptible to damage. The frontal lobes and anterior brain networks are specifically vulnerable due to their location. Therefore, it is not surprising that patients who suffer in these areas frequently present deficits in executive-function. Although there are many factors intervening in the capacity of the executive function, there are a wide variety of tests (Trail Making Test, Verbal Fluency Test, Clock Drawing Test, Wisconsin Card Sorting Test…) and tasks to evaluate the degree of affectation (Stuss, 2011). Only a few studies have found differences between patients suffering from TBI and healthy controls in some of these tasks (Milner, 1963; Stuss, 2011). For example, Wisconsin Card Sorting Test which requires the ability to sort items according to a given category and then to modify the sorting criteria when the rule changes and TBI patients exhibit a loss of conceptualization and set-shifting ability. (Milner, 1963).

3.2.4.7. Social cognition.

Social cognition can be defined as the procedure in which people process, remember, and use information in social contexts to explain and predict their own behaviour and that of others (Bulgarelli & Molina, 2016). Thus, understanding the behaviour of other people and reacting accordingly in social situations becomes a complex task for TBI patients. Furthermore, patients show impairment in various aspects of social cognition, such as the perception of emotions (ability to recognize socially relevant information and/or facial expressions of emotion); understanding the internal state of other people and empathy. However, the relationship between this decline or impairment in social cognition and more global cognitive deficits are still under debate (McDonald, 2013).

3.2.4.8. Behavioural changes.

The recovery of the cognitive and the behavioural characteristics that defines the identity are crucial factors for the quality of life of TBI survivors, as it facilitates the reintegration into their family, their social and vocational life and in general. After TBI, many behavioural changes have been reported such as lack of self-control (disinhibition, impulsivity, irritability, aggressiveness) or lack of drive (apathy, reduced initiative, poor motivation). From all these changes, irritability is one of the most frequent problems described in 67% of the cases, followed by lack of initiative (44% of cases) and socially inappropriate behaviour (26% of cases) (Benedictus et al., 2010; Ponsford et al., 2008). Recent studies have proposed new multidimensional approaches to take into account the complexity of the underlying psychological, cognitive, social, environmental and personal processes, and the dynamic nature of such problematic behaviours (Arnould et al., 2016; Rochat et al., 2013).

3.2.4.9. Mild TBI, post-concussive syndrome and concussion.

A high percentage of TBI events end up being diagnosed as mild-TBIs when the initial GCS score ranks between 13-15. While the majority of cases out of this mild-TBIs fully recover within a few weeks or months, it appears that a significant minority of patients may experience long-lasting symptoms (15%) (Tapia & Eapen, 2017). As we mentioned previously, these symptoms include headache, dizziness, fatigue, attentional and memory failure, irritability, apathy etc. It is important to recall these data since, for a proper diagnose of *post-concussion syndrome* (PCS) also known as *persistent post-concussive symptoms*, the presence of at least three of these symptoms at either 1 or 3 months after injury is required. It is defined as a syndrome that occurs when concussion symptoms last beyond the expected recovery period after the initial injury. However, PCS has been highly debated and questioned for several reasons including the absence of particular symptom specificity, recall bias and the influence of psychosocial factors and/or pre-morbidity on outcome. The symptoms are not specific to PCS alone but are present in both healthy and TBI affected population (Fear et al., 2009; Greiffenstein & Baker, 2008; Iverson & Lange, 2003).

Concussive injuries are often viewed as mild TBIs without any gross structural damage secondary to a nonpenetrating TBI (Kelly & Rosenberg, 1997). Concussion has been used synonymously with the term mTBI. Emerging classification mechanisms specific to concussion promote

separation of these terms. Mild injuries likely account for more than 80% of all TBI, although the true incidence is difficult to ascertain because many patients who sustain these injuries do not seek medical attention and therefore, they are not documented or tracked. Most research and subsequent assessment tools are focused on sports-related concussion (McCrea et al., 2017).

3.2.4.10. Chronic Traumatic Encephalopathy: *dementia pugilistica.*

Repetitive mTBI may lead to a delayed manifestation known as chronic traumatic encephalopathy (CTE). This entity gained popular attention in the media as one of the unfortunate consequences of CTE are the psychiatric disturbances, ultimately leading to suicidal behaviour in a number of high-profile athletes in professional sports. Athletes of combat or collision sports have a greater risk of multiple mTBIs than the general population, however, the cumulative effects of impacts showed conflicting results in different studies. Indeed, CTE was initially designated as *dementia pugilistica* or punch-drunk disease in boxers in the first half of twentieth century. It was described in athletes either in active status or already retired after all their professional career exposed to multiple and repetitive high intensity contact, collisions or combat sports.

The initial event that triggers the onset is often a concussion, however, the athletes may return to play prematurely and sustain a second concussive event in the middle of the recovery from their first injury (Bey & Ostick, 2009). This casuistry is also observed in non-sport related scenarios like young active military personnel service and retired veterans (Mckee et al., 2018; Tharmaratnam et al., 2018).

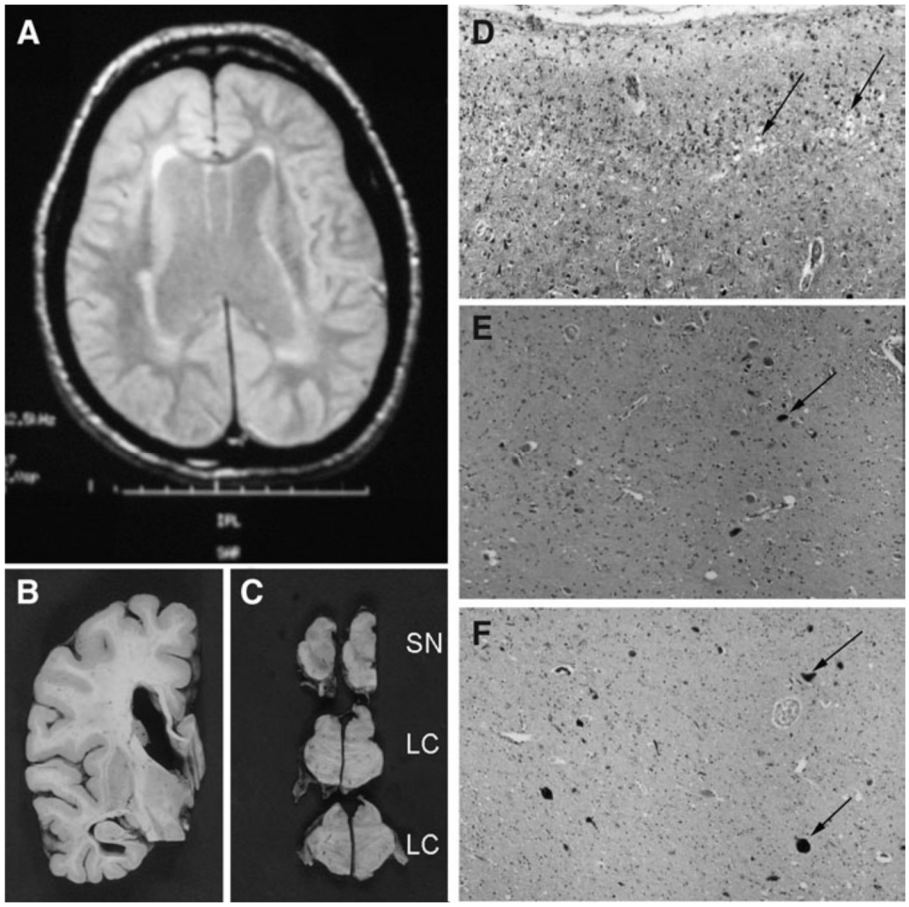


Figure I11. General characteristics of the dementia pugilistica (DP) in a patient. **A.** Magnetic resonance image showing sidened ventricles and a mild-to-moderate degree of white matter changes. **B.** Coronal section of the brain showing ventricular enlargement, absence of the septum pellucidum, a thinned corpus callosum and minimal cortical atrophy. **C.** Reduction of neuromelanin pigmentation in the SN and LC. **D.** Rarefaction (see arrows) within cortical layer 2 in temporal gyurs. **E.** Loss of neuromelanin in substantia nigra (arrows). **F.** Apparition of neurofibrillary tangles in the locus ceruleus. D-F images taken at 10X. (Source of images: Saing et al., 2012). SN: Substantia nigra; LC: Locus Ceruleus.

CTE is considered a chronic and progressive neurodegenerative primary tauopathy developed after a single, episodic or repetitive head trauma which leads to dementia or depression. Its pathophysiological hallmark is characterized by a build-up of hyperphosphorylated tau (p-tau) protein (and other pathological proteins, such as amyloid-β, α-synuclein and TDP-43), neurofibrillary tangles, abnormal neurites and perivascular astrocyte inclusions.

The axonal injury resulting from head impact which leads to chronic neuroinflammation and decreased neuroregeneration in the context of inadequate recovery from the primary TBI event and leading to tau pathology. Tau acetylation is believed to be the initiating event leading to a vicious circle of repetitive damage and imperfect repair with tau phosphorylation as the second hit resulting in p-tau aggregation and microtubule destabilization (Tharmaratnam et al., 2018;

Vile & Atkinson, 2017). There is a latency period in the development of CTE which corresponds to tau propagation from focal to widespread areas (Tharmaratnam et al., 2018).

CTE is expressed as three common syndromes: a behaviour-predominant, mood-predominant and parkinsonism (Perry et al., 2016; Tharmaratnam et al., 2018). It usually begins at mid-life with some of these different manifestations: dysarthria (a motor speech disorder in which the muscles that are used to produce speech are damaged, paralyzed or weakened), intentional tremor, pyramidal cerebellar and extrapyramidal signs, epilepsy and parkinsonism, emotional and cognitive dysfunctions (Gavett et al., 2011).

Nevertheless, the notion of CTE as a separate disease from TBI is disputed by some authors (Ziino & Ponsford, 2005). Surprisingly, longitudinal long-term studies state that retired professional American football players in fact have a lower standard mortality rates and lower risk of suicide than general population (Randolph, 2018). Some confounding factors like chronic pain and substance abuse also should be considered (Manley et al., 2017; Mckee et al., 2018). In general, TBI is seen as an aggravating risk for earlier clinical manifestation of an underlying neurodegenerative disease (like AD) due to diminished neurocognitive reserve (Fleminger, 2003; LoBue et al., 2018). However, the exact mechanism linking TBI and dementia is not known yet.

3.2.4.11. Post-traumatic stress disorder.

Post-traumatic stress disorder (PTSD) is an anxiety disorder suffered by patients exposed to a threatening event which causes fear, horror or helplessness. Symptoms include intrusive memories, nightmares, trauma reliving and psychological distress that often end up in avoidance of thoughts, feelings or reminders of trauma, withdrawal from others or emotional numbing (Bell, 1994). Several recent studies revealed that post-traumatic stress disorder (PTSD) is frequently associated with mild TBI. There is increasing evidence suggesting that mTBI can increase the risk for PTSD and that persistent impairment after mTBI is largely related to stress reactions (Bryant, 2011).

3.2.5. Modelling TBI.

TBI is not one single physiological event but it has been described as a cascade of complex processes that run in parallel including both focal and diffuse brain damage (Mckee & Daneshvar, 2015). As we previously mentioned, brain injuries occurring in human patients

present many variations involving different factors such as the severity, the nature of the TBI, the location etc. Furthermore, other factors regarding the individual also play a role in the development of the damage like the age, the gender, pre-existing pathologies and more. This has made the study of TBI at the preclinical level extremely complex. To understand the overall context of TBI, it is necessary to establish models that mimic the event under controlled conditions (Cernak, 2005). Even though some of these models do not reproduce with fidelity the causes of the damage. Nevertheless, modelling TBI allows a reconstruction of some of its aspects and can offer specific and important knowledge about the pathophysiology.

3.2.5.1. Mathematical and network models.

Although its potential is yet to be exploited, mathematical and network models are a powerful tool to study the human brain in complex situations. By combining recent developments in non-invasive neuroimaging technology together with the establishment of known circuits and information flows between them, we can infer mental disorders and different pathologies and their consequences (Sporns, 2011). The brain's structural connectivity also known as connectome comprises a map of the brain's hubs or nodes (Sporns, 2013), which support integrative processing and adaptative behaviour. It has been predicted that these hubs may have a predominant role in pathological brain regions (Crossley et al., 2014). It is known that TBI produces damage in the long-distance axonal projections and therefore could disconnect these long-range communications between hubs (Crossley et al., 2014). A deeper study from this perspective is necessary to get a wholesome view of brain functioning and be able to predict specific outcomes depending on the type of injury produced.

3.2.5.2. *In vitro* models.

Given the complex nature of TBI and the many factors that take part in it, *in vitro* models constitute an excellent platform to study specific aspects in a way that is easily repeatable, well controlled, isolated from the environment, less expensive and with less ethical implications. We can take advantage of these models to study pathophysiological cascades without having confounders such as inflammation or hypoxia (Kumaria & Tolias, 2008; Morrison et al., 2011).

Immortalized cell lines, dissociated primary cultures and organotypic cultures all have been used to mimic particular aspects of TBI (Adamchik et al., 2000; Kane et al., 2011; Silva et al., 2006). Some examples of *in vitro* TBI models are transection, compression or acceleration/deceleration

(Kumaria & Tolias, 2008). In brief, transection consists of the use of a plastic stylet or a rotating scribe to damage cells attached to the culture dish. It can simulate different types of TBI such as puncture wounds or penetrating skull fracture (Morrison III et al., 1998). Compression can simulate focal wounds that cause laceration and it is performed by using a weigh-drop (Church & Andrew, 2005; Sieg et al., 1999). Finally, in acceleration/deceleration models, neurons and glia are subjected to different types of accelerations while they are adhered to a substrate (Morrison et al., 2006).

3.2.5.3. *In vivo* models.

3.2.5.3.1. Species.

A large number of relevant animal models have been developed to gain knowledge of TBI. When choosing among the wide variety of *in vivo* models of TBI, two major factors must be considered: the animal species and the type of model to mimic TBI.

The study of TBI has been vastly extended through the use of a wide variety of animal species (Hutchinson et al., 2016; King et al., 2010; Zhang et al., 2015). These species are different to each other in terms of anatomy, morphology, metabolism, neurobiology and life-span and it is due to these huge inter-species differences that translational research becomes even more complicated. For example, differences in the shape of the skull may influence the distribution of mechanical force, resulting in discriminative responses to similar level of TBI on different animals (Anderson et al., 2003).

The rodent brain is mostly used in TBI research due to their modest cost, small size and standardized outcome measurements. However, brains of larger animals are closer to humans' in terms of size, gyrification, grey and white matter ratio and also developmental patterns or degree of myelination, i.e., swine, ferrets and primates (Conrad et al., 2012; Empie et al., 2015; Flynn, 1984; Shultz et al., 2017; Zhang et al., 2015). All these factors have profound effects on the tolerance of the brain to different injuries. To date, large animal models have been widely used for studying TBI in different circumstances: cats (Hayes et al., 1987), rabbits (Wei et al., 2011; Zhang et al., 2015), dogs (Millen et al., 1985), sheep (Lewis et al., 1996), ferrets (Lighthall, 1988), swine (Browne et al., 2011) and non-human primates (King et al., 2010). However, these species also come with a handful of drawbacks, besides accompanying ethical issues, not present in rodents that cannot be ignored. Briefly, they are more difficult to house and handle and therefore require more effort regarding their breeding and living conditions. There is also a

higher cost derived from adapting specialized TBI-equipment to work with them. The fact that there are fewer animal studies on these species comes with a worse characterization compared with rodents at many levels (including motor or cognitive characterization, biomarkers or inflammatory responses). This deficiency in the species-dependent knowledge together with the limited sample size or the lack of transgenic models in larger animals makes it hard to work and increases the difficulty to be used in research.

Leaving aside the more traditional laboratory animals, there are other rare species which adapted biologically to extreme environments developing specific protections to avoid or reduce brain trauma. Studying these adaptations and whether they indeed result in a successful avoidance of brain trauma, would also be interesting and inspiring for the design of similar protective mechanisms for humans. Some anecdotical examples are the headbutting of bighorn sheep (*Ovis canadensis*) and other bovids (Drake et al., 2016; Huang et al., 2017; Johnson, 2016), the woodpecker's pecking (Farah et al., 2018; May, 1979) and the whale's high-pressure deep dives as well as their exposure to high-amplitude underwater sounds (DeRuiter et al., 2013; Todd et al., 1996).

3.2.5.3.2. TBI models.

Due to the heterogeneous nature of TBI, it has not been possible to establish a single *in vitro* or *in vivo* model which recapitulates all of the detailed features of its pathophysiology. Instead, a variety of animal models have been developed to study specific aspects of TBI. By controlling some variables, a model can offer specific and important knowledge about TBI. Therefore, when choosing an animal model of TBI, one must consider what aspect is to be examined and then also choose the most suitable species. The development of a model would be therefore always linked to the goal of the study: the severity, the anatomical structures which are analysed, the physical mechanism causing the damage are all factors that should be taken into account when designing a specific project. The most used models are summarized here.

3.2.5.3.3. Fluid Percussion Injury.

The fluid percussion injury (FPI) is inflicted by administering a rapid saline pressure pulse to the exposed dura via a craniotomy, which is made centrally over the sagittal suture (middle FPI) (Floyd et al., 2002) or laterally over the parietal cortex (lateral FPI, LFPI). The lesion will be achieved by using a pendulum, a piston or a pulse delivered by a fluid **(Fig I11A)**. Currently, the

LFPI model is the most extensively used and characterized device of experimental TBI, due to its high reproducibility (Alder et al., 2011; Xiong et al., 2013). The percussion produces a brief displacement and consequent deformation of the nerve tissue. The animals are previously anesthetized and subjected to a craniotomy, taking care that the dura remains intact. The severity of the injury can be adjusted by modifying the height of the pendulum which defines the strength of the fluid pressure.

FPI models do not replicate clinical TBI with skull fracture (Thompson et al., 2005). However, this is a characteristic that very commonly appears in moderate or severe human TBI. FPI can, however, replicate intracranial haemorrhage, brain swelling and progressive grey matter damage, which are all pathophysiological hallmarks of human TBI (Graham et al., 2000). However, FPI models have high mortality compared with other models, which is probably due to the brainstem-compromised prolonged apnoea (Cernak, 2005).

3.2.5.3.4. Controlled cortical impact.

The controlled cortical impact (CCI) model uses a pneumatic or electromagnetic impact device to drive a rigid impactor onto the exposed, intact dura and mimics cortical tissue loss, acute subdural haematoma, axonal injury; concussion, blood-brain barrier (BBB) dysfunction and even coma **(Fig I11B)** (Dixon et al., 1991; Kabadi et al., 2010; Lighthall, 1988; Lighthall et al., 1990). The impact is delivered to the intact dura through a unilateral craniotomy lying most often between bregma and lambda. The damage will be widespread, including acute cortical, hippocampal and thalamic degeneration (Hall et al., 2005).

The most important advantage of this model over other TBI models is the accuracy that can be achieved when reproducing it, since almost every variable intervening in the injury can be modulated. The time, velocity and depth of the impact can all be adjusted and this makes it more useful than the FPI model for biomechanical studies (Albert-Weissenberger & Sirén, 2010; Cernak, 2005). An additional strength of this model is the lack of risk of a rebound injury that can be seen in gravity-driven devices.

3.2.5.3.5. Weight-drop model.

This kind of injury delivers a guided weight to the restrained head using a humane stunner, a calibrated pendulum or a dropped weight **(Fig I11C)** (Anderson et al., 2003; Lewis et al., 1996). The severity in this case can be regulated by altering the mass of the weight or the fall distance. It was originally developed to reproduce closed-head injury. The falling object usually hits a

helmet or other attachment either made of metal or a rigid material placed over the animal's head (Colak et al., 2012; D. dos S. Silva et al., 2012). By using a closed-head injury model, a higher similarity to human TBI is achieved, however, the reproducibility between experiments decreases importantly. Furthermore, without proper design, this model can cause other injuries, such as injury to the cervical region.

3.2.5.3.6. High-speed missile models.

Several names have been given to this type of model that simulate the impact of a bullet. Also referred as penetrating craniocerebral gunshot wounds (PCGWs), they are caused by gun projectiles or bomb shrapnel which produces a temporary cavity in the brain **(Fig I11E)** (Carey et al., 1989; Davis et al., 2010). They permit the analysis of morphological alterations such as lacerations, haemorrhage, and the dispersion of the injury, which are due to compression by the missile, penetration and distortion of the blood vessels and nerve fibres of the brain and tissue displacement (Finnie, 1993). Although they rarely occur in civilian practice, the rate of mortality after them is still high (Carey et al., 1989; Williams et al., 2007). This high mortality is reproduced in the animal models and makes it therefore, very complicated to study these injuries in the laboratory. And even when a model with lower mortality is achieved, the large differences in brain structure between small animals and humans impede carrying out more profound studies in PCGWs.

3.2.5.3.7. Blast injury model.

In contrast to the effects of gunshot wounds in which nature of bullet-induced brain wounds is focal along the bullet trajectory, blast injuries are multi-focal and poly-traumatic, such as oedema or vasospasm (de Lanerolle et al., 2011; Rafaels et al., 2011). Blast-induced neurotrauma is considered a common wound in military efforts and terrorist activity (Connell et al., 2011; Ling & Ecklund, 2011; Warden, 2006). A powerful shock wave emanating from explosive device encounters the animal head and the blast wave is successively transmitted through the skull, cerebral spinal fluid and brain tissue **(Fig I11D)**. The severity of brain insult in this case is associated with the distance and the position of the body relative to the explosive device, the power level of the explosion as well as the experimental environment (de Lanerolle et al., 2011; Rafaels et al., 2011).

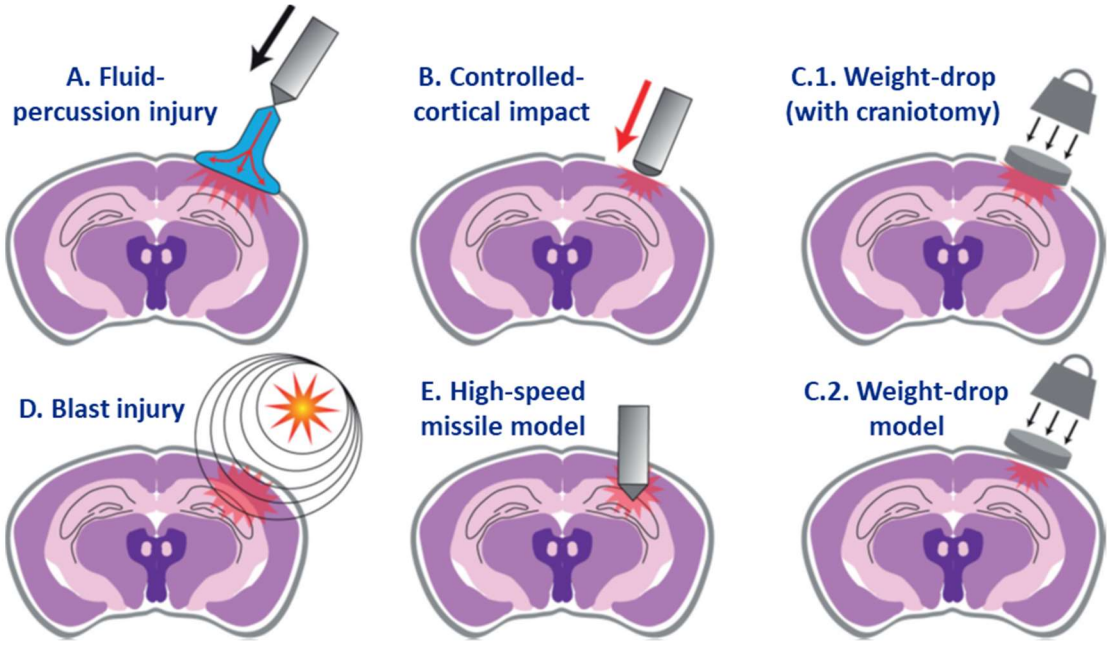


Figure I11. Schematic representative images from different TBI mice models**. A.** Lateral-FPI administering a saline pulse to the exposed dura. **B.** CCI drives a pneumatic or electromagnetic impactor onto the exposed dura. **C.** In the weight-drop model a guided weight is delivered to the restrained head using a pendulum or a dropped weight either directly to the dura (with previous craniotomy, **C.1**) or to the skull (no craniotomy, **C.2**). **D.** Blast injury produces a shock wave that is transmitted through the skull. **E.** High-speed missile models are caused by gun projectiles that produce a temporary cavity in the brain. Modified from (Pitt & Leung, 2015).

3.2.6. The hippocampus after TBI.

The high prevalence of memory deficits after injury point to a relative vulnerability of the hippocampus and mesial temporal structures as compared to the rest of the brain (Comper et al., 2005; Hamm et al., 1993). Hippocampal atrophy due to cellular and synaptic loss is a well-known consequence of TBI (Hicks et al., 1993; Kotapka et al., 1991). The atrophy of the hippocampus is often not limited to the ipsilateral side, but it also involves the contralateral one (Serra-Grabulosa, 2005; Tate & Bigler, 2000; Tomaiuolo, 2004; Tran et al., 2006). Particular areas of the hippocampus are more vulnerable than others: dentate hilar neurons, CA2 pyramidal cells and newborn neurons in the inner granule layer of the dentate gyrus are selectively lost after TBI (Gao et al., 2008a; Grady et al., 2003; Lowenstein et al., 1992; Witgen et al., 2005). It has been proved that there is a correlation between the amount of hippocampal atrophy due to cellular loss and the degree of hippocampal-dependent learning impairment during CNS injury (Tomaiuolo, 2004). However, lesion studies have found that a loss of at least 20-30% of the dorsal hippocampus and at least 39-52% of the ventral hippocampus is required to begin to observe significant impairments in hippocampal-dependent learning tasks (E. Moser et al.,

1993). In fact, just 26% of the hippocampus is capable of supporting hippocampal-dependent water maze spatial learning after injury (E. Moser et al., 1993). Therefore, functional impairment may stem from an alteration in the activity of certain subpopulations of cells within the hippocampus.

Systemic changes after TBI are also well documented. During stress response, glucocorticoids (GCs) bind to their receptors and modulate hippocampal function affecting numerous signalling and metabolic systems (Gulyaeva, 2015). Different receptors of GCs are extensively expressed in the hippocampus and their distribution and properties may underlie the vulnerability of hippocampal neurons to different types of damage (Prager & Johnson, 2009). GCs also modulate glutamate receptors and may be involved in initial effects of TBI, including exacerbation of excitotoxicity (Sorrells et al., 2014). Glutamate is particularly important, as this excitatory neurotransmitter is known to play a strong role in secondary injury mechanisms throughout the brain (Chamoun et al., 2010; Folkersma et al., 2011). In the hippocampus, glutamate decreases following moderate to severe temporal cortex injury (J. L. Harris et al., 2012). Acutely after TBI there is a large increase in intracellular calcium in both the cortex and hippocampus (Fineman et al., 1993). This global change in calcium signalling through glutamate excitotoxicity and potassium depolarization waves stimulates several calcium-dependent cell-signalling pathways. Protein kinases CaMKI, II and IV, as well as ERK1/2 and PKC are activated within hours after TBI (Atkins et al., 2006; Dash et al., 2002; Hu et al., 2004; Yang et al., 1993). In contrast, cAMP levels and PKA activation are decreased for a short period of time (Atkins et al., 2007).

Another important compound altered in the hippocampus after TBI is the neurotransmitter GABA. The expression of various GABA receptor subunits is changed, causing reductions of inhibitory postsynaptic currents and increases in excitatory post-synaptic currents after TBI in rodents (Almeida-Suhett et al., 2014; Drexel et al., 2015; Hunt et al., 2011).

Different electrophysiological changes have also been characterized after TBI in the hippocampus. It is important to establish how individual neurons and subpopulations of neurons contribute to network activity and how these networks respond to injury. In the CA1 area, a basal excitatory synaptic transmission depression has been reported by measuring the excitatory postsynaptic potential (EPSP) from hours to days after TBI (D'Ambrosio et al., 1998; Miyazaki et al., 1992; Norris & Scheff, 2009; Reeves et al., 2000; Witgen et al., 2005). An increase in the population spike amplitude and a decrease in the population spike threshold have also been reported, suggesting an imbalance between excitation and inhibition (Akasu et al., 2002; Miyazaki et al., 1992; Reeves et al., 1997, 2000; Witgen et al., 2005). There is a lack of long-term

studies of this matter, so it is still not known if either inhibitory or excitatory basal synaptic transmission is altered in the months to years after TBI (Mtchedlishvili et al., 2010). Conversely, an enhancement of basal EPSPs has been observed in the perforant path to the DG (Santhakumar et al., 2000, 2001; Witgen et al., 2005; B. Zhang et al., 2011) and a depression of basal inhibitory postsynaptic currents (Bonislawski et al., 2007; Hunt et al., 2011; Mtchedlishvili et al., 2010).

3.2.6.1. AHN after TBI.

As it has been previously explained in sections **3.1.1.** and **3.1.2.**, AHN is a process which takes place in the dentate gyrus of most mammals (Altman, 1962a). As it happens with other areas of the hippocampus, the DG and more specifically AHN is affected by TBI. However, the specific effect that brain injury produces over neurogenesis is still unclear. Data for elucidating neurogenesis after TBI are contradictory. Although most of the studies performed in this matter reported an increased (Braun et al., 2002; D. Sun et al., 2005, 2007) or unchanged level of neurogenesis after TBI (Chirumamilla et al., 2002; Gao et al., 2009; Gao & Chen, 2013), some of them also described a decrease in this process (Rola et al., 2006). In addition to the contradicting results regarding the measuring of neurogenesis itself, there is also an important lack of knowledge about the maturation and integration of these newborn neurons into the hippocampal circuitry. Studies focused in the functional integration of these neurons or even their final outcome in hippocampal-related tasks are scarce (Redell et al., 2020; Sun et al., 2007; Villasana et al., 2015).

Both the contradictory results and the lack of more profound studies make it difficult to reach a clear conclusion about the role of neurogenesis after TBI. In general, physiological neurogenesis is considered a beneficial process with important cognition and mood-related roles, as it has been previously discussed. However, different pathologies have been reported to either decrease or disturb this process by producing what has been denominated “aberrant” neurogenesis (Martín-Suárez et al., 2020; Sierra et al., 2015). A more exhaustive characterization of neurogenesis post-TBI is necessary in order to know if it can be targeted and used as a potential source of new neurons compensating for the neuronal loss following TBI.

HYPOTHESES AND OBJECTIVES

4. HYPOTHESES AND OBJECTIVES.

Adult neurogenesis is tightly linked to the level hippocampal circuitry activity. Consequently, pathologies interceding in the level of neural activity end up disturbing neurogenesis in different ways (Segi-Nishida et al., 2008; Hüttman et al., 2003). For example, both neural hyperexcitability and seizure-susceptibility both have been described after TBI (Neuberger et al., 2017; Akasu et al., 2002; Reeves et al., 2000).

Rnd2 is a protein member of the Rho small GTPases family which is known to regulate distinct morphological cellular changes and cell migration (Nakamura et al., 2006; Pacary et al., 2011). Indeed, Rnd2 is the only member of this family whose expression is selectively enriched in the SGZ area, where AHN takes place. In fact, it contributes at tissular level promoting both cell survival of newborn neurons and adjusting cellular morphology as well as in a cognitive way, controlling anxiety – a function directly related to the development and maturation of newborn hippocampal neurons – as previously reported (Kerloch et al., 2021).

Our **general hypothesis** is that TBI generates neural hyperexcitation which in turn affects to NSCs and the maturation process of newborn neurons, as it happens in other pathologies. Rnd2 could play a role not only in physiological conditions in AHN but also in some of the newborn neuronal alterations observed after TBI.

Based on these assumptions and previous data, we hypothesize that:

- TBI produces hyperexcitation, altering NSCs morphology and proliferation.
- TBI alters the process of neurogenesis changing the properties of newborn neurons and therefore producing aberrant neurogenesis.
- The consequences of TBI over NSCs and neurogenesis are not restricted to the acute phase. They could also undermine the long-life neurogenic potential of this population and contribute to the chronification of the general pathology.
- Rnd2 plays a role in the alterations related to the newborn neurons after TBI.
- In physiological conditions, overexpression of Rnd2 alters newborn neurons' properties. This has an effect in the behavioural and learning functions depending on the proper integration of these neurons that will therefore, also be modified.

Our **general objective** then is to characterize the alterations caused by TBI into the neurogenic cascade and study a model of Rnd2 overexpression in physiological conditions together with its effects in behaviour.

Objective 1. To characterize the CCI model of TBI and its suitability for the study.

Objective 1.1. To assess the level of neuronal hyperactivation in the DG after TBI.

Objective 1.2. To characterize macroscopic morphologic alterations after the impact including both damage of cortical structures and anatomical changes in the DG.

Objective 1.3. To determine changes of cellular proliferation in the DG.

Objective 2. To study the changes of TBI over NSCs in both the acute and the chronic phase after the impact.

Objective 2.1. To assess the level of reactivity acquired by NSCs after TBI. For this purpose, we will use a transgenic mouse model in which EGFP is driven by the expression of Nestin. We will analyse the Nestin-GFP positive population and their changes in morphology and expression of some factors at different time-points.

Objective 2.2. To characterize the changes in NSC proliferation rate. Taking advantage of proliferation markers and the previously mentioned transgenic animals, the proliferation of the NSC population will be assessed to investigate possible changes during the time course of the pathology.

Objective 2.3. To study the progression of the NSC pool in the long term. NSCs changes including their proliferation may have an impact in the overall niche cell population and its depletion in different pathologies. Therefore, an exhaustive analysis of NSCs population is necessary to characterize long term changes in neurogenesis after TBI.

Objective 3. To analyse the population of newborn neurons during their integration into the hippocampal circuitry after TBI.

Objective 3.1. To study the population of adult born neurons after TBI using cellular markers. Several characteristics like the fluctuations in terms of cell numbers, migration and morphology will be analysed.

Objective 3.2. To characterize morphological, migration and synaptic changes specifically in hippocampal neurons born after TBI. For this purpose, we will use a novel strategy involving the development of retroviral-vectors containing a fluorescent protein (Venus) to label newborn cells after the impact.

Objective 4. Correcting the aberrant phenotype in the migration and morphology of newborn neurons. Using a combination of two procedures: TBI mouse model and retroviral-vector

injection which overexpresses Rnd2 together with a fluorescent protein (DsRed), we evaluate the protection or reversion of the pathological phenotype observed in newborn neurons.

Objective 5. To characterize the effects of overexpressing Rnd2 in newborn neurons in physiological conditions.

Objective 5.1. To study the behavioural and memory alterations after Rnd2 overexpression in newborn neurons. For this purpose, we will perform a bilateral injection of retroviral-vector containing Rnd2 together with DsRed and later proceed to a battery of behavioural and memory analysis of tasks that are traditionally influenced by the survival and integration of newborn neurons into the hippocampus.

EXPERIMENTAL PROCEDURES

5. EXPERIMENTAL PROCEDURES.

5.1. ANIMALS.

All animals used were on a C57BL/6 background. Mice were housed with ad libitum food and water access, in a colony room at a constant temperature (19-22ºC) and humidity (40-50%) on a 12:12h light/dark cycle. Both males and females were used and pooled together in all experiments except for those including behavioural analyses where only males were used. All procedures were approved by the University of the Basque Country EHU/UPV Ethics Committees (Leioa, Spain) and Diputación Foral de Bizkaia under protocol M20/2015/236. All procedures followed the European directive 2010/63/UE and NIH guidelines.

The experiments were carried out in either Nestin-GFP^{+} transgenic mice or C57BL/6 WT mice (Nestin-GFP^{-}). Nestin-GFP transgenic mice were generated in the laboratory of Dr. Grigori Enikolopov at Cold Spring Harbor Laboratory (Cold Spring Harbor, NY, USA) (Mignone et al., 2004). The strain was kindly provided by Dr Enikolopov and were crossbred with C57BL/6 mice for at least 10 generations. In Nestin-GFP mice, the fluorescent protein is expressed under the regulatory elements of the intermediate filament Nestin, expressed in neural stem and progenitor cells. Nestin-GFP animals were used for the adult characterization of the DG neurogenic niche.

Behavioural experiments were carried out in the Neurocentre Magendie (Bordeaux). In this case, only females C57Bl6/J were used. Mice were housed, bred and treated according to the European directive 2010/63/EU and French laws on animal experimentation. All procedures involving animal experimentation and experimental protocols were approved by the Animal Care Committee of Bordeaux (CEEA50) and the French Ministry of Higher Education, Research and Innovation.

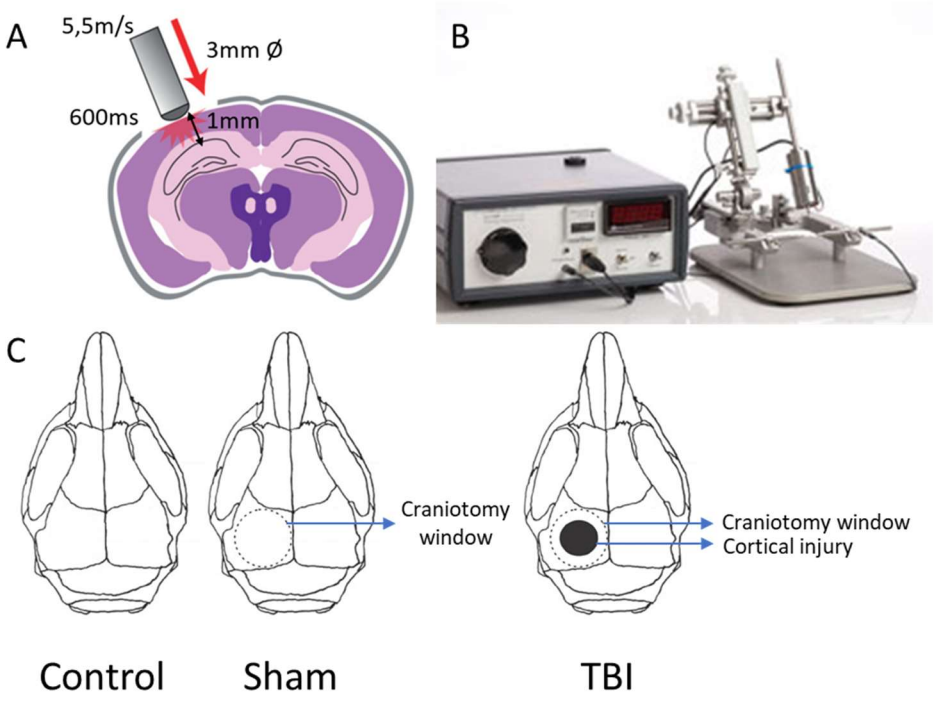
5.2. SURGICAL PROCEDURES.

5.2.1. Traumatic Brain Injury.

For the Controlled Cortical Impact procedure, mice were anesthetized with Ketamine/Medetomidine intraperitoneal mix (75mg/kg, Ketamine, #581140; 1mg/kg medetomidine; Sedastart, Pfizer) and received a single subcutaneous dose of analgesic

buprenorphine (1mg/kg; Buprecare, Animalcare Lted., #582039). Mice were ensured to be in deep anaesthesia by checking for regular, relaxed respiration and the lack of response to tail/toe pinch.

In brief, the hair over the scalp was shaved and 10% povidone iodine was applied to clean and disinfect. Also, Vaseline was applied to the eyes of the mouse to prevent drying out during surgery. The mice were positioned in the stereotactic apparatus (Kopf model 900), and their head was fixed using the ear bars and bite plate **(Fig. E1 B)**. A longitudinal incision was made in the skin over the scalp to localize Bregma. Then, the centre of the craniotomy was localized at AP -2.5mm and LL +2.5mm and a surrounding 4 mm diameter circle around this point was marked. Craniotomy was performed at this area, carefully keeping dura intact, using a 0.6mm micromotor high-speed drill. The bone was removed by using forceps and the dura mater was exposed. CCI was induced with a 1-mm deformation (5.5m/sec; 600msec dwell) delivered directly onto the exposed dura using an electromagnetic impactor with a 3-mm cylindrical tip (ImpactOne, Leica Microsystems) **(Fig. E1 A-B)**. Sham mice underwent the same surgery and anaesthesia including the craniotomy, but excluding the impact. Control animals did not undergo any type of surgery **(Fig. E1 C)**. After CCI or sham treatment, the blood was removed carefully using cotton swabs without applying any pressure and the bone was placed back to its place and helped to keep its position with a drop of glue. Then, the scalp was closed using suture (6/0 BBraun, #C0762067). The mice were placed on a warm pad to maintain body temperature while recovering.



←Previous page. **Figure E1. A.** Schematic summary of the variables intervening in the CCI model. **B.** Picture of the impactor device (ImpactOne, Leica Mycrosystems). **C.** Recapitulation of experimental groups for the TBI-characterization experiments.

5.2.2. Intrahippocampal retroviral-vector (RV) injection + TBI.

Intrahippocampal injections were performed in combination with TBI surgery. For this type of procedure two different stereotaxic stations were used: one to locate the area of interest to inject the RV and another one to proceed with the TBI impact.

5.2.2.1. First station: RV injection.

For the intrahippocampal injections, mice were subjected to the same surgical preparation: intraperitoneal injection of a mixture of ketamine/medetomidine anaesthetics (75mg/kg, Ketamine, #581140; 1mg/kg medetomidine; Sedastart, Pfizer); and a single subcutaneous dose of buprenorphine analgesic (1mg/kg; Buprecare, Animalcare Lted., #582039). Once the animal was anesthetized, its head was cleaned and shaved for scalp incision, betadine was applied in the area and the cornea of the eyes was protected with vaseline to prevent eyes from over-drying. Briefly, mice were positioned in the stereotaxic apparatus, an incision was made in the skin over the scalp to localize bregma. Next, the hippocampal region to inject was localized using the coordinates: anteroposterior (AP) -1.8mm, latero-lateral (LL) +1.6mm and dorsoventral (DV) -2.1. Then the skull was perforated using a 0.6mm micromotor high-speed drill.

Depending on the experiment, three different RV were injected: for newborn neurons characterization LeGo-SFFV-RV-Venus (spleen-focus forming virus γ-retrovirus), kindly given by Diego Gómez-Nicola (University of Southampton) (Gomez-Nicola et al., 2015; Weber et al., 2012) was used and for migration experiments CAG-i-DsRed ($2.1 \cdot 10^{10}$ TU/mL) or CAG-i-Rnd2-DsRed ($6.7 \cdot 10^{10}$TU/ml). A total volume of 500nL of retroviral solution was delivered using a nanoinjector (Nanoinject II, Drummond Scientific, #3-000-205A) attached to a pooled glass microcapillar filled with mineral oil as previously described (Sierra et al., 2015). To favour the proper distribution of the volume throughout the tissue, the total volume was injected slowly: 18.4nL/30sec. After injection was finished, the cannula was left for 2 minutes inside the tissue to avoid the reflux along the cannula tract and then withdrawn from the tissue.

5.2.2.2. Second station: Traumatic Brain Injury

After finishing the RV injection, the mice were switched from the first stereotactic station to the second one, where the impactor would be located, their head was fixed using the ear bars and bite plate and then TBI or sham surgery as described above (see **5.2.1 Traumatic Brain Injury**) would be performed. In this case, control animals would be injected with the corresponding retroviral vector and no further surgery. Later, the mice were placed on a warm pad to maintain body temperature while recovering.

5.2.3. Bilateral intrahippocampal RV injection.

For behavioural analyses, 1.5µl the retroviral solution (CAG-i-DsRed ($2.1 \cdot 10^{10}$ TU/ml); CAG-i-Rnd2-DsRed ($6.7 \cdot 10^{10}$ TU/ml) was injected into the DG of 12-week-old male C57BL/6 mice. High-titers of retroviruses were prepared with a human 293-derived retroviral packaging cell line (293GPG) and kindly provided by Dr. Emilie Pacary. Mice were anaesthetized by isoflurane (5% for induction and 2% for maintenance) and Vaseline was applied to prevent eyes from over-drying. A cranial subcutaneous injection of 0.1 µl of lidocaine (20mg/ml; Lurocaïne, Vetoquinol) and a dorsal subcutaneous injection of 0.1 µl of meloxicam (0.5mg/ml; Metacam, Boehringer Ingelheim) were performed before settling the mouse into the stereotaxic frame. Betadine was applied, then the skin was cut and a pulled microcapillary glass tube (1-5 µl, Sigma) was placed above bregma. Coordinates of the injection site from bregma were: anteroposterior: -2 mm, mediolateral: ±1.8 mm, dorsoventral: -2.2 mm. A small hole was made on each of the hemispheres of the skull using a 0.6mm micromotor high-speed drill, the microcapillary was loaded with the retroviral solution and introduced within the hole to reach the DG and stayed in place for one minute. Then 1 µl of retrovirus was injected at the rate of 0.25µl/minute and the microcapillar was kept there for two minutes after the end of infusion to avoid liquid reflux. Then, the same protocol was followed to inject the other hemisphere. Finally, the skin was stitched using absorbable sutures and the mouse was placed in a recovery chamber (37ºC) until it woke up.

5.3. TREATMENTS.

5.3.1. BrdU (5-bromo-2'-deoxyuridine) administration.

BrdU (Sigma, #B5002-1G) was diluted in sterile saline (0.1% NaOH and PBS) and administered intraperitoneally at 150mg/kg. Three injections were administered to label the proliferating cells following the paradigm: 0h after surgery, +16hours after surgery and +24hours after surgery for the neurogenic cascade characterization. For behavioural analyses mice received two injections instead: 0h after surgery and 24hours after surgery.

5.4. PERFUSION AND TISSUE COLLETION.

Animals were deeply anesthetized using 600µL of 2.5% 2,2,2-Tribromoethanol (Avertin; T48402 Sigma Aldrich). Mice were transcardially perfused with 25mL of PBS 1X followed by 25mL of a 4% paraformaldehyde (PFA) solution in PBS, pH 7.4. Next, the brains were extracted from the skull. In case TBI was performed, special care must be taken with the injured area. Then whole brains were post-fixed through immersion at room temperature in the same fixative solution (PFA 4%). Afterwards, they were transferred to PBS-0.2% PFA and kept at 4ºC until use. Left hemispheres were cut into serial 50 µm-thick sagittal sections using a Leica VT 1200S vibrating blade microtome (Leica Microsystems GmbH). Dividing into six series, whole-hemisphere representations were obtained in each of the series, since the distance between one slide to the next one is approximately 250 µm, corresponding to five sections.

5.5. IMMUNOFLUORESCENCE.

For immunofluorescence (IF), one series of free-floating tissue was incubated with blocking and permeabilization solution: PBS containing 0.25% Triton-X 100 and 3% bovine serum albumin (BSA) for 3 hours at room temperature shaking and then incubated overnight with the primary antibodies diluted in the same solution at 4ºC and also shaking. Next, the primary antibody solution was removed and the sections were washed three times with PBS for 5 minutes and then once with blocking solution again for 15 minutes. Once the sections were properly washed, blocking buffer with fluorochrome-conjugated secondary antibody plus DAPI (5mg/ml) was added and incubated for 2-3 hours in agitation in the dark at room temperature. Finally, sections were washed with PBS three times for 5 minutes and mounted on gelatine coated slides with Fluorescent Mounting Medium (DakoCytomation, #S302380).

For the BrdU analysis, sections were treated with 2M chlorohydric acid (HCl) during 20 minutes at 37ºC and immediately incubated with 0.1M tetraborate for 10 minutes at room temperature before staining. Afterwards, the sections were washed with PBS and the staining followed as

described above. GFP signal from Nestin-GFP transgenic mice, as well as the Venus signal from the SFFV-RV, was detected with an antibody against GFP for the enhancement and better visualization. Likewise, DsRed signal from the Rnd2-RV was detected with an antibody against DsRed for enhancement and visualization. The complete list of primary and secondary antibodies is indicated in **Table E1** and **Table E2**.

5.6. IMAGE ANALYSIS.

Fluorescence immunostaining images were collected using a Leica SP8 laser scanning confocal microscope with a 40x or 63X oil-immersion objective and a 0.7 µm z-step. For the quantification of total areas of the DG the 20x objective was used to completely visualize the DG in each section. For the pictures of whole-hemisphere damage after TBI, the 3DHistech Panoramic Digital Slide Scanner was used. The signal from each fluorochrome was collected sequentially and brightness, contrast and back ground were adjusted equally for the entire image using the software of the confocal microscopy Leica LAS X Life Science or ImageJ. For dendritic spine density analysis, images were subjected to a posterior deconvolution process using Huygens Professional software.

5.7. QUANTITATIVE ANALYSIS.

5.7.1. Quantitative analysis of cell populations.

Quantitative analysis of cell populations in vivo was performed by design-based (assumption free, unbiased) stereology using a modified optical fractionator sampling scheme as previously described (Encinas et al., 2006, 2011a; Encinas & Enikolopov, 2008).

For cell densities, quantifications were done maintaining the same z-stack size between conditions. The values were normalized to the volume of the SGZ+GCL in the counted area. For total numbers, the whole area of the DG was determined for every slice of a single series and then multiplied by the thickness of the GCL in each slice. The obtained value was multiplied by 6 (number of series) to obtain the volume of the whole DG.

For all the experiments including TBI, the area of analysed tissue is strictly restricted to the directly impacted place. This includes the dorsal-septal hippocampus: the dorsal blade of the septal dentate gyrus. This is anatomically the most affected area by the injury, including ±1.5

mm from the centre of the place of impact, given that the diameter of the impactor is 3 mm itself.

5.7.1.1. NSCs population.

For NSCs population quantification, which lack the expression of one specific marker, several criteria were used for their identification. First, expression of the well-described NSC marker Nestin-GFP throughout all cell body and GFAP only in cellular projections and presence of a radial process extended towards the ML. These criteria were properly described in Encinas & Enikolopov, 2008 and Kronenberg et al., 2003. To identify proliferating cells, the marker Ki67 was used. NSCs which fulfil the requirements for the previous criteria together with the expression of this proliferative marker were counted as proliferating NSCs. For image analysis, 2-3 z-stacks located at random positions in the DG were collected per hippocampal section, and 4-6 sections per series were analysed, depending on the experiment. Only cells with complete soma in each of the axis appearing in the image were counted as positive and those of left-lower margin and only one of the z-edges.

5.7.1.2. Immature neurons.

Same criteria were used for the DCX population analysis. For soma size, the diameter of the cell body was measured twice in each cell: once starting from the primary process origin to the basal part of the cell and then another one perpendicular to that one. Average from these two measurements was considered as the diameter of that specific cell. In migration analysis, the GCL was divided into discrete areas (0-30 µm, 30-60 µm, 60-90 µm, 90-120 µm and more than 120 µm) and the data was expressed as the percentage of DCX-expressing cells in each of the areas.

5.7.1.3. Proliferating cells and activation of neurons.

To quantify total proliferation, immunostaining of total Ki67 and total c-fos was performed and positive cell density was quantified using same criteria as previously described and then normalising with the total volume of the DG. Only positive cells in the GCL and SGZ were counted, whereas cells in the hilus or the molecular layer were excluded.

5.7.1.4. RV-infected immature neurons.

For the experiments using RV infections all infected cells in our area of interest in the DG were quantified. Only animals that showed a total of at least 10 infected cells were used for analysis. In this case, the cell migration was analysed determining the distance between the beginning of the SGZ and the base of the soma of these cells. For changes in soma size, same protocol as for DCX-expressing cells was used.

5.7.1.4.1. Dendritic spine density.

For dendritic spine density analysis only animals with a minimum of 10 infected neurons were used for the analysis. For each neuron, ~25µm of total length were counted from 1-4 different dendrites located 100-200µm from the soma.

5.7.2. Dorsal Blade (DB) thickness.

For dorsal blade thickness analysis, DAPI staining was used to identify the SGZ+GCL. Using coordinates and spatial criteria, 6-8 slices were measured in at least 2 different points of their GCL: thickest and thinnest.

5.7.3. Area occupied by GFAP.

To allow comparisons, only images taken under the same conditions and with the same properties were used for analysing the area occupied by a fluorescent signal. Individual images were imported to ImageJ and specific ROIs including only the GCL+SGZ areas were created. We used a macro designed to serially threshold multiple images kindly provided by Dr. Jorge Valero Gómez-Lobo to quantify the area occupied by GFAP for each of the images. The results are presented as the area fraction (percentage of the area in the image with a specific value in the grayscale: 0-255) and the total area under this curve for each of the experimental groups.

5.8. BEHAVIOURAL ANALYSES.

A total of 26 male animals altogether were used for these analyses: 14 mice were injected with Rnd2-Dsred and 12 with CAG-DsRed. They were immediately separated into individual cages after surgery. For all behavioural tests, animals were placed in the test room one hour before the beginning of the experiment. All experiments were performed during light-time in the mice cycle. For all procedures, experimenters were blind to the virus injected. The behavioural sequences correspond to the ones following this order:

5.8.1. Open field test (OF).

Mice were placed in one corner of a square open field (50cm x 50cm, 200 lux). Exploratory behaviour was monitored for 10 minutes. Time spent in the centre (35cm x 35cm) and periphery of the arena, together with the distance travelled and the number of entries to the different areas were automatically measured by a video-tracking system connected to a camera (Videotrack, VieowPoint). Mice were examined during 10 minutes, although results were analysed both for the total of 10 minutes and separately in 5+5 minutes to observe differences in time. Mice were subjected to the test in rounds of two.

5.8.2. Emergence test (ET).

Same arena as in the OF was used but with lighter conditions (300 lux in the centre of the centre of the arena). Mice were placed in a dark cylinder (10cm x 6.5cm, grey PVC) facing one corner of the arena (10cm apart). Their behaviour was monitored for 5 minutes using a video-tracking system. Latency to emerge from the cylinder, number of re-entries and time spent inside were quantified. Mice were subjected to the test one by one.

5.8.3. Elevated plus maze (EPM).

We used an elevated cross maze (37cm x 6cm arms) placed 115cm above the ground. Closed arms with walls and open arms with no walls. Light conditions for this experiment were 300 lux in the centre, 400 lux in the outer part of the open arms and 80 lux in the outer part of the closed arms. Mice were placed at the centre of the cross at the beginning, facing one of the open arms and behaviour was monitored for 5 minutes. Mice were tested one by one. Time spent in open and closed arms and entries between them were quantified using a video-tracking system.

5.8.4. Light/Dark test (LD).

A light/dark chamber was used for this test composed of a strongly illuminated chamber (36cm x 36cm, 350lux) and a dark chamber (36 x 23 cm, 10lux) both separated by a wall with a door (10cm x 10cm) allowing the animals to travel freely between the two compartments. Mice were placed in the lit chamber and left exploring both areas for 5 minutes. Latency to leave the lit chamber as well as time spent in it and number of entrances into it were manually measured.

5.8.5. Morris water maze (MWM).

For this experiment a white circular swimming pool (150cm in diameter and 60cm deep) was located in a room with various distal cues (80lux). The pool was filled with water maintained at 19ºC and made opaque by the addition of a non-toxic white cosmetic adjuvant. The escape platform (14cm diameter) was hidden underwater to that its top surface was 0.5-1cm below the surface of the water. In this task, mice were required to located the hidden platform using distal cues and mice behaviour was monitored using a camera and video-tracking system (Videotrack, Viewopoint). The day prior to starting the training, mice were habituated to the pool and trained to stay at least 10-15 seconds in the platform before taking them back to their cage. Two different training protocols were tested.

5.8.5.1. Variable start.

For the entire training period, the platform was localised in the same position: mid-centre of the north-east (NE) quadrant, and mice were tested from three variable random start positions. Mice were trained 3 trials a day, each trial being separated by a 5 minutes interval. A trial was terminated when the animals climbed onto the platform and stayed there for at least 10 seconds. Mice that failed to find the platform within the 60 seconds cut-off time were placed onto the platform by the experimenter and had to stay there for 10-15 seconds before being placed back in their home cage. The releasing point differed for each trial and different sequences of releasing points were used day to day **(Fig. E2 A)**.

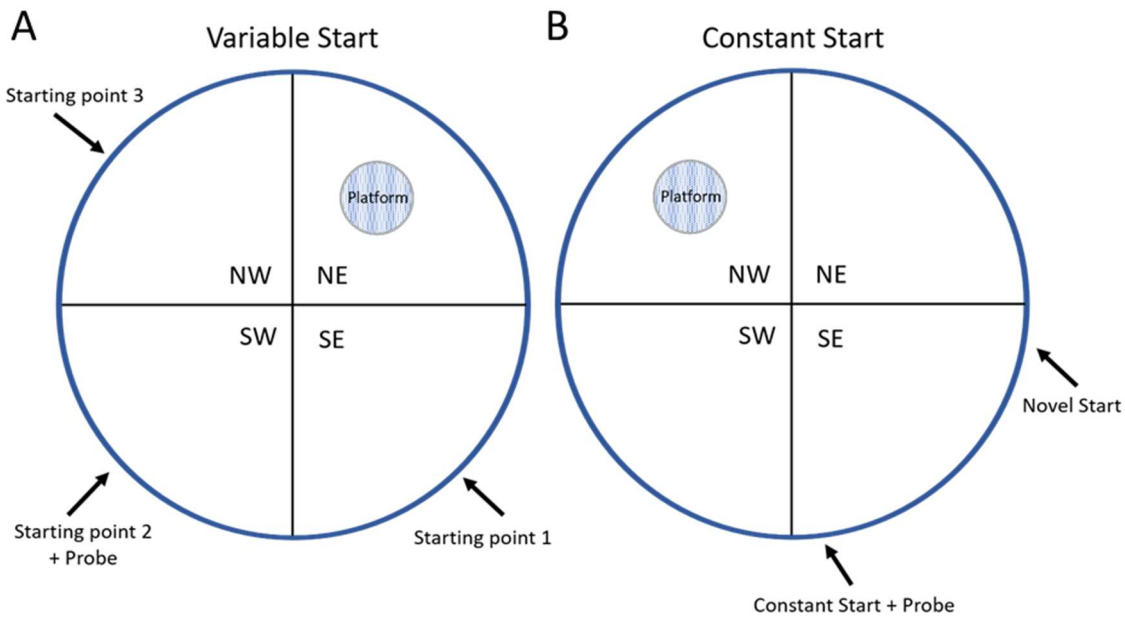
The day after the last training trial, the hidden platform was removed and the memory for the platform location was assessed during a probe test. During this test, mice were allowed to freely

swim in the water maze for 60 seconds and performances were measured through the time spent in the target quadrant where the platform used to be located, the latency to enter the area where the platform used to be and the total number of re-entries into this area.

5.8.5.2. Constant start.

Mice were trained 3 trials a day, each trial being separated by a 5 minutes interval. A trial was terminated when the animals climbed onto the platform and stayed there for at least 10 seconds. Mice that failed to find the platform within the 60 seconds cut-off time were placed onto the platform by the experimenter and had to stay there for 10-15 seconds before being placed back in their home cage. In this case, the platform was located in the north-west (NW) quadrant and kept in the same position every time. The main difference from the variable start test is that in this case, mice were always released from the same starting position.

After training a novel start test was performed. Mice were released from a different position and tested to find the platform (which is still in the same position) **(Fig. E2 B)**. The day after the novel start test, the hidden platform was removed and the memory for the platform was assessed during a probe test. During this test, mice were allowed to freely swim in the water maze for 60 seconds and performances were measured through the time spent in the target quadrant where the platform used to be located, the latency to enter this platform area and the number of re-entries into it. In this probe, the mice were released from the position used as a constant start.



←Previous page. Figure E2. Schematic representation of the MWM set up in two different conformations. The swimming pool is divided in four areas: north-west (NW), north-east (NE), south-west (SW) and south-east (SE). Visual cues would be located surrounding the whole area. **A.** For the variable start conformation the platform is located in the NE quadrant and the starting positions are randomized throughout the days/mice as it is indicated in the picture. **B.** For the constant start conformation, the platform is located in the NW quadrant, mice are always released from the same position for training and then a different starting point is tested afterwards (novel start).

5.8.6. Sucrose preference test.

In the sucrose preference test, mice were exposed in their home cage to two drinking bottles during 48 hours. During the first 24 hours, a habituation was performed with the two bottles filled with water. Consumed water for both of them was measured afterwards to test possible preferences depending on the side. For the test phase, one of the bottles was filled with 30mL of 4% sucrose water while the second bottle was filled with water. Half of these bottles were placed on the left and half of them in the right. After 24 hours the bottles were weighted. All mice were tested for this at the same time. Sucrose preference ratio was calculated as it follows.

$$Sucrose\ preference = \frac{\Delta weight_{sucrosed\ water}}{\Delta weight_{sucrosed\ water} + \Delta weight_{water}}$$

5.8.7. Forced swim test (FST).

The FST device consisted of a glass cylinder of 16cm diameter and 25cm height, filled with water to a height of 15cm. Water was maintained at 26ºC and light was 75lux. Mice were placed in the apparatus and behaviour was monitored for 6 minutes. Mice were tested in pairs. Total immobility time, immobility time/minute and total immobility in the last 4 minutes of the test were measured.

5.8.8. Criteria of exclusion.

Most tests used in this study rely on exploration and locomotor activity, therefore total distance travelled in these tests was used as an index of locomotor activity. Although no animal was excluded on the bases of the total travelled distance, several other criteria were determined to exclude mice that did not follow the rules of the designed tests. For example, mice that did not swim and instantly started floating during the MWM and the FST would be excluded for the final analysis of performances, as well as mice that did not leave the cylinder in the emergence test.

In the EPM, mice that stayed 240 seconds in the centre or less than 1 second in open or closed arms were excluded from analysis. Only animals that showed a significant number of DsRed labelled cells after euthanasia were kept for the analysis.

PRIMARY ANTIBODIES			
Antibody	**Dilution**	**Source**	**Catalog Number**
Chicken Anti-GFP	1:1000	Aves Laboratories	GFP-1020
Rabbit Anti-NeuN	1:500	Abcam	ab177487
Rabbit Anti-Ki67	1:750	Vector Laboratories	ab16667
Rabbit Anti-GFAP	1:1000	Dako	Z0334
Rat Anti-BrdU	1:1000	AbD Serotech	MCA2060GA
Goat Anti-DCX	1:1000	Sta.Cruz Biotech	sc-8067
Goat Anti-GFAP	1:2000	Abcam	ab53554
Rabbit Anti-DsRed	1:2000	Living Colors	632496
Chicken Anti-Nestin	1:1000	Aves Laboratories	NES
Rabbit Anti-GFP	1:1000	Abcam	ab6556
Goat Anti-GFAP	1:1000	Abcam	ab53554

Table E1. Primary antibodies used in the immunofluorescence.

SECONDARY ANTIBODIES			
Antibody	**Dilution**	**Source**	**Catalog Number**
Alexa Fluor 488 Goat Anti-Chicken	1:500	Molecular Probes	A11039
Alexa Fluor 488 Donkey Anti-Rabbit	1:500	Invitrogen	A21206
Alexa Fluor 568 Donkey Anti-Chicken	1:500	Invitrogen	A11042
Alexa Fluor 568 Donkey Anti-Rabbit	1:500	Invitrogen	A10042
Alexa Fluor 568 Donkey Anti-Goat	1:500	Invitrogen	A11057
Alexa Fluor 594 Goat Anti-Rat	1:500	Molecular Probes	112295167
Alexa Fluor 568 Donkey Anti-Goat	1:500	Invitrogen	A11057
Alexa Fluor Rhodamine Red X Goat Anti-Rabbit (RRX)	1:500	Jackson Immunology Research	111295144
Alexa Fluor 680 Goat Anti-Rabbit	1:500	Molecular Probes	111605003
Alexa Fluor 680 Donkey Anti-Goat	1:500	Invitrogen	21084
DAPI	1:1000	Sigma-Aldrich	32670

Table E2. Secondary antibodies used in the immunofluorescence.

RESULTS

6. RESULTS.

6.1. CHARACTERIZATION OF THE CCI MODEL AND ITS SUITABILITY FOR THE STUDY OF AHN.

The goal of the present thesis and doctoral dissertation is to unravel the relationship between AHN and TBI. Among the different TBI models (as we mentioned in section 2.5.3 of the introduction), we focused on CCI and performed its previous characterization. AHN may be affected directly or indirectly by several model-associated alterations so we resorted to assess those possible variations beforehand. Such alterations may be: the level of neuronal activity, the general anatomy and morphology of the DG and/or the level of cell proliferation in the GCL after the injury.

6.1.1. Moderate CCI enhances neuronal activity in the DG.

Alterations in AHN have been linked to neuronal hyperactivity (Martín-Suárez et al., 2020; Sierra et al., 2015). A certain level of neuronal hyperexcitation has been described shortly after TBI in different animal models (Villasana et al., 2014; Xu et al., 2018). We used the number of c-fos-expressing granule neurons in the DG as an indictor of the level of neuronal activity in the area three days post-TBI (3dpTBI). As an immediate early gene, c-fos has a quite limited expression in a control state **(Fig. R1A)** but strong expression in hyperactivated neurons. We observed an increase in c-fos in 3dpTBI animals compared to both Sham and Control groups **(Fig. R1)**. It is known that focal brain injury in mice leads to rapid dramatic increases at the c-fos protein level in several brain areas (Dragunow et al., 1990). This increase has been characterized in terms of c-fos mRNA as early as 15 minutes after the injury. Here, we demonstrate a change at protein level that lasts for at least 3 days after the injury in neuronal activity in the granule neurons of the DG **(Figure R1 D-E)**. The distribution of these c-fos positive cells (c-fos$^+$) is uniform throughout the GCL corresponding with the distribution of mature neurons in the DG **(Fig. R1 A-C)**.

TBI has been linked to several anatomical abnormalities changing the form and size of the dentate gyrus between other structures. In order to eliminate possible changes of absolute numbers estimation due to the volume of the DG or the dispersion of the GCL observed in other

related animal models, we represented the density of the c-fos-expressing cells: positive cells in a specific volume in the GCL; **(Fig. R1 D)** and then normalized the values by the total volume of the DG **(Fig. R1 E)**. Both quantifications show an important increase in the c-fos expressing cells in the TBI group.

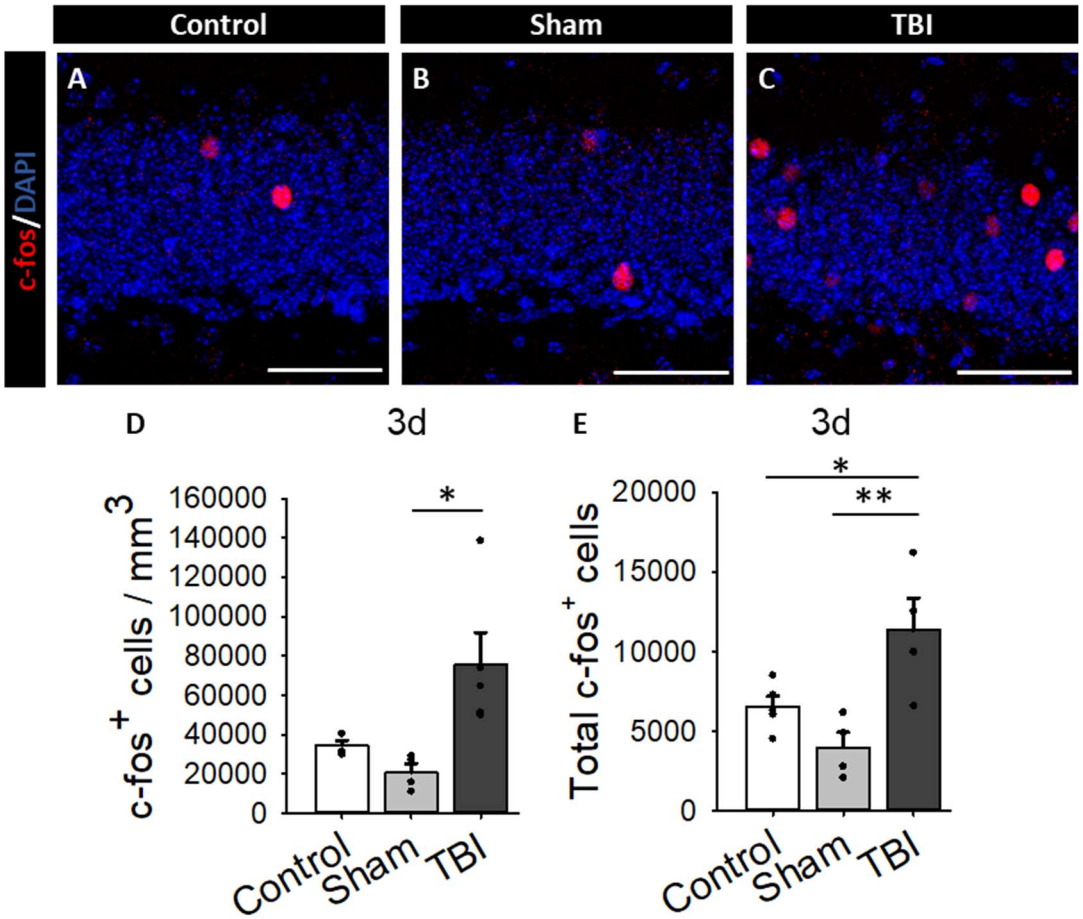


Figure R1. C-fos-expressing neurons numbers increase after TBI in the DG 3dpTBI. A-C. Representative confocal images showing c-fos$^+$ neurons in the GCL in Control (A), Sham (B) and TBI (C) groups. **D.** Quantification of cellular density of c-fos-expressing neurons per mm^3. **E.** Estimation of total c-fos$^+$ adjusted to the tissue volume. dpTBI: days post-Traumatic Brain Injury, DG: Dentate Gyrus, GCL: Granule Cell Layer. Scale bar 50 µm. *p<0,05; **p<0,01. Kruskal-Wallis followed by all-pairwise multiple comparisons (Dunn's method) in (D). One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test in (E). Bars show mean ± SEM. Dots show individual data.

6.1.2. Moderate CCI produces general anatomical changes in the whole ipsilateral hemisphere.

Animal models representing TBI often involve a plethora of anatomical abnormalities at a macroscopic level, for this reason, we sought to perform a general analysis of the complete left hemisphere before diving into specifical cellular changes.

Firstly, we designed an exhaustive experimental paradigm which involved four different timepoints that will be referred during this dissertation. The day of the surgical intervention (in case of Sham and TBI animals) was considered as the zero day. Then, a first cohort of animals was sacrificed 3 days after (3 days post-TBI, 3dpTBI) for histological evaluation of the most acute phase of the injury. Next, the second cohort was sacrificed at 15 days (15 days post-TBI, 15dpTBI) to assess AHN changes after TBI. And finally, third and fourth cohort were set to long-term timepoints: 60 days (2 months post-TBI, 2mpTBI) and 120 days (4 months post-TBI, 4mpTBI) to characterize the implications and timing of chronic consequences of TBI that still remain unclear in the literature. Due to the variability of chosen timepoints when making long-term studies (Bushnik et al., 2008; Neuberger et al., 2017), we sought to use two different ones (2mpTBI and 4mpTBI) to get a better understanding of the evolution of the injury **(Fig. R2 A)**.

As a first approach, we observed the brain at a low magnification (10X) to assess general tissue loss and damage at every timepoint **(Fig. R2 B-M)**. From a very early beginning (3dpTBI), Sham animals presented a minor cortical deformation with almost no tissue loss although there was glial activation and cell death to a smaller extent. However, animals with TBI showed strong cortical deformation with obvious cavity formation **(Fig. R2 D-M)**. Although this deformation was not present in all of the animals, in general, they showed pycnotic and necrotic nuclei highlighting a certain amount of cell death in the cortical area adjacent to the hippocampus. Over time, this region will end up creating a glial scar **(Fig. R2 M)**. In general terms, the percentage of tissue loss in our model seems to match that of similar severity models, previously reported, accounting for 15% tissue loss in moderate TBI(X. Wang et al., 2016). In addition, after TBI most animals also showed a deformation of the hippocampus towards the injured area **(Fig. R2 A'-M')** after TBI.

Next, we focused more specifically into the DG, as its subgranular region is a key niche in AHN. The most noticeable effect was a specific thickening of the Dorsal Blade (DB) of the GCL of the DG, not found in the Ventral Blade (VB). DB is the region located closer to the site of the injury. The increase of the thickness of the GCL has also been observed in other pathologies such as epilepsy (Sierra et al., 2015). However, the aetiology may be different since the increase reported in epilepsy is usually related to a dispersion of the cellular nucleus conforming the granule layer. Nevertheless, in this case the cellular density remains the same, as it can be observed in the images **(Fig. R3 A-D)**.

Histological observation of the DB at 3dpTBI does not show morphological differences between Control, Sham and TBI. However, at 15dpTBI, there is a dramatic difference in DB thickness. TBI

animals show an increase of almost 2-fold GCL thickness in DB when compared to both Control and Sham animals. This difference is still present at 2mpTBI and 4mpTBI **(Fig. R3 E-H)**. Therefore, it is an alteration that appears after the ending of the acute phase of TBI and is part of the chronic alterations developed during the pathology **(Fig. R3 I)**. Interestingly, histological observation highlights a morphologic misplacement of the tissue that makes the DB acquire a certain curvature towards the most injured area of the cortex.

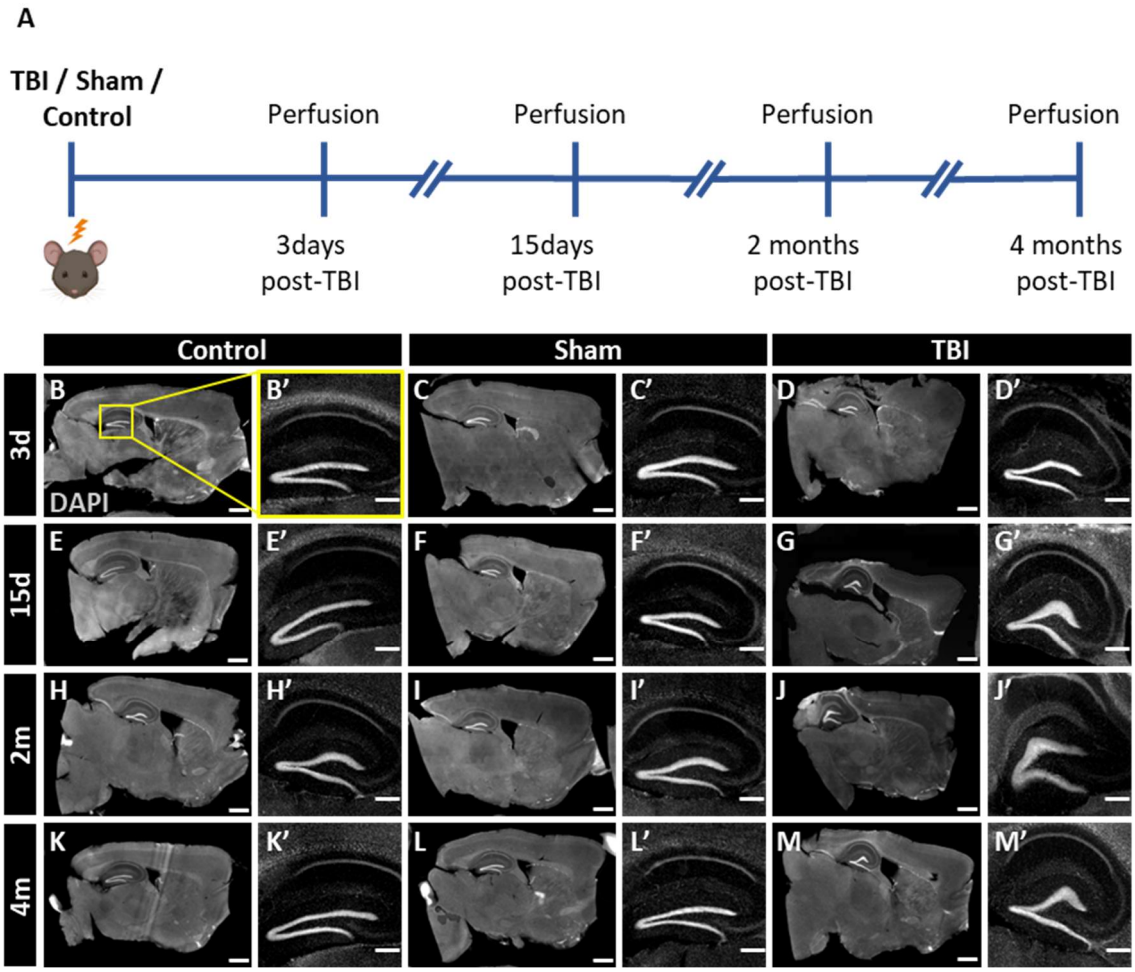


Figure R2. Anatomical changes in general in the impacted hemisphere and specifically in the DG. A. Schematic representation of the time points chosen to study changes in neurogenesis after TBI. **B-M.** Representative images of the anatomical changes produced in TBI, sham or control groups 3dpTBI (B-D), 15dpTBI (E-G), 2mpTBI (H-J) and 4 mpTBI (K-M). **B'-M'** show an amplification of the DG in each group. TBI: Traumatic Brain Injury, dpTBI: days post-TBI, DG: Dentate Gyrus. Scale bar is 1000 µm in images B-M and 300µm in images B-M'.

All the images of different experimental groups and timepoints were taken at a similar number of seriated-section corroborated by stereological coordinates of the observed brain regions (as it becomes evident by the surrounding anatomical hallmarks) so that there is no interference with the latero-lateral intrinsic brain morphology **(Fig. R2 B-M)**. Furthermore, we measured

thickness always in a central point of the DB which usually matches the thicker point in the GCL and the same number (8) slices in the surrounding area of the impact area were analysed.

From this point on, we decided to focus in this specific area that seems to be the most affected by the impact. All analyses regarding cellular densities and total cell numbers presented from now on, only include the DB of the DG (**Fig, R3, A-D**, white surrounded areas). This specific division into DB and VB is only present in the septal hippocampus.

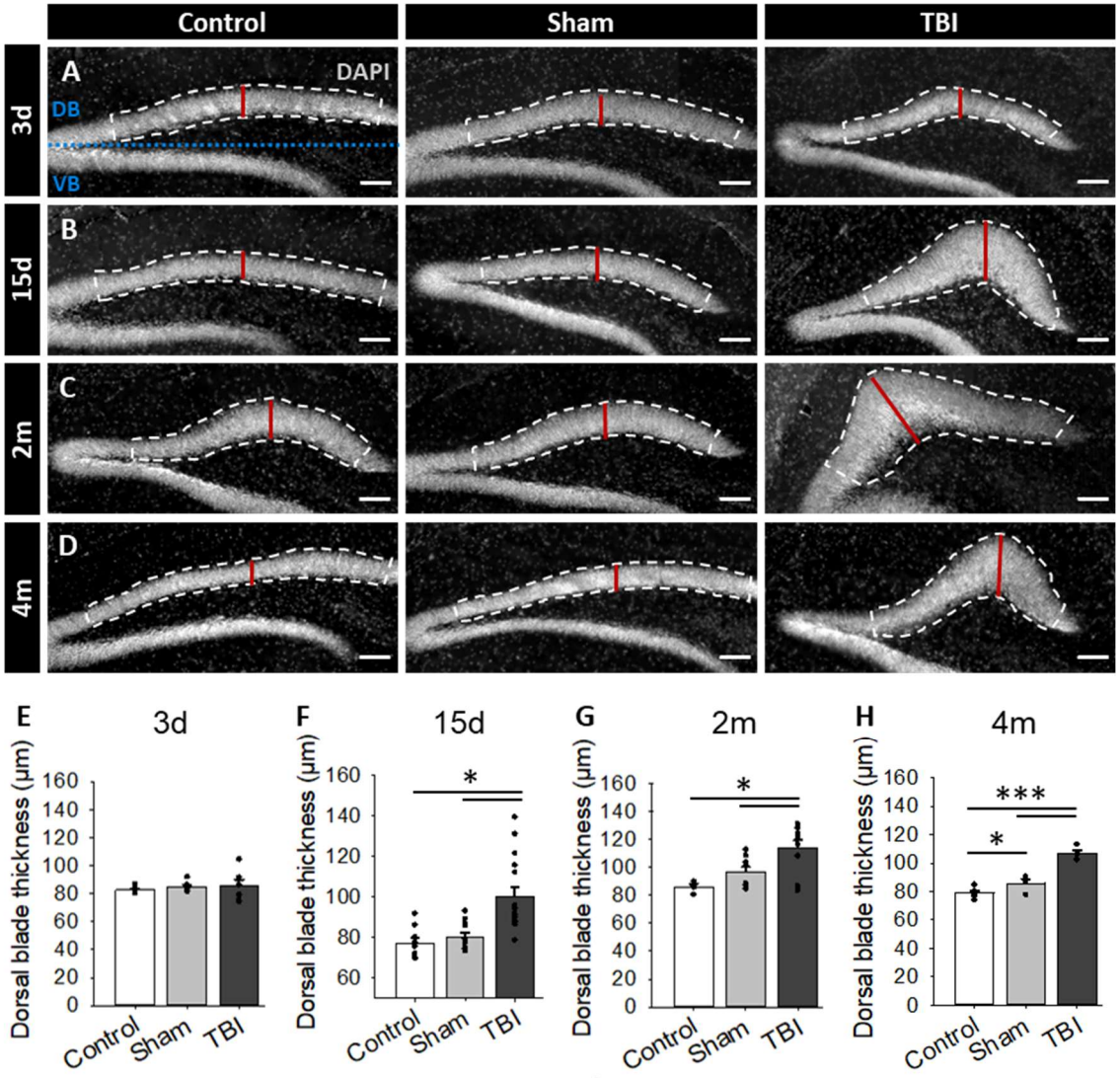


Figure R3. Evolution of the granule layer thickness in the Dorsal Blade (DB) after TBI. A-D. Representative confocal images of the thickening of the DB of the DG after TBI versus Control and Sham animals 3 days, 15 days 2 months and 4 months after the surgery. **E-H.** Quantification of DB thickening for each timepoint calculated by measuring the thickness of this layer at several different latero-

lateral coordinates. **I.** Chronological representation of the changes in the thickness of the DB after TBI. The region surrounded by white discontinuous lines comprehends the analysed area of interest. Red lines indicate the thickness of the GCL in each of the images. Dotted discontinuous line separates the DB from the VB. TBI: Traumatic Brain Injury, DG: Dentate Gyrus; GCL: Granule Cell Layer; DB: Dorsal Blade; VB: Ventral Blade. Scale bar 60 µm. *p<0,05; ***p<0,001; (*) indicates TBI vs Control differences and (#) indicates TBI vs Sham differences. Bars show mean ± SEM. Kruskal-Wallis followed by all-pairwise multiple comparisons (Dunn's method) in (E) and (F). One-way ANOVA followed by all-pairwise multiple comparisons by Holm- *Šídák* method post hoc test in (G) and (H). Dots show individual data.

6.1.3. Cell proliferation in the GCL of the DB is increased 3dpTBI.

The observed increase in the GCL thickness could be due to several reasons, however, cell dispersion was discarded since the cellular density remained the same. For this reason, we hypothesized the related increase of GCL could be due to a more elevated number of cells in the area. Thus, we checked cellular proliferation using Ki67 immunolabelling across the GCL throughout the different timepoints to get an idea of the progression of the number of cell divisions in the area. Ki67 is a nuclear protein associated with cellular proliferation which is often used as a biomarker of it (Gerdes et al., 1984).

Three days after the injury there is a dramatic increase in the cellular density of $Ki67^+$ cells in the GCL **(Fig. R4 A,E)**. As it can be observed, this increase was not located in any part of the GCL specifically but distributed all across it only after TBI **(Fig. R4 A)**. Control and Sham animals showed discrete $Ki67^+$ cells restricted to the SGZ where the neurogenic niche is located. Although it is not the main goal of this study, it should be noted an increase of cellular proliferation in other surrounding areas such as the hilus and the molecular layer.

We were interested in assessing whether this increase in the density of proliferative cells was only restricted to the acute phase after TBI or could be prolonged over time so we run the same analysis in the rest of the timepoints. By 15dpTBI and 2mpTBI, the proliferative cells in the three experimental groups were equivalent **(Figure R4 B-C and F-G)**. In this case, the location of the proliferating cells in TBI animals was in a way similar to that of Control and Sham. All three groups showed proliferation specifically in the neurogenic area while the rest of the GCL, ML and Hilus showed no relevant proliferation. It is interesting to note that by 4mpTBI the density of $Ki67^+$ cells showed a higher tendency in the Control group than in Sham, however there is no statistical difference compared to TBI. Nevertheless, when we check the graph taking into account the dots (specific animals in the experiment) we can observe a tendency for Control

animals to be also different from TBI. At this later timepoint, the distribution of the proliferative cells is similar to that 15dpTBI and 2mpTBI: almost specifically located in the SGZ. Taking this into account, it could be deduced and we could hypothesize that this difference in the proliferation at least in this timepoint may concern specifically to NSCs and therefore related to a loss of the neurogenic potential.

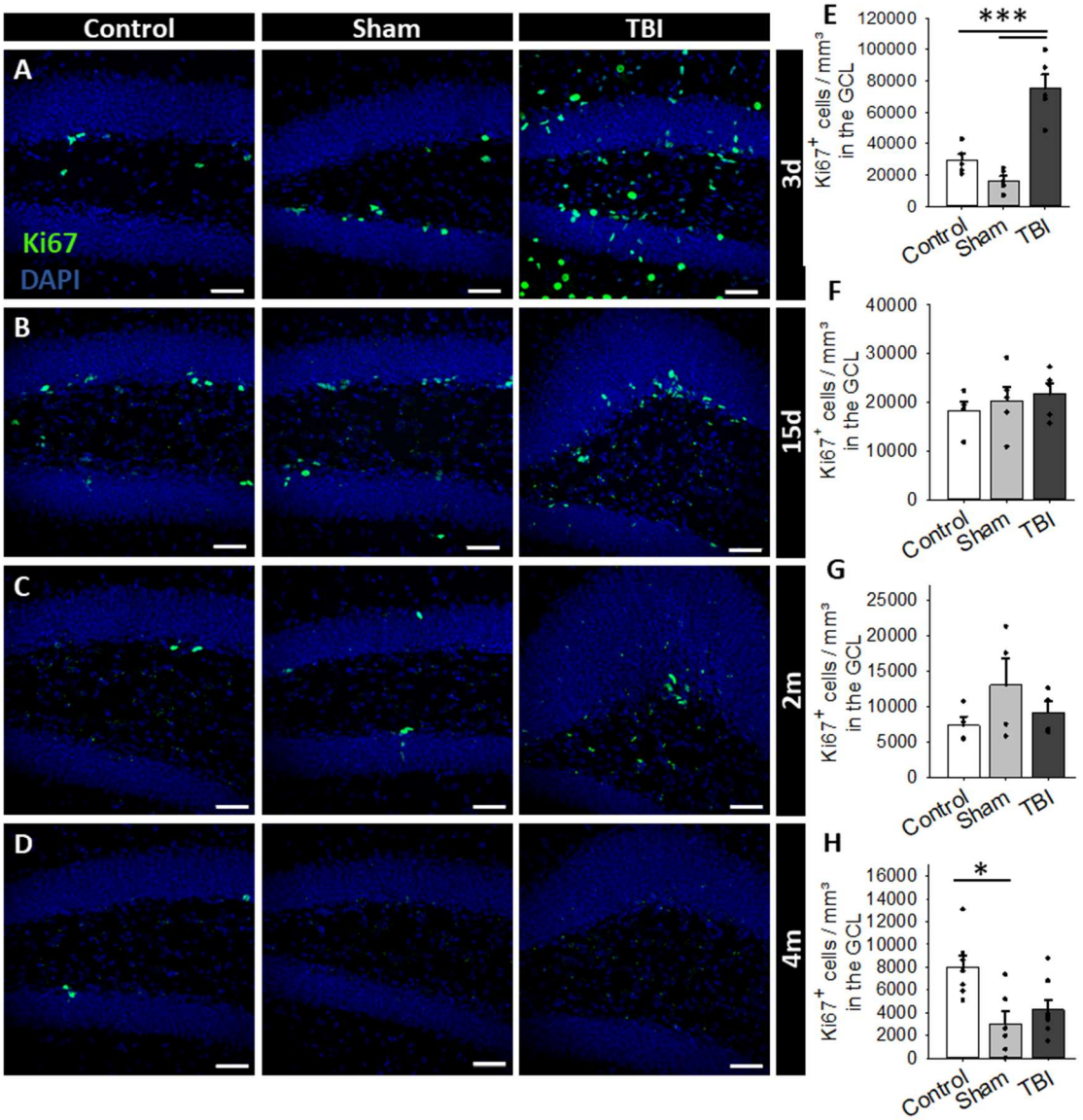


Figure R4. The density of proliferating cells in the DB of the GCL, represented as Ki67⁺ cells, increases at 3dpTBI but then remains unchanged in later timepoints. A-D. Representative confocal images of the density of Ki67⁺ cells in the DB of the DG in Control, Sham and TBI animals 3dpTBI (A), 15dpTBI (B), 2mpTBI (C) and 4mpTBI. **E-H.** Quantification of cellular density of Ki67⁺ cells in the DB of the DG measured in number of cells per mm^3 for each timepoint. The number of Ki67⁺ cells is higher in the TBI group compared to both Sham and Control 3dpTBI (E) indicating an increase in the proliferating rate by this time. However, the numbers are not significantly different in the three groups by 15dpTBI (F) and 2mpTBI (G). By 4mpTBI there is a switch in the numbers and there is actually a lower density of Ki67⁺ in Sham animals compared to Controls, but there is no difference with the TBI group (H). DB: Dorsal Blade, GCL: Granule Cell Layer, TBI: Traumatic Brain Injury, dpTBI: days post-TBI, DG: Dentate Gyrus. Scale bar 50 µm. *p<0,05;

***p<0,001. One-way ANOVA followed by all-pairwise multiple comparisons by Holm-*Šídák* method post hoc test in (E) and (F). Kruskal-Wallis followed by all-pairwise multiple comparisons (Dunn's method) in (G) and (H). Bars show mean ± SEM. Dots show individual data.

It has been previously demonstrated and also we corroborate that this model of moderate CCI induces an increase in the thickness of the GCL, producing changes in the whole volume of the DG. In this context, to measure a certain cell population in terms of cellular density (number of cells / mm^3) could mask relevant information. Indeed, the changes in the GCL thickness are not necessarily uniform across animals or even across the latero-lateral axis of each animal, producing a certain degree of additional variability to the quantified results. Hence, to solve this problem, we resorted to measure the total volume of the DG for each animal and then extrapolate the number of cell density into a total number of positive cells per animal.

Using this methodological approximation, we determined that precise volume estimations did not bias the results of $Ki67^+$ cells counts. When changing from cellular density to total numbers, the differences remained the same. The total number of $Ki67^+$ cells in the DB of the DG 3dpTBI were significantly higher than after Sham or Control treatment **(Fig. R5 A)**. However, by 15dpTBI and 2mpTBI, the total number of proliferating cells remained equal in all the groups **(Fig. R5 B-C)**. By 4mpTBI, there was again a difference only between Control and Sham animals. By this time, Control animals have a higher total number of proliferating cells in the GCL than Sham animals **(Fig. R5 D)**. The main purpose of using both Control and Sham animals is to be able to isolate and determine the effect of the impact itself without the craniotomy. The fact that there is a difference with Sham animals but not with TBI could meaning that the craniotomy itself has an effect over cell proliferation which is different from the effect the impact could have (as it will be discussed later).

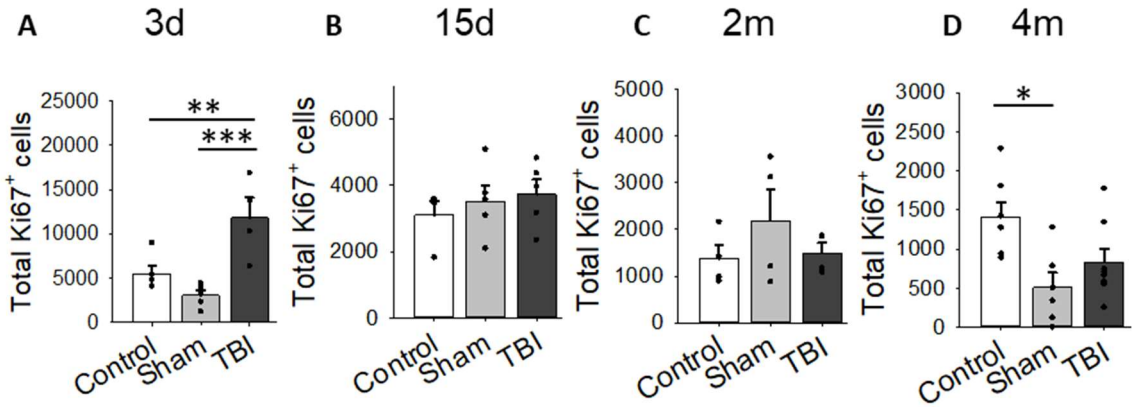


Figure R5. The total number of $Ki67^+$ cells in the analysed area is higher in the TBI animals 3dpTBI but it does not change in this group for the rest of the timepoints. A-D. Quantification of total numbers of $Ki67^+$ cells in the DB of the DG for each of the timepoints. The results were obtained by multiplying the density

of the cells by the total volume of the analysed area. There is a dramatic increase in the total number of proliferating cells 3dpTBI (A) in the TBI group compared to both Sham and Control animals. However, the numbers remain similar in the rest of the different timepoints of TBI animals (B-C). Noteworthy, fewer proliferating cells in Sham animals compared to Controls were observed at 4mpTBI (D). TBI: Traumatic Brain Injury, dpTBI: days post-TBI, DG: Dentate Gyrus. *p<0,05; **p<0,01; ***p<0,001. Bars show mean ± SEM. One-way ANOVA followed by all-pairwise multiple comparisons by Holm-*Šídák* method post hoc test in (A) and (D). Kruskal-Wallis followed by all-pairwise multiple comparisons (Dunn's method) in (C) and (D). Dots show individual data.

Despite Ki67 has been used as a marker of cell proliferation as a cell proliferation marker (Gerdes et al., 1984), it should be taken into account that it has certain limitations. One of them is that we can only get a picture of what is happening in the post-traumatic brain at a certain point coinciding with the moment of animal sacrifice. Because we were interested in getting to know how the general cell proliferation evolves during the acute phase after the injury (previously to the moment of the animal euthanasia), we designed an experimental paradigm injecting intraperitoneally several doses of saturating BrdU from the very early beginning after the injury until 3dpTBI **(Fig. R6 A)**. This strategy allowed us to identify if certain areas were proliferating more than others during these first days by incorporating this analogue of the nucleoside thymidine during the S phase.

The result obtained using BrdU resembled what we previously observed with Ki67. There is an increase in terms of cell proliferation all in the Hilus, the ML and the GCL **(Fig. R6 B-D)**. When we specifically look at the GCL, the cells that got activated and started proliferating during these first 3 days are not specifically located in any area, but dispersed across the GCL **(Fig. R6 D-E)**.

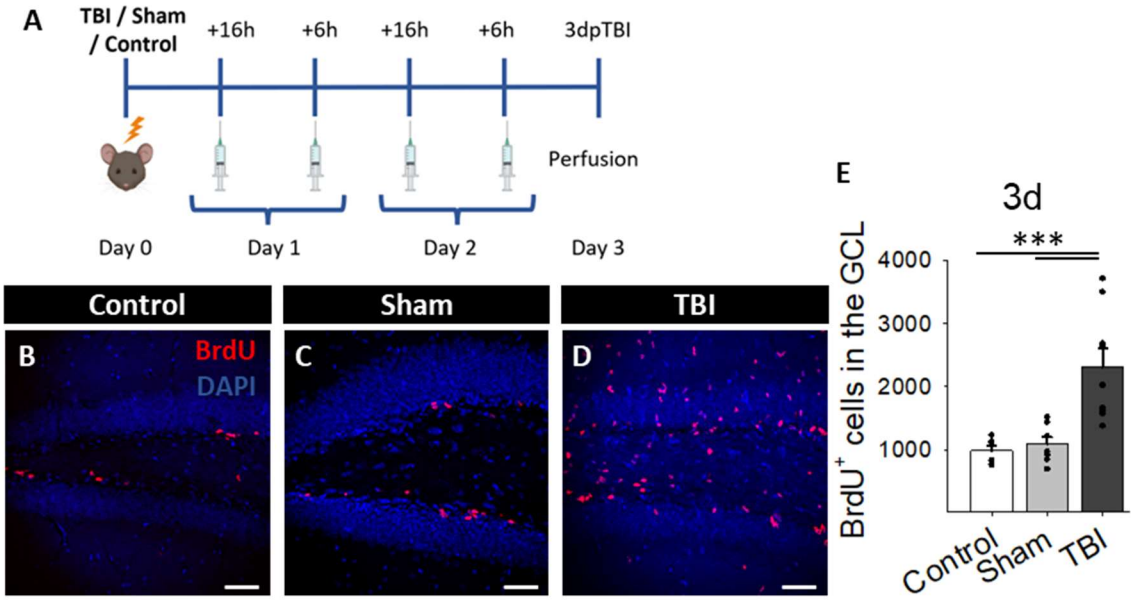


Figure R6. Cell proliferation increases in general throughout the whole GCL in the first three days after TBI. A. Schematic representation of the timeline of the BrdU administration to the animals during the first three days after the injury. **B-D.** Representative confocal images showing BrdU$^+$ cells throughout the DB

of the DG in Control (B), Sham (C) and TBI (D) conditions three days after the treatment. **E.** Quantification of total $BrdU^+$ cells in the DB in the three experimental groups. Cell proliferation increases dramatically in the TBI group compared to the other two. GCL: Granule Cell Layer, BrdU: Bromodeoxyuridine, DB: Dorsal Blade, DG: Dentate Gyrus, TBI: Traumatic Brain Injury. Scale bar 50 µm. ***p<0,001. Kruskal-Wallis followed by all-pairwise multiple comparisons (Dunn's method). Bars show mean ± SEM. Dots show individual data.

6.2. EVOLUTION OF THE NSC POPULATION AFTER TBI IN TERMS OF REACTIVITY, PROLIFERATION AND POOL EXHAUSTION.

DG has been previously reported to be one of the most affected areas after TBI (Grady et al., 2003; Kotapka et al., 1991). We demonstrated that cell proliferation is increased all across the GCL of the DB during the acute phase of TBI (3dpTBI). NSCs are located in the SGZ and remain unresponsive in normal conditions until they get activated and start to proliferate and differentiate into mature neurons (Obernier & Alvarez-Buylla, 2019). Our next goal was to examine the morphology of these cells and their proliferation to assess the funcitonality of AHN after TBI. To do this, we used a transgenic mouse model in which EGFP is driven by the expresion of Nestin.

6.2.1. NSCs become reactive shortly after TBI changing their morphology and their expression of certain markers.

NSCs show a chracteristic morphology with a isngle thin radial process emerging from the soma located in the SGZ. To measure the level of NSCs reactivity, different parameters can be used: (1) the presence of several primary processes that emerge from an enlarged soma; (2) the thickening of those primary processes or (3) the increase in the expression of several cell markers like Nestin-GFP and GFAP (Sofroniew & Vinters, 2010). In this study, we used the second one (thickness of the primary processes) as well as the expression of Nestin-GFP to assess the possible reactivity in this population.

The double labeling of the primary processes with both GFAP (red) and Nestin-GFP (green) allowed us to visualize its increase in thickness after TBI **(Fig. R7 C-D)**. The images in **Figure R7 A-C** illustrate specifically the effects of this in the acute phase after the injury (3dpTBI). However, we also monitored this change for the rest of the experimental timepoints. NSCs reactivity is

known to be an, often temporary, consequence of several pathological processes cursing with inflammation (Sierra et al., 2015). However, in our case we observed how the increase of the primary processess thickness was conserved still 15dpTBI compared to both Control and Sham animals. By 2mpTBI the change was only significant when comparing TBI to Controls and by 4mpTBI there was no statistical difference among the groups **(Fig, R7 D-H)**. The effect of NSC reactivity fades over time. It is noticeable, however, that the trend for Control and Sham animals is the opposite. As they get older, the thickness of their NSC primary processes increases as an effect of aging as it was previously reported (Martín-Suárez et al., 2019b). Our results confirmed moreover that NSCs acquire a reactive phenotype after TBI which is preserved long time after the injury has occurred.

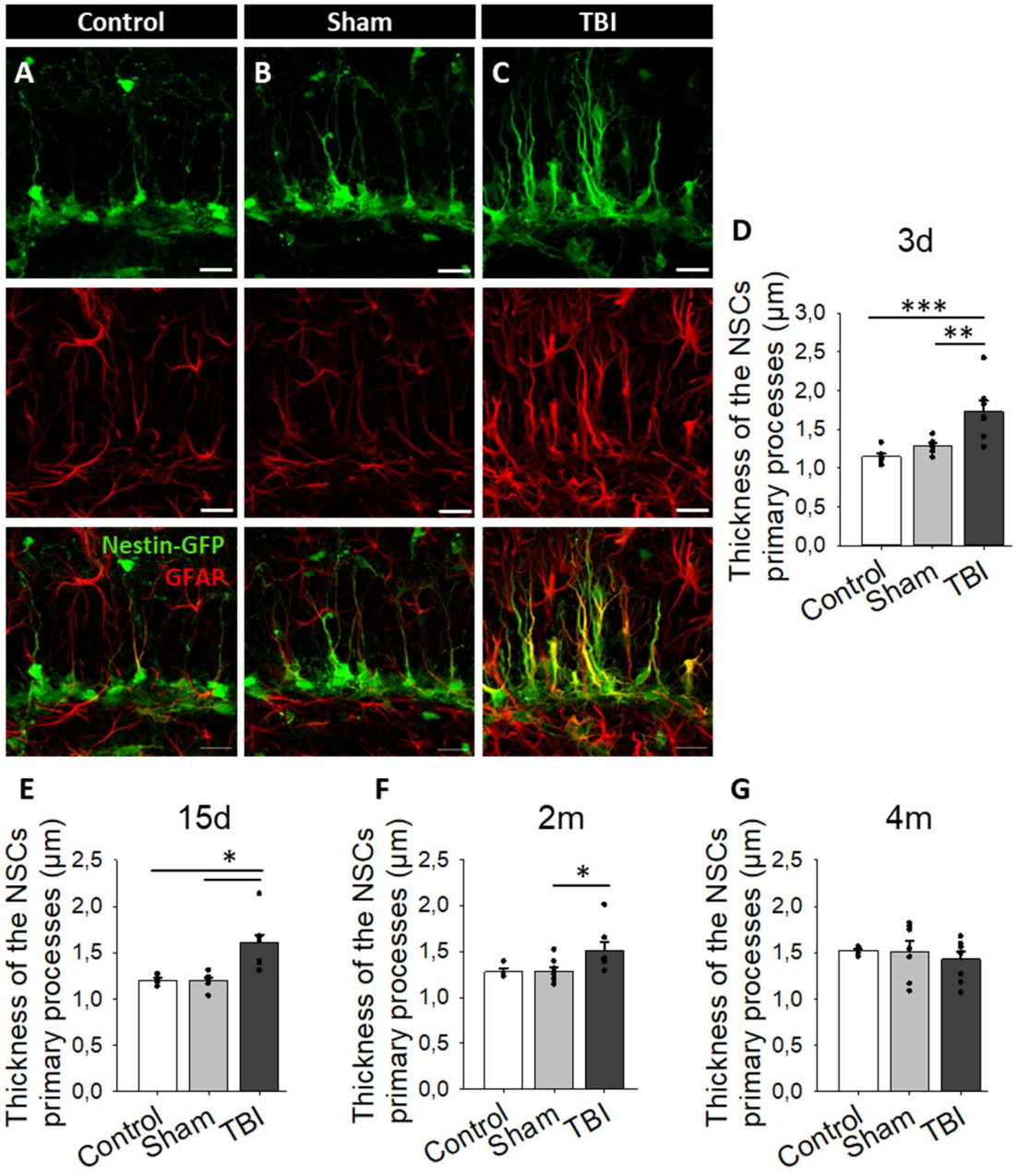
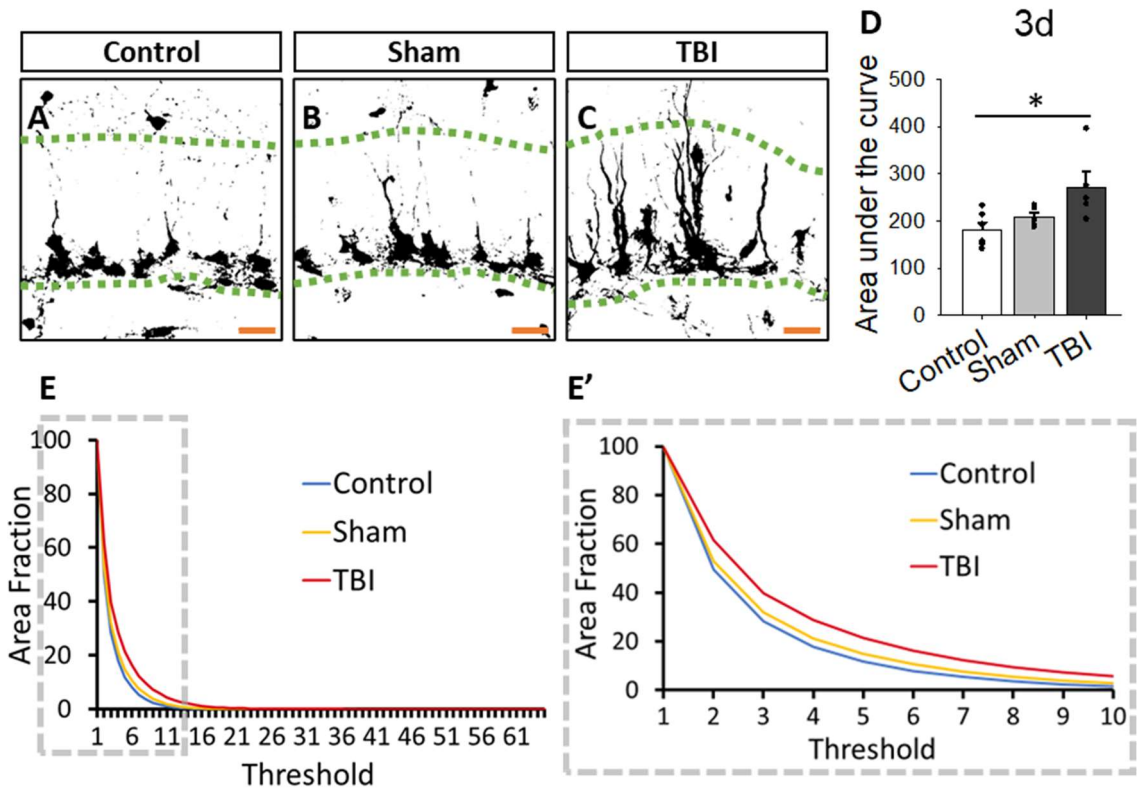


Figure R7. NSCs acquire a reactive phenotype 3dpTBI by increasing the thickness of their primary processes. A-C. Representative confocal images showing morphology of NSCs in the DB of the GCL 3dpTBI in Control (A), Sham (B) and TBI (C) groups. Nestin-GFP (in green) and GFAP (in red) are two NSC markers that label the whole cell and only its processes respectively. Co-labelling of these two markers in the primary processes of the cells allows us to measure precisely their thickness. **D-G.** Quantification of thickness of the primary processes of NSCs 3dpTBI (D), 15dpTBI (E), 2mpTBI (F) and 4mpTBI (G). Average thickness is increased in TBI compared to both Control and Sham groups 3dpTBI. By 15dpTBI the difference is only restricted to the TBI versus Sham and later there are no differences among the groups anymore. **H.** Chronological representation of the changes in the thickness

of the NSCs primary processes after TBI. NSCs: Neural Stem Cells; dpTBI: days post Traumatic Brain Injury; mpTBI: months post-TBI; DB: dorsal blade; GCL: Granule Cell Layer; TBI: Traumatic Brain Injury. Scale bar 20 µm. **p<0,01; ***p<0,001. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test was used in (D). A logarithmic transformation was performed in this case in order to comply with ANOVA assumptions (normality and homoscedasticity). In (E) and (F) no normality was achieved for the data so Kruskal-Wallis followed by all-pairwise comparisons (Dunn's method) was performed. Finally, in (G), One-way ANOVA was performed with no statistical difference. Bars show mean ± SEM. Dots show individual data.

As a second indicator of NSCs reactivity, we also measured the area occupied by Nestin-GFP in the GCL as an indirect measurement of its expression 3dpTBI. By using same-parameterized-thresholded images **(Fig, R8 A-C)** we could get to know the area fraction of this marker. When we represented this area coverage in the three experimental groups we could see how animals with TBI showed a higher percentage of area fraction than Control animals regarding Nestin-GFP in the GCL **(Fig. R8 E-E')**. To quantify this, we calculated the area under the curve which is a representation of this area fraction **(Fig. R8 D)**. We can conclude that the expression of Nestin-GFP is increased in the GCL 3dpTBI compared to Control animals, but not with Sham. By observing other reactivity markers like GFAP we can guess that there is also an increment of their expression, as it seems in the images **(Fig. R7 A-C)**, however in this case it was not quantified, since the labeling is less specific for the pure population of NSCs specially in injuried transgenic animals dominating the general astrogliosis.



←Previous page Figure R8. NSCs acquire a reactive phenotype 3dpTBI by increasing the expression of markers such as Nestin-GFP. A-C. Representative confocal images showing the area occupied by the marker Nestin-GFP 3dpTBI in Control (A), Sham (B) and TBI (C) groups. Dotted green line encloses the area corresponding to the GCL which is the analysed area for the Nestin-GFP labelling. Scale bar is 20 µm. **D.** Quantification of the area under the curve measured using the area fraction. In TBI animals the area occupied by Nestin-GFP in the GCL is higher than in Control animals, however there is no difference when comparing to Sham. **E.** Curve representing the area fraction labelled by Nestin-GFP depending on the threshold (0-255) in Control (blue), Sham (yellow) and TBI (red) animals. **E')** Amplification of the slope from the graph in (E). NSCs: Neural Stem Cells; dpTBI: days post Traumatic Brain Injury; GCL: Granule Cell Layer; TBI: Traumatic Brain Injury. *p<0,05. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test was used in (D). Bars show mean ± SEM. Dots show individual data.

6.2.2. NSCs get activated and increase their proliferation in the acute phase after TBI.

Together with the changes in cell morphology and markers expression, the activation of NSCs comes with an increase in their proliferation rate which might end up generation several cell types (Encinas et al., 2011a). To test this level of proliferation we administered four saturating doses of intraperitoneal BrdU throughout the first two days of the acute phase at +16 hours, +22hours, +38hours +44hours into Nestin-GFP transgenic animals and then sacrifice them at 3dpTBI **(Fig. R9 A)**. By testing the colocalization of BrdU together with both Nestin-GFP and GFAP we were able to identify specifically the NSCs that were activated shortly after the impact and started proliferating during the window of time of BrdU administration **(Fig. 9 B-D)**. Our results show that NSCs in both Control and Sham animals remained at a division rate matching that of physiological conditions (3-4% of $BrdU^+$ NSCs). However, NSC in TBI animals boosted their proliferation reaching almost a two-fold change (7-8% of $BrdU^+$ NSCs). In light of the results, we

concluded that at least part of the increase in BrdU⁺ labelling previously mentioned **(Fig. R6)** is due to an activation of NSCs.

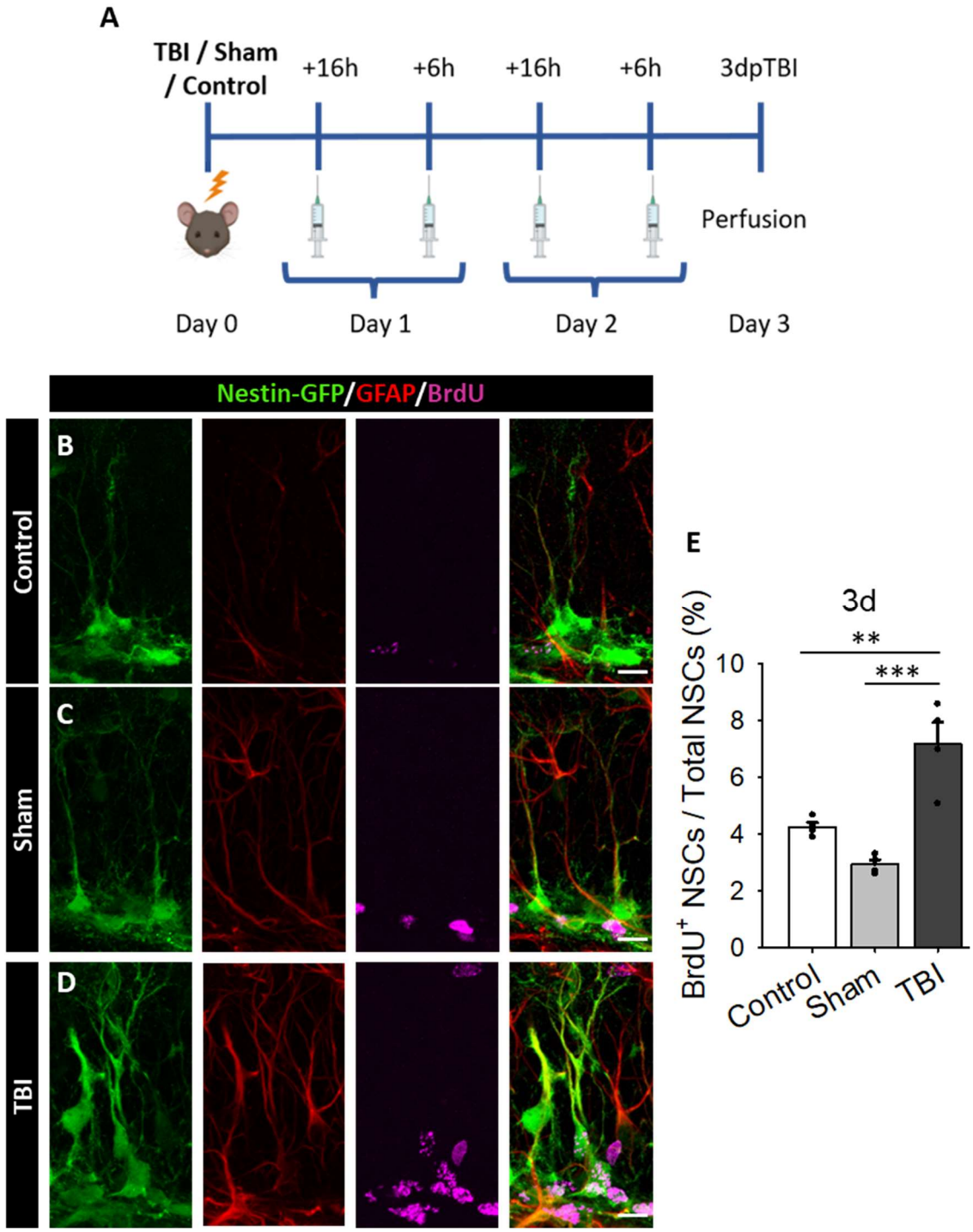


Figure R9. The increase of NSC proliferation takes place during at least the first three days after the injury. A. Schematic representation of the timeline of the BrdU administration to the animals during the first 3dpTBI. **B-D.** Representative confocal images showing NSCs in Nestin-GFP transgenic animals by the co-expression of Nestin (green) together with GFAP (red). BrdU⁺ labelling of these cells (pink) shows the proliferating cells in the area. **E.** Quantification of percentage of proliferating NSCs calculated by dividing the BrdU⁺ NSCs over the total NSCs. After TBI, NSCs increase their rate of proliferation compared to both Sham and Control. NSCs: Neural Stem Cells; BrdU: Bromodeoxyuridine; TBI: Traumatic Brain Injury. Scale

bar 10 µm. **p<0,01; ***p<0,001. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test. Bars show mean ± SEM. Dots show individual data.

Apart from NSCs, there are other cell types (astrocytes, ANPs, blood vessels, etc) that could be also contributing to this boost of general proliferation. As a matter of fact, we could observe great accumulation of BrdU in the SGZ surrounding NSCs suggesting an activation of other cell types, like ANPs, residing in this are **(Fig. R9 D)**. In fact, ANPs have also been reported to increase their division under similar pathologic conditions (Sierra et al., 2015). Nevertheless, in this study we are only focusing in the contribution of NSCs to AHN after TBI.

6.2.3. The proliferation of NSCs decreases with time.

Reactivity and proliferation are two consequences of acute TBI. However, there is still a lack of studies regarding the long-term effects of TBI over NSCs, in both their morphology and activation/division. We previously described how the reactive state of these cells in terms of thickness of their primary processes decreases over time reaching normal levels by 4mpTBI. Then, we next asked how does the proliferation of this cell type evolve in time after TBI. To achieve this, we identified NSCs in Nestin-GFP using Ki67 co-immunolabelling since it labels all the phases of cell cycle except quiescent G0. Once again, we performed the experiment for all the previously described timepoints. We evaluated the dividing NSCs and assessed this population in three different terms: their density in the GCL, their total numbers (taking into account the changes in volume of the DG) and the total proliferating ratio (proliferating NSCs / total NSCs). In general, similar results were obtained for the three different approaches but we will comment on them separately.

The density of $Ki67^+$ dividing NSCs in the DB of the DG at 3dpTBI corroborated the previous results of BrdU. Indeed, we determined a higher density of dividing NSCs per mm^3 during acute TBI **(Fig. R10 A-C, M)** compared to Control and Sham animals. However, one of the most striking results in this study was finding out that this enhancement is still maintained at 15dpTBI **(Fig. R10 D-F, N)**. NSCs activation has been described mostly as a temporary acute event but the long-term activation of these cells is rarely reported. Furthermore, in both of these timepoints, there was also labelling of Ki67 in the surrounding areas of NSCs suggesting an activation and division of ANPs too. Nevertheless, by 2mpTBI, the density of dividing NSCs in the DG is equal among all experimental groups. At this timepoint, the level of dividing NSCs dropped in the TBI animals **(Fig. R10 G-I, O)**. However, by 4mpTBI there is a decrease in the density of dividing NSCs

compared only to controls in this case. This decrease is also present, surprisingly, when we compared Sham to Control animals **(Fig. R10 J-L, P)**.

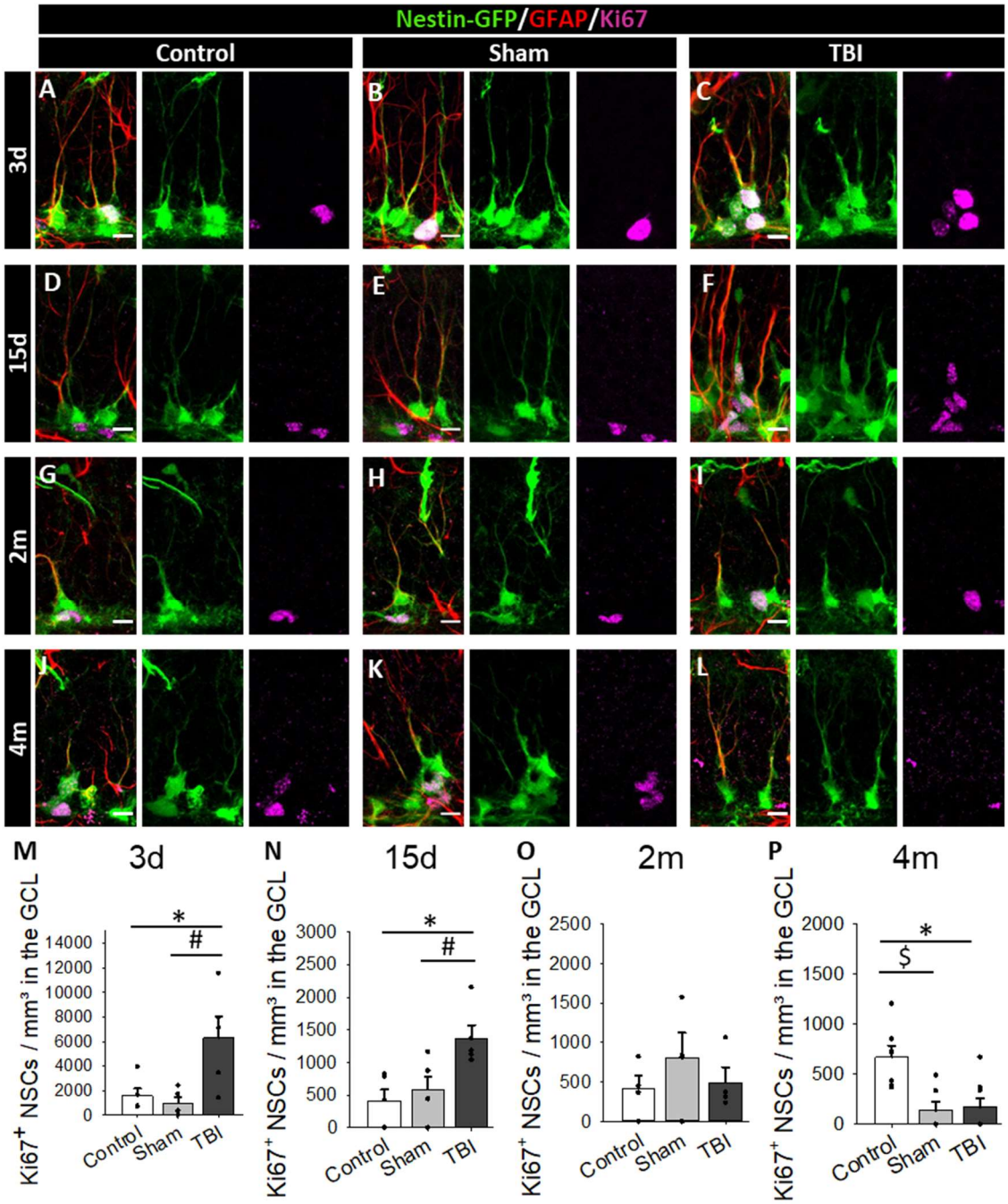


Figure R10. NSCs proliferation increases at 3dpTBI and 15dpTBI, drops to normal levels by 2mpTBI and reduces by 4mpTBI respect to Control animals. A-D. Representative confocal images of the density of Ki67$^+$ NSCs (labelled as Nestin-GFP$^+$ and GFAP$^+$) in the DB of the DG in Control, Sham and TBI animals 3dpTBI (A-C), 15dpTBI (D-F), 2mpTBI (G-I) and 4mpTBI (J-L). **E-H.** Quantification of proliferative NSCs density in the DB of the DG measured in number of Ki67$^+$ NSCs per mm^3 for each timepoint. **I.** Chronological representation of the changes the density of proliferative NSCs after TBI. NSCs: Neural Stem Cells; dpTBI: days post-Traumatic Brain Injury; mpTBI: months post-TBI; DB: Dorsal Blade; DG: Dentate Gyrus; mm: millimetre. Scale bar 10 µm. *p<0,05; (*) indicates TBI vs Control differences; (#) indicates TBI vs Sham differences and ($) indicates Sham vs Control differences. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test in (M) and (N). One-way ANOVA with no statistical differences in (O). Kruskal-Wallis followed by all-pairwise comparisons (Dunn's method) in (P). Bars show mean ± SEM. Dots show individual data.

At this point, we speculated that several changes are taking place in the DG after TBI that could be affecting these numbers. It is difficult to interpret changes in cellular densities when we know that the volume of DG itself is being affected too. So all analyses were repeated taking into account these volume changes to study the total number of the NSC population in the DB of the DG **(Fig. R11)**. When we counted the total number of dividing (as Ki67$^+$ cells) NSCs in the analysed area, we obtained the same results. At 3dpTBI there is an enhancement in the proliferating NSCs that is maintained at least until 15dpTBI and then reduced by 2mpTBI **(Fig. R11 A-C, E)**. This change is inverted when we get to 4mpTBI when TBI animals, as well as Sham, show a lower number of total dividing NSCs than Control animals **(Fig. R11 D-E)**.

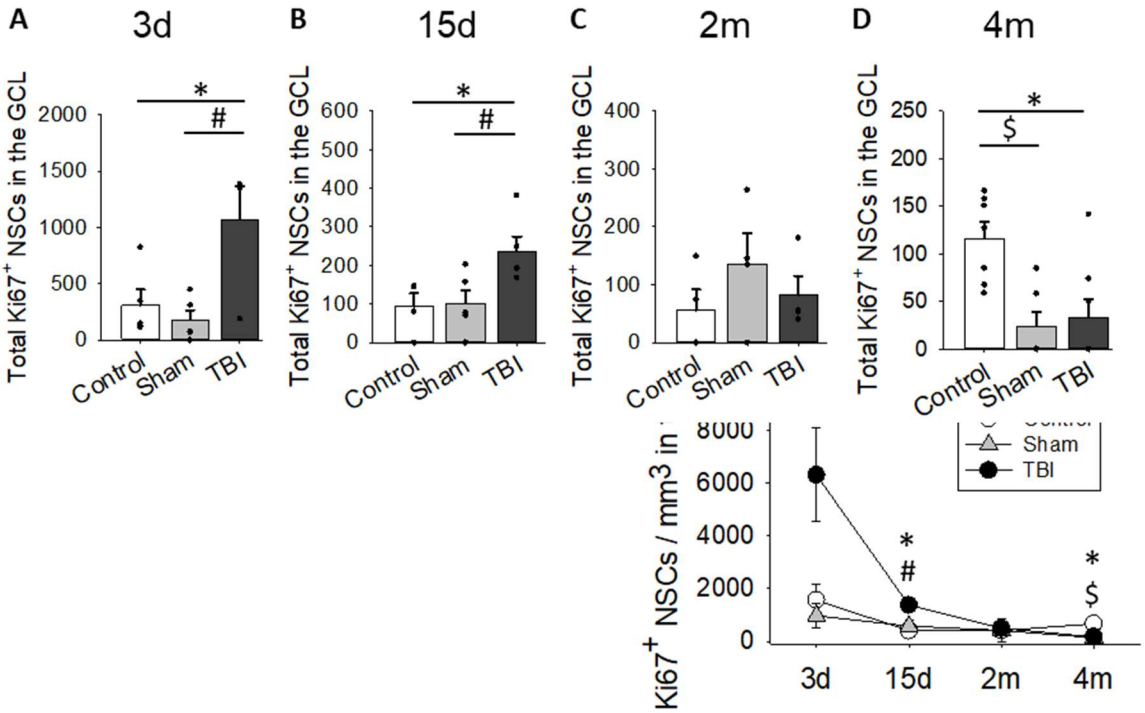
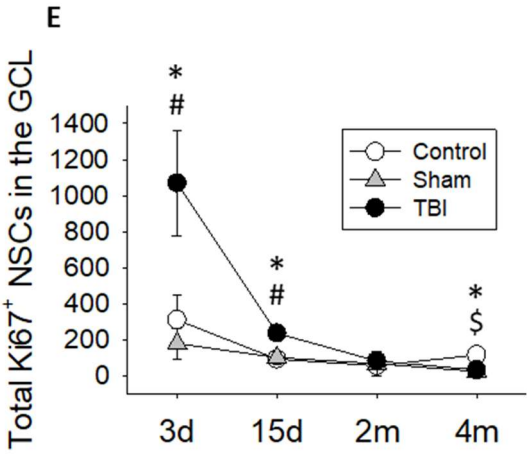


Figure R11. The total number of proliferating NSCs in the analysed area is higher after injury both 3dpTBI and 15dpTBI, then normalizes by 2mpTBI and is lower than in Control animals by 4mpTBI. A-D. Quantification of total numbers of proliferating NSCs measured using Ki67 expression in the DB of the DG for each of the timepoints. The results were obtained by multiplying the density of the cells by the total volume of the analysed area. Proliferating NSCs increase after TBI in the short-time after the impact (3 days and 15 days), then become normal 2mpTBI and finally are fewer than in Controls 4mpTBI. **E.** Chronological representation of the changes in the total number of proliferating NSCs after TBI throughout time. NSCs: Neural Stem Cells; dpTBI: days post-Traumatic Brain Injury; mpTBI: months post-Traumatic Brain Injury; DB: Dorsal Blade; DG: Dentate Gyrus. *p<0.05. (*) indicates TBI vs Control differences, (#) indicates TBI vs Sham differences and ($) indicates Sham vs Control differences. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test in (A) and (B). One-way ANOVA with no statistical differences in (C). Kruskal-Wallis followed by all-pairwise comparisons (Dunn's method) in (D). Bars show mean ± SEM. Dots show individual data.

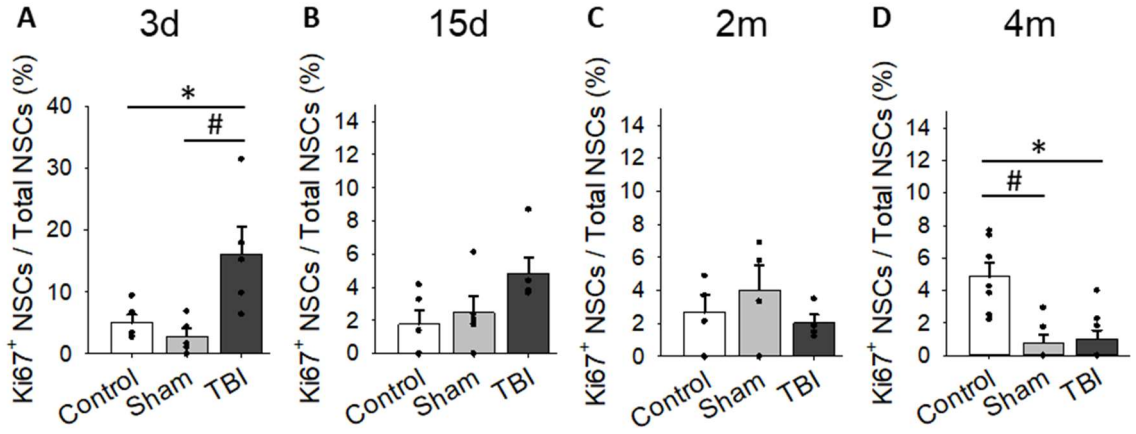


The NSC population changes throughout the life of an animal (Encinas et al., 2011a). The adult NSC pool is established during development and from a certain age it starts to decrease through aging (Martín-Suárez et al., 2019b). Despite the changes in the total numbers of NSCs in the DG, their rate of activation and proliferation remains at a constant level under physiological conditions around 4-5% (Encinas et al., 2011a). Therefore, this percentage of dividing NSCs is a useful indicator independent of the size of the pool, or the total number of NSCs at one certain timepoint. Hence, we sought to calculate this index by dividing Ki67$^+$ NSCs (proliferating NSCs) by the total number of NSCs (proliferating and non-proliferating) in order to get an independent value from the pool size **(Fig. R12)**. Another advantage is that this result mainly reproduces those from the density and total numbers of active NSCs throughout the experimental timeline. Thus, we estimated that the proliferating index was drastically increased at 3dpTBI reaching around a 16% of NSCs activation **(Fig. R12 A)**. This percentage was even higher than the one obtained from the BrdU$^+$ NSCs (7%) **(Fig. R9E)**. These observed differences may have several different explanations as we will discuss later. In conclusion, there is an enhancement of NSC proliferation after TBI but not in Sham or Control conditions.

The proliferating index at 15dpTBI did not show a significant difference among TBI, Sham and Control groups. However, a tendency of increase can be observed for TBI animals still at this timepoint **(Fig. R12 B)**. Hence, in general terms, we conclude that the NSCs percentage of proliferation is only enhanced for a shorter period of time than 15 days. Next timepoint, 2mpTBI,

showed no difference in terms of NSC activation or proliferation index confirming our previous results **(Fig. R12 C)**. Finally, at 4mpTBI, we determined a reduction of the proliferative index in both TBI and Sham compared to Controls. Considering the age of the animals at this point, Control condition had a similar rate of proliferation as they did for their entire lifespan (around 5%). However, most of the animals in Sham and specifically TBI treatment showed an even lower percentage (about 1%) of activated NSCs **(Fig. R12 D)**.

Putting all these results together we may conclude that NSCs get activated in the acute phase after TBI boosting their proliferation for at least 3 days. Later, this proliferation level will decrease in time (15dpTBI and 2mpTBI) until the point where it will become lower in TBI than in Control animals (4mpTBI).



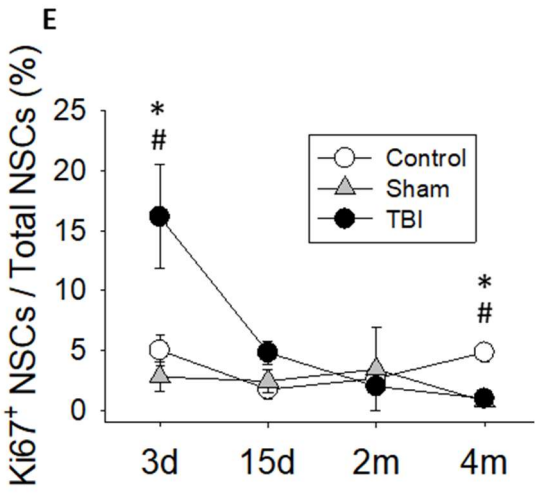


Figure R12. NSCs activation and proliferation properties changes respect the elapsed time post-TBI. A-D. Quantification of the percentage of Ki67$^+$ proliferating NSCs respect to total NSCs population in the DB of the DG for each timepoint. **E.** Representation of the changes in the percentage of proliferating NSCs after TBI throughout time. NSCs: Neural Stem Cells; dpTBI: days post-Traumatic Brain Injury; mpTBI: months post-Traumatic Brain Injury; DB: Dorsal Blade; DG: Dentate Gyrus. *p<0,05. (*) indicates TBI vs Control differences and (#) indicates Sham vs Control differences. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test in (A). Kruskal-Wallis with no statistical differences in (B). One-way ANOVA with no statistical differences in (C). Kruskal-Wallis followed by all-pairwise comparisons (Dunn's method) in (D). Bars show mean ± SEM. Dots show individual data.

6.2.4. The activation of NSCs after TBI does not involve a faster depletion of the pool.

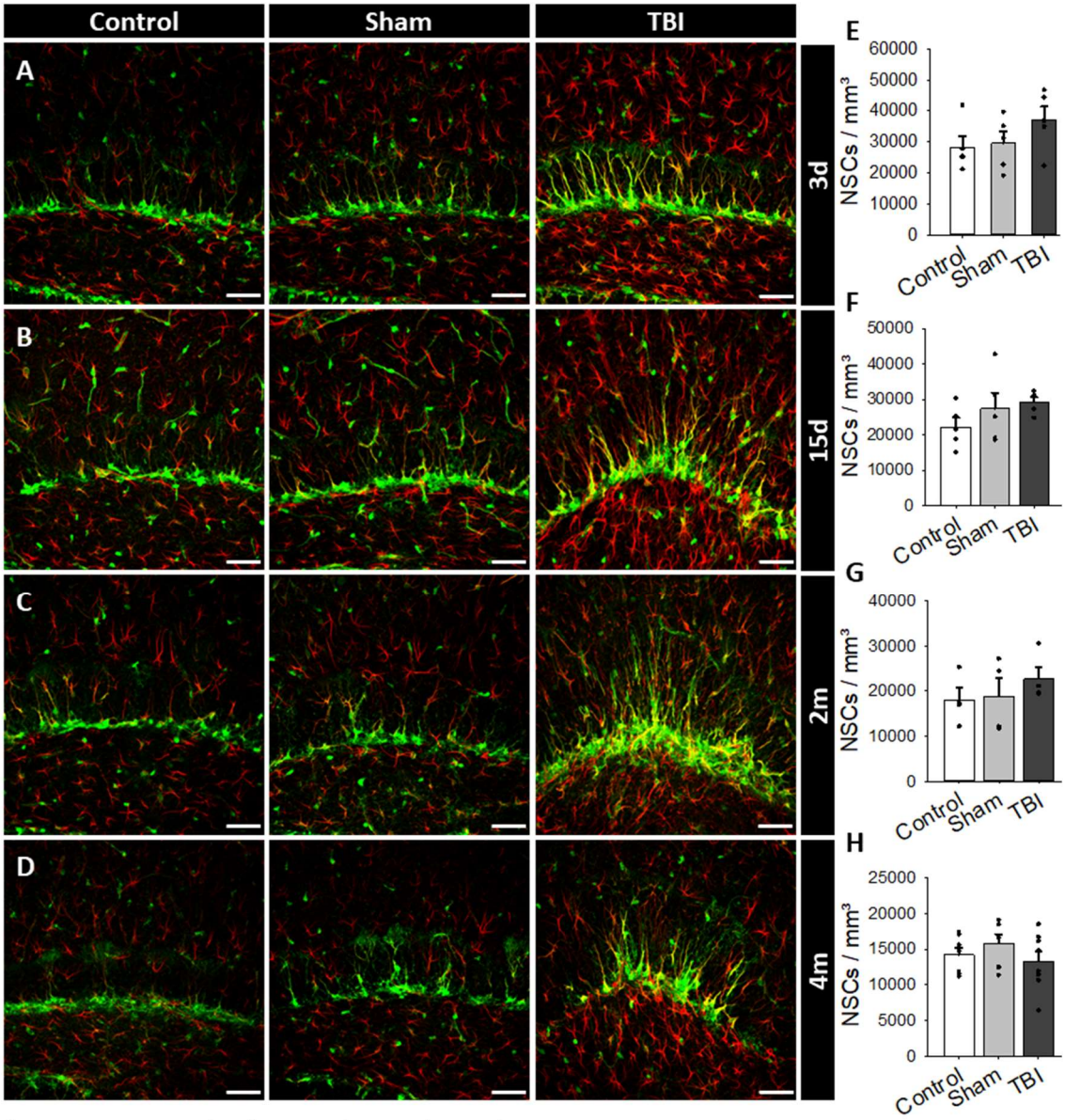
As we mentioned before, NSCs are a limited source in the adult brain of mice. Their activation in physiological conditions comes with a form of asymmetric division which generates two different daughter cells, one ANP and one NSC (Encinas et al., 2011a). This form of division, however, is the most common one but is not unique. Once we described an increase in the proliferation of NSCs after TBI, we sought to investigate how this enhancement affected the general pool of NSCs in terms of total numbers. To do this, we examined the density of this population and their total number through the complete extent of the experiment. The decrease of this population in physiological conditions is characterized by a steep slope in young adults that becomes lighter with age (Encinas et al., 2011a).

In our study, all experimental groups (Control, Sham and TBI) replicated this decrease from an early age both in terms of cellular density **(Fig. R13)** and total number **(Fig. R14)**. The fact that the percentage of active NSCs is usually maintained constant along the age of the animals makes the depletion of the NSC pool a progressive process that becomes slower with time: as the pool size grows smaller, the total number of proliferating cells will be lower in time since the percentage is unchanged.

Hence, it can be deduced that a dramatic activation of NSCs proliferation would end up in a faster depletion of the pool, since they are non-renewable. Unexpectedly, this is evolution changes after TBI. Even though we described in the previous sections a short activation of NSCs in the acute phase, the whole population seems to follow the normal trend of depletion. The density and total numbers of NSCs in the longest timepoints (2mpTBI and 4mpTBI) are comparable in TBI with both Control and Sham animals **(Fig. R13 and Fig. R14)**. It should be considered that the way of division could change temporarily after the injury and during the healing process. However, then NSCs must divide in a symmetric manner giving rise to two NSCs and therefore amplifying the pool more than if they generated just one NSC and one ANP. Nevertheless, this would mean that in the short-term timepoints, the numbers of NSCs should be higher after TBI, which does not happen **(Fig. R13 and Fig. R14)**. Other explanations should also be examined. For example, the fact that the proliferative rate becomes lower at 4mpTBI could be considered as a mechanism for slowing down the depletion progress in TBI animals and keeping the size of the population. Another factor that is to be taken into account is that the level of activation of NSCs necessary to see an impact in the total of the pool could be higher than the one that takes place after TBI. This would make it harder to see a difference in the pool

under these conditions. A deeper insight into all these possibilities will be argued in the discussion section.

In general, we can conclude that the NSC pool remains unchanged after TBI even in the chronic stages both in terms of cellular density and total numbers. NSCs, despite proliferating more than in the control condition, follow a normal trend of depletion after the injury.



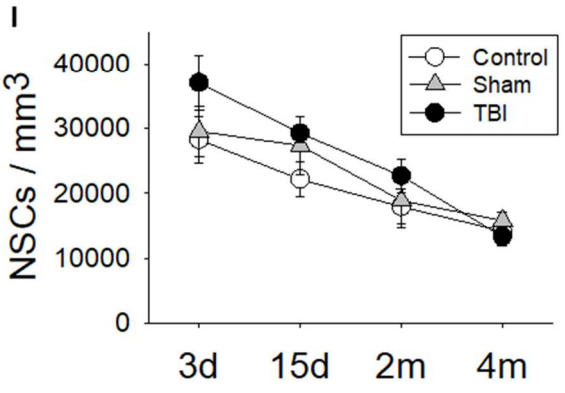


Figure R13. NSCs density remains unchanged after TBI. A-D. Representative confocal images of the density of NSCs in the DB of the DG in Control, Sham and TBI animals 3dpTBI (A), 15dpTBI (B), 2mpTBI (C) and 4mpTBI (D). **E-H.** Quantification of NSCs density in the DB of the DG measured in number of cells per mm^3 for each of the timepoints. **I.** Chronological representation of the changes in NSCs density after TBI. NSCs: Neural Stem Cells; TBI: Traumatic Brain Injury; DB: Dorsal Blade; DG: Dentate Gyrus; dpTBI: days post-Traumatic Brain Injury; mm: millimeter. Scale bar 50 µm. One-way ANOVA. No statistical differences were found in these results. Bars show mean ± SEM. Dots show individual data.

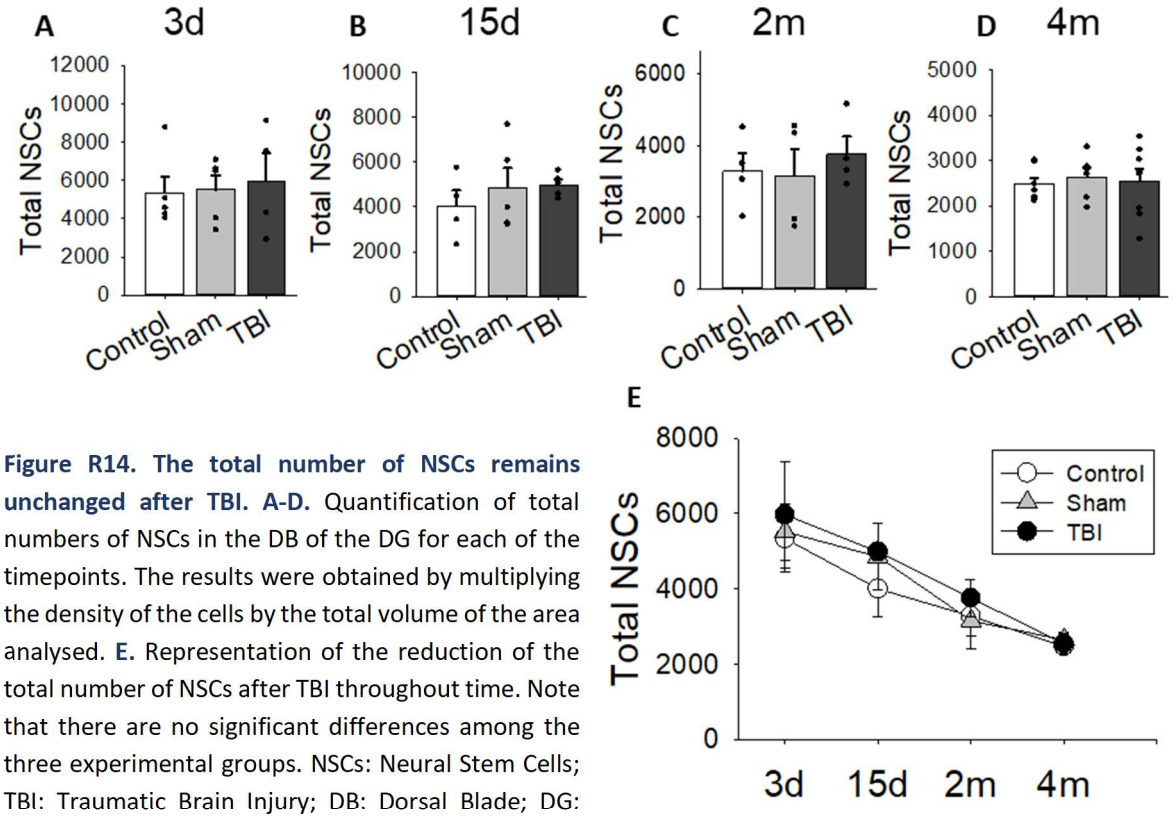


Figure R14. The total number of NSCs remains unchanged after TBI. A-D. Quantification of total numbers of NSCs in the DB of the DG for each of the timepoints. The results were obtained by multiplying the density of the cells by the total volume of the area analysed. **E.** Representation of the reduction of the total number of NSCs after TBI throughout time. Note that there are no significant differences among the three experimental groups. NSCs: Neural Stem Cells; TBI: Traumatic Brain Injury; DB: Dorsal Blade; DG: Dentate Gyrus. One-way ANOVA. No statistical differences were found in these results. Bars show mean ± SEM. Dots show individual data.

6.3. IMMATURE NEURONS GENERATION AFTER TBI: EVOLUTION OF THE POPULATION, MORPHOLOGY, MIGRATION AND SPINE DENSITY.

After characterizing the increase of NSCs cell division conserving its same total numbers after TBI, we wanted to unravel what kind of cells were being produced. Previous data describing physiological conditions state that NSCs divide asymmetrically generating two daughter cells, one NSC and one ANP (Encinas et al., 2011a). These ANPs will later divide again eventually giving rise to new adult-born immature neurons (Kempermann et al., 2004). Nevertheless, it is still unclear whether TBI could be altering these conditions in a way that the neurogenic cascade would switch. Therefore, we resorted to perform an exhaustive study of the immature neuronal population in the DB of the DG after TBI. By taking advantage of doublecortin (DCX), marker that identifies developing newborn neurons, we were able to examine, not just the potential increase or decrease of this population but also several features which are determining in their development and integration into the hippocampal circuitry.

6.3.1. The immature neurons population becomes expanded after TBI but its density decreases in the chronic phase.

DCX is a microtubule-associated protein related to neuronal differentiation which is expressed during the developing process of ANPs into mature neurons (Francis et al., 1999). Its expression will start increasing as soon as ANPs loose their ability to divide and become neuroblasts. Then, it will be kept high during approximately 21 days until their expression of more mature neuronal markers starts (Merz & Lie, 2013). This lapse of time involves many cellular changes at different levels: from ANPs to neuroblasts to mature neurons, but at this point, the cellular fate of these cells is already determined and therefore, they can all be considered as immature neurons (IN), the term that we will use from this point on to describe the population labelled by this marker. Again, since volumetric changes are taking place in the DB of the DG, all measurements were made in terms of cellular density and then extrapolated to the total number of cells in the analysed area.

The number of DCX^+ cells 3dpTBI is no different to the one in Sham or Control animals **(Fig. R15 A, E)**. This timepoint gives us information about possible alterations in the expression of the marker itself, but not about the generation of new neurons. As it was mentioned before, NSCs or ANPs proliferation together with the maturating process of neuroblasts takes a few days until DCX starts to be expressed. Therefore, we would need to wait a bit longer to see changes in neurogenesis after TBI. An important read out from this result is that there is no major effect on cellular death of the DCX^+ population specifically after the injury, since all three groups have similar number of densities.

By 15dpTBI, there is an increase in immature neuron density **(Fig. R15 B, F)** in TBI animals but only compared to Controls. In this case, the Sham group shows an intermediate degree of enhancement in neurogenesis that seems to be somewhere in between the other two groups **(Fig. R15, F)**. We must state that the NSC population that was dividing during the acute phase after TBI, at this temporal point, had enough time to undergo the proliferative and developing process to reach the immature neuronal phenotype. Hence, it would be an appropriate timepoint to examine the newborn neuron population. However it should be noted that there is also a high variability in terms of acquired data specifically in the TBI group: some of the animals show a level of immature neuron density of 3-fold the one from the Controls while others look very similar to them. Despite being an appropriate timepoint to study acute neurogenesis after TBI, 15dpTBI is also an intermediate moment with overlapping molecular and cellular events (not considered acute neither chronic phase).

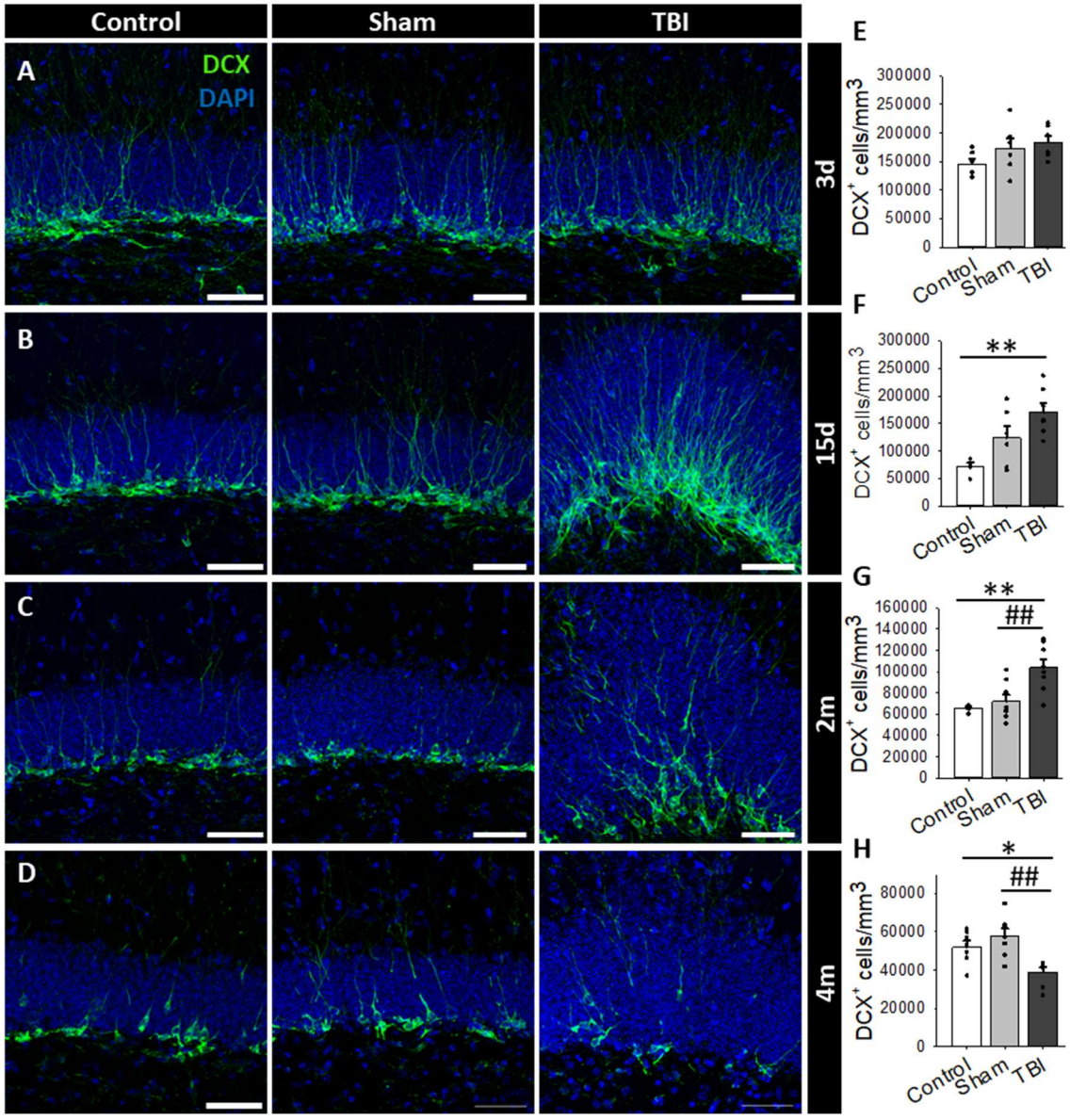


Figure R15. The density of newborn neurons increases 15dpTBI and 2mpTBI however it is drastically reduced by 4mpTBI. A-D. Representative confocal images of the density of DCX-expressing cells in the DB of the DG in Control, Sham and TBI animals 3dpTBI (A), 15dpTBI (B), 2mpTBI (C) and 4mpTBI (D). **E-H.** Quantification of cellular density of DCX-expressing cells in the DB of the DG measured in number of cells per mm^3 for each of the timepoints. **I.** Representation of the changes in immature neuron density after TBI throughout time. TBI: Traumatic Brain Injury; dpTBI: days post-TBI; DG: Dentate Gyrus. Scale bar 50 µm. *p<0,05; **p<0,01; (*) indicates TBI vs Control differences and (#) indicates TBI vs Sham differences. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test in E-I. Bars show mean ± SEM. Dots show individual data.

Focusing at 2mpTBI we can observe a higher density of immature neurons in TBI compared to both Sham and Controls in this case **(Fig. R15 C, G)**. These neurons were actually born long time -about 4 to 6 weeks- after the impact. This could mean that there is still a high level of progenitors' proliferation at that point, developing a higher number of neurons. However, another possibility could be that the percentage of surviving neurons to the two critical periods during their development was enhanced, therefore allowing a bigger amount of these cells to remain in the GCL. Also, we cannot rule out possible alterations relating TBI directly to DCX expression (as we deduced from data from 3dpTBI). Indeed, changes regarding the development of these immature neurons specially during the lasts steps of maturation may also be considered as a cause of accumulation of newborn neurons after TBI. All these different possibilities will be later expanded in the discussion section.

Finally, at 4mpTBI, the TBI animals show a decreased immature neuron density in the analysed area **(Fig. R15 D, H)**. This result would correlate with the fact that at this time NSCs show a lower proliferation rate **(Fig. R12, D)**. A decreased level of proliferation by NSCs in the chronic phase would end up producing a lower number of immature neurons generated.

When we consider the overall changes in the immature neuron population after TBI along the experimental timeline we can see that there are no changes in DCX-expressing cells in the acute phase, however, there is an increase in their cellular density 15dpTBI which lasts until at least 2mpTBI. By 4mpTBI there is a lower density of these cells in the analysed area **(Fig. R15 I)**.

Taking into account the volumetric changes in the DG, when we extrapolate the result to get an idea of the total number of this population after the injury, we observe a slightly different picture. At 3dpTBi, 15dpTBI and 2mpTBI, the results are basically the same. At first immature neurons do not seem to be affected and then the population increases at least until the two months timepoint **(Fig. R16 A-C)**. However, the numbers are even by 4mpTBI. The difference in the cellular density at this point is not reflected in the total number of the population **(Fig. R16 D)**. This could be due to a dilution of the effect. The specific area where the main alterations take place is quite reduced compared to the totality of the DB of the DG. It could also happen that the effect of the decrease in the cell density was compensated by the increase in the total volume of the DG, resulting in an equal number of the total population overall **(Fig. R16 E)**.

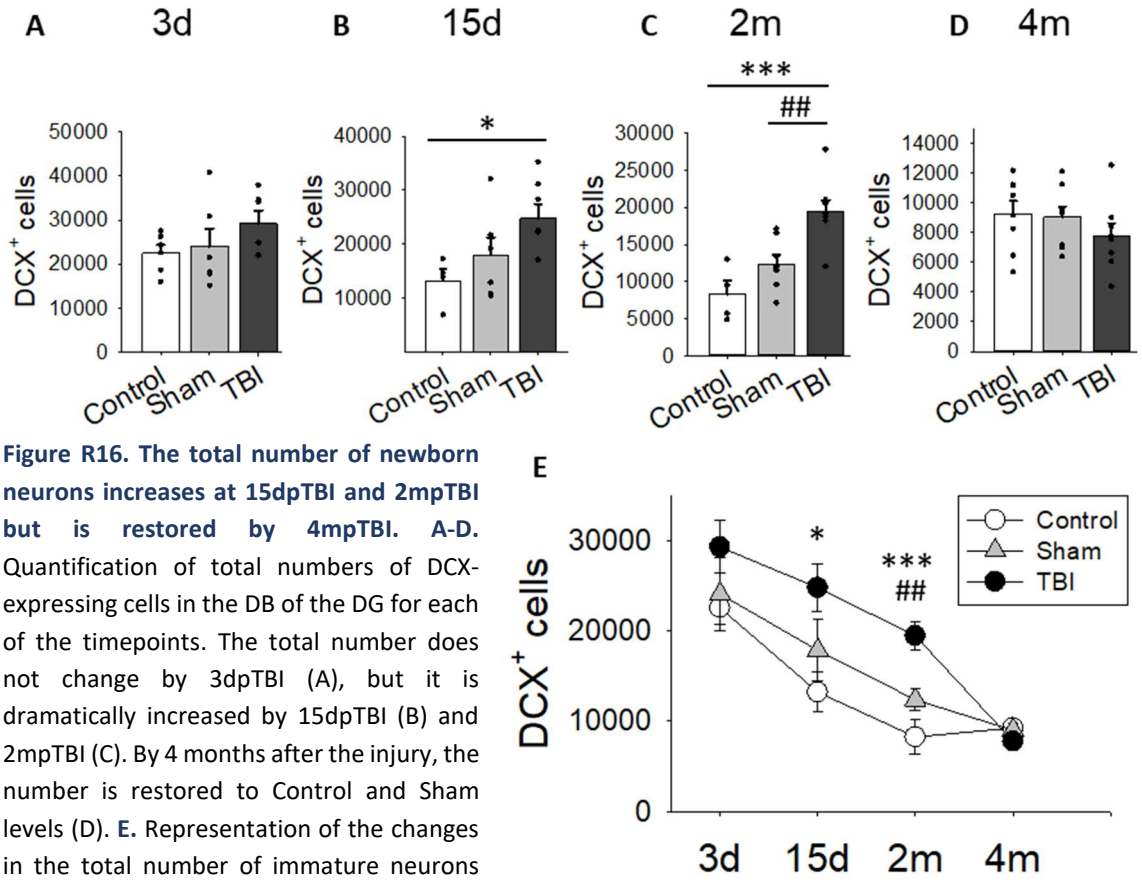


Figure R16. The total number of newborn neurons increases at 15dpTBI and 2mpTBI but is restored by 4mpTBI. A-D. Quantification of total numbers of DCX-expressing cells in the DB of the DG for each of the timepoints. The total number does not change by 3dpTBI (A), but it is dramatically increased by 15dpTBI (B) and 2mpTBI (C). By 4 months after the injury, the number is restored to Control and Sham levels (D). **E.** Representation of the changes in the total number of immature neurons after TBI throughout time. TBI: Traumatic Brain Injury; dpTBI: days post-TBI; DG: Dentate Gyrus. *p<0,05; **p<0,01; ***p<0,001; (*) indicates TBI vs Control differences and (#) indicates TBI vs Sham differences. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test in E-I. Bars show mean ± SEM. For the analysis of the 15dpTBI data, a logarithmic transformation was performed in order to comply with ANOVA assumptions (normality and homoscedasticity). Dots show individual data.

6.3.1.1. he population of immature neurons born up to 2mpTBI shows an increase in soma size.

During their maturating process, adult-born neurons undergo many morphological changes until they can be considered mature fully integrated neurons. Any altered conditions in the neurogenic niche may affect this journey by changing the properties of these neurons. Furthermore, the changes that happen in this process could take a toll both in their functionality during integration, which is crucial for the hippocampal circuitry and afterwards, once they are already mature (X. Jin, 2016; C. Zhao, 2006). For all these reasons, we decided to perform an exhaustive histological analysis. We wondered whether these new DCX$^+$ neurons could be participating in the increased thickness of the GCL in their soma size. By calculating the soma diameter of these DCX+ cells we got an idea of their cell body size at the different timepoints **(Fig R17)**. Briefly, we averaged the measurements from the base of the soma to the primary

process origin and the diameter perpendicular to the primary process, as it is represented in **Fig. R17**. We observed that immature neurons display an expanded soma diameter than both Control and Sham at 3dpTBI **(Fig. R17 A, E)**. DCX-labelled cells at this timepoint are actually immature neurons that were generated before the injury. Therefore, we deduce that this morphologic alteration affects at least cells that were in the developmental process when the injury took place. In the longer timepoints, (15dpTBI and 2mpTBI), immature neurons also showed an increased diameter of the cell soma compared to Control animals without significant differences respect to the Sham group **(Fig. R17, B-C, F-G)**. Indeed, the Sham group showed an intermediate phenotype between Control and TBI. Interestingly, this point evidences a transient alteration of their soma generated after the impact since at 4mpTBI all three experimental groups show equivalent soma sizes **(Fig. R17 D, H)**. However, it is interesting to note that the degree of cell maturity during the neuronal differentiation also could affect to the soma size as we will discuss later.

In conclusion, the population of adult-born immature neurons either suffering the impact or becoming generated in the following weeks after, showed increased soma size, only becoming restored by 4mpTBI when this effect completely disappears **(Fig. R17, I)**.

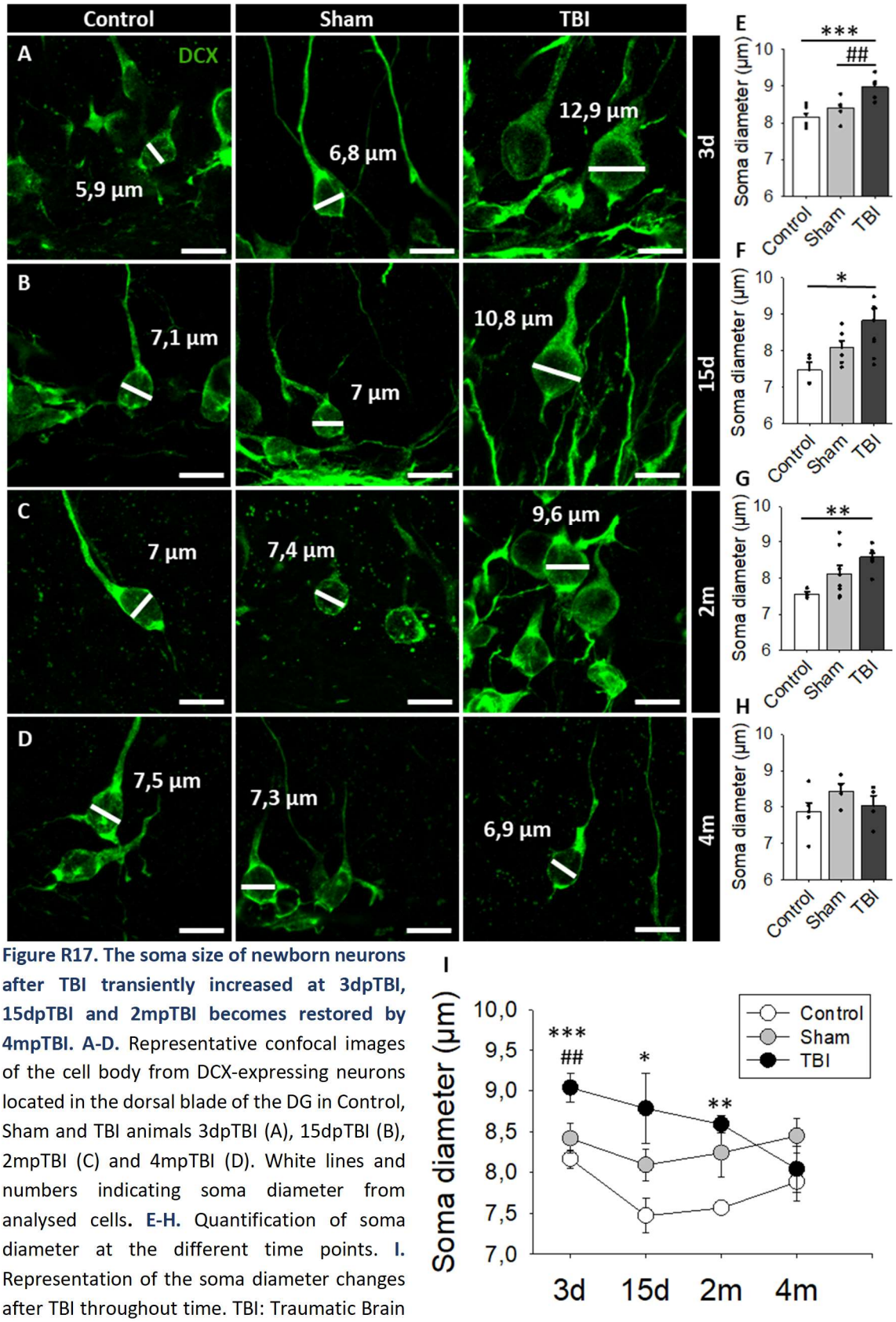


Figure R17. The soma size of newborn neurons after TBI transiently increased at 3dpTBI, 15dpTBI and 2mpTBI becomes restored by 4mpTBI. A-D. Representative confocal images of the cell body from DCX-expressing neurons located in the dorsal blade of the DG in Control, Sham and TBI animals 3dpTBI (A), 15dpTBI (B), 2mpTBI (C) and 4mpTBI (D). White lines and numbers indicating soma diameter from analysed cells. **E-H.** Quantification of soma diameter at the different time points. **I.** Representation of the soma diameter changes after TBI throughout time. TBI: Traumatic Brain Injury; dpTBI: days post-TBI; DG: Dentate Gyrus. Scale bar 10 µm. *p<0,05; **p<0,01; ***p<0,001; (*) indicates TBI vs Control differences and (#) indicates TBI vs Sham differences. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test in E-I. Bars show mean ± SEM. Dots show individual data.

6.3.1.2. The population of immature neurons born up to 2mpTBI exhibits an abnormal pattern migration in the GCL.

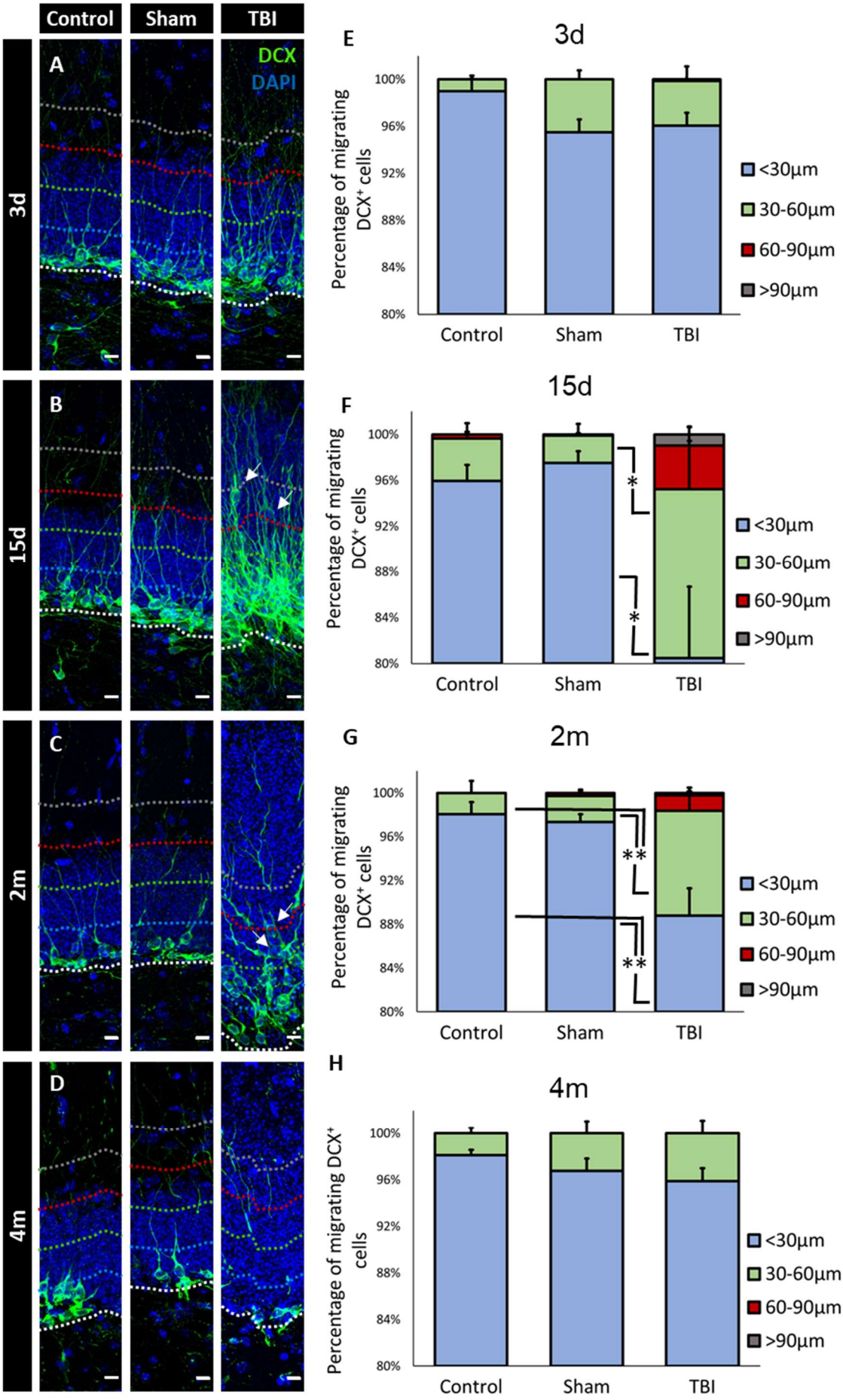
The migration of newborn neurons across the GCL in the DG is also crucial for their development. Immature neurons need to move through the DG parenchyma to participate in the hippocampal circuitry properly they usually remain close to the SGZ and do not enter very deeply into the GCL (G. J. Sun et al., 2015; C. Zhao, 2006). However, having determined already previous alterations in the morphology of DCX^+ cells, we hypothesized that their migration capabilities could also be affected. To get an idea of the location of these cells in the GCL we quantified their migrated distance from the SGZ and grouped them into different clusters: (1) DCX^+ cells that migrated less than 30 microns, which is where adult born neurons usually remain (white to blue dotted lines in the pictures, **Fig. R18**, A-D); (2) DCX^+ cells that migrated 30 to 60 microns (blue to green dotted line); (3) DCX^+ cells that migrated 60 to 90 microns (green to red dotted line); and (4) DCX^+ cells that migrated more than 90 microns (red to green dotted line and beyond). The total number of these cells conform the 100% of the immature neurons in the DB of the GCL and the result was expressed as a percentage of the total in each of the subdividing areas **(Fig. R18, E-H)**.

At 3dpTBI, the distribution of the DCX^+ cells in TBI animals is equivalent to that in Control and Shams. The vast majority of the cells (96-98%) remain closer to the SGZ **(Fig. R18 A, E)**. Unlike it happens with the soma size, TBI does not seem to have any type of effect over the migration pattern of the developing neurons at this short time after the injury.

Interestingly, by 15dpTBI, with the increased number of immature neurons, we determined that around 15% of DCX^+ cells were found located in the 30-60 microns area from the SGZ. We also observed that even some of the cells moved further and were positioned in the area from 60 to 90 microns or even more than 90 microns away from the SGZ. This means that the percentage of these DCX^+ cells that remain properly located close to the GCL, is reduced (80%) compared to the Control group **(Fig. R18 B, F)**. It should also be noticed that without the change in the thickness of the GCL, these distances in normal physiological conditions would correspond to the outer area of the GCL o even into the ML.

At 2mpTBI the previously observed pattern is still present **(Fig. R18 C, G)**. The percentage of DCX^+ cells located closer to the SGZ is reduced while there are more of them specially in the 30 to 60 microns area. Since DCX is expressed only in cells of about 7 to 21 days old, this would mean that even cells that were born weeks after the impact show an altered pattern of migration. However, by 4mpTBI, immature neurons have already recovered their normal distance of cell migration and most of them remain close to the SGZ **(Fig. R18 D, H)**.

In conclusion, TBI alters the migration of new neurons that are generated after the injury, but it does not affect those that were developing during the injury. This alteration is temporary and it involves neurons that are born during, at least, the two first months after the insult, but not those that are born four months after, as this effect completely disappears.


Control
Sham
TBI
3d
15d
2m
4m
A
B
C
D
DCX
DAPI
E
3d
F
15d
G
2m
H
4m
Percentage of migrating DCX+ cells
Percentage of migrating DCX+ cells
100%
96%
92%
88%
84%
80%
<30µm
30-60µm
60-90µm
>90µm
*
*
**
**
Control
Sham
TBI

← Previous page. Figure R18. Aberrant migration of immature neurons located in the DB of the DG is observed as early as 15dpTBI and is still present 2mpTBI. A-D. Representative confocal images of DCX^+ neurons in the DB of the DG 3dpTBI (A), 15dpTBI (B), 2mpTBI (C) and 4mpTBI (D) and their location throughout the GCL. Dotted lines in the GCL indicate the separation of the following interval distances (white line sets the lower limit of the GCL); white-to-blue space 0-30µm; blue-to-green space 30-60µm, green-to-red 60-90µm and red-to-grey >90µm in the GCL. White arrows indicate further migrated immature neurons. **E-H.** Quantification of migrated distance of DCX^+ neurons along the GCL in the analysed area expressed as percentage of DCX^+ cells that fall into each of the previously mentioned ranges. DB: Dorsal Blade; DG: Dentate Gyrus; dpTBI: days post-TBI; GCL: Granule Cell Layer; SGZ: Subgranular Zone. Scale bar 50 µm. *p<0,05; **p<0,01. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test in (G – 30 to 60µm). Kruskal-Wallis followed by all-pairwise comparisons (Dunn's method) in (F – less than 30µm and 30 to 60µm; and G – less than 30µm). One-way ANOVA without statistical differences in the rest of the analyses. Bars show mean ± SEM. Dots show individual data.

6.3.2. The alterations in immature neurons after TBI can be reproduced in a model with retroviral infection.

Using cell markers like DCX has both advantages and disadvantages when interpreting these results. Being able to identify the whole population gives us an idea of how variable this population is and it allows us to have a bigger picture of adult neurogenesis. However, since DCX is expressed during several weeks in immature neurons, it becomes difficult to discern apart neurons that were born before and after the injury, which makes a major difference in this model.

To characterize alterations in new neurons that were specifically born during or after TBI, we resorted to use a technology that allowed us to label only cells that had been born in a very specific narrow window of time. Prior to TBI surgery (and also to Sham and Control conditions), we administered one single intrahippocampal injection of a retroviral vector (RV) containing the sequence of a fluorescent (Venus) protein which would specifically infect dividing cells. Taking together the short lifespan of the viral particles and the short elapsed time (around 10 minutes) between stereotaxic injection and TBI insult (or Sham operation), we can assume that the infected cells will specifically be those that were dividing during TBI and in the first few hours afterwards.

Therefore, by combining the labelling with RV-Venus together with the localization, morphology and colocalization with other neuronal markers, we can identify specifically neurons that were born in this very narrow period of time.

Since the main objective of this study is to characterize the alterations during the development of these new neurons, the animals in this experiment were sacrificed 3 weeks (21 days) after the injection and surgery **(Fig. R20 D)**. This is a crucial timepoint during neuronal development when newborn neurons have already extended processes through the GCL and into the ML and started making synaptic connexions inside the hippocampal circuitry (R. L. Faulkner et al., 2008; X. Jin, 2016). We proceeded then to characterize the soma size and migration alterations, adding a synaptic density study in our Venus-labelled newborn neurons.

6.3.2.1. Immature neurons born specifically during a TBI event display an enlarged soma diameter.

Using the same quantifying technique as in previous experiments, we analysed the diameter of the infected cells 3 weeks post-TBI (3wpTBI), Sham or Control conditions. We observed again the increased soma diameter after TBI when comparing only to Controls, while Sham animals show an intermediate phenotype, in line with the phenotype previously described in immature DCX^+ neurons **(Fig. R19)**. Thus, we can conclude that the effect of TBI over soma size is not only restricted to neurons that were born previously to the impact (as previously shown at 3dpTBI), but it also involves neurons born during and after the insult.

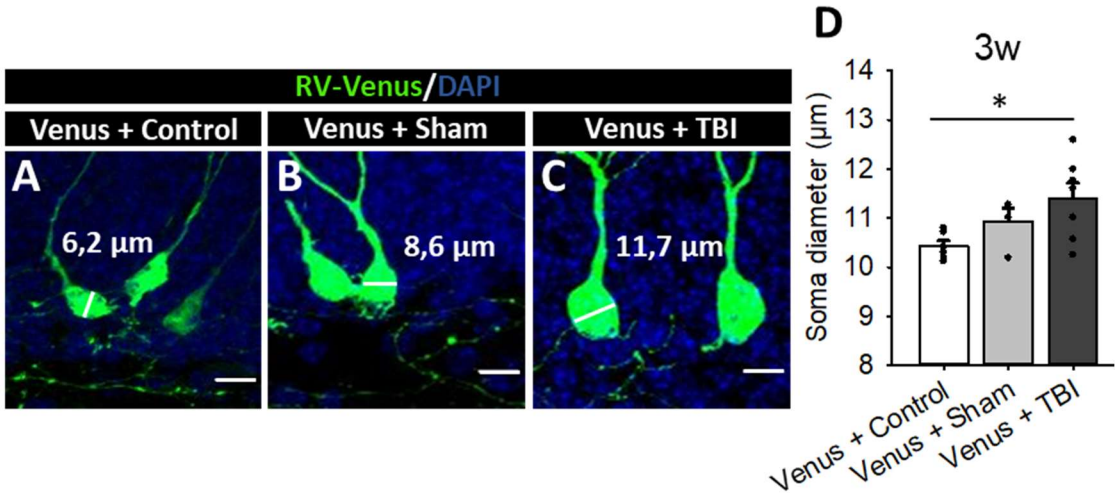


Figure R19. Neurons born shortly after TBI show a bigger soma size than those in Control animals. A-C. Representative confocal images show RV-Venus infected somata of neurons in the GCL in Control (A), Sham (B) and TBI (C) animals. White lines and numbers indicating soma diameter from analysed cells. **D.** Quantification of soma diameter (as previously done in **Fig. R17**). TBI: Traumatic Brain Injury; GCL: Granule Cell Layer; RV: Retrovirus. Scale bar 10 µm. *p<0,05. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test. Bars show mean ± SEM. Dots show individual data.

6.3.2.2. Immature neurons born specifically during a TBI event show an aberrant pattern of migration through the GCL.

Next, we characterized the distance of cell migration for the Venus-labelled neurons. It should be noted that other cell types that were in the process of division after TBI could also be infected. Therefore, in all cases for this and the previous experiment, we checked that the analysed cells were also positive for cell makers of immature neurons (DCX) **(Fig. R20, red)**.

In this case, instead of measuring the different range of distances (0-30 microns, 30 to 60 microns, etc) through the GCL, the specific travelled distance was measured for each cell starting from the SGZ baseline, obtaining a cell-individualized result **(Fig. R20 E)**.

We observed that 3wpTBI Venus neurons showed an aberrant pattern of migration through the GCL compared to both Sham and Controls **(Fig. R20 A-C)**. We determined that actually, many of these neurons migrate a distance that is close to the 30 microns, what was previously described in the DCX experiments **(Fig. R18 F-G)**. Using the combination of this technical and methodological application, we conclude that neurons born specifically after TBI migrate further than those of Sham and Control animals.

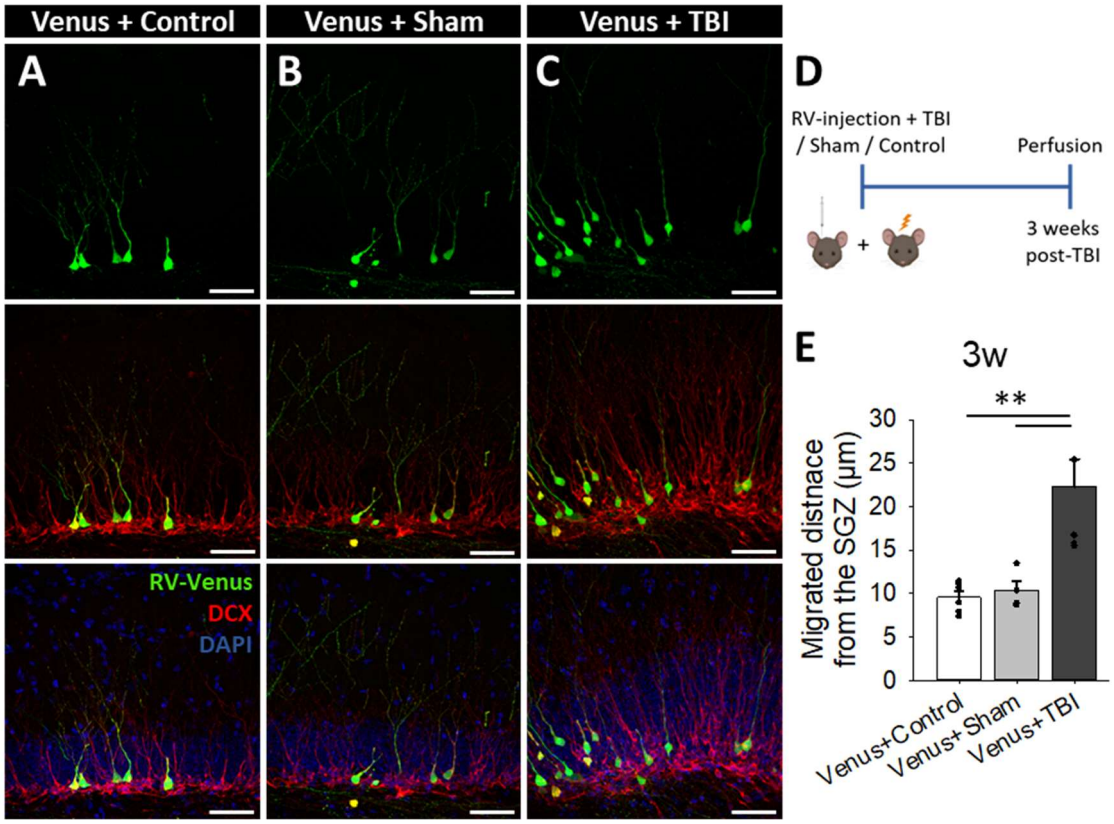


Figure R20. Neurons born shortly after TBI migrate further in the GCL than those in Controls and Shams. **A-C.** Representative confocal images show RV-Venus infected cells after Control (A), Sham (B) or TBI (C) conditions after the combined surgery 3wpTBI colocalizing with the marker for immature neurons DCX. **D.** Schematic representation of the combined surgery of retroviral injection followed by TBI, Control or

Sham conditions and the timeline followed in the experiment. **E.** Quantification of the total distance of migration of RV-Venus infected neurons across the GCL. TBI: Traumatic Brain Injury; GCL: Granule Cell Layer, RV: Retrovirus. Scale bar 50 µm. **p<0,01. One-way ANOVA followed by all pairwise multiple comparisons by Holm-*Šídák* post hoc test. Bars show mean ± SEM. Dots show individual data.

6.3.2.3. Immature neurons born specifically during a TBI event show no alterations in their dendritic spine density.

Another advantage offered by the use of retroviral vectors is the high resolution of the complete cell body we can get, including its projections. A very precise tracing of the extending processes and even the synaptic buttons can be depicted. It is known that newborn neurons of three weeks of age are at a critical point in terms of circuit integration and synaptic establishment (R. L. Faulkner et al., 2008). We hence used the resolution offered by the viral infection to identify and quantify dendritic spines of at this timepoint in our model **(Fig. R21)**. Neurons generated post-TBI showed no differences in terms of synaptic density compared to neither Sham nor Controls. Therefore, we conclude that synaptic density in the neurons born during the acute phase of TBI is not affected by the injury.

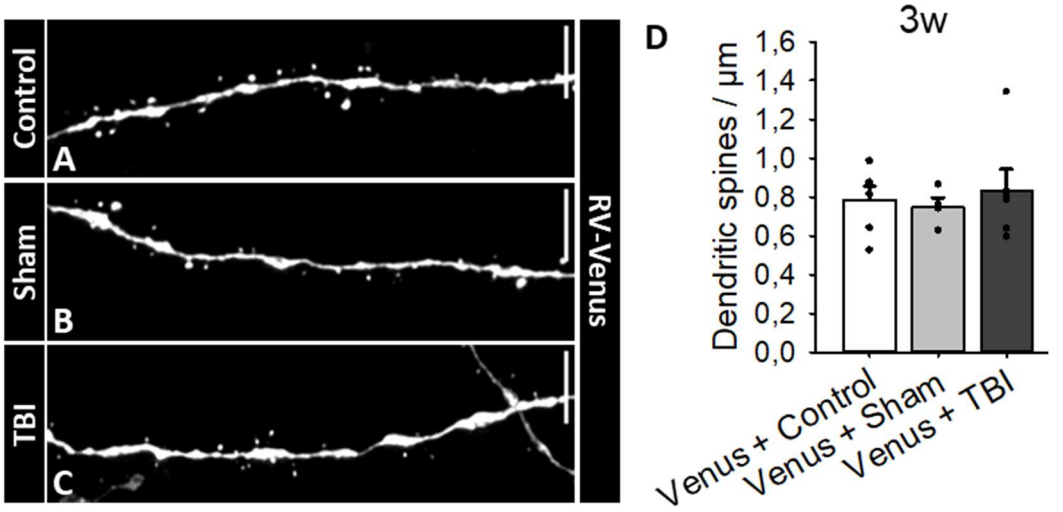


Figure R21. Neurons born shortly after TBI show no differences in spine density compared to Shams or Controls. A-C. Representative confocal images show dendrites of RV-Venus infected neurons in Control (A), Sham (B) and TBI (C) animals 3 weeks post-infection. **D.** Quantification of dendritic spine density in the three experimental groups. TBI: Traumatic Brain Injury; RV: Retrovirus. Scale bar 5 µm. One-way ANOVA. These results showed no statistical differences. Bars show mean ± SEM. Dots show individual data.

6.4. RND2 IS A CANDIDATE FOR CORRECTING THE ABERRANT PHENOTYPE SHOWN BY NEWBORN NEURONS AFTER TBI.

Rnd2 is a small GTPase highly enriched in the adult SGZ that acts as a key regulator of cell cytoskeleton and migration. The deletion of Rnd2 in adult newborn neurons has been shown to increase cell body area and neuronal migration in the adult DG (Kerloch et al., 2021; Pacary et al., 2011). The similarity of this altered phenotype with our TBI phenotype led us to hypothesize whether an alteration affecting Rnd2 could underlie our model, opening the door for a possible molecular reestablishment towards a healthy neuronal phenotype.

Next, we resorted to use a similar strategy using viral infection to correct the morphological and migratory defects in our newborn neurons after TBI. We took advantage of a RV-vector that held both a DsRed fluorescent protein and the sequence for the Rnd2 gene to induce its expression. Here, we needed two different RV constructs.

- Rnd2-DsRed: The neurons infected by the RV containing this construct overexpress Rnd2 in addition to their endogenous Rnd2 levels and are labelled thanks to the fluorescent protein DsRed.
- DsRed: Control condition in which the infected neurons are labelled with fluorescent protein DsRed and only express the endogenous levels of Rnd2 expression.

Both are pieced together with our previous three experimental groups obtaining the following combinations:

- Control + DsRed.
- Control + Rnd2-DsRed.
- Sham + DsRed.
- Sham + Rnd2-DsRed.
- TBI + DsRed.
- TBI + Rnd2-DsRed.

Our experimental paradigm allows us to specifically identify the effects of, not only Rnd2 and TBI, but the combination of the overexpression and each of the experimental groups in any of the cases.

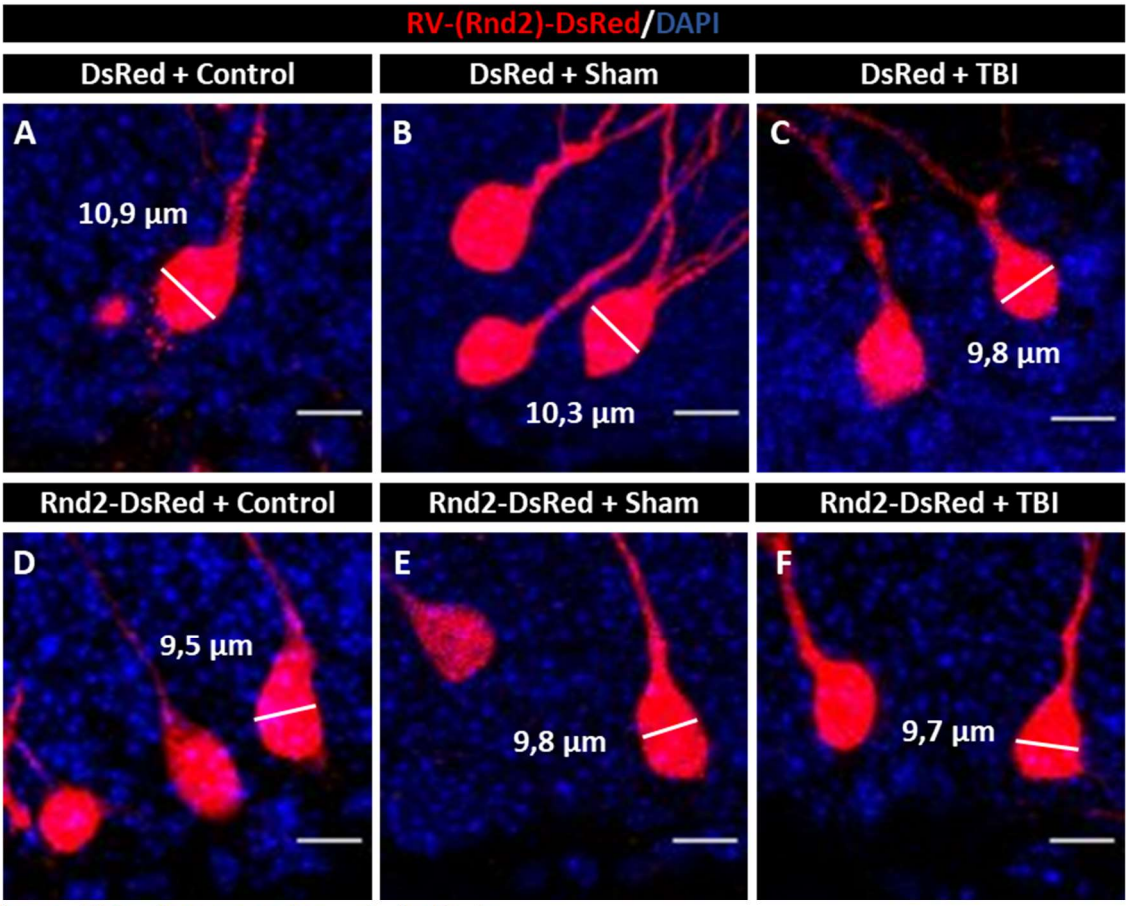
Taking into account the results of both the migration and soma size 3 weeks after the RV-Venus infection and TBI, we decided to repeat the same experimental approach for the RV-Rnd2 experiments **(Fig. R23 G)**.

6.4.1. Rnd2 overexpression is not enough to correct the soma size enlargement in new neurons after TBI.

We previously demonstrated an increase of cell soma size after TBI and we wanted to unravel if Rnd2 overexpression could affect to this alteration. None of the experimental groups with RV-infected newborn neurons showed a significant difference in their soma size compared among them **(Fig. R22)**. In general, all the groups infected in the control RV (RV-DsRed) seem to display a slightly bigger soma size than the groups that were infected by the RV expressing Rnd2 (RV-Rnd2-DsRed). However, there are no differences depending on the TBI, Sham or Control group.

It should be noted that when infecting with this specific retrovirus, the alterations in the soma in general were not reproduced as in previous experiments. When we compared separately the groups that were infected by the RV-DsRed among them: Control + DsRed, Sham + DsRed and TBI + DsRed; or Control + Rnd2-DsRed, Sham + Rnd2-DsRed and TBI + Rnd2-DsRed, we could observe that there was not an enlargement of TBI somata versus the rest of the conditions as it would be expected. These unexpected results of reproducibility make it impossible for us to correct the aberrant phenotype.

We know that in physiological (equivalent to Control conditions in this case), lack of Rnd2 is translated into bigger soma size. However, there is no data on whether the overexpression could actually act in a mirroring way. The data presented here suggest that regardless of the Control, Sham or TBI treatment, in all conditions there is a tendency for the overexpression of Rnd2 to reduce the area of the RV-infected somata **(Fig. R22 G)**.



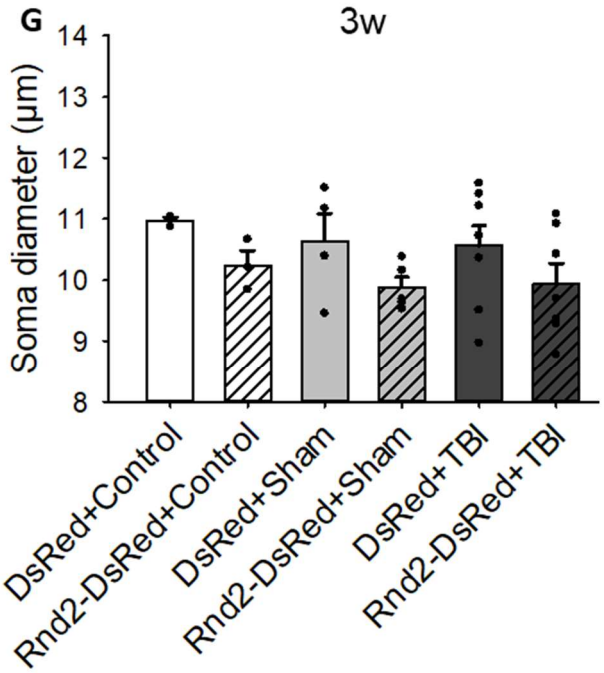


Figure R22. Overexpression of Rnd2 in neurons born shortly after TBI does not affect changes in their soma size. A-F. Representative confocal images of RV-DsRed or RV-Rnd2-DsRed infected neurons showing their somata and processes in the GCL in Control (A & D), Sham (B & E) and TBI (C & F) animals. White lines and numbers indicate soma diameter from analysed cells. Tissue was obtained 3 weeks post-infection. **G.** Quantification of soma diameter calculated measuring as it was described in **Fig. R17**. TBI: Traumatic Brain Injury; GCL: Granule Cell Layer; RV: Retrovirus. Scale bar 10 µm. One-way ANOVA. No statistical differences were found in these results. Bars show mean ± SEM. Dots show individual data.

6.4.2. Rnd2 overexpression is able to correct the aberrant neuronal migration after TBI.

Neuronal migration is known to be regulated by several members of the Rho GTPase family during development and also in adult stages (Ridley, 2001b). Rnd2 is the only protein in this family enriched specifically in the adult SGZ (Miller et al., 2013). To test the hypothesis whether Rnd2 plays a role in promoting migration of immature precursors we overexpressed the protein using the RV as previously described. Animals received an intrahippocampal injection of a solution containing the vector and were sacrificed 3 weeks post infection to analyse the tissue **(Fig. R23 G)**. We assured that the analysis was made only in immature neurons by colocalizing the DsRed$^+$ cells with DCX **(Fig. R23 A-F)**.

We were successful at replicating this experiment with this specific RV. Animals that were all injected with the "control" RV showed the migration difference we had previously described with DCX$^+$ cells and Venus$^+$ cells **(Fig. R18 and Fig. R20)**. Neurons infected by RV-DsRed in TBI animals migrate further than RV-DsRed in Sham or in Control animals **(Fig. R23)**. Furthermore, the approximated distance of migration was similar to the one previously determined in the RV-Venus experiment.

Interestingly, when we overexpressed Rnd2 in TBI animals, their neurons did not migrate as long as in the RV-DsRed + TBI. We observed that the migration in Rnd2-DsRed + TBI animals was equivalent to that of Control animals. Thus, the overexpression of Rnd2 after TBI in newborn neurons is therefore sufficient to recover the normal migration phenotype **(Fig. R23 E-F)**. Moreover, we could also observe a reduction in the migration between DsRed + Sham and Rnd2-DsRed + Sham. These groups have shown an intermediate level of migration. By overexpressing Rnd2 we are able to reduce the distance of migration of newborn neurons to the level of Control animals. When comparing TBI, Sham and Control regardless of the injected RV, we could appreciate the general trend in the same direction as observed in our previous experiments: TBI is the condition that most boosts neuronal migration. Also, the fact that Rnd2 seems to be reducing neuronal migration in Control and Sham animals too, suggests that the overexpression of this protein in physiological conditions could have the opposite role from its deletion.

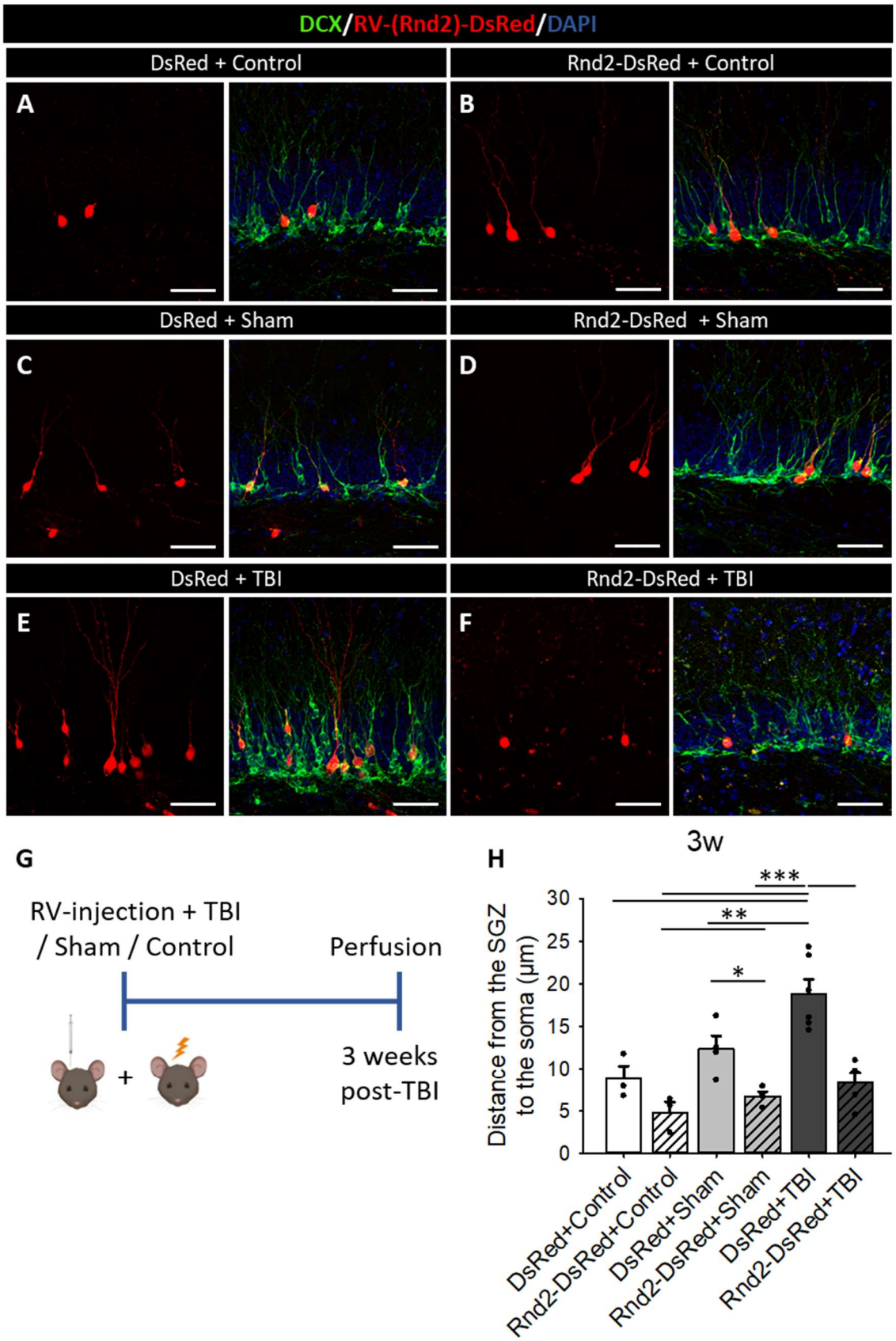


Figure R23. Overexpression of Rnd2 is able to correct the aberrant migration phenotype in neurons born shortly after TBI. A-F. Representative confocal images show RV-DsRed or RV-Rnd2-DsRed infected cells after Control (A-B), Sham (C-D) or TBI (E-F) treatment colocalizing with the marker for immature neurons DCX. Tissue was obtained 3 weeks after the combined surgery of retroviral injection followed by TBI, Sham

or Control treatment. **G.** Schematic representation of the experimental paradigm and the timeline followed in the experiment. **H.** Quantification of the total migrated distance travelled by RV-DsRed or RV-Rnd2-DsRed infected neurons across the GCL. TBI: Traumatic Brain Injury; GCL: Granule Cell Layer; RV: Retrovirus. Scale bar 50 µm. *p<0,05; **p<0,01; ***p<0,00. One-way ANOVA followed by all pairwise multiple comparisons by Fisher-LSD post hoc test. Bars show mean ± SEM. Dots show individual data.

6.5. RND2 IS A POTENTIAL CANDIDATE FOR REDUCING ANXIETY-LIKE BEHAVIOUR AND IMPROVING SPATIAL LEARNING AND MEMORY IN PHYSIOLOGICAL CONDITIONS.

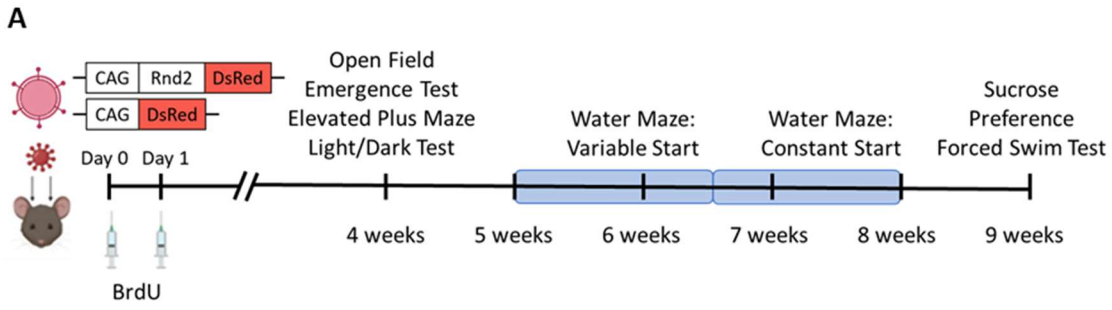
Prior to investigating the role of Rnd2 in a pathologic context, it is necessary to know how it works in physiological conditions. A recent study describes the effects of removing Rnd2 from newborn neurons of the adult hippocampus (Kerloch et al., 2021), reporting among other effects, a decrease in neuronal survival, and an increase in both cell migration and cell body area. It is noteworthy to mention that these alterations at a cellular level also had functional repercussions, animals with a deletion of Rnd2 in adult-born neurons showed an increased anxiety-like behaviour in tests such as the Elevated Plus Maze (EPM) and Light/Dark Test (LT).

Hence, we hypothesised that Rnd2 overexpression could act in the opposite way by increasing neuronal survival, migration and cell body area and also functionally by decreasing anxiety-like behaviour and changing other cognitive tasks highly depending on AHN, such as the spatial learning and/or memory and depressive-like behaviour.

Next set of experiments was performed in collaboration with Dr. Emilie Pacary during a stay at the laboratory of Neurogenesis and Neuropathology in the Neurocentre Magendie. Unfortunately, the results regarding the histological study are not yet available so we will only focus in the results concerning the functional and behavioural experiments.

To generate animals which overexpressed Rnd2 in newborn neurons, we used a total of 26 male animals altogether were used for these analyses. They were separated into two cohorts and injected randomly with either one of two RV: RV-DsRed (12 mice) or RV-Rnd2-DsRed (14 mice). In this case, the vector was injected bilaterally (2 injections per mouse) using specific coordinates to target the DG. To analyse neuronal survival, two doses of intraperitoneal BrdU were administered on Day 0 and Day 1 after the intrahippocampal injections **(Fig. R24 A)**. Animals were let to habituation and recovery after the surgery for 4 weeks in individual cages before

starting the analyses. A complete battery of tests was designed in order to characterize the exploratory, anxiety, depression, learning and memory tasks of the animals. The tests were performed in the specified sequence of the scheme **(Fig. R24 A)**. In the first week after habituation, we performed the exploratory and anxiety testing in the following order: Open Field Test (OF), Emergence Test (ET), Elevated Plus Maze (EPM) and Light/Dark Test (LD). The next four weeks were exclusively dedicated to spatial learning and memory testing: first a Variable Start (VS) version of the Morris Water Maze (MWM) and then a Constant Start (CS) version. Finally, the nineth week we focused in the depression testing: Sucrose Preference Test (ST) and Forced Swim Test (FST). Afterwards, the animals were sacrificed for histopathological analysis the day after the last cognitive test. We analysed the tissue in order to quantify the total number of infected cells per animal **(Fig. R24 B)**. Animals that showed no infected cells or that had a low number of infected cells (not enough to produce a functional change) were discarded for all the analyses **(Figure R24 B)**.



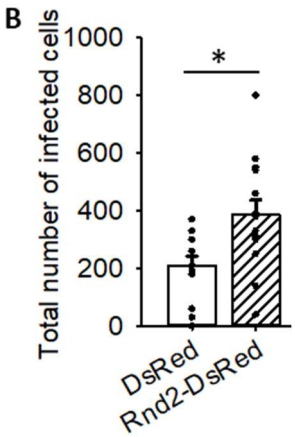


Figure R24. Experimental design to assess Rnd2 role in animal behaviour and efficiency of the viral infection. A. Schematic representation of experimental design to unravel behavioural abnormalities due to Rnd2 overexpression. BrdU was injected IP the same day of RV-injection and after 24 hours. After 4 weeks behavioural analyses started in the specific order shown in the panel A. Perfusion was performed 24 hours after the last test was finished. **B.** After perfusion, histological sections were used to assess the number of infected cells in each of the animals for both experimental groups. Animals with a low number of infected cells were discarded for the analyses. BrdU: Bromodeoxyuridine; IP: intraperitoneal, RV: retrovirus. Student's t-test. *p<0,05. Bars show mean ± SEM. Dots show individual data.

6.5.1. Rnd2 overexpression does not alter exploratory behaviour in mice during Open Field test.

We studied the behaviour of both experimental groups (RV-DsRed and RV-Rnd2-DsRed-injected) during the Open Field (OF) test prior to testing for more specific hippocampal-related functions.

The OF arena is a white square surrounded with walls in which the centre has brighter conditions (200 lux) than the periphery. The field was divided into sub-areas that were used as a measurement to trace the mice tracks during the test **(Fig. R25 A)**. We tested two animals at the same time in adjacent arenas for a total period of 10 minutes **(Fig. R25 B)**. We monitored the movement of the animals through the arena using a video-tracking system in order to study velocity, placement preferences and travelled distance both in general and in each specific sub-area. **Figure R25 C** shows an example of specific path followed by one animal in each group.

Two parameters were used to study the suitability of the animals for this test: the total travelled distance and the walking velocity throughout the totality of the time tested in the whole arena. The potential identification of animals that refused to participate or showed any type of inability to walk and explore the environment is important for the rest of the tests. However, all animals in both groups walked a similar total distance at similar velocity **(Fig. R25 D-E)**, so any of them got discarded. In specific occasions, mice may show different behaviour along the time of the experiment. To solve that, we performed first of all a general data collection from 10 minutes of recordings and later we compared with separated data from the first and last 5 minutes of the test. No differences were found either (data not shown).

The luminous difference between the “artificial” compartments in the OF arena is sometimes used as a general measurement for anxiety-like behaviour. In normal conditions, mice tend to spend more time in the periphery of the area and less in the centre. We measured this with three different parameters: not just the travelled distance in the centre but also the time spent there and the number of entries towards this area. In all cases, the parameters were not significantly different between the two groups **(Fig. R25 F-H)**. Control mice and Rnd2-overexpressing mice both prefer to spend more time near the periphery than in the centre of the arena. We can therefore conclude that Rnd2 overexpression in hippocampal adult-born neurons does not affect mice exploratory behaviour or cause gross locomotor abnormalities during OF test.

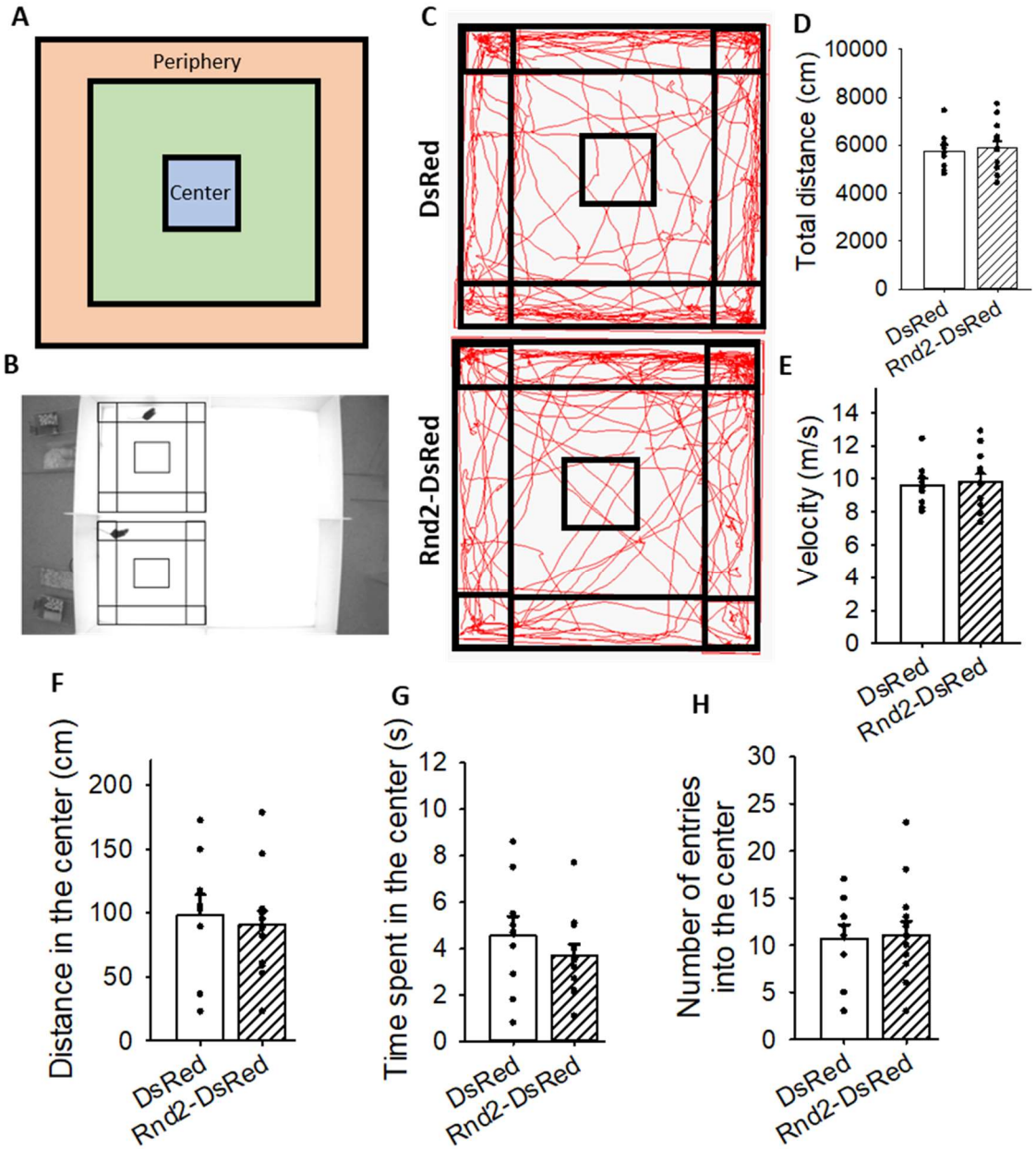


Figure R25. Rnd2 overexpression in adult-generated neurons does not affect the animals' exploratory behaviour during OF testing. A. Schematic representation of the experimental arena used during OF test and the areas characterized as periphery (red) or centre (blue) used in the analyses. **B.** Image representing the experimental set up used during OF testing. Two animals were tested at the same time in two adjacent arenas. A video-tracking system was used to both record and analyse the results. **C.** Representative exploratory paths followed by a Ds-Red (top) or a Rnd2-DsRed (bottom) injected mouse. **D.** Total distance travelled by each of the animals during the 10 minute-test in both experimental groups. **E.** Walking velocity of the animals during the test. **F.** Total travelled distance in the centre of the arena in both of the experimental groups. **G.** Total time spent in the centre of the arena by both DsRed and Rnd2-DsRed injected animals. **H.** Total number of entries into the central part of the arena during the test in both experimental groups. Student's t-test. No statistical differences were found in any of the tests to assess the performance of both groups during the OF task. OF: Open Field. Bars show mean ± SEM. Dots show individual data.

6.5.2. Rnd2 overexpression in adult-born neurons does not affect anxiety-like behaviour during the Emergence Test.

Emergence Test (ET) is a preliminary test often used to identify changes in anxiety-like behaviour. The arena in this case is the same as in the OF, however, in ET the animals are firstly placed into a dark cylinder which is then positioned inside the arena near one of the walls **(Fig. R26 A-B)** and 5 minutes of time are allowed to the animals to explore the arena. Mice tend to avoid luminous conditions in open spaces during what is considered as anxiety-like behaviour. Thus, the measurement of the latency until they first emerge from the tube, the total time they spend inside it and the number or re-entries inside the tube are considered as parameters for quantifying anxiety-like behaviour in ET **(Fig. R26 A-B)**.

The total travelled distance through the arena was taken as a basal line reference for the exploratory behaviour of the mice **(Fig. R26 C)**. In this case, one subject was removed for the analysis due to the excessive time it took to emerge from the tube.

In general, no differences were found between the two groups in ET for the parameters of: latency to emerge, time inside the tube of re-entries to the tube **(Fig. R26 D-F)**. However, the suitability of this test for these kind of experiments is questionable for several reasons. There is a thin line separating anxious behaviour from exploratory behaviour towards a new object in this case. This will be later commented in the discussion.

We may conclude that Rnd2 overexpression does not produce alterations in anxiety-like behaviour detectable using ET.

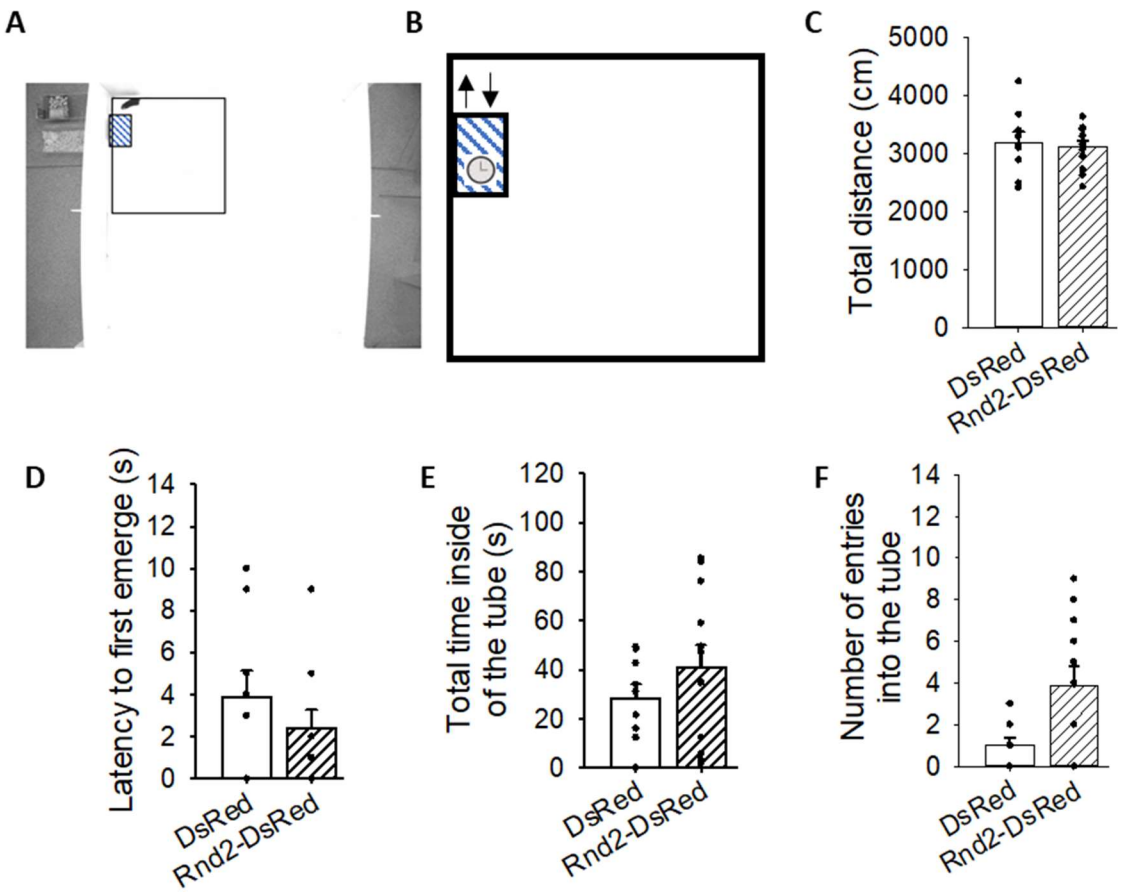


Figure R26. Rnd2 overexpression in adult-generated neurons does not affect anxiety-like behaviour during ET. A. Image representing the experimental set up used during ET. Animals were placed in a small dark cylinder (marked in blue in the scheme) and introduced into the arena. **B.** Schematic representation of the experimental arena used during ET. The cylinder containing the animal was placed near one of the corners of the arena and latency to first emerge from the cylinder was quantified together with other parameters like the number of entries back inside the tube. **C.** Total distance travelled in the arena by the animals in both experimental groups during the five minutes of the testing. **D.** Time to first emerge from the cylinder once the animals were placed inside the arena. **E.** Total time spent inside the tube during the whole span of the test. It includes the time of the latency together with the time spent during the re-entries. **F.** Number of re-entries back inside the tube during the experiment. Student's t-test. No statistical differences were found in any of the tests to assess the performance of both groups during the ET task. ET: Emergence Test. Bars show mean ± SEM. Dots show individual data.

6.5.3. Rnd2 overexpression in adult-born neurons does not affect anxiety-like behaviour during Elevated Plus Maze.

A more sensible and specific test for anxiety-like behaviours is using the Elevated Plus Maze (EPM). In this maze, animals tend to spend more time in the closed arms (CA) which have walls and provide a darker more protected environment for them **(Fig. R27 A)**. In terms of both time and travelled distance, in control conditions, mice tend to spend a lower amount of time and walk a shorter distance in the OA compared to the CA. **Fig. R27 B** shows an example path of a

random video-tracked animal. The path is shown in red. Walked distance in the CA (black) is significantly larger than walked distance in the OA (red).

As a reference, the total walked distance during the 5 minutes of the testing was similar between the two experimental groups **(Fig. R27 C)**. When we specifically studied the anxiety-behaviour we could see how both groups spent more time and walked a longer distance in the CA compared to the OA **(Fig. R27 D, F)**. Only about the 30% of the time is spent in OA and about 20% of their walked distance. However, there are no significant differences in these terms between the DsRed and Rnd2-DsRed groups. We also checked the number of re-entries into the OA to evaluate their exploration activity and no differences were identified here neither **(Fig. R27 E)**. In conclusion, Rnd2 overexpression in hippocampal adult-born neurons does not produce any type of detectable alterations in anxiety-like behaviours evaluated with the EPM test.

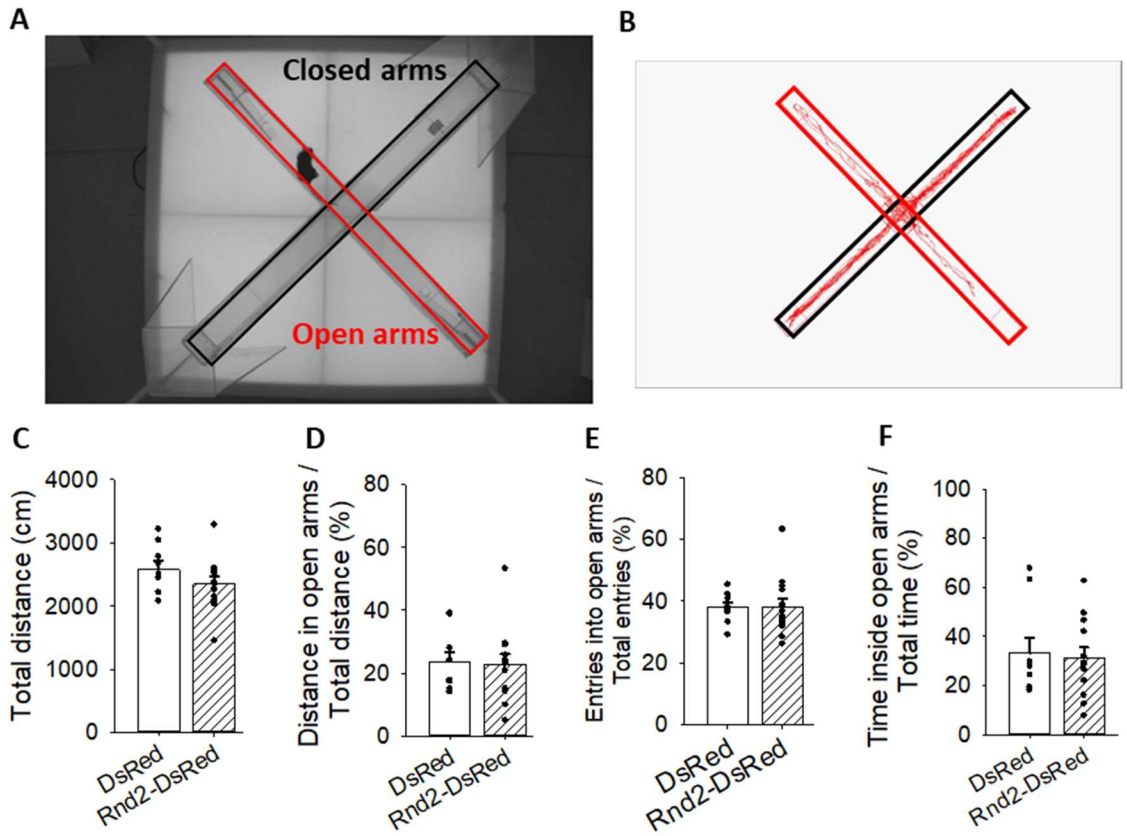


Figure R27. Rnd2 overexpression in adult-generated neurons does not affect anxiety-like behaviour in EPM test. A. Image representing the experimental set up used during EPM. Animals were placed in the centre of the crossed arms and left to explore for a total time of five minutes. OA (with no walls) are labelled in red and CA (with walls) are labelled in black. **B.** Schematic representation of the experimental maze used during EPM. Representative exploratory path followed by a Rnd2-DsRed injected mouse. **C.** Total distance travelled in the arms by the animals in both experimental groups during the five minutes of the testing. **D.** Percentage of distance travelled in the OA out of the total travelled distance during the experiment in both groups. **E.** Number of entries into the OA area out of the total number of entries during

the whole experiment. **F.** Time spent inside the OA out of the total time of the experiment. Student's t-test. No statistical differences were found in any of the tests to assess the performance of both groups during the EPM test. EPM: Elevated Plus Maze; OA: Open Arms; CA: Closed Arms. Bars show mean ± SEM. Dots show individual data.

6.5.4. Rnd2 overexpression in adult-born neurons decreases anxiety-like behaviour during the light/dark test.

One of the most specific behavioural tests designed to identify changes in anxiety-like behaviour is the light/dark (LD) test. Here, an animal is placed in a light big chamber with a very high luminous condition (350 lux). This chamber communicates with a smaller darker chamber (10 lux) **(Fig. R28 A-B)**. Mice are left free to travel from one chamber to the other for 5 minutes and several parameters are taken as anxiety-indicators: (1) the latency to first escape from the light room, (2) the total time spent in the light room throughout the experiment and (3) the number of re-entries back into the light room **(Fig. R28 C-E)**. We performed this test in our groups of mice and analysed the results separately.

The time to first escape from the light room is not significantly different between boths groups **(Fig. R27 C)**. As it happens with the ET, movement response to new stimuli is usually very fast in mice so it makes it hard to see differences in tests like this that are consistent in just a few seconds. Although none of the groups tend to escape faster from the light room, Rnd2-overexpressing animals ended up spending more time in total through the experiment in this room **(Fig. R27 D)**. The increased latency of the animal in a brighter environment, that should be perceived as dangerous, is usually associated to a decrease in anxiety. It is also important to discern animals that could be spending more time in the light room during paralyzing behaviour by fear. To discard this ambiguity, we measured the number of re-entries inside the light room. We found that Rnd2 overexpressing animals entered back into the light room more often indicating two things: first, they were moving and exploring (not paralized by fear) so therefore the whole time that they spent in the light room formed part of the exploratory behaviour, and second, they tended to come back to an environment which therefore was not perceived as scary **(Fig. R27 E).**

Hence, we can conclude that Rnd2 overexpression in adult-born hippocampal neurons reduces anxiety-behaviour during the LD test.

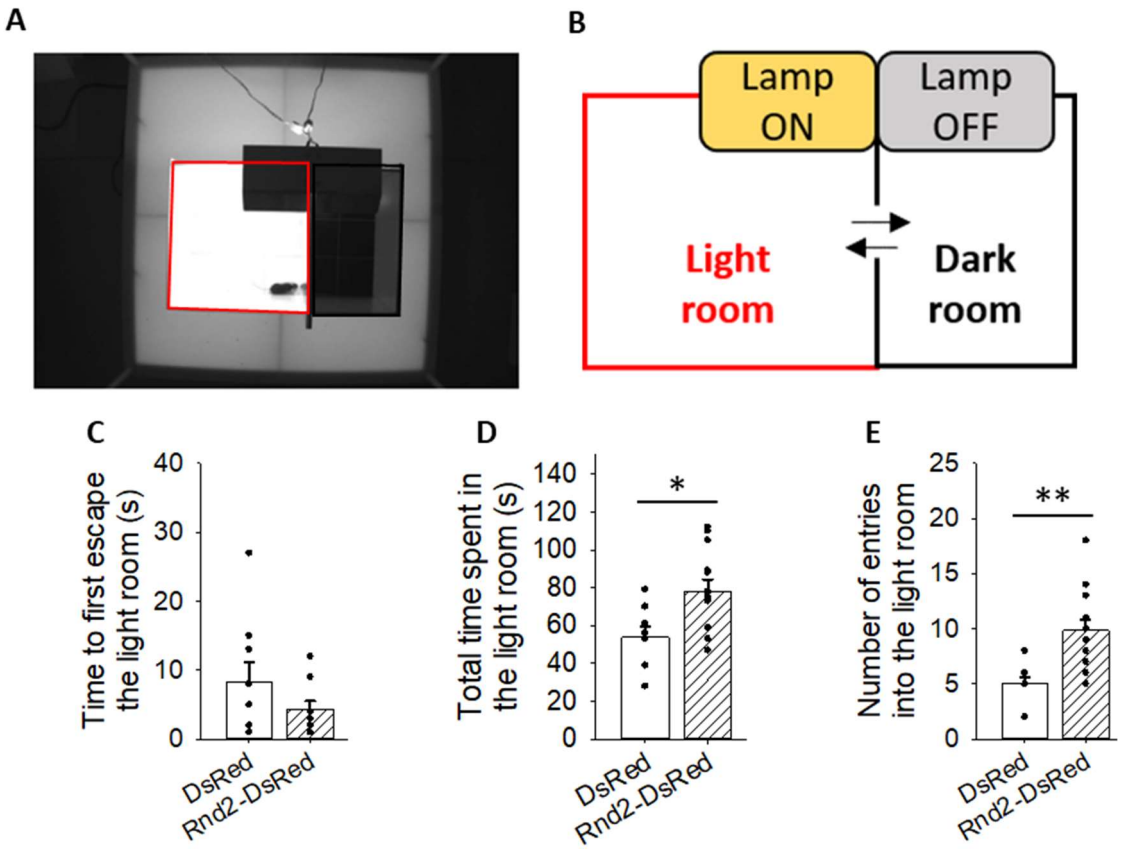


Figure R28. Rnd2 overexpression in adult-generated neurons decreases anxiety-like behaviour during LD test. A. Image representing the experimental set up used during LD test. Animals were placed in a light chamber (in red) that was communicated with a dark chamber (in black). They were left there to explore for a total of five minutes. **B.** Schematic representation of the experimental arena used during LD. The light chamber/room is placed to the left with high light conditions (350 lux) and it is connected with a dark chamber or room to the right very dim twilight (10 lux). **C.** Time until first escape from the light room. **D.** Total time spent in the light room during the whole experiment showed that Rnd2-DsRed injected mice spend significantly more time in the light room than the DsRed-injected ones. **E.** Number of entries into the light room. Rnd2-DsRed-injected animals entered more often back into the light room than the group of DsRed-injected ones. LD: Light/Dark. Student's t-test. *p<0,05; **p<0,01. Bars show mean ± SEM. Dots show individual data.

6.5.5. Rnd2 overexpression in adult-born hippocampal neurons does not affect depressive-like behaviour reflected in the Sucrose Preference test.

Sucrose Preference Test (ST) detects anhedonia-like behaviour. This mood disturbance is often a comorbid symptom linked with depression as well as other mental health disorders. Indeed, anhedonia is defined as the lack of ability to feel pleasure. To assign a value to this test, the animal is exposed to a pleasant stimulus, usually a sweet solution of 4% sucrose in water. Control stimulus is a basic stimulus of non-sweet water. Healthy animals tend to choose the pleasant

stimulus while depressive animals after developing anhedonia do not display any preference. Thus, the experimental paradigm was designed to exclude environmental factors from the main result. First of all, animals were habituated to the presence of two bottles in their cage for 24 hours. Initially, these two bottles contained only water. Both bottles were weighted before and after their placement to calculate the total consumption of water during 24 hours **(Fig. R29 A)**. Then, we changed the solution in one of the bottles for 4% sucrose in water. The position of the bottles was randomised in a way that assured that half of them had their sucrose on the left and the other half on the right to avoid preferences over one specific drinking spot. Finally, after another 24-hour period the bottles were taken out and weighted again **(Fig. R29 A)**. It is noteworthy to mention that one of the animals was removed from the analyses since one of the bottles in its cage was empty after the first 24 hours indicating a fluid leakage.

The total liquid consumption of mice in both groups was not significantly different during the duration of the test **(Fig. R29 B)**. When we specifically focused at the ingested volumed of water with sucrose, we could determine again similar values in both groups **(Fig. R29 C)**. The sucrose preference ratio is calculated by dividing the consumed volume of 4% sucrose water into the total consumed liquid. Both groups showed a high preference for sucrose (around 80%), so neither of them manifested anhedonia **(Fig. R29 D)**. In conclusion, Rnd2 overexpression in hippocampal adult-born neurons does not produce anhedonia, therefore there are no proves that it triggers this type of depression-like behaviour.

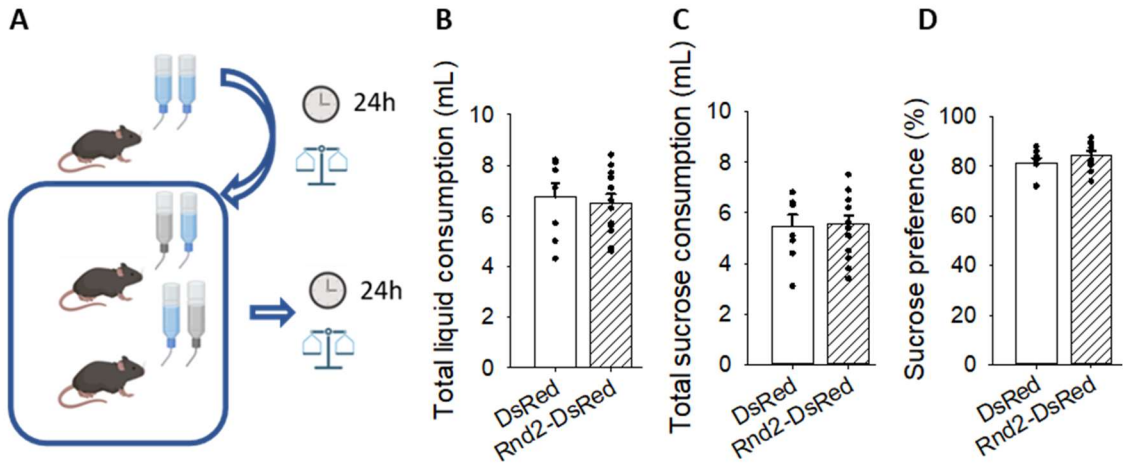


Figure R29. Rnd2 overexpression in adult-generated neurons does not have an effect over depression-like behaviour during ST. A. Schematic representation of the ST experimental design. Mice of both experimental groups (DsRed and Rnd2-DsRed injected animals) were exposed to two bottles of water for 24 hours of habituation. After that, one of the bottle's solution was switched for 4% sucrose. Half the bottles were positioned in the right side and the other half on the left side in both experimental groups. After 24 hours, both bottles were weighted to calculate the volume of liquid consumed in each of them. **B.** Total liquid consumption when the animals were already exposed to the 4% sucrose bottle. **C.** Total sucrose consumption in both DsRed and Rnd2-Dsred injected animals during the day of exposure to the

4% sucrose solution. **D.** Sucrose preference is calculated by dividing the consumed volume of 4% sucrose in the total consumed liquid (water + 4% sucrose). Student's t-test. No statistical differences were found in any of the tests to assess the performance of both groups during the ST task. ST: Sucrose Test. Bars show mean ± SEM. Dots show individual data.

6.5.6. Rnd2 overexpression in adult-born hippocampal neurons does not induce depression-like behaviour during Forced Swim Test.

Another alternative and complementary test to assess depression-like behaviours is by using the Forced Swim Test (FST). Here, mice are exposed to a stressing inescapable situation by placing them inside a small glass cylinder filled with warm water (26ºC) and their mobility is monitored for a total of 6 minutes.

Two animals were evaluated at the same time during this test **(Fig. R30 A)**. Several parameters are useful to analyse the resilience of the animals in this context. Among them, the most common ones are: the latency to immobility (how much time it takes them to show immobility for the first time) and the total immobility time in the last 4 minutes of the test. Both experimental groups showed a similar result in these parameters **(Fig. R30 B-C)**. It took them around 70 seconds to trigger the immobility behaviour and spent a total of about 100-110 seconds immobile in the last 4 minutes. The result suggests that there is not a significative difference in the depressive-like behaviour between control and Rnd2-injected animals. However, when we assess the immobility time per minute of the test, we can observed that during the second minute, DsRed-injected animals spend a higher amount of time immobile than Rnd2-DsRed-injected animals **(Fig. R30 D)**. Therefore, although there is not a major difference in the overall depressive behaviour, animals overexpressing Rnd2 in their adult-born neurons display a slightly slower trigger to the immobility response.

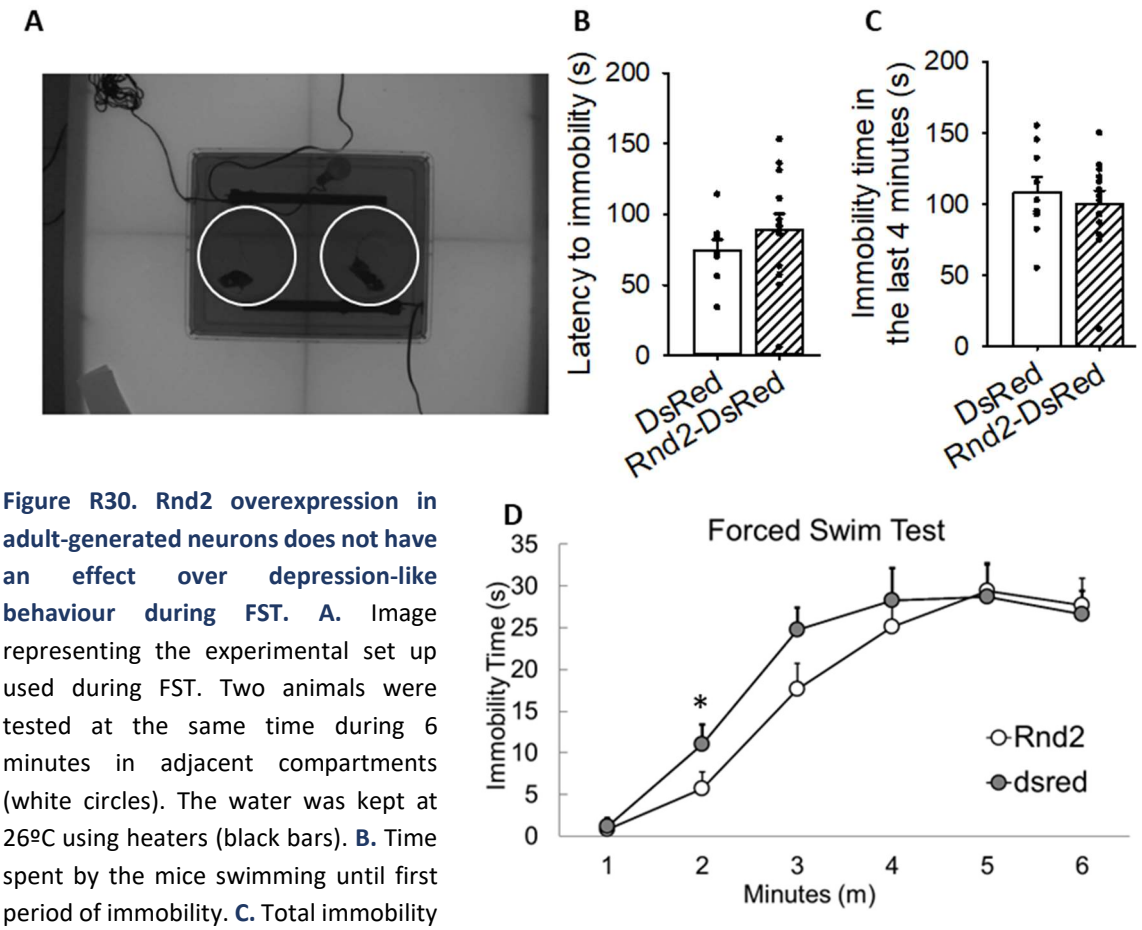


Figure R30. Rnd2 overexpression in adult-generated neurons does not have an effect over depression-like behaviour during FST. A. Image representing the experimental set up used during FST. Two animals were tested at the same time during 6 minutes in adjacent compartments (white circles). The water was kept at 26ºC using heaters (black bars). **B.** Time spent by the mice swimming until first period of immobility. **C.** Total immobility time in the last 4 minutes of the test by both experimental groups. **D.** Immobility time of each group per minute of the test. DsRed-injected mice show increased time of immobility than Rnd2-DsRed-injected mice only in the second minute of the test. U-Mann-Whitney analysis. *p<0,05. (*) indicates differences between DsRed and Rnd2-DsRed. No statistical differences were found in the rest of the analyses for the FST evaluation. FST: Forced Swim Test. Bars show mean ± SEM. Dots show individual data.

6.5.7. Spatial learning and memory assessing after Rnd2 overexpression in newborn neurons.

The presence of new neurons in the hippocampus influences, not just the mentioned mood-related tasks but also spatial navigation (Dupret et al., 2008; Jessberger et al., 2009). The most common strategy to study spatial learning is by using the Morris Water Maze (MWM), in which animals learn the location of a hidden platform by using distal visual cues.

The animals reach the hidden platform using multiple strategies in parallel, which require the integrity of the hippocampus at different levels. Depending on the type of memory that we want to assess, we will use one variation of training (hippocampal-dependent) or the other (hippocampal independent). Therefore, the strategies that the animals will develop depend on the type of training that is used (Eichenbaum et al., 1990; Morris et al., 1982). In this study we

decided to evaluate both of them: hippocampal dependent and independent memory. In our hypothesis, the alteration of Rnd2 expression in newborn neurons would only affect the hippocampal-dependent learning and memory. However, by evaluating also the independent one we can get a more complete overview of other possible alterations that could take place at a higher network level.

6.5.7.1. Rnd2 overexpression in newborn neurons does not alter spatial learning during a spatial navigation hippocampal-dependent task but it slightly impairs memory recall.

To detect hippocampal-dependent procedures, the experimental design at the beginning of the training phase requires that the underwater platform is maintained hidden and the releasing point for the animals is changed at each of the three daily trials **(Fig. R31 A)**. This is known as a Variable Start training (VS). Thus, in order to find the hidden platform, mice will have to use an allocentric mental mapping strategy that consists of the learning of all the positional relationships among multiple independent environmental cues (spatial relational memory). This relational representation is needed to support the flexible use of learned discriminative cues in novel situations (i.e., changing starting position), and it is consequently necessary to solve the task. This cognitive ability is one of the most well-studied hippocampus-dependent abilities, since this task cannot be learned if the hippocampus is impaired (Eichenbaum et al., 1990).

Therefore, to train mice in this hippocampal-dependent task we designed a specific maze layout **(Fig. R31 A)** and a specific learning protocol. Mice were trained 3 times a day for 12 days until it was considered they learned the position of the platform (it took them to get there around 10 seconds or less). **Fig. R31 B** shows an example of the path followed by a specific mouse injected with the Rnd2-DsRed vector in one of the trials during 3 days of learning (Day 1, Day 4 and Day 12).

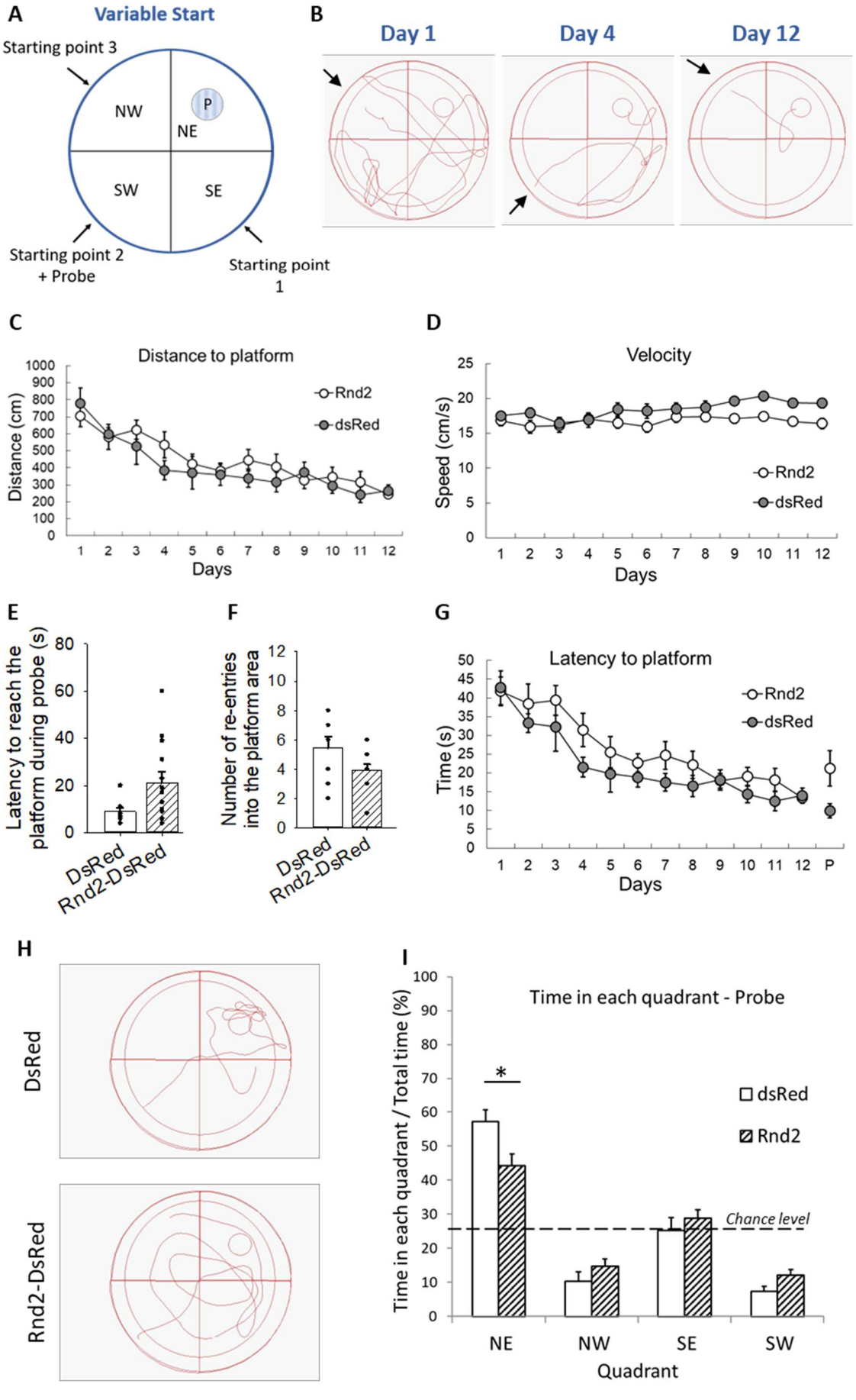
Two parameters were used to assess their hippocampal-dependent learning capacity: the distance they swam from the releasing point into the platform and the elapsed time until completion of the task. Both of the experimental groups followed a similar learning pattern throughout the experiment by progressively improving their performance **(Fig. R31 C, E)**. As a measure for their correct involvement in the task, we also calculated the velocity for each of them to rule out non-participating mice. All animals seemed to be interested in learning the task and did not show any evidence of thigmotaxic behaviour (the tendency to remain close to the

walls) so none of them had to be discarded **(Fig. R31 D)**. In general, there are no alteration caused by the overexpression of Rnd2 in newborn neurons in the spatial learning assessed during a VS strategy in MWM.

To test memory recall, we perform what is defined as a Probe after the training. For this experimental design, the platform is removed and we measure several indicators of memory: (1) the latency for the animals to reach the place where the platform used to be; (2) number of re-entries into the (non-existing) platform area and (2) total time spent in the quadrant where the platform used to be located (in our case North-East, NE situation).

In terms of the latency to reach the platform we did not find differences between the two experimental groups **(Fig. R31 F)**. However, after careful examination of the data represented in the graph, a tendency for Rnd2-DsRed-injected mice to increase the elapsed time needed to reach the platform is observed. This is more evident if we compare two example tracks of one of the animals in each of the groups **(Fig. R31 H)**. However, the number of re-entries inside the platform area is not significantly different **(Fig. R31 G)**. Finally, when they were tested for the percentage of the time spent in the target quadrant, we could see that Rnd2-DsRed injected animals spent less time there **(Fig. R31 I)**. This value is illustrative for the persistence in the memory of these animals: whether they remain in the area they know is correct or switch rapidly to a different quadrant. This is also reflected in the example track shown in **Fig. R31 H**.

Thus, we conclude that Rnd2 overexpression in adult-hippocampal newborn neurons does not alter spatial learning in a VS approach of MWM but it slightly impairs memory recall. Hippocampal-dependent learning remains intact while memory recall is altered.


A
Variable Start
Starting point 3
NW
NE
P
SW
SE
Starting point 2
+ Probe
Starting point
1
B
Day 1
Day 4
Day 12
C
Distance to platform
Distance (cm)
Days
Rnd2
dsRed
D
Velocity
Speed (cm/s)
Days
Rnd2
dsRed
E
Latency to reach the platform during probe (s)
DsRed
Rnd2-DsRed
F
Number of re-entries into the platform area
DsRed
Rnd2-DsRed
G
Latency to platform
Time (s)
Days
Rnd2
dsRed
H
DsRed
Rnd2-DsRed
I
Time in each quadrant - Probe
Time in each quadrant / Total time (%)
*
dsRed
Rnd2
Chance level
NE
NW
SE
SW
Quadrant

←Previous page Figure R31. Rnd2 overexpression in adult-generated neurons does not affect spatial memory during MWM in VS training. However, a deficit is observed in the probe test showing abnormalities in memory retention and recall. A. Schematic representation of the experimental arena used during MWM with variable start test. The pool is divided in four quadrants: NW, NE, SW and SE. The target platform is always located in the NE quadrant. Mice starting points are randomized throughout the process. **B.** Representative exploratory paths followed by Rnd2-DsRed-injected animals during different days throughout the learning process. **C.** Total distance the animals of each experimental group swim each day of the learning process to get to the platform. **D.** Swimming velocity of the animals per day. **E.** Time to get to the platform during the probe in both of the experimental groups. **F.** Number of entries inside the platform area during the probe. **G.** Latency to get to the platform during the training days and also during the probe in both of the experimental groups. **H.** Representative exploratory paths followed by a DsRed (top) or a Rnd2-DsRed (bottom) injected mouse during the Probe. **I.** Percentage of time spent in each of the four quadrants during the Probe. Discontinuous line shows the chance level of spending time in a random quadrant. DsRed injected animals spend longer time in the target quadrant (NE) than Rnd2-DsRed-injected animals. Two-way ANOVA followed by all pairwise comparisons by Holm-*Šídák* post-hoc test. *p<0.05. No statistical differences were found in the rest of the analyses. MWM: Morris Water Maze; VS: Variable Start; NW: North West; NE: North East; SW; South West; SE: South East. Bars show mean ± SEM. Dots show individual data.

6.5.7.2. Rnd2 overexpression in newborn neurons does not alter spatial navigation in a hippocampal-independent task.

To assess hippocampal-independent memory we a different variable of MWM: the Constant Start procedure (CS). In this design, we also hid the platform from the beginning of the training but the starting point was maintained constant for all trials **(Fig. R32 A)**. In this case, although the development of an allocentric mapping strategy is not prevented, the animal can also learn the position of the platform using egocentric strategies consisting of, for example, an association of an invariant configuration of spatial cues to the escape platform (place learning). Egocentric strategies are known to be hippocampal-independent and thus, if the starting point is maintained constant, animals can solve the water maze task even after a complete impairment of the hippocampus (Eichenbaum et al., 1990). Here, after nine days of training sessions, two tests were performed to assessed two types of memory. Firstly, mice were released just once from a Novel Start position (NS) to test for flexibility and assess whether they also established an allocentric strategy. Then, we removed the platform and performed a second Probe to test for memory recall.

The parameters used to evaluate the performance during the learning process are the same as in the previous strategy: latency to teach the platform and swam distance. Both groups showed a similar, although irregular curve of learning **(Fig. R32 D, F)** as we will later discuss. Their

swimming speed remained normal throughout the process **(Fig. R32 E)**. Both groups exhibited an efficient performance and found the platform properly even in NS conditions. In conclusion, there were no significant differences in their latency to reach the platform during the NS **(Fig. R32 G)**.

We obtained the same result later with the Probe. Both groups spent a similar amount of time reaching the area where the platform used to be **(Fig. R32 B)** and tried to re-enter this area several times **(Fig. R32 C)**. Also, the time tend to spent consistently more time in the quadrant where the platform used to be located **(Fig. R32 H)**. No differences were found between the groups in any of the parameters regarding the Probe. In conclusion, Rnd2 overexpression in hippocampal adult-born neurons does not alter hippocampal-independent memory, as it is demonstrated by the CS variation of MWM.

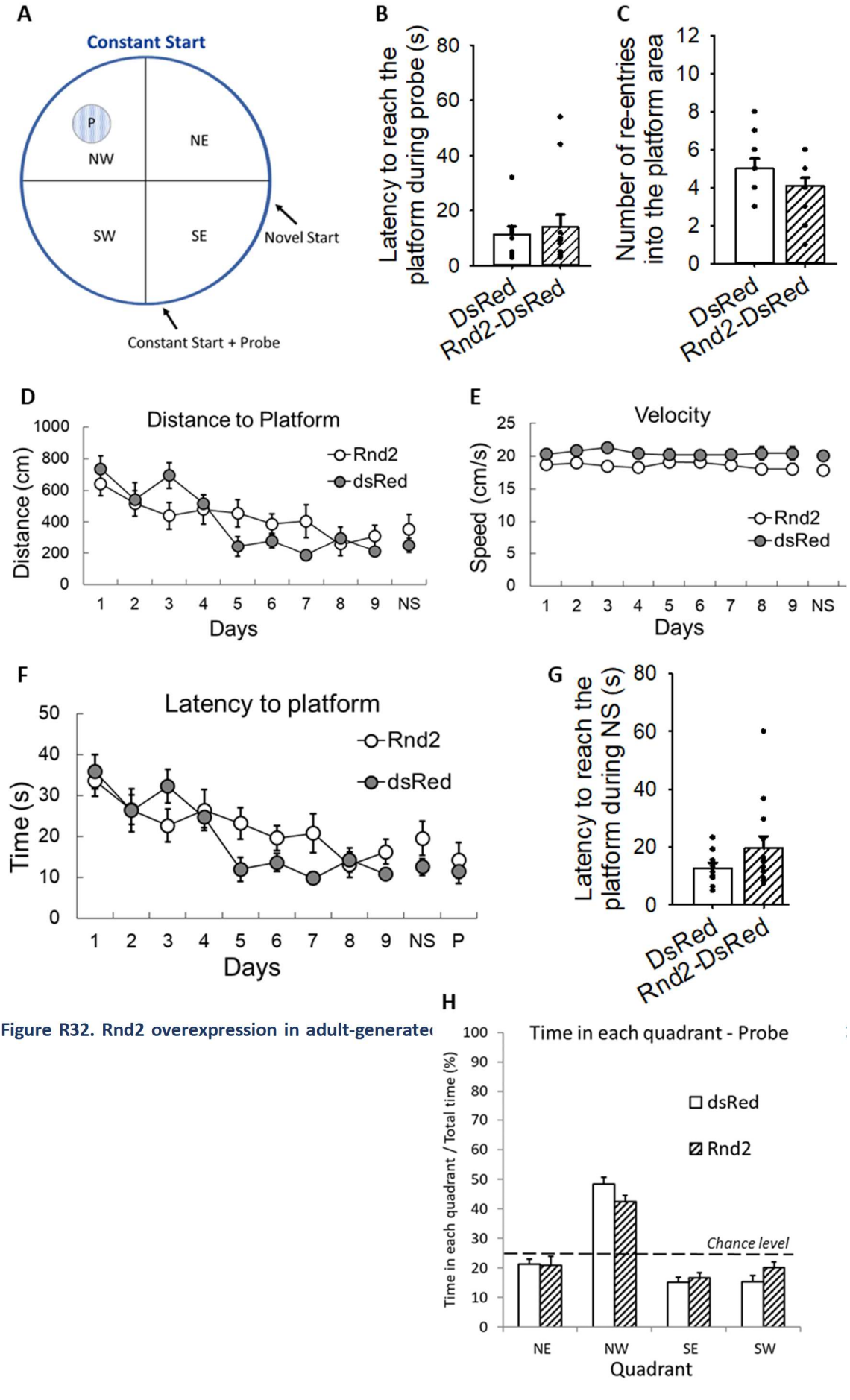


Figure R32. Rnd2 overexpression in adult-generate

MWM in CS training. A. Schematic representation of the experimental arena used during MWM with CS. The pool is divided in four quadrants: NW, NE, SW and SE. The target platform is always located in the NW quadrant. There is only one point of release for mice during training labelled as CS. Later, a novel start will be used when the training is done. **B.** Time to get to the platform during the probe in both of the experimental groups. **C.** Number of entries inside the platform area during the Probe. **D.** Total distance the animals of each experimental group swim each day of the learning process to reach the platform. **E.** Swimming velocity of the animals per day. **F.** Latency to reach the platform during the training days and also during the probe and the NS in both of the experimental groups. **G.** Time elapsed to reach the platform during the NS in both of the experimental groups. **H.** Percentage of time spent in each of the four quadrants during the Probe. Discontinuous line shows the chance level of spending time in a random quadrant. Two-Way ANOVA. No statistical differences were found in any of the tests. MWM: Morris Water Maze; CS: Constant Start; NW: North West; NE: North East; SW; South West; SE: South East; NS: Novel Start. Bars show mean ± SEM. Dots show individual data.

DISCUSSION

7. DISCUSSION.

7.1. CCI AS AN APPROPIATE MODEL OF TBI.

The efficacy of modelling TBI has been questioned on multiple occasions generating an intense debate. The heterogeneous nature of this pathology together with the extensive list of factors that may interfere during its development create a highly complicated scenario to replicate (Mckee & Daneshvar, 2015).

Instead of paralyzing in front of the magnitude of this problem, the scientific community has responded by creating a myriad of solutions adapted to each of the situations. Starting with *in vitro* models, we can try to simplify the most complex issue to the minimum unit. For example, studying the effect of compression over one specific cell type (Kane et al., 2011; R. F. M. Silva et al., 2006). By adding levels of complexity to this specific issue we can keep piling on useful information that helps us to build the bigger picture: what would happen in the same scenario to organotypic cultures? Or in living animals? What specific areas are the most affected ones? What are the different consequences over more severe or milder forms of injury?

In this same line, the Controlled-Cortical Impact model, which is the chosen one in the present study, is a smart solution that allows us to set specific boundaries in an animal model that can be customized at convenience. By choosing the area that will be injured, the depth and the velocity of the impact, and its dwelling time, we can very much model the severity of any TBI and replicate it under controlled conditions (Albert-Weissenberger & Sirén, 2010).

Another characteristic of TBI that has been found to be decisive for the progression of the pathology is the presence or absence of skull break. Apart from determining the previously mentioned parameters, the fact that the skull may crack during an injury adds another level of complexity to the issue. This is the reason why many studies resolve to use a Sham group as a control (Almeida-Suhett et al., 2014; Hall et al., 2005). Sham animals are subjected to a craniotomy. During this surgery, the part of the skull that is located right above the coordinates where the injury will take place is removed carefully without damaging the dura and later replaced back on (Romine et al., 2014). On the other hand, the animals named as Control in this study do not undergo any type of surgical procedure. By using a combination of both of these groups, we are able to extract more useful information. Not only can we determine the consequences of TBI over AHN, but we can also observe how much the craniotomy contributes

to those consequences. The level of inflammation and damage produced by the craniotomy has been very often underestimated since most studies only take the Sham group as a baseline and discard Control animals. By comparing all of these three groups, we can determine the repercussion and contribution of skull break into TBI to the whole aftermath of the injury.

7.2. NEURONAL ACTIVITY AFTER TBI: CONSEQUENCES ON THE HIPPOCAMPAL CIRCUITRY.

Hippocampal vulnerability towards TBI has been consistently reported (Gao et al., 2008b; Grady et al., 2003; Lowenstein et al., 1992; Witgen et al., 2005). Between the many effects of TBI in the hippocampus, a common one comprehends hilar neurons loss (Lowenstein et al., 1992). Although they comprise a small percentage of the whole hippocampal neurons, hilar neurons are an important component of the local inhibitory circuitry that modulates dentate granule cell excitability (Grady et al., 2003). Decreased hilar inhibitory neuron number may lead to increased DG net synaptic efficacy (Witgen et al., 2005). In this context, adult-born neurons play an important role in modulating activity at the circuitry level. For example, adult-born neurons innervate tens of basket interneurons which in turn inhibit hundreds of mature granule cells **(Fig. D1)** (Freund & Buzsáki, 1998). Newborn neurons are also known to innervate hilar mossy cells, which also activate many mature granule cells contralaterally. There are many published studies supporting a role of adult-born neurons in inhibiting recurrent network activity in the DG, likely through regulation of interneurons (Lacefield et al., 2012; Song et al., 2012).

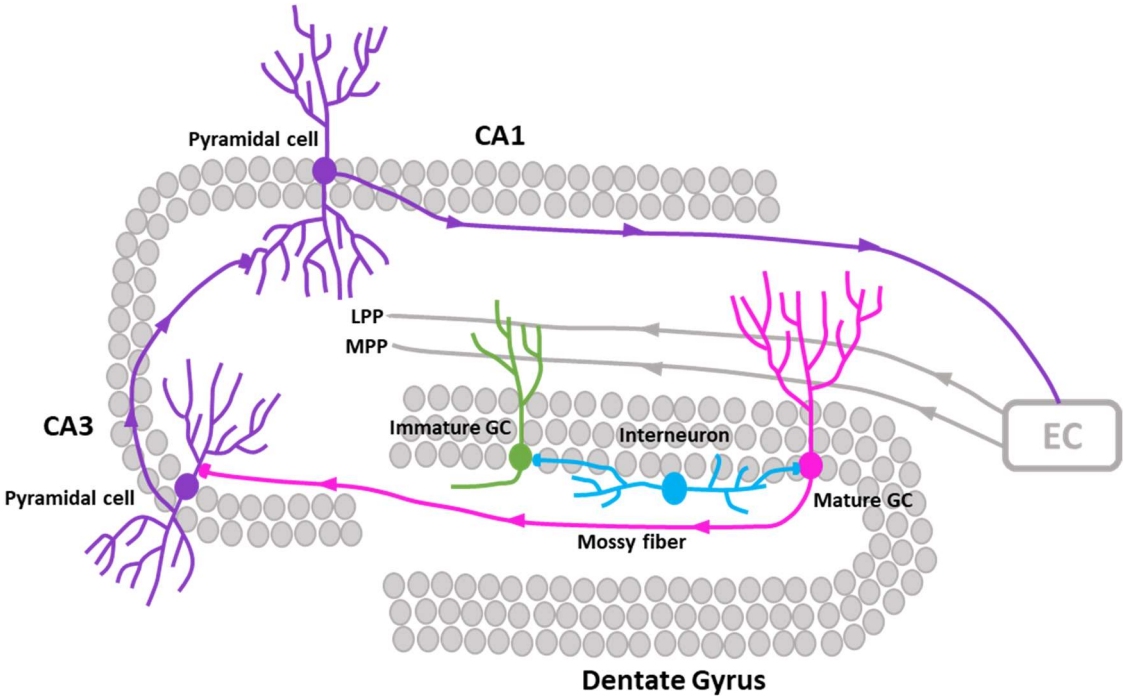


Figure D1. Diagram representing the trisynaptic circuitry in a transversal section of the hippocampus. Mature and newborn granule cells (pink and green respectively) are located in the GCL of the DG where they receive their major synaptic input from the Medial and Lateral Perforant Path (MPP and LPP) that originates from the Entorhinal Cortex (EC). These GC send their axons (mossy fibers) to the CA3 pyramidal cells which in turn send their axons to CA1 pyramidal cells. These CA1 pyramidal cells finally project back to the EC. Inhibitory interneurons (such as basket cells) provide a dense network of GABergic synaptic boutons within the GCL and SGZ, innervating both mature and newborn GCs.

In our study, we validate an increase in mature granule cells activity demonstrated by the enhancement in the c-fos expression in this population. In light of the above mentioned information, we can assume that this change in neuronal activity may have a variety of both causes and repercussions inside the hippocampal circuitry. Although we cannot make any claims on how this could affect the pre-existing population of adult-born neurons in the DG, we know that through the innervation of basket interneurons and hilar mossy cells, adult-born neurons are communicating with mature granule cells **(Fig. D1)** (Song et al., 2012). It is clear that the boost in neuronal activity could be altering the whole functioning of the hippocampal circuitry, including its effects in the adult-born neuronal population.

7.3. CORTICAL INJURY CONTRIBUTES TO THE PATHOLOGICAL TBI OUTCOME.

We consider important to include the conclusions of the present work in the context of an injured brain with multiple affected areas (either directly or indirectly). TBI produces an

immediate cell death due to mechanical forces. Furthermore, the progressive atrophy of surviving cells and/or white matter degeneration will later exacerbate the tissue loss (Gale et al., 1993, 1995; Lifshitz et al., 2007). To compensate this, the absent parenchyma is replaced with cerebrospinal fluid filling the generated cavity. This produces a hydrocephalia ex vacuo, but TBI may also lead to increased ventricular volume caused by what is known as compensatory atrophy. To facilitate comparisons among different degrees of lesions, the amount of tissue loss is often used as an objective assessment of injury severity (X. Wang et al., 2016). The lost volume is estimated by measuring its area in the brain sections together with the distance between them (Cavalieri Principle) (Henery & Mayhew, 1989; Michel & Cruz-Orive, 1988).

In our study, we establish the severity of the injury prior to the surgery instead of doing it post hoc. By adjusting the depth of the impact (1mm), its velocity (5m/s) and dwell time (600ms) we reproduce a moderate form of CCI. Cavity volume is not specifically measured, however, we could observe that it was concordant to other published models with similar severity (Villasana et al., 2015; X. Wang et al., 2016). In human patients, anatomical and response-dependent scales are used as a method to quantify severity after TBI (**see 3.2.2. Severity Measurement**). However, there is no such a thing for animal models. Severity, in this case, is set depending on the parameters of the model and later double-checked with the level of damage to the tissue.

It should be noted when interpreting the neurobehavioural outcome of these studies that the cortex is another vulnerable area for TBI. It modulates executive functions such as working memory, decision making, problem solving and mental flexibility (McAllister, 2011). Nevertheless, depending on the impacted area, the affected functions may change drastically. For example, injuries in the occipital cranial region can lead to long-lasting visual impairments after impairment of the occipital cortex (Frankowski et al., 2021). On the other hand, impact on the parietal region damaging the somatosensory cortex could lead to motor and sensory deficits (Carron et al., 2016).

7.4. THE INCREASE IN THE DB THICKNESS AS A CENTRAL FACTOR OF THE INJURY.

The vast majority of the GCL neurons are born after birth (Angevine, 1965; Bayer, 1980; Bayer & Altman, 1974). Postnatal neurogenesis follows a sharp peak early after birth and then it drops off later by the second week (Bayer & Altman, 1974). Once developmental neurogenesis is over, only adult neurogenesis remains active in the mouse hippocampus. The number of neurons that

are being added continuously to the brain due to this adult neurogenesis sharpy decreases in the first few weeks in young adults. As well as the number of added neurons, the occupied space by these neurons is also rapidly reduced and they hardly contribute to an increase in the total volume of the DG.

In our TBI model, we describe a progressive increase in the thickness of the DB of the GCL which becomes evident at 15 days post-injury and that remains present for 4 months. The timing of this event suggests that the observed variation could be due to the generation and integration of new neurons into the DG. On the other hand, the fact that the alteration is still present long-time after the injury suggests that a high percentage of these new neurons could be surviving and integrating into the circuitry after the initial period of cell death. We may also expect that the enlargement of the soma size observed in new neurons could also contribute to the DB thickness increase. However, the definitive cause for this increase in the thickness of the GCL is still not clear and we consider it could be the consequence of multiple factors converging after the injury.

It is important to take into account two characteristics of the CCI model when evaluating the changes in the DB thickness. First of all, the depth of the impact is set at 1mm from dura. This means that the directly impacted area is restricted to several cortical layers. Therefore, any alterations observed in the hippocampus will always be an indirect consequence of the mechanical forces. And second, the diameter of the impactor is set to 3mm. The area located just under this 3mm diameter will be the most affected one, placed immediately at the centre of the injury. We characterized the effects at close neuroanatomical level and we found a progressive trend in which the maximum damage was located in the area closest to the impact, decreasing progressively as we get further from the impacted area. **(Fig. D2)**. This is the main reason why we limited a very specific region where all studies were performed. The main effect of TBI over the DG is locally reduced to the impacted area and close surroundings.

The fact that the experimental group of animals with TBI shows an increased thickness of the DB (even though the affected area was a very limited one), could eventually lead to an increase in the total volume of the GCL. Therefore, we decided to reflect every result in terms of both cellular densities and total numbers in the analysed area in order to take into account the possible changes in volume of the DG **(Fig. D2)**. In general, changes in any population in terms of cellular density are correlated with changes in the total number of that population in the DG. The fact that the DB thickness is increased is not enough to alter the total number of the NSC or the immature neuron population in most of the cases. The only result in which DB thickness

seems to have an effect on is the total number of immature neurons 4mpTBI. In this case, there is a decrease in cellular density which is not reflected in total numbers: the total population of DCX^+ at this point is not significantly different among the three experimental groups **(Fig. R15 and Fig. R16)**. At this point the change in the DB thickness is enough to compensate for the decrease in immature neurons after TBI and therefore, the total number of the population is unaltered.

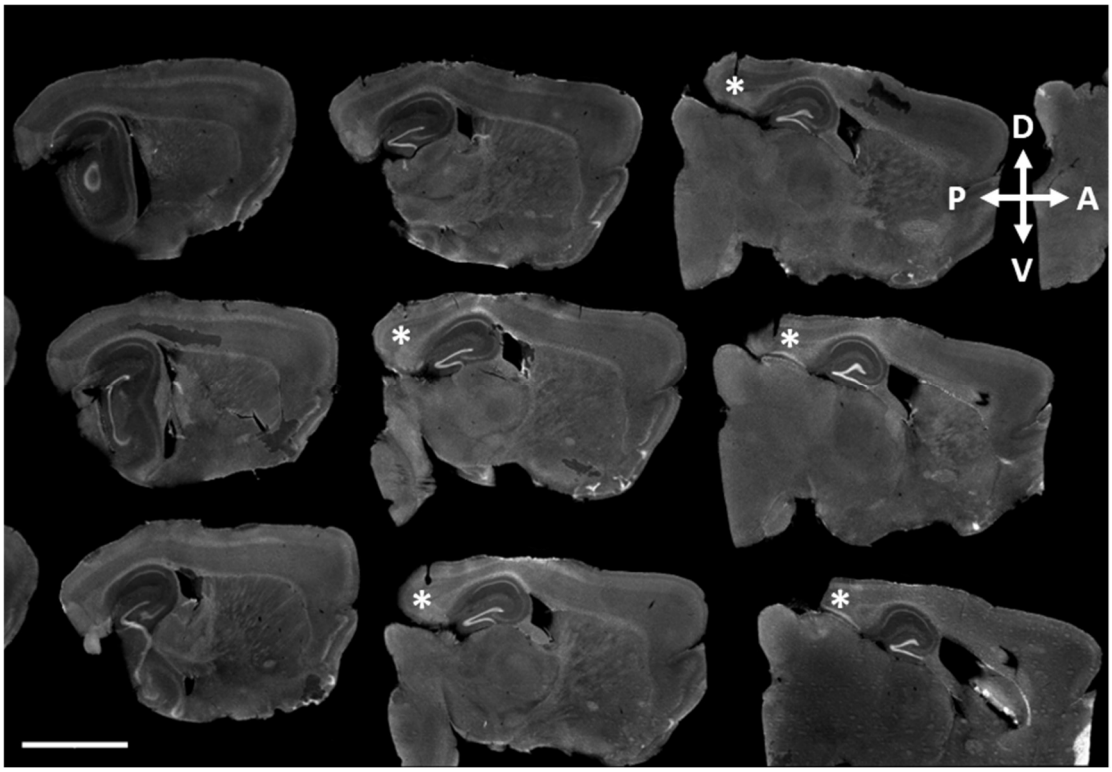


Figure D2. Illustration of a serial cut reconstruction of a 4mpTBI lesion in an injured brain. Left hemisphere is cut sagittally into 50µm thick slices and divided into six series. The image shows 6 slices of one series. (*) labels the mainly affected slices by the TBI, which comprehend the analysed area for the cellular studies. Scale bar is 3000µm. D: Dorsal, V: Ventral, P: Posterior, A: Anterior.

7.5. GLIAL CONTRIBUTION TO CELL PROLIFERATION AFTER TBI.

Proliferation in the hippocampus is executed by several different cell types: microglia, astrocytes, blood vessels and others. All of these cell types have been reported to activate and enter into cell division after injury (R. G. Mira et al., 2021). In our model, cell division is activated in the GCL in the acute phase after TBI persisting over time until 15dpTBI and then fading away.

We demonstrate the presence of proliferating cells after TBI by immunofluorescence against both Ki67 and BrdU, observing proliferating cells distributed throughout the whole GCL (both

Dorsal and Ventral Blades) and the SGZ. We also observe more proliferating cells in the Hilus and the ML. By 4mpTBI, cell proliferation is clearly higher in Control animals than in Sham but there is no difference with TBI. This suggests a possible effect of the surgical craniotomy over cell proliferation irrespective of the impact itself. Now, we will comment briefly on several events of different cell types that may lead to the general cell proliferation increase in the DG.

Astrocytes are a type of cell glia that produces astrogliosis during an injury. Astrogliosis is a process that has been widely documented after TBI of different severities (Chen et al., 2014; Luo et al., 2014; Mouzon et al., 2014). The formation of an astroglial scar is a protective mechanism to avoid spreading of secondary damage to other brain regions (J. R. Faulkner, 2004). The formation of this scar gives important inflammatory mediators to remove damaged tissue (Sofroniew, 2005). However, they could also exacerbate negative outcomes, for example contributing to excitotoxicity, spreading the damage to distal sites of the lesion, neuroinflammation and oedema.

Microglia are the resident macrophages of the central nervous system (CNS). Activated microglia release cytokines and other factors that may promote inflammation resulting also in further tissue damage (Bodnar et al., 2018). Microglia response can be observed chronically by weeks or months after brain injury (Henry et al., 2020). Furthermore, both glial responses to TBI are not isolated, but probably integrated and with each other. This issue has been barely studied but it would be interesting to know how astrocytes and microglia coordinate during injury response.

Another cell type present in the CNS are oligodendrocytes, which act as myelin producers, promoting axonal support. TBI produces oligodendrocyte apoptosis for up to 5 weeks after TBI (Dent et al., 2015) promoting demyelination and oligodendrocyte loss (Lotocki et al., 2011). Polydendrocyte (oligodendrocyte precursor cells, $NG2^{+}$ cells) proliferation has also been characterized after TBI (Kim et al., 2012; Mierzwa et al., 2015).

7.6. NSCs AFTER TBI.

Adult hippocampal neurogenesis is a process highly sensitive to different experiences like physical exercise, environmental enrichment or stress (Cope & Gould, 2019; Kempermann et al., 1998; Yun et al., 2016). Therefore, it represents an adaptive mechanism which responds to demands of the external environment. Neurogenesis and NSCs also respond to internal stimuli such as pathological conditions adapting and changing several of their intrinsic characteristics

like their expression markers, their morphology, their type of cell division and eventually even their pool size and their subsequent progeny. For all these reasons, it is fundamental to consider NSCs as an important part of the whole adult neurogenic process.

7.6.1. NSCs reactivity as a chronic trait of TBI.

NSCs conform a very heterogeneous population. This heterogeneity is reflected in different traits like their proliferating rate, markers expression, morphology and others (Ibrayeva et al., 2021). It should be considered, therefore, that TBI could modify differently the NSCs subpopulations depending on their vulnerability level, altering the dynamic of the whole pool and also their progeny.

The intrinsic changes of NSCs morphology and function after the exposure to pathological conditions lead to NSC reactivity in a similar manner to astrocytic reactivity. Astrocytes have also been described to respond to TBI by proliferating but also increasing their size. The hypertrophy in these cells has been related to aberrant neurogenesis in the hippocampus including ectopic growth and migration of newborn neurons (Robinson et al., 2016). When we measure the area occupied by GFAP in the GCL, we cannot discard that part of this increase may be due to astrocytes residing in this area together with the NSCs

We assessed the level of NSCs reactivity after TBI using two different parameters. The first one is the thickness of their primary processes. These processes show an increment in their thickness as early as 3dpTBI and it is conserved until at least 2mpTBI. By 4mpTBI, however, there are no significant differences between the experimental groups. The second parameter consists is the area occupied in the DG by GFAP which is significantly higher at least 3dpTBI compared to both Control and Sham animals.

NSCs become reactive in the acute phase after TBI **(Fig. D3)** and they remain in a reactive state for a very long time (2mpTBI), although seems to fade away with time (4mpTBI). It is important to notice that the level of reactivity measured by thickness of the primary processes also increases in Control and Sham animals, specifically in the last checked timepoint (4mpTBI) **(Fig. R7 H)**. The NSC pool is very dynamic and it changes throughout the lifespan of mice. A switch in this population in aged animals has already been described (Martín-Suárez et al., 2019a). The remaining NSCs in aged brains in Nestin-GFP mice show a more complex morphology correlating with the increase in NSC reactivity observed in the Control group (6 months old animals) and therefore, masking the persistence of reactivity after TBI. It should be noted that NSCs reactivity

is not just an effect taking place during the acute phase after TBI, but it also seems to be, at least to some degree, a chronic transformation. In other pathological conditions, such as epilepsy, once NSCs become reactive and start to divide, their daughter cell conserves the reactive phenotype (Sierra et al., 2015).

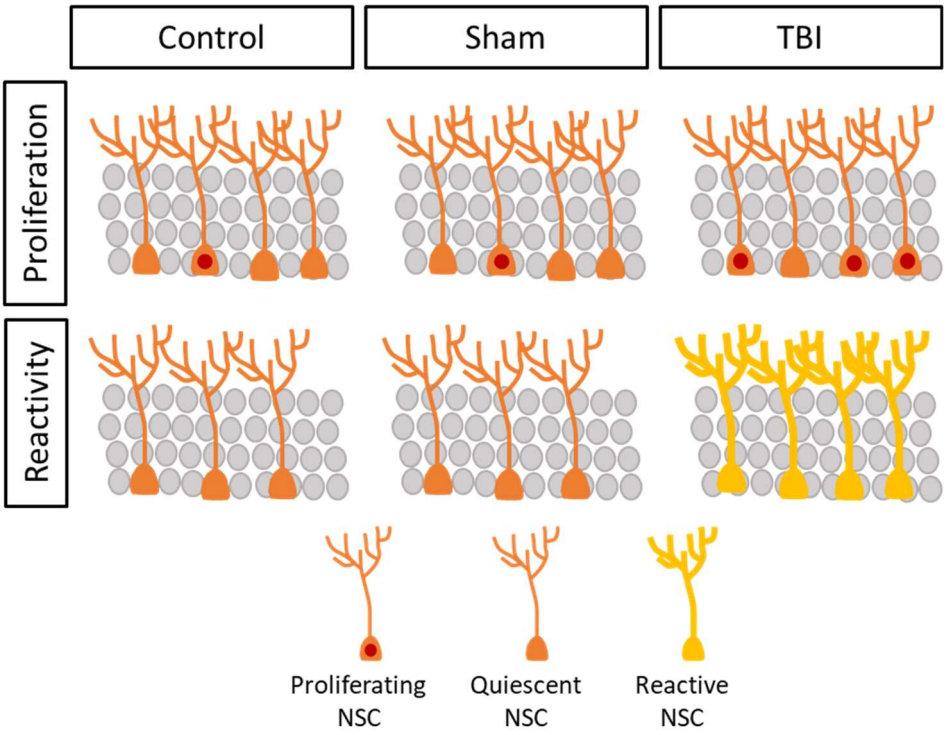


Figure D3. Diagram summarizing the described effects of TBI over NSCs during the acute phase. NSCs display an increased proliferative rate and reactivity 3dpTBI. This reactive state is manifested in the form of thicker primary processes and increased expression of GFAP in the GCL.

7.6.2. NSCs acute proliferation after TBI depends on multiple factors.

NSCs enhance their proliferation in the acute phase after TBI (3dpTBI), as we demonstrate using BrdU as a marker for cell division **(Fig.D3)**. Regarding previous publications there is a lot on controversy on whether TBI increases, decreases or does not affect NSC proliferation. In a publication in 2016, Wang and colleagues examine the effects on neurogenesis of three different models of CCI -mild, moderate and severe- by increasing the depth of the impact **(Fig. D4)**. They conclude that while mild TBI does not affect neurogenesis, moderate TBI promotes NSC proliferation but without increasing neurogenesis. Finally, only the severe model of TBI is able to enhance NSC proliferation, immature neuron number and mature neuron generation (X.

Wang et al., 2016). Therefore, severity seems to be directly related to the level of activation of the neurogenic cascade (the higher the damage, the more likely it is for neurogenesis to take place) **(Fig. D4)**. Another study carried out by using a Fluid Percussion Injury (FPI) model in rats focuses in the acute effect of the injury and the proliferative activity in the hippocampus in the first 48 h. They found in this interval of time a mainly gliogenic response. Using BrdU to label proliferation after injury and trace their cellular fate, they unravel that the predominant cell types making up for this proliferative response are microglia/macrophages and astrocytes (Chirumamilla et al., 2002). In a similar study, Gao and Chen, using a moderate CCI model in mice they find out that NSC proliferation is transiently increased shortly after the injury (48-72 hours) and the majority of the surviving progeny analysed at 5 weeks are astrocytes coming from NSCs. Thus, they conclude that this moderate CCI model enhanced proliferation of NSCs without significantly increasing neurogenesis in the adult hippocampus, suggesting the possibility of selective susceptibility of immature newborn neurons (Gao & Chen, 2013).

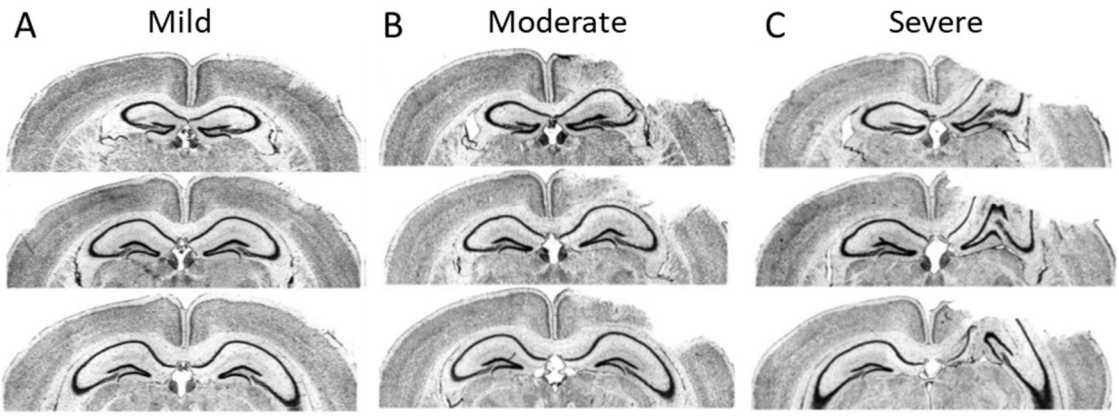


Figure D4. Different TBI severities from a CCI model varying injury depth shown by Nissl staining 48 hours after the impact. Serial coronal sections from mild **(A)** moderate **(B)** and severe **(C)** and TBI. Increasing levels of cortical disruption and cavity formation are obvious. Modified from (X. Wang et al., 2016).

The hypothesis behind the activation and proliferation of NSCs after TBI is related to the neural death that takes place in the hippocampus after the injury. Mossy cells, which reside in the hilar region of the hippocampus are glutamatergic and are known to form synapses with hilar inhibitory interneurons (Scharfman & Myers, 2013). Chemo-genetic activation of mossy cells causes GABA release (via stimulation of inhibitory neurons) onto NSCs, promoting a quiescent state. In contrast, mossy cell inhibition facilitates proliferation by reducing GABA release onto stem cells (Yeh et al., 2018). TBI is known to produce death of hilar GABAergic neurons (Lowenstein et al., 1992; Santhakumar et al., 2000; J. Zhao et al., 2018). Therefore, the loss of these inhibitory neurons is likely to remove the inhibitory constraint over NSCs and facilitate their proliferation after injury.

In conclusion, although many publications agree on an increase in AHN after TBI, which involves an activation of NSCs, there is still no consensus on this issue. What is clear is that there are many factors (i.e., the severity, the used model, the brain areas which area affected, the ratio of primary/secondary injury produced, etc) which could have an impact on the level of cell number driven by the neurogenesis after injury **(Fig. D5)**. In our hands, NSC proliferation boosts in the acute phase after the injury.

TBI model	Animal model	Time after injury	Effect over neurogenesis
CCI	Mice	1, 3, 7 days	Increased NSC proliferation
CCI	Mice	1-28 days	Transient increased proliferation. Decreased newborn neurons
CCI	Mice	1-8 weeks	Ectopic newborn neurons. Impaired dendritic arborization.
CCI	Mice	2, 4 weeks	Increased newborn neurons. Increased dendritic complexity
FPI	Mice	1, 7 days	Increased newborn neurons. Ectopic newborn neurons.
CCI	Rat	1-21 days	Transient increased proliferation. Increased newborn neurons
TBI	Human	< 1 day	Increased NSC proliferation
CCI	Mice	2 days	Increased proliferation. Severity-dependent increased newborn neurons
FPI	Rat	3, 7, 30, 90 days	Increased proliferation. Transient increased newborn neurons

Figure D5. Table summarizing the variability of the results of previous works of TBI over neurogenesis. Data illustrates the TBI and the animal model and the time after the impact when the histopathological characterization was performed (data from Bielefeld et al., 2019).

7.6.3. NSCs proliferation throughout time.

In our study, apart from characterizing the initial enhancement of NSC division and cell proliferation in the acute phase, we also check long-term effects after the injury. Quantifying the cellular density and total number of dividing NSCs (counted as Ki67+ NSCs), we observe an increase at 15dpTBI. This result would suggest a persisting activation of NSCs after the injury. However, calculating the proliferation index by dividing the proliferating NSCs by the total population of NSCs, we obtain a more precise idea of cell activation, and we can see how the previous differences vanish becoming just a trend **(Fig. R11-R13)**. Therefore, even if the total

NSC pool at this timepoint is slightly bigger in TBI animals, the proportion of the activated NSCs is the same as in Control and Sham conditions. By 2mpTBI there are no differences at all between the groups in terms of NSC proliferation and finally, by 4mpTBI there is a decreased density, total number and proliferative index of NSCs. Interestingly, at 4mpTBI, sham animals display a lower NSC proliferation. This decrease could be due to a long-lasting effect to the craniotomy which could be related to a certain amount of inflammation. Thus, NSCs proliferative potential could be impaired in the chronic stages. An increase in NSC proliferation could be followed by an early decrease and loss of the neurogenic capacity. The plastic heterogeneity of NSCs tends to contribute to their preservation during aging. Previous research show how after several cycles of cell activation, active NSCs disappear with age and are substituted by a more quiescent NSCs population (L. Harris et al., 2021; Ibrayeva et al., 2021). In agreement with these observations, in our laboratory, we previously found different ways of NSCs division over aging depending on the implicated subpopulation of NSCs (Martín-Suárez et al., 2019a). Extrapolating this to our results, we might think that TBI could also selectively impair specific NSCs subpopulations. What we can conclude at this point is that different subpopulations of NSCs in the hippocampus can, not only behave differently, but also change their dynamics with time accordingly to previous published data (Ibrayeva et al., 2021).

7.6.4. Progression of NSCs population throughout time after TBI.

Neurogenesis may be enhanced at different levels from the neurogenic cascade. Knowing that NSC proliferation is altered in our TBI model, we hypothesized that the NSC population could be exhausted over time due to its increase of proliferation leading to its depletion (Encinas et al., 2011a). Unexpectedly, we do not observe any changes in the size of the NSC pool throughout the studied timepoints. However, we observe a similar progression of the population in all of the groups: the age-related decrease. In conclusion, at the observed time points, TBI does not produce an early exhaustion of the NSC pool.

A possible explanation could be that the increase in NSC proliferation is only moderate and not high enough to produce a massive early exhaustion of the NSC pool. In other pathological models, NSC proliferative boost has already been characterized. For example, in a Mesial Temporal Lobe Epilepsy model (MTLE), using a similar methodological approach of intraperitoneal BrdU similar to the one used in this study, NSC proliferation raises to 15% compared to the basal level of 3-4% determined in Control animals (Sierra et al., 2015). In our

case, after TBI, NSC proliferation increases to a 7-8%. This means that we would need to analyse at a longer timepoint post-injury to be able to observe similar effects, if there were any.

Another explanation related to this issue is that TBI could have an effect over the NSC type of cell division. In the previously mentioned publication with the MTLE model, the depletion of the NSC pool is mainly caused by an increase in the percentage of symmetric division of NSCs which leads to the production of reactive astrocytes instead of neurons (Sierra et al., 2015). To characterize way may happen in this TBI model, we should check the number of generated neurons, reactive astrocytes and proportion of symmetric/asymmetric divisions in NSCs after TBI.

Other cell types that could be also contributing to the general boost of cell proliferation are the intermediate progenitors (ANPs). A study led by Neuberger and collaborators characterized the population of ANPs (as $Tbr2^+$ cells) and NSCs (as $Sox2^+$/$GFAP^+$ cells) at 3 days and 90 days after an injury. They found that the proliferation rate and the total population of ANPs increased by 3 days and then dropped by 90 days in the injured group. They observe similar effects for DCX^+ immature neurons. However, no changes are observed in the NSC population: the proliferation index is not measured and the only reduction in the population is due to the age difference between the two timepoints and therefore unrelated to the Sham/TBI groups (Neuberger et al., 2017). In this case, the neurogenic burst taking place shortly after the impact is considered as a negative consequence since it impairs the long-term neurogenic capacity by ablating dividing ANPs. In order to try to correct these changes they attempt to reduce this neurogenic burst and return it to physiological levels instead of completely ablating neurogenesis as it was traditionally done. By using a vascular endothelial growth factor receptor 2 (VEGFR2) antagonist, they are able to reduce this abrupt increase in neurogenesis and conserve the dividing population of ANPs for a longer time (Neuberger et al., 2017). The effects of VEGFR2 suppression are not only restricted to the level of neurogenesis, but they also reduce seizure susceptibility detected in the animals (Neuberger et al., 2017), although this has not been necessarily linked directly to newborn neurons.

In our TBI model, NSC preservation seems to be prioritized allowing early proliferation without producing a depletion of the pool.

7.7. PROGRESSION OF THE NEWLY GENERATED NEURONS THROUGHOUT TIME AFTER TBI.

The increase in NSC proliferation (and maybe also ANPs) translates in a higher cellular density of DCX-expressing immature neurons 15dpTBI **(Fig. D6)**. Previously, at 3dpTBI no changes in this population were observed, suggesting that the present population of developing neurons in the DG at the moment of the injury was not significantly damaged. The increase in immature neurons is maintained at least 2mpTBI, pointing to a sustained in time potentiation of neurogenesis. However, by 4 months, the density of developing neurons in the DB of the DG is actually lower in TBI animals compared to both Sham and Controls, coinciding with the decrease in the NSC proliferating index **(Fig. D6)**. When we normalise these results by the total volume of the DG, the decrease of the number of DCX-expressing cells at 4mpTBI is no longer observed. Due to the increase in the total volume of the DG, even though the cellular density decreases, the total number of these cells is not significantly different respect to Sham or Control animals. Therefore, in our hands, neurogenesis is boosted after TBI for several weeks until at least 2mpTBI. By 4 months, both NSC proliferation and density of immature neurons in the DG decrease, but not in total numbers.

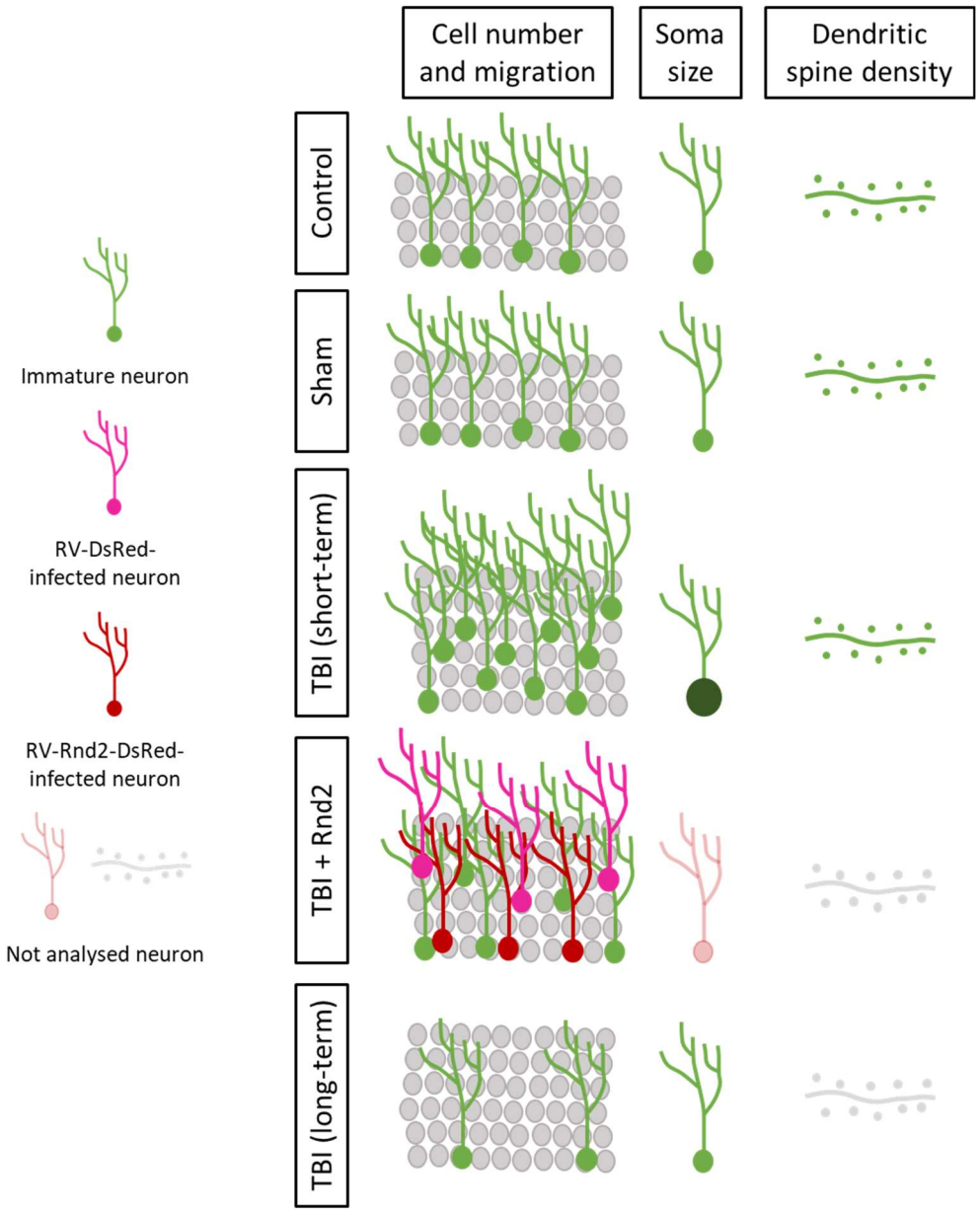


Figure D6. Diagram summarizing the described effects of TBI over adult-generated newborn neurons. In the short-terms after the injury, neurogenesis is increased in numbers and new neurons show an aberrant migration and bigger soma size. Rnd2 overexpression in these neurons is able to correct the aberrant migration. However, the increased soma size phenotype is not replicated under these conditions. Long time after the injury, the number of newly-born neurons is decreased but their migration and cell body size is normal. Throughout all the timepoints, the dendritic spine density remains unaltered.

As it was mentioned previously, published results regarding neurogenesis after TBI are contradictory. For example, Rola et al. described in a study a loss of SGZ precursor cells which translates into a later decrease in new neuron production in a moderate model of CCI. In this case, the decrease is attributed to a higher sensitivity of these cells to the injury (Rola et al., 2006). In other works, like the one from Ga & Chen, instead of pointing to the high sensitivity of precursor cells, they present newborn neurons as the main vulnerable population (Gao & Chen, 2013). In the scenario they present, in order to successfully repair damage to the brain caused

by TBI, additional events would be required to increase not only proliferation of NSCs, but also to prevent newborn neurons from dying. Other studies claim that this difference on the production of new neurons after injury may be related to the type of damage produced. By using a mild model of TBI, which is focused in producing specifically axonal injury (Traumatic Axonal Injury, TAI), Bye and colleagues propose that despite inducing a robust stimulation of cell proliferation in the neurogenic regions, TAI does not result in an ultimate increase in the number of new granule neurons (Bye et al., 2011) unlike it would happen in other focal TBI models (Sun et al., 2005, 2007; Yu et al., 2008). Now, in the opposite direction, several studies provide evidence of an increase of adult hippocampal neurogenesis after TBI. Braun and collaborators were one of the first ones to report this in 2002 using βIII-Tubulin expression as a marker for newborn neurons. Newly-generated neurons in the DG contain high amounts of βIII-Tubulin in their microtubules which plays a major role in the polymerization and depolymerization of the intracellular cytoskeleton, key for cell migration in neuroblasts (Braun et al., 2002; Moskowitz & Oblinger, 1995). Further maturation into differentiated neurons is accompanied with a sharp decline of βIII-Tubulin expression. In this study, they describe particularly numerous neurogenic cells in the dentate gyrus which localization coincides with the areas of the tissue that show a better recovery (Braun et al., 2002).

It could be argued, however, that the study does not follow these newborn cells throughout their maturation process and is only focused in the increase in neuroblasts. Nevertheless, there are other studies which use the expression of the neuronal nuclear protein (NeuN) together with the incorporation of BrdU to investigate the actual production of mature neurons that get incorporated into the hippocampal circuitry (Sun et al., 2005). For example, Sun et al., 2005 investigate age-related differences in the cell proliferative response of the rat brain following TBI, determining that the production of neurons is two times higher in the juveniles compared to adults. Interestingly, the increase of neuronal differentiation is linked to a larger pool of proliferative cells consequence of the injury (Sun et al., 2005). The period of neuronal formation and integration would strongly correlate with the data of Prins and Hovda obtained with the Morris Water Maze demonstrating that juvenile rats experience fewer cognitive deficits and have a higher index of recovery than adult rats (Prins & Hovda, 1998). Integrating the results of both works, we may deduce that injury-induced neurogenesis may try to replace the damaged neurons in an attempt to the repair of damaged neuronal circuits.

Most of these published works take advantage of the use of different cellular markers that are associated to diverse maturing neuronal states (βIII-Tubulin, DCX…) (Braun et al., 2002; Ibrahim et al., 2016). However, these limitations have perpetuated the doubt on whether the changes

on the level of neurogenesis after injury are real or they could be misread with changes in neuronal maturation that may delay or shorten the neurogenic process. To tackle this issue, Yu and colleagues designed a strategy where they used a Nestin-delta-HSV-TK transgenic mice together with a ganciclovir treatment (Yu et al., 2008). Specifically, NSCs proliferation was ablated during 4 weeks before the injury (the necessary time for the DCX-expressing immature neurons present in the DG to maturate and stop expressing DCX). The end of ganciclovir treatment was followed by CCI and seven days later, more DCX-expressing cells were observed in the SGZ and the GCL in injured brains compared to uninjured controls. These results suggest that injury enhances neurogenesis by stimulating proliferation of NSCs, but it does not accelerate neuronal maturation (Yu et al., 2008).

7.8. ADULT-BORN NEURONS PLAY AN IMPORTANT ROLE IN THE HIPPOCAMPAL CIRCUITRY DURING THEIR DEVELOPMENT.

Adult-born neurons up to 7 weeks after generation have different electrophysiological properties than their developmentally-born partners in the DG. The newly generated neurons predominately activate inhibitory neurons that regulate the activity of mature granule neurons within the DG (Drew et al., 2016). However, once they reach their full maturation, these cells are no longer distinguishable from the other resident granule neurons. It has been proposed that their differential maturating properties, may play a role in how they participate or regulate specific behaviours (Gage, 2000). Most of the changes reported after TBI produced in these newborn neurons coincide with that critical period of maturation and therefore they could have consequences at the future function.

7.8.1. Adult-generated neurons display morphological alterations after TBI.

During neurogenesis, an extensive and dynamic remodelling of the cell cytoskeleton is promoted, especially during the development and maturation of neuroblasts. When studying possible morphological changes in newborn neurons after TBI we found an important increase in the cell body size.

The area of the cell body in developing neurons of the hippocampus increases after TBI as early as 3dpTBI **(Fig. D6)**. By this time, the DCX-expressing cells present in the DG are cells that were generated previously to the injury. Therefore, TBI causes alterations not just in neurons that would be born after the injury but also in the ones that were maturating at the time of the damage. The vulnerability of developing neurons to injury has been repeatedly demonstrated (Grady et al., 2003; Kotapka et al., 1991; Witgen et al., 2005). However, the increase in the soma size is still present 15dpTBI and even 2mpTBI. The DCX-expressing cells at these times were certainly born after the impact. However, neurons that are developing 4mpTBI show average cell body area compared to Controls and Shams **(Fig. D6)**. In order to make sure whether new neurons born after TBI were affected by this alteration we sought to use RV-labelling for these cells. Using this tool, we observed that neurons born at the moment of the injury showed an increased cell body diameter 3 weeks after the impact compared to Control animals.

The increase in the cell body size could be due to a general enlargement of the soma or to a more specific expansion of the nucleus. To determine a potential enlargement of the nucleus, a specific marker for it should be measured. With our data, we cannot make any claims about the main reason of this alteration. If the increase was specifically due to the soma, an osmotic imbalance could be favouring the entrance of extracellular liquid, therefore producing an increase of the volume occupied by the cytoplasm. However, if the change was due to an enlargement of the nucleus, as the images of DCX^+ cells suggest, more specific mechanisms regarding the compaction of the DNA or structural changes in the intermediate filaments should be considered.

The alteration of morphological development of newborn neurons has been previously described after injury. Some studies report a selective increase in the number of branches close to the soma, more dendritic branches and increased total dendritic length (Villasana et al., 2015). However, others studies using a similar methodology show a decrease in dendritic complexity (Ibrahim et al., 2016). Thus, there is contradictory data describing the cellular alterations produced after TBI and more studies are necessary to complete this cellular characterization.

7.8.2. Alterations in migration of adult-generated neurons may alter the overall hippocampal circuitry.

Another alteration that has been often reported in different models of TBI is a misplacement of newborn neurons along the GCL (Ibrahim et al., 2016; Shapiro, 2017; Villasana et al., 2015). A number of these neurons born after TBI often appear placed at the outer GCL when they are physiologically always located in the inner-third of the GCL (Sun et al., 2015b). This indicates an aberrant cell migration. In our model, we reproduced it at 15dpTBI and even 2mpTBI suggesting that the changes that cause this phenotype prevail in the DG for, at least, 6-8 weeks, impairing therefore an important amount of newly-generated neurons **(Fig. D6)**. To corroborate the aberrant migration, we used a RV-vector to infect and label proliferating cells at the time of the impact. The NSCs or ANPs that were activated during the impact and started proliferating would likely become reactive as it was previously demonstrated in this study and later end up giving rise to aberrantly migrating RV-labelled neurons.

How adult-born neurons reach their final destinations in the DG in a question still unanswered. It is known, however, that the radial glial scaffold forming the DG plays an important role during granule neuron migration. Studies in mutants with defects in reelin signalling cascade provide evidence for a role of this radial glial scaffold in the proper migration of granule cells. In these mutants, the radial glial scaffold is altered to a varying extent and this is accompanied by migration defects of GCs (Förster et al., 2002, 2006; K. H. Weiss et al., 2003). Another factor which has been reported to be required for relaying positional signals is Disrupted-In-Schizophrenia 1 (Disc1). However, its participation is related to a general aspect in maturation and it does not intervein directly in neuronal migration (Duan et al., 2007). Other centrosome-associated proteins such as PCM-1 and the length of cilia have been reported to affect indirectly to the migration of subventricular DCX^+ cells (Pineda et al., 2013). However, if DCX^+ cellular primary cilia or subgranular DCX^+ cell migration could be affected remains to be elucidated.

There are a bunch of processes and specific properties of neurons which are directly linked to their proper regulated migration: arrangement of axon projections in layers, orientation, target connection, neuronal subtype specification or activity-dependent refinement are some examples (Hatten, 1999, 2002). Bringing cells into appropriate spatial relationships and establishing the bases for the subsequent wiring of neural circuitries is fundamental. As a consequence, wrong cellular positioning could end up producing inappropriate connections with nearby targets in the pre-existing neural networks and performing inappropriate functions. Thus, aberrant migration has been shown to cause certain disorders such as epilepsy, dyslexia and schizophrenia (Marín & Rubenstein, 2003).

Moreover, the organization of granule neurons into laminae in the DG allows them to receive different neural input from different regions of the brain (Hatten, 2002). The commissural/associational fibres, which originate from the mossy cells in the contralateral and ipsilateral hilar region, terminate in the inner Molecular Layer (iML), whereas entorhinal fibres, which originate from projection neurons in the superficial layers of the entorhinal cortex, terminate in the outer Molecular Layer (oML) (99, 100). This lamina-specific termination of fibre projections results in a subcellular specificity of afferent input **(Fig. D7)**. This way, commissural/associational fibres contact only proximal dendritic segments, whereas entorhinal fibres make synaptic contacts with only the distal dendritic segments of the same target cells **(Fig. D7 B)**. Following this trend, the dendrites of granule neurons located at the first inner third of the GCL mainly distribute at the ML immediately adjacent to the GCL (iML). They form synapses with and receive input from ipsilateral and contralateral hippocampal commissural and associational fibres. While the dendrites of granule neurons located in the outer two-thirds of the GCL mainly distribute at the oML and form synapses with fibres from the enthorhinal cortex **(Fig. D7 C)** (Blackstad, 1956; Förster et al., 2006; Zimmer, 1971). Putting together this information with our results, we hypothesize that, while in healthy conditions newly born neurons are located near the SGZ and receive input from hippocampal commissural and associational fibers, after TBI, these same neurons would be more likely to receive input from the entorhinal cortex instead. Improper integration of these neurons could lead into functional impairment of learning and memory, one common complaint from patients who have suffered TBI (Piolino et al., 2007; Russell & Smith, 1961).

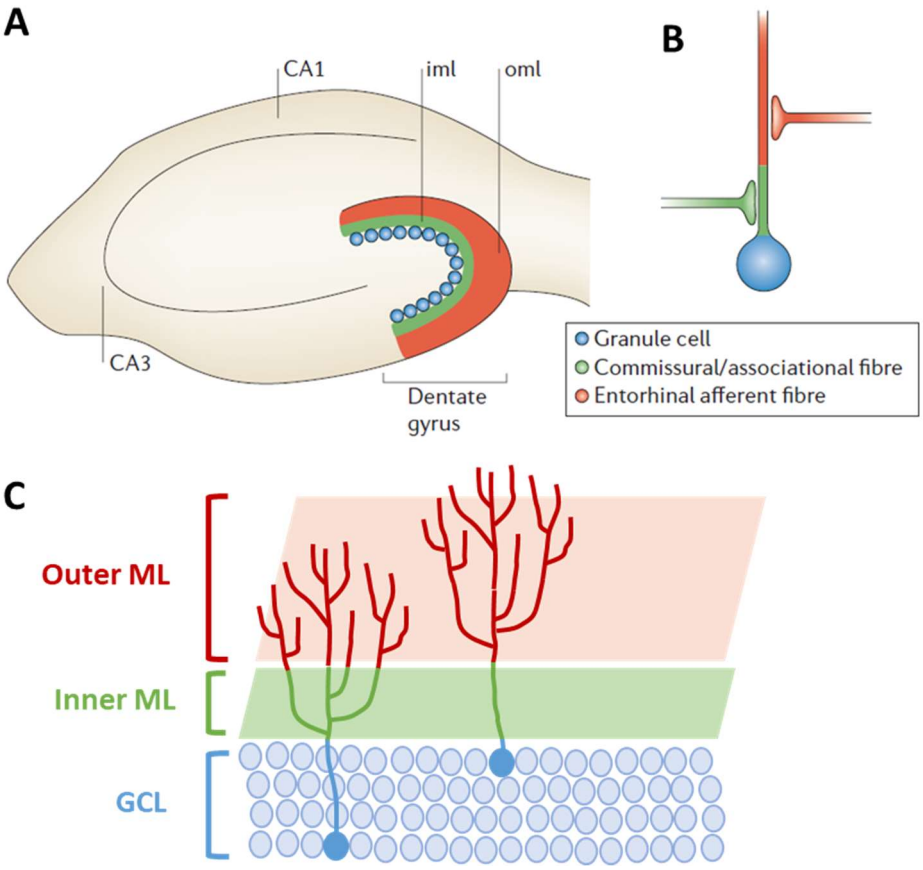


Figure D7. Afferent fibre projections terminate in distinct hippocampal layers which depend directly on its proper lamina-specific organization. **A.** The architecture of the hippocampus is organized in a layer-specific manner. The DG includes a compact GCL containing the cell bodies of mature and immature GCs and the ML is mainly formed by the projection of these and other cell types being innervated by the fibres arising from distant locations. This ML can be divided into an inner (iML) and an outer (oML) region. **B.** The different subcellular compartments of the GCs are innervated by fibres coming from distinct sources depending on their location on the ML. The proximal segment of the GC is mainly in contact with commissural/associational fibres while the more distant segment communicates with entorhinal afferent fibres. **C.** The specific location of the GCs through the GCL may alter the specificity in the innervation of different fibres to distinct subcellular compartments. Modified from (Förster et al., 2006). iML: inner Molecular Layer; oML: outer Molecular Layer; GCL: Granule Cell Layer; DG: Dentate Gyrus; GC: Granule Cell.

7.8.3. Dendritic spine density is not altered in neurons born during TBI.

Alterations in neuronal maturation could also impair the process of integration into the hippocampal network. A fundamental step within this process is the establishment of dendritic spines. Because dendritic spines are the major post-synaptic sites of granule neurons to receive glutamatergic inputs, the timing of spine formation may reflect one of the important physiological transitions during their maturation. Thus, the number and shape of these dendritic

spines are indicative of the connectivity of these cells. Spine growth starts around 16 days after birth in adult-born neurons and it continues to increase for weeks (Denoth-Lippuner & Jessberger, 2021; Zhao, 2006). By the time neurons are three weeks old, they already show a dendritic density of around 1 spine/µm in physiological conditions. Therefore, analysing dendritic spine density at 3wpTBI (equivalent to 21 days), gives us very precise information about their degree of integration into the hippocampal circuitry: a delay in maturation would be shown by an absence of dendritic spines while a quicker integration would be evidenced by a higher dendritic spine density. With our model, neurons born during the impact showed no alteration of dendritic spine density at this point, indicating that there is not an alteration of the timing of integration of these neurons in the circuitry at 3wpTBI **(Fig. D6)**. This results are in correlation with the few previous studies on dendritic spine density after TBI, which does not seem to be affected (Villasana et al., 2015).

Electrophysiological properties of these neurons and their integration into the hippocampal circuitry have been previously discussed. Synaptic innervation and proper balance between excitatory and inhibitory synaptic activity has been reported (Villasana et al., 2015). Still, the fact that there could be an increase in the number of new neurons being incorporated in a short time to the hippocampal circuitry could be having long term effects, even if these cells integrate properly. Brain injury leads to loss of hilar inhibitory and excitatory neurons but not granule cells (Santhakumar et al., 2000; Toth et al., 1997). Since newborn neurons are mainly excitatory, they are unlikely to compensate for the loss of hilar inhibitory regulation after TBI. Instead, they are adding more excitatory neurons to a network that has already been challenged by a massive neuronal death and are therefore likely to contribute to an increased dentate excitability (Gupta et al., 2012; Neuberger et al., 2017).

7.9. HIPPOCAMPAL-RELATED FUNCTIONAL CONSEQUENCES OF TBI.

Some studies have also addressed the functional consequences of either impairing or promoting neurogenesis in the injured brain. Enhancing neurogenesis through the dispensation of different pro-neurogenic compounds: administration of nitric oxide donor DETA/NONOate (Lu et al., 2003); infusion of S100B (Kleindienst et al., 2005) or administration of erythropoietin, antidepressants, statins or others (Blaya et al., 2014; Han et al., 2011; Lu et al., 2005; Yoshimura et al., 2003) is generally associated with improved neurological outcome (improved

performance in Morris Water Maze and other tests). Of course, the possibility that these treatments have effects in other cell types cannot be ruled out and therefore the improvement would not be necessarily directly related to neurogenesis. On the other hand, disruption of neurogenesis after TBI results in exacerbated hippocampus-dependent tasks dysfunction (Blaiss et al., 2011; Sun et al., 2015a).

In our study we present the possibility of aberrant neurogenesis being the cause for some of the detrimental alterations in the hippocampus after TBI. Therefore, enhancing or ablating neurogenesis as a tool to restore the neurogenic potential in the adult mice brain would be a too simplistic solution to a very much complex issue. Instead, we lean towards the hypothesis that promoting physiologically-like neurogenesis in order to generate healthy neurons would be a much appropriate solution to try to prevent hippocampal damage. We, therefore, went deep into the study of a molecular candidate for the previously characterized alterations to try to prevent aberrant migration and increased soma size in newborn neurons after TBI.

7.10. RND2 AS A CANDIDATE TO RESTORE PHYSIOLOGICALLY-LIKE NEUROGENESIS AFTER TBI.

Cell migration requires a dynamic plasma membrane remodelling (Keren, 2011). Furthermore, the soma size and the translocation of neuronal nucleus after neurogenesis require dynamic changes of the cell cytoskeleton (Taverna & Huttner, 2010). The Rho family of small GTPases are an extensive family of key regulators of cell cytoskeleton in various cell types, including neurons, (Ridley, 2001a). For this reason, we considered them as good candidates that could regulate the altered properties of new neurons after TBI. We focused specifically in Rnd2 since, out of the many members of this family, only this one is selectively enriched in the adult SGZ (Miller et al., 2013).

Rnd2 expression is mainly found in endosomes (Fujita et al., 2002; Tanaka et al., 2002; Wakita et al., 2011) and endosomal signalling has been strongly associated to cell migration (Schiefermeier et al., 2011). Its cellular distribution makes it difficult to detect it at a protein level using commercial antibodies. Measuring its mRNA levels could be an indirect approximation to identify changes over its expression. Unfortunately, we lacked transgenic animals that allowed us to identify and isolate newly-generated neurons specifically, to study the expression of Rnd2. Even if mRNA levels remained unchanged, post-transcriptional modifications and interaction with other molecules could be the reason of possible unexpected alterations, which again would

not be reflected in the level of expression. Therefore, the main objective in this study is not to determine whether Rnd2 is responsible for the alterations in newborn neurons in the hippocampus after TBI. Instead, our interest is getting to know whether, regardless of being the main cause or not, Rnd2 may play a role by increasing its levels and could revert the aberrant phenotype of newborn immature neurons after TBI.

Under physiological conditions, Rnd2 is cell-intrinsically required for the survival and maturation (migration, neurite outgrowth) of adult-born neurons (Kerloch et al., 2021). We will now discuss these functions separately.

7.10.1. Potential role of Rnd2 in cell soma size.

Previous studies reported, an increase of cell body size in absence of Rnd2, specifically in newborn neurons (Kerloch et al., 2021). Because a similar phenotype is observed after TBI, we hypothesize that increasing the expression of Rnd2 in neurons born during TBI, could revert the phenotype. Unexpectedly, the soma size increase -which was previously described using DCX as a maker and then reproduced with RV-Venus infection- was not then replicated with the RV-(Rnd2)-DsRed construction. Control animals injected with the RV-DsRed showed a similar soma size when compared to Sham and TBI injected with the same RV. Future insight into the repercussion of this specific RV in cell dynamics would be necessary to identify the cause of the absence of the increased-soma phenotype in this case.

Nevertheless, when we compare DsRed-injected (control RV) with the Rnd2-DsRed injected (Rnd2 overexpressing) groups in general, we can observe a tendency. However, Rnd2 overexpression in these cells does not translate into a significant decrease in their cell body area at 15 days post-infection. It is important to note that the increment in the cell body size after the deletion of Rnd2 in physiological conditions has only been detected in 21 days old neurons, suggesting that maybe the absence of this protein is required for a longer time to provoke measurable change over time.

7.10.2. Rnd2 expression is sufficient to correct the aberrant migration of newborn neurons after TBI.

On the other hand, the alteration of cell migration after TBI is correctly reproduced using the RV-(Rnd2)-DsRed injection strategy. DsRed-injected animals in combination with TBI showed an

abnormal migration through the GCL, higher than DsRed + Sham and DsRed + Control conditions. Labelling the specific cell population to track is an elegant and precise approximation to study the alteration in cell migration. When Rnd2 is overexpressed in cells after TBI, the excessive cell migration is reduced to an equivalent level observed in Control animals **(Fig. D6)**. Moreover, all the other animal groups also show a decrease of migration when Rnd2 overexpression is compared within the same groups (Control + DsRed vs Control + Rnd2-DsRed and Sham + DsRed vs Sham + Rnd2-DsRed). All these results suggest that the role of Rnd2 in controlling neuronal migration in physiological conditions is related directly to its expression. In the opposite, the lack of Rnd2 provokes an excessive migration (Kerloch et al., 2021).

At intracellular signalling level, Rnd2 promotes migration partially through the inhibition of RhoA. However, the RhoA inhibition does not seem to be involved in Rnd2-mediated neuronal survival (Kerloch et al., 2021; Pacary et al., 2011). On the other hand, Rnd2 produces an accumulation of F-actin in neuronal processes as well as in the cell body. Actin dynamics also have been reported to be important for cell migration (Schaks et al., 2019). It is possible that the accumulation of F-actin upon Rnd2 knock down (Pacary et al., 2013) together with the plasma membrane trafficking could impair normal cell migration. This suggests that different roles of Rnd2 could converge in the observed phenotype, although intracellular signalling pathways mediating Rnd2 action, which are poorly understood, may vary (Azzarelli et al., 2015).

In physiological conditions, AHN is considered beneficial for the individual and so are the stimuli that enhance it. Promoting neurogenesis results in an improvement of functional hippocampal-related tasks, as it happens with physical exercise or environmental enrichment (Hodge et al., 2008; Lee et al., 2000; Nilsson et al., 1999). These external stimuli promote neurogenesis without altering any of the properties of the generated neurons. However, whether enhancing AHN in pathological conditions is beneficial or detrimental is still unknown. While some consider that it could work as a mechanism of brain repair and regeneration, others doubt of the ability of the new neurons developing under altered conditions to properly integrate and accomplish their function (Yu et al., 2016).

In this study we propose that the level of neurogenesis and the quality of the newly-generated neurons are two separated matters. Not every stimulus promoting neurogenesis is able to provide functional new neurons, and therefore the enhancement of neurogenesis should not always be considered as beneficial. We present here an approach to maintain the potentiation of neurogenesis after TBI but correcting the alterations of the neurons generated after the

injury. Instead of focusing in the increase of neurogenesis after the impact, we aim to assure a proper development and integration of the new neurons.

7.11. EFFECTS OF RND2 OVEREXPRESSION IN ADULT-GENERATED NEURONS IN ANIMAL BEHAVIOUR.

Rnd2 role is not limited to survival and maturation of adult-born neurons. It is also critical for the control of anxiety-like behaviour (Kerloch et al., 2021). The deletion of Rnd2 in adult newborn neurons impacts anxiety-like behaviour while depression-like behaviour is not affected. Neither are the reference memory and behavioural pattern separation. It still remains to be unravelled whether the key reason for this behaviour abnormalities is due to the death of newborn neurons, their mispositioning, their abnormal morphology or all of these traits together.

In our study we next focus in the behavioural effects of overexpressing Rnd2 in physiological conditions. By increasing the level of this protein in a significant number of newly generated neurons, we aim to assess if there are any behavioural changes on hippocampal-related tasks.

It is important to remark that only animals that showed a level of infection that was considered enough to produce a functional change in hippocampal tasks that involved newly generated neurons were considered for the experiments. Those showing little to no cells infected were discarded. When quantifying the number of labelled neurons, we could observe that the animals injected with Rnd2-DsRed displayed an increase number of infected neurons in general. This result would correlate with the hypothesis that Rnd2 overexpression promotes neuronal survival as opposite to what happened with Rnd2 depletion, where neurons died in higher numbers during their development (Kerloch et al., 2021). Nevertheless, the title of these two viral solutions were not equivalent complicating the estimation of the precise absolute quantification of infected cells. New experiments would be necessary to determine if this increase in infected cells in the Rnd2 groups is due to the higher viral title or an increase in neuronal survival directly produced by the protein.

7.11.1. Rnd2 overexpression in adult-generated neurons does not affect exploratory behaviour.

The most widely accepted behavioural test to determine exploratory alterations is the OF test. As it was expected, no major differences were found between DsRed and Rnd2-DsRed-injected mice. In previous studies, it was demonstrated that specific ablation of adult-born hippocampal neurons does not affect the simplest forms of spatial knowledge (Gray & McNaughton, 1983; O'Keefe & Nadel, 1978) and the habituation to novel environments is neither impaired after neurogenesis inhibition (Dupret et al., 2008).

7.11.2. Rnd2 overexpression in adult-generated neurons decreases anxiety-like behaviour only in very specific conditions.

Next, we moved to the anxiety-like tests. The ET has been used to identify increases in anxiety-like behaviour (Whitney, 1970). The main parameter to measure this behaviour is the latency to first emerge from the tube. Considering that animals with an increased fear of exposed bright environments would take longer time to leave from the tube than Control healthy animals, our hypothesis is that animals with an overexpression of Rnd2 in their adult-born hippocampal neurons would display a decreased anxiety-like behaviour, contrary to what occurs when Rnd2 is deleted. Detecting a decrease in anxiety would mean identifying animals that leave the protective environment faster than controls (usually less than five seconds of time). Considering the narrow window of time, measuring a potential reduction is complicated. We conclude that this test is not the optimal to identify subtle decreases in anxiety-like behaviours. Furthermore, the other parameters measured during the test like the total time inside the tube and the number of re-entries may be confusing to interpret since they can either be read as anxiety (going back into the tube) or exploratory behaviours (investigating the only new object present in the arena). For all these reasons, we complemented the analysis using other complementary and more specific tests.

One of the most commonly used tests is the EPM, widely used to characterize the increase in anxiety-like behaviour in mice (Kerloch et al., 2021). In our case, no differences were found when overexpressing Rnd2 in the EPM. However, it should be considered, due to the characteristics of the maze, switching to a different type to identify milder differences. For example, in our maze the open arms had no walls and the closed arms had walls that were transparent. This trait influences the result in two different ways. First of all, it is harder for mice to identify the fact that there are walls, so the environment could still be perceived as dangerous, just like in the

open arms, therefore minimizing the difference. And second, the light condition that is used in this test is usually very high in the open arms and then darker in the closed arms. Dark walls help this by creating shadow in the closed arms. That is not the case in our experiments where open arms are just as bright as the closed ones and then again, perceived like just as dangerous. Finally, we conclude that EPM does not show any differences in anxiety-like behaviour in animals overexpressing Rnd2 in newborn neurons. However, it should be considered to repeat this experiment particularly using a different type of maze that increases the differences between closed and open arms.

Finally, another test considered the most specific one to identify anxiety-like behaviour is the light/dark test. In this case, mice overexpressing Rnd2 bilaterally in adult-generated hippocampal neurons shows a decreased anxiety-like behaviour by spending a bigger amount of time in the big bright room and re-entering more often than DsRed injected animals **(Fig. D8)**. However, no difference is found in the latency to first escape from the bright room. The difference between what is characterized as a dangerous and protective environment is very polarized so that even if the differences between the groups are subtle, they can still be identified.

In previous studies, when neurogenesis was specifically inhibited by ablating hippocampal NSCs, anxiety was impaired, as demonstrated by bad performances in all of the above mentioned tests: ET, EPM and LD (Revest et al., 2009). During this experiments, almost complete ablation of neurogenesis is achieved. In our case, however, we start from an unknown effect on neurogenesis. Rnd2 overexpression is hypothesized to alter survival, morphology and migration of new neurons rather than directly ablating cell numbers. Our results show that anxiety-like behaviour is decreased only in a context where the dangerous/not dangerous environment are very polarized, suggesting that Rnd2 overexpression might contribute to some degree for a proper development of these new neurons.

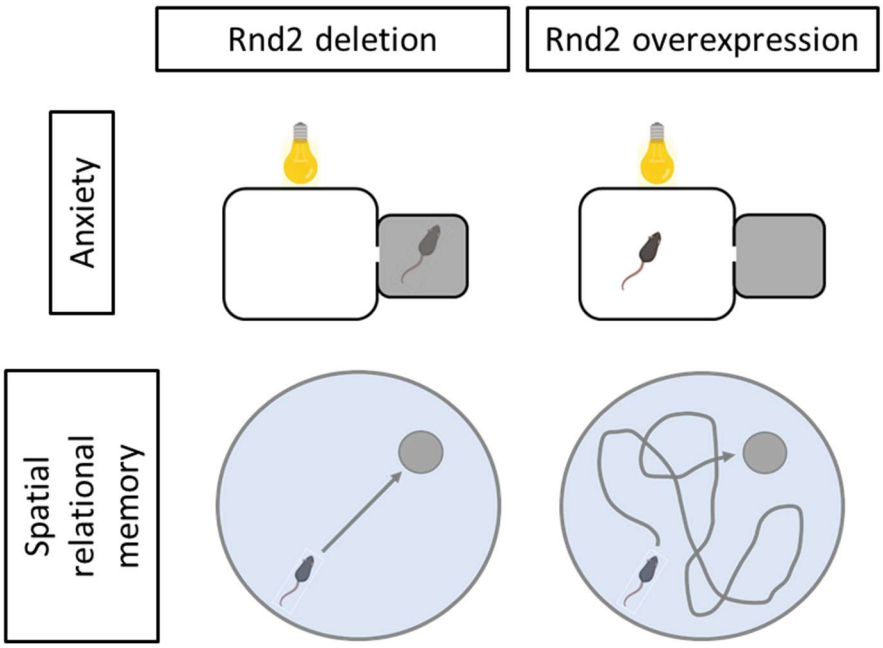


Figure D8. Diagram summarizing the described effects of Rnd2 loss of function and gain of function in adult-born neurons over behaviour. While the deletion of the gene produces increases in anxiety-like behaviour, it does not affect the spatial learning and memory capacity. Meanwhile, the overexpression of Rnd2 in adult-born neurons generates decreases in anxiety-like behaviour and mildly impairs spatial relational memory, but it has no effects on the learning process.

7.11.3. Depression-like behaviour is not altered by Rnd2 overexpression in adult-generated hippocampal neurons.

One of the most impeding behavioural alteration apart from anxiety is depression. Depression is another task which has been suggested to be strongly regulated by adult-born hippocampal neurons (Y. Li et al., 2008; Santarelli et al., 2003; Surget et al., 2008). In our ST and FST, no major differences were determined in depressive-like behaviour when overexpressing Rnd2 in these neurons. A tendency for Rnd2 overexpressing mice to take a bit more time in developing immobility during the FST was identified, though. During the second minute of this test, DsRed-injected mice spent more time immobile than their Rnd2-injected partners. It is unclear however how relevant this difference may be, since none of the major parameters showed any differences: total immobility time or latency to immobility. In general, no major differences were identified in depression-like behaviour in mice overexpressing Rnd2 in adult-born neurons.

It should be mentioned, however, that the link between depression and neurogenesis is still unclear. Many studies relate the use of antidepressant with an increase in hippocampal neurogenesis and an improvement in hippocampal-related tasks. However, there is not clear

evidence proving a causal relationship between alterations in neurogenesis and depression (Santarelli et al., 2003; Surget et al., 2008).

7.11.4. Spatial relational memory is mildly impaired after Rnd2 overexpression in adult-born neurons.

Water maze is another test in which inhibition of neurogenesis in the DG selectively impairs spatial relational memory while simpler forms of spatial navigation are spared (Dupret et al., 2008).

Initially, mice were tested in a hippocampal-dependent procedure. Here, they are required to develop an allocentric mapping strategy consisting in learning of the positional relationships among multiple independent environmental cues (spatial relational memory). This relational representation is needed to support the flexible use of learned discriminative cues in novel situations and consequently necessary to solve the task. In our case, animals are able to properly learn the location of the platform, as demonstrated by the evolution during the training sessions, which is equivalent in both experimental groups. The latency to reach the platform during the Probe is not affected either. Nevertheless, during the probe, Rnd2-overexpressing mice spent less time in the target quadrant (where the platform should be) than the DsRed-injected animals. This alteration could be reflecting a defect in memory recall or a change in flexibility since these animals switch rapidly their searching strategy instead of remaining in the area where the platform used to be located **(Fig. D8)**. Our results suggest that Rnd2 overexpression in newborn neurons alters to some extent spatial relational memory without completely impairing it, in a similar manner as what happens with anxiety. However, more experiments should be considered to fully corroborate these changes.

7.11.5. Hippocampal-independent memory is not affected by Rnd2 overexpression in adult-born neurons.

For the characterization of hippocampal-independent procedure (Constant Start), mice are able to use an egocentric strategy consisting of, for example, an association of an invariant configuration of spatial cues to the escape platform (place learning). No alterations were identified during the learning process, nor during the Probe after the training proving that Rnd2 does not affect hippocampal-independent learning tasks in correlation with our previously

obtained data. Then, at the end of this test the platform was moved to a different location (Novel Start) and the animals were still able to find it successfully. The training for this procedure was carried out after the Variable Start training, therefore, mixed strategies may be present in these animals. New configuration for the trainings should be considered for future experiments.

As an important note, it has been previously suggested that memory formation and anxiety could rely on different areas of the hippocampus. Several studies show complementary information in this matter when they ablate either the dorsal or the ventral hippocampus and check for different behavioural tasks **(Fig. D9)**. The dorsal subregion, defined as 50% of hippocampal volume starting at the septal pole (Bannerman et al., 1999), has a preferential role in spatial learning and memory while the ventral region, defined as 50% of hippocampal volume starting at the temporal pole, may have a preferential role in anxiety-related behaviours **(Fig. D9)** (Gray & McNaughton, 2007). There are clear differences in afferent and efferent connectivity along the septotemporal extent of the hippocampus that support this (Hughes, 1965; Nadel, 1968; Stevens & Cowey, 1973). Dorsal hippocampus receives most of the highly pre-processed visuo-spatial information from sensory modalities (M.-B. Moser & Moser, 1998). Meanwhile, the ventral region projects to the prefrontal cortex (Goldman-Rakic et al., 1984; Jay & Witter, 1991) and it is closely connected to the bed nucleus of the stria terminals and the amygdala (Henke, 1990; Krettek & Price, 1977) as well as other subcortical structures which are associated with the hypothalamic pituitary-adrenal (HPA) axis (Jacobson & Sapolsky, 1991; Siegel & Tassoni, 1971). The strong connectivity between ventral hippocampus and both the hypothalamus and the amygdala, makes it tempting to propose a role for the ventral subregion in fear and/or anxiety.

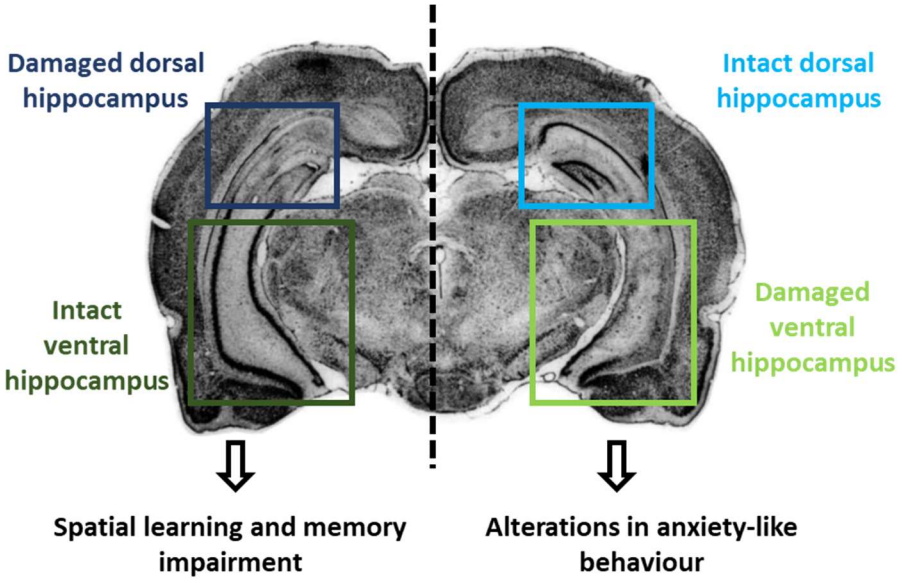


Figure D9. Coronal section showing cell loss 7 days after a unilateral dorsal hippocampal lesion on the left side and a unilateral ventral hippocampal lesion on the right side. Modified from (Bannerman et al., 1999).

Altogether, we can hypothesize then that altering neurogenesis at different locations within the hippocampus could produce changes in specific tasks while not in others. In our hands, Rnd2 overexpression in new neurons produces what could be considered as an improvement (decrease) in anxiety-like behaviour while spatial relational memory is slightly damaged. This raises new questions on whether the expression of one specific gene could have opposed effects depending on the area of the hippocampus that is affected by it and therefore the functions depending on that area.

CONCLUSIONS

8. CONCLUSIONS.

CHARACTERIZATION OF NSCs AND NEUROGENESIS AFTER TBI.

1. **Moderate CCI recapitulates the characteristics of other similar-severity TBI models.**
   - 1.1. Moderate CCI enhances neuronal hyperactivation in the DG during the acute phase after the injury.
   - 1.2. Moderate CCI produces a chronic increase in the thickness of the DB of the DG, but induces no changes in the VB, pointing to a very locally-restricted effect of the injury.
   - 1.3. Cell proliferation increases throughout the GCL in the acute phase after moderate CCI.
2. **The NSC population undergoes several different changes regarding their activation and morphology from the acute until the chronic stages after TBI.**
   - 2.1. NSCs become reactive shortly after TBI changing their morphology and their expression of certain markers.
   - 2.2. NSCs get activated and increase their proliferation in the acute phase after TBI.
   - 2.3. The proliferation rate of NSCs decreases with time until it becomes lower than control age-matching mice.
   - 2.4. The activation of NSCs after TBI does not involve a faster depletion of the pool.
3. **Neurogenesis is altered after TBI in terms of both the quantity of the newly-generated neurons and their maturating process.**
   - 3.1. The immature neuronal population becomes expanded after TBI but its density decreases in the chronic phase.
   - 3.2. These new neurons born after TBI show abnormalities in their soma size and their migrating pattern through the GCL.
   - 3.3. Neurons born specifically during the acute phase of TBI resemble the previously described alterations as the general population of immature neurons during their development in terms of cell body size and migration.
   - 3.4. Neurons born during the acute phase of TBI, however, show no alterations in their dendritic spine density.

POTENTIAL OF RND2 TO CORRECT TBI ALTERATIONS IN NEWBORN NEURONS.

4. **Rnd2 is an appropriate molecular target to correct the alterations produced by TBI in newborn neurons.**

   4.1. Rnd2 overexpression in adult-born neurons does not allow to mimic the changes in cell body size and therefore is not a good model to correct this specific alteration.

   4.2. Rnd2 overexpression in adult-generated neurons is enough to correct their aberrant migration after TBI.

ROLE OF RND2 IN NEWBORN NEURONS REGARDING MOOD-CONTROL AND SPATIAL MEMORY.

5. **Rnd2 is a potential candidate for reducing anxiety-like behaviour and improving spatial learning and memory in physiological conditions.**

   5.1. Rnd2 overexpression in adult-generated neurons does not alter exploratory behaviour in mice during OF.

   5.2. Rnd2 overexpression in adult-born neurons does not affect anxiety-like behaviour during the ET or EPM, however, it decreases anxiety-like behaviour during the LD test.

   5.3. Rnd2 overexpression in adult-born hippocampal neurons does not affect depressive-like behaviour reflected in the ST or the FST.

   5.4. Rnd2 overexpression in newborn neurons does not alter spatial learning during a spatial navigation hippocampal-dependent task but it impairs memory recall.

   5.5. As expected, Rnd2 overexpression in newborn neurons does not alter spatial navigation in a hippocampal-independent task.

BIBLIOGRAPHY

9. BIBLIOGRAPHY.

A.

Adamchik, Y., Frantseva, M. V., Weisspapir, M., Carlen, P. L., & Perez Velazquez, J. L. (2000). Methods to induce primary and secondary traumatic damage in organotypic hippocampal slice cultures. *Brain Research Protocols*, *5*(2), 153-158. https://doi.org/10.1016/S1385-299X(00)00007-6

Adams, J. H., Doyle, D., Ford, I., Gennarelli, T. A., Graham, D. I., & Mclellan, D. R. (1989). Diffuse axonal injury in head injury: Definition, diagnosis and grading. *Histopathology*, *15*(1), 49-59. https://doi.org/10.1111/j.1365-2559.1989.tb03040.x

Aimone, J. B., Deng, W., & Gage, F. H. (2011). Resolving New Memories: A Critical Look at the Dentate Gyrus, Adult Neurogenesis, and Pattern Separation. *Neuron*, *70*(4), 589-596. https://doi.org/10.1016/j.neuron.2011.05.010

Akasu, T., Muraoka, N., & Hasuo, H. (2002). Hyperexcitability of hippocampal CA1 neurons after fluid percussion injury of the rat cerebral cortex. *Neuroscience Letters*, *329*(3), 305-308. https://doi.org/10.1016/S0304-3940(02)00707-3

Albert-Weissenberger, C., & Sirén, A.-L. (2010). Experimental traumatic brain injury. *Experimental & Translational Stroke Medicine*, *2*(1), 16. https://doi.org/10.1186/2040-7378-2-16

Alder, J., Fujioka, W., Lifshitz, J., Crockett, D. P., & Thakker-Varia, S. (2011). Lateral Fluid Percussion: Model of Traumatic Brain Injury in Mice. *Journal of Visualized Experiments*, *54*, 3063. https://doi.org/10.3791/3063

Almeida-Suhett, C. P., Prager, E. M., Pidoplichko, V., Figueiredo, T. H., Marini, A. M., Li, Z., Eiden, L. E., & Braga, M. F. M. (2014). Reduced GABAergic Inhibition in the Basolateral Amygdala and the Development of Anxiety-Like Behaviors after Mild Traumatic Brain Injury. *PLoS ONE*, *9*(7), e102627. https://doi.org/10.1371/journal.pone.0102627

Altman, J. (1962a). Are New Neurons Formed in the Brains of Adult Mammals? *Science*, *135*(3509), 1127-1128. https://doi.org/10.1126/science.135.3509.1127

Altman, J. (1962b). Autoradiographic study of degenerative and regenerative proliferation of neuroglia cells with tritiated thymidine. *Experimental Neurology*, *5*(4), 302-318. https://doi.org/10.1016/0014-4886(62)90040-7

Altman, J., & Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *The Journal of Comparative Neurology*, *124*(3), 319-335. https://doi.org/10.1002/cne.901240303

Alvarez-Buylla, A., & García-Verdugo, J. M. (2002). Neurogenesis in Adult Subventricular Zone. *The Journal of Neuroscience*, *22*(3), 629-634. https://doi.org/10.1523/JNEUROSCI.22-03-00629.2002

Ampuero, E., Jury, N., Härtel, S., Marzolo, M.-P., & van Zundert, B. (2017). Interfering of the Reelin/ApoER2/PSD95 Signaling Axis Reactivates Dendritogenesis of Mature Hippocampal Neurons: REELIN SIGNALING MAINTAINS ADULT NETWORK STABILITY. *Journal of Cellular Physiology*, *232*(5), 1187-1199. https://doi.org/10.1002/jcp.25605

Anderson, R. W. G., Brown, C. J., Blumbergs, P. C., McLean, A. J., & Jones, N. R. (2003). Impact Mechanics and Axonal Injury in a Sheep Model. *Journal of Neurotrauma*, *20*(10), 961-974. https://doi.org/10.1089/089771503770195812

Angevine, J. B. (1965). Time of neuron origin in the hippocampal region. An autoradiographic study in the mouse. *Experimental Neurology. Supplement*, Suppl 2:1-70.

Arnould, A., Dromer, E., Rochat, L., Van der Linden, M., & Azouvi, P. (2016). Neurobehavioral and self-awareness changes after traumatic brain injury: Towards new multidimensional approaches. *Annals of Physical and Rehabilitation Medicine*, *59*(1), 18-22. https://doi.org/10.1016/j.rehab.2015.09.002

Ashkenazi, A., & Dixit, V. M. (1998). Death Receptors: Signaling and Modulation. *Science*, *281*(5381), 1305-1308. https://doi.org/10.1126/science.281.5381.1305

Atkins, C. M., Chen, S., Alonso, O. F., Dietrich, W. D., & Hu, B.-R. (2006). Activation of Calcium/Calmodulin-Dependent Protein Kinases after Traumatic Brain Injury. *Journal of Cerebral Blood Flow & Metabolism*, *26*(12), 1507-1518. https://doi.org/10.1038/sj.jcbfm.9600301

Atkins, C. M., Oliva, A. A., Alonso, O. F., Pearse, D. D., Bramlett, H. M., & Dietrich, W. D. (2007). Modulation of the cAMP signaling pathway after traumatic brain injury. *Experimental Neurology*, *208*(1), 145-158. https://doi.org/10.1016/j.expneurol.2007.08.011

Azouvi, P. (2000). Neuroimaging correlates of cognitive and functional outcome after traumatic brain injury: *Current Opinion in Neurology*, *13*(6), 665-669. https://doi.org/10.1097/00019052-200012000-00009

Azouvi, P., Couillet, J., Leclercq, M., Martin, Y., Asloun, S., & Rousseaux, M. (2004). Divided attention and mental effort after severe traumatic brain injury. *Neuropsychologia*, *42*(9), 1260-1268. https://doi.org/10.1016/j.neuropsychologia.2004.01.001

Azzarelli, R., Guillemot, F., & Pacary, E. (2015). Function and regulation of Rnd proteins in cortical projection neuron migration. *Frontiers in Neuroscience*, *9*. https://doi.org/10.3389/fnins.2015.00019

Azzarelli, R., Pacary, E., Garg, R., Garcez, P., van den Berg, D., Riou, P., Ridley, A. J., Friedel, R. H., Parsons, M., & Guillemot, F. (2014). An antagonistic interaction between PlexinB2 and Rnd3 controls RhoA activity and cortical neuron migration. *Nature Communications*, *5*(1), 3405. https://doi.org/10.1038/ncomms4405

B.

Bannerman, D. M., Yee, B. K., Good, M. A., Heupel, M. J., Iversen, S. D., & Rawlins, J. N. P. (1999). Double dissociation of function within the hippocampus: A comparison of dorsal, ventral, and complete hippocampal cytotoxic lesions. *Behavioral Neuroscience*, *113*(6), 1170-1188. https://doi.org/10.1037/0735-7044.113.6.1170

Barker, J. M., Wojtowicz, J. M., & Boonstra, R. (2004). Where's my dinner? Adult neurogenesis in free-living food-storing rodents: Neurogenesis in food-storing rodents. *Genes, Brain and Behavior*, *4*(2), 89-98. https://doi.org/10.1111/j.1601-183X.2004.00097.x

Bauer, S., & Patterson, P. H. (2005). The cell cycle–apoptosis connection revisited in the adult brain. *Journal of Cell Biology*, *171*(4), 641-650. https://doi.org/10.1083/jcb.200505072

Bayer, S. A. (1980). Development of the hippocampal region in the rat I. Neurogenesis examined with3H-thymidine autoradiography. *The Journal of Comparative Neurology*, *190*(1), 87-114. https://doi.org/10.1002/cne.901900107

Bayer, S. A., & Altman, J. (1974). Hippocampal development in the rat: Cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation. *The Journal of Comparative Neurology*, *158*(1), 55-79. https://doi.org/10.1002/cne.901580105

Bayer, S. A., Yackel, J. W., & Puri, P. S. (1982). Neurons in the Rat Dentate Gyrus Granular Layer Substantially Increase During Juvenile and Adult Life. *Science*, *216*(4548), 890-892. https://doi.org/10.1126/science.7079742

Beccari, S., Valero, J., Maletic-Savatic, M., & Sierra, A. (2017). A simulation model of neuroprogenitor proliferation dynamics predicts age-related loss of hippocampal neurogenesis but not astrogenesis. *Scientific Reports*, *7*(1), 16528. https://doi.org/10.1038/s41598-017-16466-3

Beckervordersandforth, R., Ebert, B., Schäffner, I., Moss, J., Fiebig, C., Shin, J., Moore, D. L., Ghosh, L., Trinchero, M. F., Stockburger, C., Friedland, K., Steib, K., von Wittgenstein, J., Keiner, S., Redecker, C., Hölter, S. M., Xiang, W., Wurst, W., Jagasia, R., … Lie, D. C. (2017). Role of Mitochondrial Metabolism in the Control of Early Lineage Progression

and Aging Phenotypes in Adult Hippocampal Neurogenesis. *Neuron*, *93*(3), 560-573.e6. https://doi.org/10.1016/j.neuron.2016.12.017

Bell, C. C. (1994). DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. *JAMA: The Journal of the American Medical Association*, *272*(10), 828. https://doi.org/10.1001/jama.1994.03520100096046

Belmont, A., Agar, N., Hugeron, C., Gallais, B., & Azouvi, P. (2006). Fatigue et traumatisme crânien. *Annales de Réadaptation et de Médecine Physique*, *49*(6), 283-288. https://doi.org/10.1016/j.annrmp.2006.04.017

Benedictus, M. R., Spikman, J. M., & van der Naalt, J. (2010). Cognitive and Behavioral Impairment in Traumatic Brain Injury Related to Outcome and Return to Work. *Archives of Physical Medicine and Rehabilitation*, *91*(9), 1436-1441. https://doi.org/10.1016/j.apmr.2010.06.019

Benveniste, E. N. (1998). Cytokine Actions in the Central Nervous System. *Cytokine & Growth Factor Reviews*, *9*(3-4), 259-275. https://doi.org/10.1016/S1359-6101(98)00015-X

Berg, A. T., Berkovic, S. F., Brodie, M. J., Buchhalter, J., Cross, J. H., van Emde Boas, W., Engel, J., French, J., Glauser, T. A., Mathern, G. W., Moshé, S. L., Nordli, D., Plouin, P., & Scheffer, I. E. (2010). Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia*, *51*(4), 676-685. https://doi.org/10.1111/j.1528-1167.2010.02522.x

Bergami, M., Masserdotti, G., Temprana, S. G., Motori, E., Eriksson, T. M., Göbel, J., Yang, S. M., Conzelmann, K.-K., Schinder, A. F., Götz, M., & Berninger, B. (2015). A Critical Period for Experience-Dependent Remodeling of Adult-Born Neuron Connectivity. *Neuron*, *85*(4), 710-717. https://doi.org/10.1016/j.neuron.2015.01.001

Bergami, M., Rimondini, R., Santi, S., Blum, R., Götz, M., & Canossa, M. (2008). Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. *Proceedings of the National Academy of Sciences*, *105*(40), 15570-15575. https://doi.org/10.1073/pnas.0803702105

Bergsneider, M., Hovda, D. A., Lee, S. M., Kelly, D. F., McARTHUR, D. L., Vespa, P. M., Lee, J. H., Huang, S.-C., Martin, N. A., Phelps, M. E., & Becker, D. P. (2000). Dissociation of Cerebral Glucose Metabolism and Level of Consciousness During the Period of Metabolic Depression Following Human Traumatic Brain Injury. *Journal of Neurotrauma*, *17*(5), 389-401. https://doi.org/10.1089/neu.2000.17.389

Bergsneider, M., Hovda, D. A., McArthur, D. L., Etchepare, M., Huang, S.-C., Sehati, N., Satz, P., Phelps, M. E., & Becker, D. P. (2001). Metabolic Recovery Following Human Traumatic Brain Injury Based on FDG-PET: Time Course and Relationship to Neurological Disability:

Journal of Head Trauma Rehabilitation, *16*(2), 135-148. https://doi.org/10.1097/00001199-200104000-00004

Bergsneider, M., Hovda, D. A., Shalmon, E., Kelly, D. F., Vespa, P. M., Martin, N. A., Phelps, M. E., McArthur, D. L., Caron, M. J., Kraus, J. F., & Becker, D. P. (1997). Cerebral hyperglycolysis following severe traumatic brain injury in humans: A positron emission tomography study. *Journal of Neurosurgery*, *86*(2), 241-251. https://doi.org/10.3171/jns.1997.86.2.0241

Bey, T., & Ostick, B. (2009). Second impact syndrome. *The Western Journal of Emergency Medicine*, *10*(1), 6-10.

Bielefeld, P., Durá, I., Danielewicz, J., Lucassen, P. J., Baekelandt, V., Abrous, D. N., Encinas, J. M., & Fitzsimons, C. P. (2019). Insult-induced aberrant hippocampal neurogenesis: Functional consequences and possible therapeutic strategies. *Behavioural Brain Research*, *372*, 112032. https://doi.org/10.1016/j.bbr.2019.112032

Blackstad, T. W. (1956). Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. *The Journal of Comparative Neurology*, *105*(3), 417-537. https://doi.org/10.1002/cne.901050305

Blaiss, C. A., Yu, T.-S., Zhang, G., Chen, J., Dimchev, G., Parada, L. F., Powell, C. M., & Kernie, S. G. (2011). Temporally Specified Genetic Ablation of Neurogenesis Impairs Cognitive Recovery after Traumatic Brain Injury. *Journal of Neuroscience*, *31*(13), 4906-4916. https://doi.org/10.1523/JNEUROSCI.5265-10.2011

Blaya, M. O., Bramlett, H. M., Naidoo, J., Pieper, A. A., & Dietrich, W. D. (2014). Neuroprotective Efficacy of a Proneurogenic Compound after Traumatic Brain Injury. *Journal of Neurotrauma*, *31*(5), 476-486. https://doi.org/10.1089/neu.2013.3135

Bodnar, C., Morganti, J., & Bachstetter, A. (2018). Depression following a traumatic brain injury: Uncovering cytokine dysregulation as a pathogenic mechanism. *Neural Regeneration Research*, *13*(10), 1693. https://doi.org/10.4103/1673-5374.238604

Boldrini, M., Fulmore, C. A., Tartt, A. N., Simeon, L. R., Pavlova, I., Poposka, V., Rosoklija, G. B., Stankov, A., Arango, V., Dwork, A. J., Hen, R., & Mann, J. J. (2018). Human Hippocampal Neurogenesis Persists throughout Aging. *Cell Stem Cell*, *22*(4), 589-599.e5. https://doi.org/10.1016/j.stem.2018.03.015

Bonaguidi, M. A., Wheeler, M. A., Shapiro, J. S., Stadel, R. P., Sun, G. J., Ming, G., & Song, H. (2011). In Vivo Clonal Analysis Reveals Self-Renewing and Multipotent Adult Neural Stem Cell Characteristics. *Cell*, *145*(7), 1142-1155. https://doi.org/10.1016/j.cell.2011.05.024

Bond, A. M., Peng, C.-Y., Meyers, E. A., McGuire, T., Ewaleifoh, O., & Kessler, J. A. (2014). BMP Signaling Regulates the Tempo of Adult Hippocampal Progenitor Maturation at Multiple Stages of the Lineage. *Stem Cells*, *32*(8), 2201-2214. https://doi.org/10.1002/stem.1688

Bonislawski, D. P., Schwarzbach, E. P., & Cohen, A. S. (2007). Brain injury impairs dentate gyrus inhibitory efficacy. *Neurobiology of Disease*, *25*(1), 163-169. https://doi.org/10.1016/j.nbd.2006.09.002

Bouilleret, V., Ridoux, V., Depaulis, A., Marescaux, C., Nehlig, A., & Le Gal La Salle, G. (1999). Recurrent seizures and hippocampal sclerosis following intrahippocampal kainate injection in adult mice: Electroencephalography, histopathology and synaptic reorganization similar to mesial temporal lobe epilepsy. *Neuroscience*, *89*(3), 717-729. https://doi.org/10.1016/S0306-4522(98)00401-1

Bramlett, H. M., & Dietrich, W. D. (2007). Progressive damage after brain and spinal cord injury: Pathomechanisms and treatment strategies. En *Progress in Brain Research* (Vol. 161, pp. 125-141). Elsevier. https://doi.org/10.1016/S0079-6123(06)61009-1

Braun, H., Schäfer, K., & Höllt, V. (2002). *β* III Tubulin-Expressing Neurons Reveal Enhanced Neurogenesis in Hippocampal and Cortical Structures after a Contusion Trauma in Rats. *Journal of Neurotrauma*, *19*(8), 975-983. https://doi.org/10.1089/089771502320317122

Breunig, J. J., Arellano, J. I., Macklis, J. D., & Rakic, P. (2007). Everything that Glitters Isn't Gold: A Critical Review of Postnatal Neural Precursor Analyses. *Cell Stem Cell*, *1*(6), 612-627. https://doi.org/10.1016/j.stem.2007.11.008

Browne, K. D., Chen, X.-H., Meaney, D. F., & Smith, D. H. (2011). Mild Traumatic Brain Injury and Diffuse Axonal Injury in Swine. *Journal of Neurotrauma*, *28*(9), 1747-1755. https://doi.org/10.1089/neu.2011.1913

Bryant, R. (2011). Post-traumatic stress disorder vs traumatic brain injury. *Dialogues in Clinical Neuroscience*, *13*(3), 251-262. https://doi.org/10.31887/DCNS.2011.13.2/rbryant

Büki, A., & Povlishock, J. T. (2006). All roads lead to disconnection? – Traumatic axonal injury revisited. *Acta Neurochirurgica*, *148*(2), 181-194. https://doi.org/10.1007/s00701-005-0674-4

Bulgarelli, D., & Molina, P. (2016). Social Cognition in Preschoolers: Effects of Early Experience and Individual Differences. *Frontiers in Psychology*, *7*. https://doi.org/10.3389/fpsyg.2016.01762

Bullock, R., Myseros, J., & Marmarou, A. (1998). Factors affecting excitatory amino acid release following severe human head injury. *J. Neurosurg.*, *89*, 12.

Bullock, R., Zauner, A., Myseros, J. S., Marmarou, A., Woodward, J. J., & Young, H. F. (1995). Evidence for Prolonged Release of Excitatory Amino Acids in Severe Human Head Trauma.: Relationship to Clinical Events. *Annals of the New York Academy of Sciences*, *765*(1 Neuroprotecti), 290-297. https://doi.org/10.1111/j.1749-6632.1995.tb16586.x

Bushnik, T., Englander, J., & Wright, J. (2008). Patterns of Fatigue and Its Correlates Over the First 2 Years After Traumatic Brain Injury. *Journal of Head Trauma Rehabilitation*, *23*(1), 25-32. https://doi.org/10.1097/01.HTR.0000308718.88214.bb

Bye, N., Carron, S., Han, X., Agyapomaa, D., Ng, S. Y., Yan, E., Rosenfeld, J. V., & Morganti-Kossmann, M. C. (2011). Neurogenesis and glial proliferation are stimulated following diffuse traumatic brain injury in adult rats. *Journal of Neuroscience Research*, *89*(7), 986-1000. https://doi.org/10.1002/jnr.22635

C.

Cahill, S. P., Yu, R. Q., Green, D., Todorova, E. V., & Snyder, J. S. (2017). Early survival and delayed death of developmentally-born dentate gyrus neurons. *Hippocampus*, *27*(11), 1155-1167. https://doi.org/10.1002/hipo.22760

Cameron, H. A., & Glover, L. R. (2015). Adult Neurogenesis: Beyond Learning and Memory. *Annual Review of Psychology*, *66*(1), 53-81. https://doi.org/10.1146/annurev-psych-010814-015006

Cameron, H. A., Woolley, C. S., McEwen, B. S., & Gould, E. (1993). Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience*, *56*(2), 337-344. https://doi.org/10.1016/0306-4522(93)90335-D

Capizzi, A., Woo, J., & Verduzco-Gutierrez, M. (2020). Traumatic Brain Injury. *Medical Clinics of North America*, *104*(2), 213-238. https://doi.org/10.1016/j.mcna.2019.11.001

Carey, M. E., Sarna, G. S., Farrell, J. B., & Happel, L. T. (1989). Experimental missile wound to the brain. *Journal of Neurosurgery*, *71*(5), 754-764. https://doi.org/10.3171/jns.1989.71.5.0754

Carron, S. F., Alwis, D. S., & Rajan, R. (2016). Traumatic Brain Injury and Neuronal Functionality Changes in Sensory Cortex. *Frontiers in Systems Neuroscience*, *10*. https://doi.org/10.3389/fnsys.2016.00047

Caruncho, H. J., Brymer, K., Romay-Tallón, R., Mitchell, M. A., Rivera-Baltanás, T., Botterill, J., Olivares, J. M., & Kalynchuk, L. E. (2016). Reelin-Related Disturbances in Depression: Implications for Translational Studies. *Frontiers in Cellular Neuroscience*, *10*. https://doi.org/10.3389/fncel.2016.00048

Cernak, I. (2005). Animal models of head trauma. *NeuroRX*, *2*(3), 410-422. https://doi.org/10.1602/neurorx.2.3.410

Chamoun, R., Suki, D., Gopinath, S. P., Goodman, J. C., & Robertson, C. (2010). Role of extracellular glutamate measured by cerebral microdialysis in severe traumatic brain injury: Clinical article. *Journal of Neurosurgery*, *113*(3), 564-570. https://doi.org/10.3171/2009.12.JNS09689

Chardin, P. (2006). Function and regulation of Rnd proteins. *Nature Reviews Molecular Cell Biology*, *7*(1), 54-62. https://doi.org/10.1038/nrm1788

Chen, Y., Mao, H., Yang, K. H., Abel, T., & Meaney, D. F. (2014). A Modified Controlled Cortical Impact Technique to Model Mild Traumatic Brain Injury Mechanics in Mice. *Frontiers in Neurology*, *5*. https://doi.org/10.3389/fneur.2014.00100

Cheng, M.-F. (2013). Hypothalamic neurogenesis in the adult brain. *Frontiers in Neuroendocrinology*, *34*(3), 167-178. https://doi.org/10.1016/j.yfrne.2013.05.001

Chirumamilla, S., Sun, D., Bullock, M. R., & Colello, R. J. (2002). Traumatic Brain Injury Induced Cell Proliferation in the Adult Mammalian Central Nervous System. *Journal of Neurotrauma*, *19*(6), 693-703. https://doi.org/10.1089/08977150260139084

Choi, D. (1987). Ionic dependence of glutamate neurotoxicity. *The Journal of Neuroscience*, *7*(2), 369-379. https://doi.org/10.1523/JNEUROSCI.07-02-00369.1987

Church, A. J., & Andrew, R. D. (2005). Spreading Depression Expands Traumatic Injury in Neocortical Brain Slices. *Journal of Neurotrauma*, *22*(2), 277-290. https://doi.org/10.1089/neu.2005.22.277

Cifu, D. X., & Eapen, B. C. (2019). *Rehabilitation After Traumatic Brain Injury*. Elsevier.

Clark, R. S. B., Kochanek, P. M., Watkins, S. C., Chen, M., Dixon, C. E., Seidberg, N. A., Melick, J., Loeffert, J. E., Nathaniel, P. D., Jin, K. L., & Graham, S. H. (2001). Caspase-3 Mediated Neuronal Death After Traumatic Brain Injury in Rats. *Journal of Neurochemistry*, *74*(2), 740-753. https://doi.org/10.1046/j.1471-4159.2000.740740.x

Colak, T., Cine, N., Bamac, B., Kurtas, O., Ozbek, A., Bicer, U., Sunnetci, D., & Savlı, H. (2012). Microarray-based gene expression analysis of an animal model for closed head injury. *Injury*, *43*(8), 1264-1270. https://doi.org/10.1016/j.injury.2012.01.021

Comper, P., Bisschop, S. M., Carnide, N., & Tricco, A. (2005). A systematic review of treatments for mild traumatic brain injury. *Brain Injury*, *19*(11), 863-880. https://doi.org/10.1080/02699050400025042

Connell, S., Gao, J., Chen, J., & Shi, R. (2011). Novel Model to Investigate Blast Injury in the Central Nervous System. *Journal of Neurotrauma*, *28*(7), 1229-1236. https://doi.org/10.1089/neu.2011.1832

Conrad, M. S., Dilger, R. N., & Johnson, R. W. (2012). Brain Growth of the Domestic Pig ***(Sus scrofa)*** from 2 to 24 Weeks of Age: A Longitudinal MRI Study. *Developmental Neuroscience*, *34*(4), 291-298. https://doi.org/10.1159/000339311

Cope, E. C., & Gould, E. (2019). Adult Neurogenesis, Glia, and the Extracellular Matrix. *Cell Stem Cell*, *24*(5), 690-705. https://doi.org/10.1016/j.stem.2019.03.023

Coras, R., Siebzehnrubl, F. A., Pauli, E., Huttner, H. B., Njunting, M., Kobow, K., Villmann, C., Hahnen, E., Neuhuber, W., Weigel, D., Buchfelder, M., Stefan, H., Beck, H., Steindler, D. A., & Blumcke, I. (2010). Low proliferation and differentiation capacities of adult hippocampal stem cells correlate with memory dysfunction in humans. *Brain*, *133*(11), 3359-3372. https://doi.org/10.1093/brain/awq215

Corotto, F. S., Henegar, J. A., & Maruniak, J. A. (1993). Neurogenesis persists in the subependymal layer of the adult mouse brain. *Neuroscience Letters*, *149*(2), 111-114. https://doi.org/10.1016/0304-3940(93)90748-A

Crossley, N. A., Mechelli, A., Scott, J., Carletti, F., Fox, P. T., McGuire, P., & Bullmore, E. T. (2014). The hubs of the human connectome are generally implicated in the anatomy of brain disorders. *Brain*, *137*(8), 2382-2395. https://doi.org/10.1093/brain/awu132

D.

D'Ambrosio, R., Maris, D. O., Grady, M. S., Winn, H. R., & Janigro, D. (1998). Selective loss of hippocampal long-term potentiation, but not depression, following fluid percussion injury. *Brain Research*, *786*(1-2), 64-79. https://doi.org/10.1016/S0006-8993(97)01412-1

Danzer, S. C., Kotloski, R. J., Walter, C., Hughes, M., & McNamara, J. O. (2008). Altered morphology of hippocampal dentate granule cell presynaptic and postsynaptic terminals following conditional deletion of TrkB. *Hippocampus*, *18*(7), 668-678. https://doi.org/10.1002/hipo.20426

Dash, P. K., Mach, S. A., & Moore, A. N. (2002). The role of extracellular signal-regulated kinase in cognitive and motor deficits following experimental traumatic brain injury. *Neuroscience*, *114*(3), 755-767. https://doi.org/10.1016/S0306-4522(02)00277-4

Davis, A. R., Shear, D. A., Chen, Z., Lu, X.-C. M., & Tortella, F. C. (2010). A comparison of two cognitive test paradigms in a penetrating brain injury model. *Journal of Neuroscience Methods*, *189*(1), 84-87. https://doi.org/10.1016/j.jneumeth.2010.03.012

de Lanerolle, N. C., Bandak, F., Kang, D., Li, A. Y., Du, F., Swauger, P., Parks, S., Ling, G., & Kim, J. H. (2011). Characteristics of an Explosive Blast-Induced Brain Injury in an Experimental

Model. *Journal of Neuropathology & Experimental Neurology*, *70*(11), 1046-1057. https://doi.org/10.1097/NEN.0b013e318235bef2

Del Bigio, M. R. (1999). Proliferative status of cells in adult human dentate gyrus. *Microscopy Research and Technique*, *45*(6), 353-358. https://doi.org/10.1002/(SICI)1097-0029(19990615)45:6<353::AID-JEMT3>3.0.CO;2-M

Deng, W., Saxe, M. D., Gallina, I. S., & Gage, F. H. (2009). Adult-Born Hippocampal Dentate Granule Cells Undergoing Maturation Modulate Learning and Memory in the Brain. *Journal of Neuroscience*, *29*(43), 13532-13542. https://doi.org/10.1523/JNEUROSCI.3362-09.2009

Dennerll, T. J., Lamoureux, P., Buxbaum, R. E., & Heidemann, S. R. (1989). The cytomechanics of axonal elongation and retraction. *Journal of Cell Biology*, *109*(6), 3073-3083. https://doi.org/10.1083/jcb.109.6.3073

Denoth-Lippuner, A., & Jessberger, S. (2021). Formation and integration of new neurons in the adult hippocampus. *Nature Reviews Neuroscience*, *22*(4), 223-236. https://doi.org/10.1038/s41583-021-00433-z

Dent, K. A., Christie, K. J., Bye, N., Basrai, H. S., Turbic, A., Habgood, M., Cate, H. S., & Turnley, A. M. (2015). Oligodendrocyte Birth and Death following Traumatic Brain Injury in Adult Mice. *PLOS ONE*, *10*(3), e0121541. https://doi.org/10.1371/journal.pone.0121541

DeRuiter, S. L., Southall, B. L., Calambokidis, J., Zimmer, W. M. X., Sadykova, D., Falcone, E. A., Friedlaender, A. S., Joseph, J. E., Moretti, D., Schorr, G. S., Thomas, L., & Tyack, P. L. (2013). First direct measurements of behavioural responses by Cuvier's beaked whales to mid-frequency active sonar. *Biology Letters*, *9*(4), 20130223. https://doi.org/10.1098/rsbl.2013.0223

Deshpande, A., Bergami, M., Ghanem, A., Conzelmann, K.-K., Lepier, A., Götz, M., & Berninger, B. (2013). Retrograde monosynaptic tracing reveals the temporal evolution of inputs onto new neurons in the adult dentate gyrus and olfactory bulb. *Proceedings of the National Academy of Sciences*, *110*(12). https://doi.org/10.1073/pnas.1218991110

Devarakonda, S., Malipatlolla, D. K., Patel, P., Grandér, R., Kuhn, H. G., Steineck, G., Sjöberg, F., Rascón, A., Nyman, M., Eriksson, Y., Danial, J., Ittner, E., Naama Walid, R., Prykhodko, O., Masuram, S., Kalm, M., & Bull, C. (2021). Dietary Fiber and the Hippocampal Neurogenic Niche in a Model of Pelvic Radiotherapy. *Neuroscience*, *475*, 137-147. https://doi.org/10.1016/j.neuroscience.2021.08.030

Dockree, P. M., Bellgrove, M. A., O'Keeffe, F. M., Moloney, P., Aimola, L., Carton, S., & Robertson, I. H. (2006). Sustained attention in traumatic brain injury (tbi) and healthy controls:

Enhanced sensitivity with dual-task load. *Experimental Brain Research*, *168*(1-2), 218-229. https://doi.org/10.1007/s00221-005-0079-x

Dolbeare, F. (1995). Bromodeoxyuridine: A diagnostic tool in biology and medicine, Part I: Historical perspectives, histochemical methods and cell kinetics. *The Histochemical Journal*, *27*(5), 339-369.

Dragunow, M., Goulding, M., Faull, R. L. M., Ralph, R., Mee, E., & Frith, R. (1990). Induction of c-fos mRNA and protein in neurons and glia after traumatic brain injury: Pharmacological characterization. *Experimental Neurology*, *107*(3), 236-248. https://doi.org/10.1016/0014-4886(90)90141-E

Drake, A., Haut Donahue, T. L., Stansloski, M., Fox, K., Wheatley, B. B., & Donahue, S. W. (2016). Horn and horn core trabecular bone of bighorn sheep rams absorbs impact energy and reduces brain cavity accelerations during high impact ramming of the skull. *Acta Biomaterialia*, *44*, 41-50. https://doi.org/10.1016/j.actbio.2016.08.019

Drew, L. J., Kheirbek, M. A., Luna, V. M., Denny, C. A., Cloidt, M. A., Wu, M. V., Jain, S., Scharfman, H. E., & Hen, R. (2016). Activation of local inhibitory circuits in the dentate gyrus by adult-born neurons: ACTIVATION OF LOCAL INHIBITORY CIRCUITS. *Hippocampus*, *26*(6), 763-778. https://doi.org/10.1002/hipo.22557

Drexel, M., Puhakka, N., Kirchmair, E., Hörtnagl, H., Pitkänen, A., & Sperk, G. (2015). Expression of GABA receptor subunits in the hippocampus and thalamus after experimental traumatic brain injury. *Neuropharmacology*, *88*, 122-133. https://doi.org/10.1016/j.neuropharm.2014.08.023

Duan, X., Chang, J. H., Ge, S., Faulkner, R. L., Kim, J. Y., Kitabatake, Y., Liu, X., Yang, C.-H., Jordan, J. D., Ma, D. K., Liu, C. Y., Ganesan, S., Cheng, H.-J., Ming, G., Lu, B., & Song, H. (2007). Disrupted-In-Schizophrenia 1 Regulates Integration of Newly Generated Neurons in the Adult Brain. *Cell*, *130*(6), 1146-1158. https://doi.org/10.1016/j.cell.2007.07.010

Dupret, D., Revest, J.-M., Koehl, M., Ichas, F., De Giorgi, F., Costet, P., Abrous, D. N., & Piazza, P. V. (2008). Spatial Relational Memory Requires Hippocampal Adult Neurogenesis. *PLoS ONE*, *3*(4), e1959. https://doi.org/10.1371/journal.pone.0001959

Duque, A., & Spector, R. (2019). A balanced evaluation of the evidence for adult neurogenesis in humans: Implication for neuropsychiatric disorders. *Brain Structure and Function*, *224*(7), 2281-2295. https://doi.org/10.1007/s00429-019-01917-6

E.

Eckenhoff, M., & Rakic, P. (1988). Nature and fate of proliferative cells in the hippocampal dentate gyrus during the life span of the rhesus monkey. *The Journal of Neuroscience*, *8*(8), 2729-2747. https://doi.org/10.1523/JNEUROSCI.08-08-02729.1988

Edward Dixon, C., Clifton, G. L., Lighthall, J. W., Yaghmai, A. A., & Hayes, R. L. (1991). A controlled cortical impact model of traumatic brain injury in the rat. *Journal of Neuroscience Methods*, *39*(3), 253-262. https://doi.org/10.1016/0165-0270(91)90104-8

Ehm, O., Goritz, C., Covic, M., Schaffner, I., Schwarz, T. J., Karaca, E., Kempkes, B., Kremmer, E., Pfrieger, F. W., Espinosa, L., Bigas, A., Giachino, C., Taylor, V., Frisen, J., & Lie, D. C. (2010). RBPJ -Dependent Signaling Is Essential for Long-Term Maintenance of Neural Stem Cells in the Adult Hippocampus. *Journal of Neuroscience*, *30*(41), 13794-13807. https://doi.org/10.1523/JNEUROSCI.1567-10.2010

Eichenbaum, H., Stewart, C., & Morris, R. (1990). Hippocampal representation in place learning. *The Journal of Neuroscience*, *10*(11), 3531-3542. https://doi.org/10.1523/JNEUROSCI.10-11-03531.1990

Empie, K., Rangarajan, V., & Juul, S. E. (2015). Is the ferret a suitable species for studying perinatal brain injury? *International Journal of Developmental Neuroscience*, *45*(1), 2-10. https://doi.org/10.1016/j.ijdevneu.2015.06.005

Encinas, J. M., & Enikolopov, G. (2008). Identifying and Quantitating Neural Stem and Progenitor Cells in the Adult Brain. En *Methods in Cell Biology* (Vol. 85, pp. 243-272). Elsevier. https://doi.org/10.1016/S0091-679X(08)85011-X

Encinas, J. M., Michurina, T. V., Peunova, N., Park, J.-H., Tordo, J., Peterson, D. A., Fishell, G., Koulakov, A., & Enikolopov, G. (2011a). Division-Coupled Astrocytic Differentiation and Age-Related Depletion of Neural Stem Cells in the Adult Hippocampus. *Cell Stem Cell*, *8*(5), 566-579. https://doi.org/10.1016/j.stem.2011.03.010

Encinas, J. M., Michurina, T. V., Peunova, N., Park, J.-H., Tordo, J., Peterson, D. A., Fishell, G., Koulakov, A., & Enikolopov, G. (2011b). Division-Coupled Astrocytic Differentiation and Age-Related Depletion of Neural Stem Cells in the Adult Hippocampus. *Cell Stem Cell*, *8*(5), 566-579. https://doi.org/10.1016/j.stem.2011.03.010

Encinas, J. M., & Sierra, A. (2012). Neural stem cell deforestation as the main force driving the age-related decline in adult hippocampal neurogenesis. *Behavioural Brain Research*, *227*(2), 433-439. https://doi.org/10.1016/j.bbr.2011.10.010

Encinas, J. M., Vaahtokari, A., & Enikolopov, G. (2006). Fluoxetine targets early progenitor cells in the adult brain. *Proceedings of the National Academy of Sciences*, *103*(21), 8233-8238. https://doi.org/10.1073/pnas.0601992103

Englander, J., Bushnik, T., Oggins, J., & Katznelson, L. (2010). Fatigue after traumatic brain injury: Association with neuroendocrine, sleep, depression and other factors. *Brain Injury*, *24*(12), 1379-1388. https://doi.org/10.3109/02699052.2010.523041

Engström, K. G., Angrén, J., Björnstig, U., & Saveman, B.-I. (2018). Mass Casualty Incidents in the Underground Mining Industry: Applying the Haddon Matrix on an Integrative Literature Review. *Disaster Medicine and Public Health Preparedness*, *12*(1), 138-146. https://doi.org/10.1017/dmp.2017.31

Eriksson, P. S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.-M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine*, *4*(11), 1313-1317. https://doi.org/10.1038/3305

Esposito, M. S. (2005). Neuronal Differentiation in the Adult Hippocampus Recapitulates Embryonic Development. *Journal of Neuroscience*, *25*(44), 10074-10086. https://doi.org/10.1523/JNEUROSCI.3114-05.2005

Evans, J., Sumners, C., Moore, J., Huentelman, M. J., Deng, J., Gelband, C. H., & Shaw, G. (2002). Characterization of Mitotic Neurons Derived From Adult Rat Hypothalamus and Brain Stem. *Journal of Neurophysiology*, *87*(2), 1076-1085. https://doi.org/10.1152/jn.00088.2001

F.

Fabel, K., Fabel, K., Tam, B., Kaufer, D., Baiker, A., Simmons, N., Kuo, C. J., & Palmer, T. D. (2003). VEGF is necessary for exercise-induced adult hippocampal neurogenesis. *European Journal of Neuroscience*, *18*(10), 2803-2812. https://doi.org/10.1111/j.1460-9568.2003.03041.x

Faden, A. I., Demediuk, P., Panter, S. S., & Vink, R. (1989). The Role of Excitatory Amino Acids and NMDA Receptors in Traumatic Brain Injury. *Science*, *244*(4906), 798-800. https://doi.org/10.1126/science.2567056

Farah, G., Siwek, D., & Cummings, P. (2018). Tau accumulations in the brains of woodpeckers. *PLOS ONE*, *13*(2), e0191526. https://doi.org/10.1371/journal.pone.0191526

Farioli-Vecchioli, S., Saraulli, D., Costanzi, M., Pacioni, S., Cinà, I., Aceti, M., Micheli, L., Bacci, A., Cestari, V., & Tirone, F. (2008). The Timing of Differentiation of Adult Hippocampal Neurons Is Crucial for Spatial Memory. *PLoS Biology*, *6*(10), e246. https://doi.org/10.1371/journal.pbio.0060246

Farkas, O. (2006). Mechanoporation Induced by Diffuse Traumatic Brain Injury: An Irreversible or Reversible Response to Injury? *Journal of Neuroscience*, *26*(12), 3130-3140. https://doi.org/10.1523/JNEUROSCI.5119-05.2006

Farkas, O., & Povlishock, J. T. (2007). Cellular and subcellular change evoked by diffuse traumatic brain injury: A complex web of change extending far beyond focal damage. En *Progress in Brain Research* (Vol. 161, pp. 43-59). Elsevier. https://doi.org/10.1016/S0079-6123(06)61004-2

Faulkner, J. R. (2004). Reactive Astrocytes Protect Tissue and Preserve Function after Spinal Cord Injury. *Journal of Neuroscience*, *24*(9), 2143-2155. https://doi.org/10.1523/JNEUROSCI.3547-03.2004

Faulkner, R. L., Jang, M.-H., Liu, X.-B., Duan, X., Sailor, K. A., Kim, J. Y., Ge, S., Jones, E. G., Ming, G., Song, H., & Cheng, H.-J. (2008). Development of hippocampal mossy fiber synaptic outputs by new neurons in the adult brain. *Proceedings of the National Academy of Sciences*, *105*(37), 14157-14162. https://doi.org/10.1073/pnas.0806658105

Fear, N. T., Jones, E., Groom, M., Greenberg, N., Hull, L., Hodgetts, T. J., & Wessely, S. (2009). Symptoms of post-concussional syndrome are non-specifically related to mild traumatic brain injury in UK Armed Forces personnel on return from deployment in Iraq: An analysis of self-reported data. *Psychological Medicine*, *39*(8), 1379-1387. https://doi.org/10.1017/S0033291708004595

Feliciano, D. M., Bordey, A., & Bonfanti, L. (2015). Noncanonical Sites of Adult Neurogenesis in the Mammalian Brain. *Cold Spring Harbor Perspectives in Biology*, *7*(10), a018846. https://doi.org/10.1101/cshperspect.a018846

Filippov, V., Kronenberg, G., Pivneva, T., Reuter, K., Steiner, B., Wang, L.-P., Yamaguchi, M., Kettenmann, H., & Kempermann, G. (2003). Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. *Molecular and Cellular Neuroscience*, *23*(3), 373-382. https://doi.org/10.1016/S1044-7431(03)00060-5

Fineman, I., Hovda, D. A., Smith, M., Yoshino, A., & Becker, D. P. (1993). Concussive brain injury is associated with a prolonged accumulation of calcium: A45Ca autoradiographic study. *Brain Research*, *624*(1-2), 94-102. https://doi.org/10.1016/0006-8993(93)90064-T

Finnie, J. W. (1993). Pathology of experimental traumatic craniocerebral missile injury. *Journal of Comparative Pathology*, *108*(1), 93-101. https://doi.org/10.1016/S0021-9975(08)80231-9

Fleminger, S. (2003). Head injury as a risk factor for Alzheimer's disease: The evidence 10 years on; a partial replication. *Journal of Neurology, Neurosurgery & Psychiatry*, *74*(7), 857-862. https://doi.org/10.1136/jnnp.74.7.857

Flor-García, M., Terreros-Roncal, J., Moreno-Jiménez, E. P., Ávila, J., Rábano, A., & Llorens-Martín, M. (2020). Unraveling human adult hippocampal neurogenesis. *Nature Protocols*, *15*(2), 668-693. https://doi.org/10.1038/s41596-019-0267-y

Floyd, C. L., Golden, K. M., Black, R. T., Hamm, R. J., & Lyeth, B. G. (2002). Craniectomy Position Affects Morris Water Maze Performance and Hippocampal Cell Loss after Parasagittal Fluid Percussion. *Journal of Neurotrauma*, *19*(3), 303-316. https://doi.org/10.1089/089771502753594873

Flynn, T. J. (1984). Developmental changes of myelin-related lipids in brain of miniature swine. *Neurochemical Research*, *9*(7), 935-945. https://doi.org/10.1007/BF00964525

Folkersma, H., Foster Dingley, J. C., van Berckel, B. N., Rozemuller, A., Boellaard, R., Huisman, M. C., Lammertsma, A. A., Vandertop, W. P., & Molthoff, C. F. (2011). Increased cerebral (R)-[11C]PK11195 uptake and glutamate release in a rat model of traumatic brain injury: A longitudinal pilot study. *Journal of Neuroinflammation*, *8*(1), 67. https://doi.org/10.1186/1742-2094-8-67

Fontaine, A., Azouvi, P., Remy, P., Bussel, B., & Samson, Y. (1999). Functional anatomy of neuropsychological deficits after severe traumatic brain injury. *Neurology*, *53*(9), 1963-1963. https://doi.org/10.1212/WNL.53.9.1963

Förster, E., Tielsch, A., Saum, B., Weiss, K. H., Johanssen, C., Graus-Porta, D., Müller, U., & Frotscher, M. (2002). Reelin, Disabled 1, and β_1 integrins are required for the formation of the radial glial scaffold in the hippocampus. *Proceedings of the National Academy of Sciences*, *99*(20), 13178-13183. https://doi.org/10.1073/pnas.202035899

Förster, E., Zhao, S., & Frotscher, M. (2006). Laminating the hippocampus. *Nature Reviews Neuroscience*, *7*(4), 259-268. https://doi.org/10.1038/nrn1882

Francis, F., Koulakoff, A., Boucher, D., Chafey, P., Schaar, B., Vinet, M.-C., Friocourt, G., McDonnell, N., Reiner, O., Kahn, A., McConnell, S. K., Berwald-Netter, Y., Denoulet, P., & Chelly, J. (1999). Doublecortin Is a Developmentally Regulated, Microtubule-Associated Protein Expressed in Migrating and Differentiating Neurons. *Neuron*, *23*(2), 247-256. https://doi.org/10.1016/S0896-6273(00)80777-1

Frankowski, J. C., Foik, A. T., Tierno, A., Machhor, J. R., Lyon, D. C., & Hunt, R. F. (2021). Traumatic brain injury to primary visual cortex produces long-lasting circuit dysfunction. *Communications Biology*, *4*(1), 1297. https://doi.org/10.1038/s42003-021-02808-5

Freund, T. F., & Buzsáki, G. (1998). Interneurons of the hippocampus. *Hippocampus*, *6*(4), 347-470. https://doi.org/10.1002/(SICI)1098-1063(1996)6:4<347::AID-HIPO1>3.0.CO;2-I

Fujita, H., Katoh, H., Ishikawa, Y., Mori, K., & Negishi, M. (2002). Rapostlin Is a Novel Effector of Rnd2 GTPase Inducing Neurite Branching. *Journal of Biological Chemistry*, *277*(47), 45428-45434. https://doi.org/10.1074/jbc.M208090200

G.

Gaetz, M. (2004). The neurophysiology of brain injury. *Clinical Neurophysiology*, *115*(1), 4-18. https://doi.org/10.1016/S1388-2457(03)00258-X

Gage, F. H. (2000). Mammalian Neural Stem Cells. *Science*, *287*(5457), 1433-1438. https://doi.org/10.1126/science.287.5457.1433

Gale, S. D., Burr, R. B., Bigler, E. D., & Blatter, D. (1993). Fornix degeneration and memory in traumatic brain injury. *Brain Research Bulletin*, *32*(4), 345-349. https://doi.org/10.1016/0361-9230(93)90198-K

Gale, S. D., Johnson, S. C., Bigler, E. D., & Blatter, D. D. (1995). Nonspecific white matter degeneration following traumatic brain injury. *Journal of the International Neuropsychological Society*, *1*(1), 17-28. https://doi.org/10.1017/S1355617700000060

Gao, X., & Chen, J. (2013). Moderate traumatic brain injury promotes neural precursor proliferation without increasing neurogenesis in the adult hippocampus. *Experimental Neurology*, *239*, 38-48. https://doi.org/10.1016/j.expneurol.2012.09.012

Gao, X., Deng-Bryant, Y., Cho, W., Carrico, K. M., Hall, E. D., & Chen, J. (2008a). Selective death of newborn neurons in hippocampal dentate gyrus following moderate experimental traumatic brain injury. *Journal of Neuroscience Research*, *86*(10), 2258-2270. https://doi.org/10.1002/jnr.21677

Gao, X., Deng-Bryant, Y., Cho, W., Carrico, K. M., Hall, E. D., & Chen, J. (2008b). Selective death of newborn neurons in hippocampal dentate gyrus following moderate experimental traumatic brain injury. *Journal of Neuroscience Research*, *86*(10), 2258-2270. https://doi.org/10.1002/jnr.21677

Gao, X., Enikolopov, G., & Chen, J. (2009). Moderate traumatic brain injury promotes proliferation of quiescent neural progenitors in the adult hippocampus. *Experimental Neurology*, *219*(2), 516-523. https://doi.org/10.1016/j.expneurol.2009.07.007

Gaudet, A. D., Popovich, P. G., & Ramer, M. S. (2011). Wallerian degeneration: Gaining perspective on inflammatory events after peripheral nerve injury. *Journal of Neuroinflammation*, *8*(1), 110. https://doi.org/10.1186/1742-2094-8-110

Gavett, B. E., Stern, R. A., & McKee, A. C. (2011). Chronic Traumatic Encephalopathy: A Potential Late Effect of Sport-Related Concussive and Subconcussive Head Trauma. *Clinics in Sports Medicine*, *30*(1), 179-188. https://doi.org/10.1016/j.csm.2010.09.007

Ge, S., Goh, E. L. K., Sailor, K. A., Kitabatake, Y., Ming, G., & Song, H. (2006). GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature*, *439*(7076), 589-593. https://doi.org/10.1038/nature04404

Ge, S., Yang, C., Hsu, K., Ming, G., & Song, H. (2007). A Critical Period for Enhanced Synaptic Plasticity in Newly Generated Neurons of the Adult Brain. *Neuron*, *54*(4), 559-566. https://doi.org/10.1016/j.neuron.2007.05.002

Gehrmann, J., Matsumoto, Y., & Kreutzberg, G. W. (1995). Microglia: Intrinsic immuneffector cell of the brain. *Brain Research Reviews*, *20*(3), 269-287. https://doi.org/10.1016/0165-0173(94)00015-H

Gennarelli, T. A., Thibault, L. E., Adams, J. H., Graham, D. I., Thompson, C. J., & Marcincin, R. P. (1982). Diffuse axonal injury and traumatic coma in the primate. *Annals of Neurology*, *12*(6), 564-574. https://doi.org/10.1002/ana.410120611

Gentry, L., Godersky, J., & Thompson, B. (1988). MR imaging of head trauma: Review of the distribution and radiopathologic features of traumatic lesions. *American Journal of Roentgenology*, *150*(3), 663-672. https://doi.org/10.2214/ajr.150.3.663

Gerdes, J., Lemke, H., Baisch, H., Wacker, H. H., Schwab, U., & Stein, H. (1984). Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *Journal of Immunology (Baltimore, Md.: 1950)*, *133*(4), 1710-1715.

Giachino, C., Barz, M., Tchorz, J. S., Tome, M., Gassmann, M., Bischofberger, J., Bettler, B., & Taylor, V. (2014). GABA suppresses neurogenesis in the adult hippocampus through GABAB receptors. *Development*, *141*(1), 83-90. https://doi.org/10.1242/dev.102608

Gibbons, M. B., Smeal, R. M., Takahashi, D. K., Vargas, J. R., & Wilcox, K. S. (2013). Contributions of astrocytes to epileptogenesis following status epilepticus: Opportunities for preventive therapy? *Neurochemistry International*, *63*(7), 660-669. https://doi.org/10.1016/j.neuint.2012.12.008

Goldman-Rakic, P. S., Selemon, L. D., & Schwartz, M. L. (1984). Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. *Neuroscience*, *12*(3), 719-743. https://doi.org/10.1016/0306-4522(84)90166-0

Gomez-Nicola, D., Riecken, K., Fehse, B., & Perry, V. H. (2015). In-vivo RGB marking and multicolour single-cell tracking in the adult brain. *Scientific Reports*, *4*(1), 7520. https://doi.org/10.1038/srep07520

Gonçalves, J. T., Schafer, S. T., & Gage, F. H. (2016). Adult Neurogenesis in the Hippocampus: From Stem Cells to Behavior. *Cell*, *167*(4), 897-914. https://doi.org/10.1016/j.cell.2016.10.021

Gould, E., Cameron, H., Daniels, D., Woolley, C., & McEwen, B. (1992). Adrenal hormones suppress cell division in the adult rat dentate gyrus. *The Journal of Neuroscience*, *12*(9), 3642-3650. https://doi.org/10.1523/JNEUROSCI.12-09-03642.1992

Grady, M. S., Charleston, J. S., Maris, D., Witgen, B. M., & Lifshitz, J. (2003). Neuronal and Glial Cell Number in the Hippocampus after Experimental Traumatic Brain Injury: Analysis by Stereological Estimation. *Journal of Neurotrauma*, *20*(10), 929-941. https://doi.org/10.1089/089771503770195786

Graham, D. I., McIntosh, T. K., Maxwell, W. L., & Nicoll, J. A. R. (2000). Recent Advances in Neurotrauma. *Journal of Neuropathology & Experimental Neurology*, *59*(8), 641-651. https://doi.org/10.1093/jnen/59.8.641

Gray, J. A., & McNaughton, N. (1983). Comparison between the behavioural effects of septal and hippocampal lesions: A review. *Neuroscience & Biobehavioral Reviews*, *7*(2), 119-188. https://doi.org/10.1016/0149-7634(83)90014-3

Gray, J. A., & McNaughton, N. (2007). *The neuropsychology of anxiety: An enquiry into the function of the septo-hippocampal system* (2. ed., repr). Oxford Univ. Press.

Greiffenstein, M. F., & Baker, W. J. (2008). Validity Testing in Dually Diagnosed Post-Traumatic Stress Disorder and Mild Closed Head Injury. *The Clinical Neuropsychologist*, *22*(3), 565-582. https://doi.org/10.1080/13854040701377810

Gross, C. G. (2000). Neurogenesis in the adult brain: Death of a dogma. *Nature Reviews Neuroscience*, *1*(1), 67-73. https://doi.org/10.1038/35036235

Grote, S., Böcker, W., Mutschler, W., Bouillon, B., & Lefering, R. (2011). Diagnostic Value of the Glasgow Coma Scale for Traumatic Brain Injury in 18,002 Patients with Severe Multiple Injuries. *Journal of Neurotrauma*, *28*(4), 527-534. https://doi.org/10.1089/neu.2010.1433

Gu, Y., Arruda-Carvalho, M., Wang, J., Janoschka, S. R., Josselyn, S. A., Frankland, P. W., & Ge, S. (2012). Optical controlling reveals time-dependent roles for adult-born dentate granule cells. *Nature Neuroscience*, *15*(12), 1700-1706. https://doi.org/10.1038/nn.3260

Gulyaeva, N. V. (2015). Ventral hippocampus, Stress and Psychopathology: Translational implications. *Neurochemical Journal*, *9*(2), 85-94. https://doi.org/10.1134/S1819712415020075

Gupta, A., Elgammal, F. S., Proddutur, A., Shah, S., & Santhakumar, V. (2012). Decrease in Tonic Inhibition Contributes to Increase in Dentate Semilunar Granule Cell Excitability after Brain Injury. *Journal of Neuroscience*, *32*(7), 2523-2537. https://doi.org/10.1523/JNEUROSCI.4141-11.2012

H.

Haasper, C., Junge, M., Ernstberger, A., Brehme, H., Hannawald, L., Langer, C., Nehmzow, J., Otte, D., Sander, U., Krettek, C., & Zwipp, H. (2010). Die Abbreviated Injury Scale (AIS): Potenzial und Probleme bei der Anwendung. *Der Unfallchirurg*, *113*(5), 366-372. https://doi.org/10.1007/s00113-010-1778-8

Hall, E. D., Sullivan, P. G., Gibson, T. R., Pavel, K. M., Thompson, B. M., & Scheff, S. W. (2005). Spatial and Temporal Characteristics of Neurodegeneration after Controlled Cortical Impact in Mice: More than a Focal Brain Injury. *Journal of Neurotrauma*, *22*(2), 252-265. https://doi.org/10.1089/neu.2005.22.252

Hamm, R. J., Lyeth, B. G., Jenkins, L. W., O'Dell, D. M., & Pike, B. R. (1993). Selective cognitive impairment following traumatic brain injury in rats. *Behavioural Brain Research*, *59*(1-2), 169-173. https://doi.org/10.1016/0166-4328(93)90164-L

Han, X., Tong, J., Zhang, J., Farahvar, A., Wang, E., Yang, J., Samadani, U., Smith, D. H., & Huang, J. H. (2011). Imipramine Treatment Improves Cognitive Outcome Associated with Enhanced Hippocampal Neurogenesis after Traumatic Brain Injury in Mice. *Journal of Neurotrauma*, *28*(6), 995-1007. https://doi.org/10.1089/neu.2010.1563

Harris, J. L., Yeh, H.-W., Choi, I.-Y., Lee, P., Berman, N. E., Swerdlow, R. H., Craciunas, S. C., & Brooks, W. M. (2012). Altered Neurochemical Profile after Traumatic Brain Injury: ^{1}H-MRS Biomarkers of Pathological Mechanisms. *Journal of Cerebral Blood Flow & Metabolism*, *32*(12), 2122-2134. https://doi.org/10.1038/jcbfm.2012.114

Harris, L., Rigo, P., Stiehl, T., Gaber, Z. B., Austin, S. H. L., Masdeu, M. del M., Edwards, A., Urbán, N., Marciniak-Czochra, A., & Guillemot, F. (2021). Coordinated changes in cellular behavior ensure the lifelong maintenance of the hippocampal stem cell population. *Cell Stem Cell*, *28*(5), 863-876.e6. https://doi.org/10.1016/j.stem.2021.01.003

Hatten, M. E. (1999). Central Nervous System Neuronal Migration. *Annual Review of Neuroscience*, *22*(1), 511-539. https://doi.org/10.1146/annurev.neuro.22.1.511

Hatten, M. E. (2002). New Directions in Neuronal Migration. *Science*, *297*(5587), 1660-1663. https://doi.org/10.1126/science.1074572

Hayes, R. L., Stalhammar, D., Povlishock, J. T., Allen, A. M., Galinat, B. J., Becker, D. P., & Stonnington, H. H. (1987). A new model of concussive brain injury in the cat produced by extradural fluid volume loading: II. Physiological and neuropathological observations. *Brain Injury*, *1*(1), 93-112. https://doi.org/10.3109/02699058709034449

Henery, C. C., & Mayhew, T. M. (1989). The cerebrum and cerebellum of the fixed human brain: Efficient and unbiased estimates of volumes and cortical surface areas. *Journal of Anatomy*, *167*, 167-180.

Heng, J. I.-T., Nguyen, L., Castro, D. S., Zimmer, C., Wildner, H., Armant, O., Skowronska-Krawczyk, D., Bedogni, F., Matter, J.-M., Hevner, R., & Guillemot, F. (2008). Neurogenin 2 controls cortical neuron migration through regulation of Rnd2. *Nature*, *455*(7209), 114-118. https://doi.org/10.1038/nature07198

Henke, P. G. (1990). Hippocampal pathway to the amygdala and stress ulcer development. *Brain Research Bulletin*, *25*(5), 691-695. https://doi.org/10.1016/0361-9230(90)90044-Z

Henry, R. J., Ritzel, R. M., Barrett, J. P., Doran, S. J., Jiao, Y., Leach, J. B., Szeto, G. L., Wu, J., Stoica, B. A., Faden, A. I., & Loane, D. J. (2020). Microglial Depletion with CSF1R Inhibitor During Chronic Phase of Experimental Traumatic Brain Injury Reduces Neurodegeneration and Neurological Deficits. *The Journal of Neuroscience*, *40*(14), 2960-2974. https://doi.org/10.1523/JNEUROSCI.2402-19.2020

Hermann, A., Maisel, M., Liebau, S., Gerlach, M., Kleger, A., Schwarz, J., Kim, K.-S., Antoniadis, G., Lerche, H., & Storch, A. (2006). Mesodermal cell types induce neurogenesis from adult human hippocampal progenitor cells. *Journal of Neurochemistry*, *98*(2), 629-640. https://doi.org/10.1111/j.1471-4159.2006.03916.x

Hicks, R. R., Smith, D. H., Lowenstein, D. H., Marie, R. S., & McINTOSH, T. K. (1993). Mild Experimental Brain Injury in the Rat Induces Cognitive Deficits Associated with Regional Neuronal Loss in the Hippocampus. *Journal of Neurotrauma*, *10*(4), 405-414. https://doi.org/10.1089/neu.1993.10.405

Hodge, R. D., Kowalczyk, T. D., Wolf, S. A., Encinas, J. M., Rippey, C., Enikolopov, G., Kempermann, G., & Hevner, R. F. (2008). Intermediate Progenitors in Adult Hippocampal Neurogenesis: Tbr2 Expression and Coordinate Regulation of Neuronal Output. *Journal of Neuroscience*, *28*(14), 3707-3717. https://doi.org/10.1523/JNEUROSCI.4280-07.2008

Holmin, S., Söderlund, J., Biberfeld, P., & Mathiesen, T. (1998). Intracerebral Inflammation after Human Brain Contusion. *Neurosurgery*, *42*(2), 291-298.

Hu, B., Liu, C., Bramlett, H., Sick, T. J., Alonso, O. F., Chen, S., & Dietrich, W. D. (2004). Changes in TrkB–ERK1/2–CREB/Elk-1 Pathways in Hippocampal Mossy Fiber Organization after Traumatic Brain Injury. *Journal of Cerebral Blood Flow & Metabolism*, *24*(8), 934-943. https://doi.org/10.1097/01.WCB.0000125888.56462.A1

Huang, W., Zaheri, A., Jung, J.-Y., Espinosa, H. D., & Mckittrick, J. (2017). Hierarchical structure and compressive deformation mechanisms of bighorn sheep (Ovis canadensis) horn. *Acta Biomaterialia*, *64*, 1-14. https://doi.org/10.1016/j.actbio.2017.09.043

Hughes, K. R. (1965). Dorsal and ventral hippocampus lesions and maze learning: Influence of preoperative environment. *Canadian Journal of Psychology/Revue Canadienne de Psychologie*, *19*(4), 325-332. https://doi.org/10.1037/h0082915

Hunt, R. F., Scheff, S. W., & Smith, B. N. (2011). Synaptic Reorganization of Inhibitory Hilar Interneuron Circuitry after Traumatic Brain Injury in Mice. *Journal of Neuroscience*, *31*(18), 6880-6890. https://doi.org/10.1523/JNEUROSCI.0032-11.2011

Hutchinson, E. B., Schwerin, S. C., Radomski, K. L., Irfanoglu, M. O., Juliano, S. L., & Pierpaoli, C. M. (2016). Quantitative MRI and DTI Abnormalities During the Acute Period Following CCI in the Ferret. *Shock*, *46*(3S), 167-176. https://doi.org/10.1097/SHK.0000000000000659

Huttmann, K., Sadgrove, M., Wallraff, A., Hinterkeuser, S., Kirchhoff, F., Steinhauser, C., & Gray, W. P. (2003). Seizures preferentially stimulate proliferation of radial glia-like astrocytes in the adult dentate gyrus: Functional and immunocytochemical analysis. *European Journal of Neuroscience*, *18*(10), 2769-2778. https://doi.org/10.1111/j.1460-9568.2003.03002.x

Huttner, H. B., Bergmann, O., Salehpour, M., Rácz, A., Tatarishvili, J., Lindgren, E., Csonka, T., Csiba, L., Hortobágyi, T., Méhes, G., Englund, E., Solnestam, B. W., Zdunek, S., Scharenberg, C., Ström, L., Ståhl, P., Sigurgeirsson, B., Dahl, A., Schwab, S., … Frisén, J. (2014). The age and genomic integrity of neurons after cortical stroke in humans. *Nature Neuroscience*, *17*(6), 801-803. https://doi.org/10.1038/nn.3706

I.

Ibrahim, S., Hu, W., Wang, X., Gao, X., He, C., & Chen, J. (2016). Traumatic Brain Injury Causes Aberrant Migration of Adult-Born Neurons in the Hippocampus. *Scientific Reports*, *6*(1), 21793. https://doi.org/10.1038/srep21793

Ibrayeva, A., Bay, M., Pu, E., Jörg, D. J., Peng, L., Jun, H., Zhang, N., Aaron, D., Lin, C., Resler, G., Hidalgo, A., Jang, M.-H., Simons, B. D., & Bonaguidi, M. A. (2021). Early stem cell aging in the mature brain. *Cell Stem Cell*, *28*(5), 955-966.e7. https://doi.org/10.1016/j.stem.2021.03.018

Inta, D., Cameron, H. A., & Gass, P. (2015). New neurons in the adult striatum: From rodents to humans. *Trends in Neurosciences*, *38*(9), 517-523. https://doi.org/10.1016/j.tins.2015.07.005

Iverson, G. L., & Lange, R. T. (2003). Examination of «Postconcussion-Like» Symptoms in a Healthy Sample. *Applied Neuropsychology*, *10*(3), 137-144. https://doi.org/10.1207/S15324826AN1003_02

J.

Jacobson, L., & Sapolsky, R. (1991). The Role of the Hippocampus in Feedback Regulation of the Hypothalamic-Pituitary-Adrenocortical Axis*. *Endocrine Reviews*, *12*(2), 118-134. https://doi.org/10.1210/edrv-12-2-118

Jay, T. M., & Witter, M. P. (1991). Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport ofPhaseolus vulgaris-leucoagglutinin. *The Journal of Comparative Neurology*, *313*(4), 574-586. https://doi.org/10.1002/cne.903130404

Jessberger, S., Clark, R. E., Broadbent, N. J., Clemenson, G. D., Consiglio, A., Lie, D. C., Squire, L. R., & Gage, F. H. (2009). Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learning & Memory*, *16*(2), 147-154. https://doi.org/10.1101/lm.1172609

Jin, K., Wang, X., Xie, L., Mao, X. O., Zhu, W., Wang, Y., Shen, J., Mao, Y., Banwait, S., & Greenberg, D. A. (2006). Evidence for stroke-induced neurogenesis in the human brain. *Proceedings of the National Academy of Sciences*, *103*(35), 13198-13202. https://doi.org/10.1073/pnas.0603512103

Jin, X. (2016). The role of neurogenesis during development and in the adult brain. *European Journal of Neuroscience*, *44*(6), 2291-2299. https://doi.org/10.1111/ejn.13251

Johnson, K. L. (2016). *From Horns to Helmets: Multi-Objective Design Optimization Considerations to Protect the Brain*. Mississipi State University.

Jourdan, C., Bayen, E., Pradat-Diehl, P., Ghout, I., Darnoux, E., Azerad, S., Vallat-Azouvi, C., Charanton, J., Aegerter, P., Ruet, A., & Azouvi, P. (2016). A comprehensive picture of 4-year outcome of severe brain injuries. Results from the PariS-TBI study. *Annals of Physical and Rehabilitation Medicine*, *59*(2), 100-106. https://doi.org/10.1016/j.rehab.2015.10.009

K.

Kabadi, S. V., Hilton, G. D., Stoica, B. A., Zapple, D. N., & Faden, A. I. (2010). Fluid-percussion–induced traumatic brain injury model in rats. *Nature Protocols*, *5*(9), 1552-1563. https://doi.org/10.1038/nprot.2010.112

Kalamakis, G., Brüne, D., Ravichandran, S., Bolz, J., Fan, W., Ziebell, F., Stiehl, T., Catalá-Martinez, F., Kupke, J., Zhao, S., Llorens-Bobadilla, E., Bauer, K., Limpert, S., Berger, B., Christen, U., Schmezer, P., Mallm, J. P., Berninger, B., Anders, S., … Martin-Villalba, A. (2019). Quiescence Modulates Stem Cell Maintenance and Regenerative Capacity in the Aging Brain. *Cell*, *176*(6), 1407-1419.e14. https://doi.org/10.1016/j.cell.2019.01.040

Kane, M. J., Hatic, H., Delic, V., Dennis, J. S., Butler, C. L., Saykally, J. N., & Citron, B. A. (2011). Modeling the pathobiology of repetitive traumatic brain injury in immortalized neuronal cell lines. *Brain Research*, *1425*, 123-131. https://doi.org/10.1016/j.brainres.2011.09.047

Kaplan, M. S. (1985). Formation and Turnover of Neurons in Young and Senescent Animals: An Electronmicroscopic and Morphometric Analysis. *Annals of the New York Academy of Sciences*, *457*(1), 173-192. https://doi.org/10.1111/j.1749-6632.1985.tb20805.x

Katayama, Y., Becker, D. P., Tamura, T., & Hovda, D. A. (1990). Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. *Journal of Neurosurgery*, *73*(6), 889-900. https://doi.org/10.3171/jns.1990.73.6.0889

Kawamata, T., Katayama, Y., Hovda, D. A., Yoshino, A., & Becker, D. P. (1992). Administration of Excitatory Amino Acid Antagonists via Microdialysis Attenuates the Increase in Glucose Utilization Seen following Concussive Brain Injury. *Journal of Cerebral Blood Flow & Metabolism*, *12*(1), 12-24. https://doi.org/10.1038/jcbfm.1992.3

Kelley, B. J., Farkas, O., Lifshitz, J., & Povlishock, J. T. (2006). Traumatic axonal injury in the perisomatic domain triggers ultrarapid secondary axotomy and Wallerian degeneration. *Experimental Neurology*, *198*(2), 350-360. https://doi.org/10.1016/j.expneurol.2005.12.017

Kelly, J. P., & Rosenberg, J. H. (1997). Diagnosis and management of concussion in sports. *Neurology*, *48*(3), 575-580. https://doi.org/10.1212/WNL.48.3.575

Kempermann, G. (2012). New neurons for «survival of the fittest». *Nature Reviews Neuroscience*, *13*(10), 727-736. https://doi.org/10.1038/nrn3319

Kempermann, G., Gage, F. H., Aigner, L., Song, H., Curtis, M. A., Thuret, S., Kuhn, H. G., Jessberger, S., Frankland, P. W., Cameron, H. A., Gould, E., Hen, R., Abrous, D. N., Toni, N., Schinder, A. F., Zhao, X., Lucassen, P. J., & Frisén, J. (2018). Human Adult Neurogenesis: Evidence and Remaining Questions. *Cell Stem Cell*, *23*(1), 25-30. https://doi.org/10.1016/j.stem.2018.04.004

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., & Gage, F. H. (2003). Early determination and long-term persistence of adult-generated new neurons in the

hippocampus of mice. *Development*, *130*(2), 391-399. https://doi.org/10.1242/dev.00203

Kempermann, G., Jessberger, S., Steiner, B., & Kronenberg, G. (2004). Milestones of neuronal development in the adult hippocampus. *Trends in Neurosciences*, *27*(8), 447-452. https://doi.org/10.1016/j.tins.2004.05.013

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1998). Experience-Induced Neurogenesis in the Senescent Dentate Gyrus. *The Journal of Neuroscience*, *18*(9), 3206-3212. https://doi.org/10.1523/JNEUROSCI.18-09-03206.1998

Kempermann, G., Song, H., & Gage, F. H. (2015). Neurogenesis in the Adult Hippocampus. *Cold Spring Harbor Perspectives in Biology*, *7*(9), a018812. https://doi.org/10.1101/cshperspect.a018812

Keren, K. (2011). Cell motility: The integrating role of the plasma membrane. *European Biophysics Journal*, *40*(9), 1013-1027. https://doi.org/10.1007/s00249-011-0741-0

Kerloch, T., Farrugia, F., Bouit, L., Maître, M., Terral, G., Koehl, M., Mortessagne, P., Heng, J. I.-T., Blanchard, M., Doat, H., Leste-Lasserre, T., Goron, A., Gonzales, D., Perrais, D., Guillemot, F., Abrous, D. N., & Pacary, E. (2021). The atypical Rho GTPase Rnd2 is critical for dentate granule neuron development and anxiety-like behavior during adult but not neonatal neurogenesis. *Molecular Psychiatry*, *26*(12), 7280-7295. https://doi.org/10.1038/s41380-021-01301-z

Kheirbek, M. A., Drew, L. J., Burghardt, N. S., Costantini, D. O., Tannenholz, L., Ahmari, S. E., Zeng, H., Fenton, A. A., & Hen, R. (2013). Differential Control of Learning and Anxiety along the Dorsoventral Axis of the Dentate Gyrus. *Neuron*, *77*(5), 955-968. https://doi.org/10.1016/j.neuron.2012.12.038

Kim, W. R., Kim, J. Y., Moon, Y., Kim, H. J., Kim, H., & Sun, W. (2012). Regional difference of reactive astrogliosis following traumatic brain injury revealed by hGFAP-GFP transgenic mice. *Neuroscience Letters*, *513*(2), 155-159. https://doi.org/10.1016/j.neulet.2012.02.023

King, C., Robinson, T., Dixon, C. E., Rao, G. R., Larnard, D., & Nemoto, C. E. M. (2010). Brain Temperature Profiles during Epidural Cooling with the ChillerPad in a Monkey Model of Traumatic Brain Injury. *Journal of Neurotrauma*, *27*(10), 1895-1903. https://doi.org/10.1089/neu.2009.1178

Kleindienst, A., McGinn, M. J., Harvey, H. B., Colello, R. J., Hamm, R. J., & Bullock, M. R. (2005). Enhanced Hippocampal Neurogenesis by Intraventricular S100B Infusion Is Associated with Improved Cognitive Recovery after Traumatic Brain Injury. *Journal of Neurotrauma*, *22*(6), 645-655. https://doi.org/10.1089/neu.2005.22.645

Knoth, R., Singec, I., Ditter, M., Pantazis, G., Capetian, P., Meyer, R. P., Horvat, V., Volk, B., & Kempermann, G. (2010). Murine Features of Neurogenesis in the Human Hippocampus across the Lifespan from 0 to 100 Years. *PLoS ONE*, *5*(1), e8809. https://doi.org/10.1371/journal.pone.0008809

Kohara, K., Pignatelli, M., Rivest, A. J., Jung, H.-Y., Kitamura, T., Suh, J., Frank, D., Kajikawa, K., Mise, N., Obata, Y., Wickersham, I. R., & Tonegawa, S. (2014). Cell type–specific genetic and optogenetic tools reveal hippocampal CA2 circuits. *Nature Neuroscience*, *17*(2), 269-279. https://doi.org/10.1038/nn.3614

Korr, H. (1980). *Proliferation of different cell types in the brain*. Springer.

Kosaka, T., & Hama, K. (1986). Three-dimensional structure of astrocytes in the rat dentate gyrus. *The Journal of Comparative Neurology*, *249*(2), 242-260. https://doi.org/10.1002/cne.902490209

Kotapka, M. J., Gennarelli, T. A., Graham, D. I., Adams, J. H., Thibault, L. E., Ross, D. T., & Ford, I. (1991). Selective Vulnerability of Hippocampal Neurons in Acceleration-Induced Experimental Head Injury. *Journal of Neurotrauma*, *8*(4), 247-258. https://doi.org/10.1089/neu.1991.8.247

Koura, S. S., Doppenberg, E. M. R., Marmarou, A., Choi, S., Young, H. F., & Bullock, R. (1998). Relationship between Excitatory Amino Acid Release and Outcome after Severe Human Head Injury. En A. Marmarou, R. Bullock, C. Avezaat, A. Baethmann, D. Becker, M. Brock, J. Hoff, H. Nagai, H.-J. Reulen, & G. Teasdale (Eds.), *Intracranial Pressure and Neuromonitoring in Brain Injury* (pp. 244-246). Springer Vienna. https://doi.org/10.1007/978-3-7091-6475-4_70

Kraus, J. F., & McArthur, D. L. (1996). EPIDEMIOLOGIC ASPECTS OF BRAIN INJURY. *Neurologic Clinics*, *14*(2), 435-450. https://doi.org/10.1016/S0733-8619(05)70266-8

Krettek, J. E., & Price, J. L. (1977). Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. *The Journal of Comparative Neurology*, *172*(4), 723-752. https://doi.org/10.1002/cne.901720409

Kronenberg, G., Reuter, K., Steiner, B., Brandt, M. D., Jessberger, S., Yamaguchi, M., & Kempermann, G. (2003). Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *The Journal of Comparative Neurology*, *467*(4), 455-463. https://doi.org/10.1002/cne.10945

Kuhn, H., Dickinson-Anson, H., & Gage, F. (1996). Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation. *The Journal of*

Neuroscience, *16*(6), 2027-2033. https://doi.org/10.1523/JNEUROSCI.16-06-02027.1996

Kuhn, H. G., Toda, T., & Gage, F. H. (2018). Adult Hippocampal Neurogenesis: A Coming-of-Age Story. *The Journal of Neuroscience*, *38*(49), 10401-10410. https://doi.org/10.1523/JNEUROSCI.2144-18.2018

Kuhn, H. G., Winkler, J., Kempermann, G., Thal, L. J., & Gage, F. H. (1997). Epidermal Growth Factor and Fibroblast Growth Factor-2 Have Different Effects on Neural Progenitors in the Adult Rat Brain. *The Journal of Neuroscience*, *17*(15), 5820-5829. https://doi.org/10.1523/JNEUROSCI.17-15-05820.1997

Kumaria, A., & Tolias, C. M. (2008). In vitro models of neurotrauma. *British Journal of Neurosurgery*, *22*(2), 200-206. https://doi.org/10.1080/02688690701772413

Kuwabara, T., Hsieh, J., Muotri, A., Yeo, G., Warashina, M., Lie, D. C., Moore, L., Nakashima, K., Asashima, M., & Gage, F. H. (2009). Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis. *Nature Neuroscience*, *12*(9), 1097-1105. https://doi.org/10.1038/nn.2360

L.

Lacefield, C. O., Itskov, V., Reardon, T., Hen, R., & Gordon, J. A. (2012). Effects of adult-generated granule cells on coordinated network activity in the dentate gyrus. *Hippocampus*, *22*(1), 106-116. https://doi.org/10.1002/hipo.20860

Laplagne, D. A., Espósito, M. S., Piatti, V. C., Morgenstern, N. A., Zhao, C., van Praag, H., Gage, F. H., & Schinder, A. F. (2006). Functional Convergence of Neurons Generated in the Developing and Adult Hippocampus. *PLoS Biology*, *4*(12), e409. https://doi.org/10.1371/journal.pbio.0040409

Lee, J., Duan, W., Long, J. M., Ingram, D. K., & Mattson, M. P. (2000). Dietary Restriction Increases the Number of Newly Generated Neural Cells, and Induces BDNF Expression, in the Dentate Gyrus of Rats. *Journal of Molecular Neuroscience*, *15*(2), 99-108. https://doi.org/10.1385/JMN:15:2:99

Lendahl, U., Zimmerman, L. B., & McKay, R. D. G. (1990). CNS stem cells express a new class of intermediate filament protein. *Cell*, *60*(4), 585-595. https://doi.org/10.1016/0092-8674(90)90662-X

Lewis, S. B., Finnie, J. W., Blumbergs, P. C., Scott, G., Manavis, J., Brown, C., Reilly, P. L., Jones, N. R., & McLEAN, A. J. (1996). A Head Impact Model of Early Axonal Injury in the Sheep. *Journal of Neurotrauma*, *13*(9), 505-514. https://doi.org/10.1089/neu.1996.13.505

Li, G., Fang, L., Fernández, G., & Pleasure, S. J. (2013). The Ventral Hippocampus Is the Embryonic Origin for Adult Neural Stem Cells in the Dentate Gyrus. *Neuron*, *78*(4), 658-672. https://doi.org/10.1016/j.neuron.2013.03.019

Li, Y., Luikart, B. W., Birnbaum, S., Chen, J., Kwon, C.-H., Kernie, S. G., Bassel-Duby, R., & Parada, L. F. (2008). TrkB Regulates Hippocampal Neurogenesis and Governs Sensitivity to Antidepressive Treatment. *Neuron*, *59*(3), 399-412. https://doi.org/10.1016/j.neuron.2008.06.023

Lifshitz, J., Friberg, H., Neumar, R. W., Raghupathi, R., Welsh, F. A., Janmey, P., Saatman, K. E., Wieloch, T., Grady, M. S., & McIntosh, T. K. (2003). Structural and Functional Damage Sustained by Mitochondria after Traumatic Brain Injury in the Rat: Evidence for Differentially Sensitive Populations in the Cortex and Hippocampus. *Journal of Cerebral Blood Flow & Metabolism*, *23*(2), 219-231. https://doi.org/10.1097/01.WCB.0000040581.43808.03

Lifshitz, J., Kelley, B. J., & Povlishock, J. T. (2007). Perisomatic Thalamic Axotomy After Diffuse Traumatic Brain Injury Is Associated With Atrophy Rather Than Cell Death: *Journal of Neuropathology and Experimental Neurology*, *66*(3), 218-229. https://doi.org/10.1097/01.jnen.0000248558.75950.4d

Lighthall, J. W. (1988). Controlled Cortical Impact: A New Experimental Brain Injury Model. *Journal of Neurotrauma*, *5*(1), 1-15. https://doi.org/10.1089/neu.1988.5.1

Lighthall, J. W., Goshgarian, H. G., & Pinderski, C. R. (1990). Characterization of Axonal Injury Produced by Controlled Cortical Impact. *Journal of Neurotrauma*, *7*(2), 65-76. https://doi.org/10.1089/neu.1990.7.65

Ling, G. S., & Ecklund, J. M. (2011). Traumatic brain injury in modern war. *Current Opinion in Anaesthesiology*, *24*(2), 124-130. https://doi.org/10.1097/ACO.0b013e32834458da

Lipp, H.-P., & Bonfanti, L. (2016). Adult Neurogenesis in Mammals: Variations and Confusions. *Brain, Behavior and Evolution*, *87*(3), 205-221. https://doi.org/10.1159/000446905

Llorens-Martín, M., Rábano, A., & Ávila, J. (2016). The Ever-Changing Morphology of Hippocampal Granule Neurons in Physiology and Pathology. *Frontiers in Neuroscience*, *9*. https://doi.org/10.3389/fnins.2015.00526

LoBue, C., Cullum, C. M., Didehbani, N., Yeatman, K., Jones, B., Kraut, M. A., & Hart, J. (2018). Neurodegenerative Dementias After Traumatic Brain Injury. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *30*(1), 7-13. https://doi.org/10.1176/appi.neuropsych.17070145

Lotocki, G., Vaccari, J. de R., Alonso, O., Molano, J. S., Nixon, R., Dietrich, W. D., & Bramlett, H. M. (2011). Oligodendrocyte Vulnerability Following Traumatic Brain Injury in Rats: Effect

of Moderate Hypothermia. *Therapeutic Hypothermia and Temperature Management*, *1*(1), 43-51. https://doi.org/10.1089/ther.2010.0011

Lowenstein, D., Thomas, M., Smith, D., & McIntosh, T. (1992). Selective vulnerability of dentate hilar neurons following traumatic brain injury: A potential mechanistic link between head trauma and disorders of the hippocampus. *The Journal of Neuroscience*, *12*(12), 4846-4853. https://doi.org/10.1523/JNEUROSCI.12-12-04846.1992

Lu, D., Mahmood, A., Qu, C., Goussev, A., Schallert, T., & Chopp, M. (2005). Erythropoietin Enhances Neurogenesis and Restores Spatial Memory in Rats after Traumatic Brain Injury. *Journal of Neurotrauma*, *22*(9), 1011-1017. https://doi.org/10.1089/neu.2005.22.1011

Lu, D., Mahmood, A., Zhang, R., Li, Y., & Chopp, M. (2003). Upregulation of neurogenesis and reduction in functional deficits following administration of DETA/NONOate, a nitric oxide donor, after traumatic brain injury in rats. *Journal of Neurosurgery*, *99*(2), 351-361. https://doi.org/10.3171/jns.2003.99.2.0351

Luna, V. M., Anacker, C., Burghardt, N. S., Khandaker, H., Andreu, V., Millette, A., Leary, P., Ravenelle, R., Jimenez, J. C., Mastrodonato, A., Denny, C. A., Fenton, A. A., Scharfman, H. E., & Hen, R. (2019). Adult-born hippocampal neurons bidirectionally modulate entorhinal inputs into the dentate gyrus. *Science*, *364*(6440), 578-583. https://doi.org/10.1126/science.aat8789

Luo, J., Nguyen, A., Villeda, S., Zhang, H., Ding, Z., Lindsey, D., Bieri, G., Castellano, J. M., Beaupre, G. S., & Wyss-Coray, T. (2014). Long-Term Cognitive Impairments and Pathological Alterations in a Mouse Model of Repetitive Mild Traumatic Brain Injury. *Frontiers in Neurology*, *5*. https://doi.org/10.3389/fneur.2014.00012

M.

Maas, A. I. R., Menon, D. K., Adelson, P. D., Andelic, N., Bell, M. J., Belli, A., Bragge, P., Brazinova, A., Büki, A., Chesnut, R. M., Citerio, G., Coburn, M., Cooper, D. J., Crowder, A. T., Czeiter, E., Czosnyka, M., Diaz-Arrastia, R., Dreier, J. P., Duhaime, A.-C., … Zumbo, F. (2017). Traumatic brain injury: Integrated approaches to improve prevention, clinical care, and research. *The Lancet Neurology*, *16*(12), 987-1048. https://doi.org/10.1016/S1474-4422(17)30371-X

Maier, B., Schwerdtfeger, K., Mautes, A., Holanda, M., Müller, M., Steudel, W. I., & Marzi, I. (2001). DIFFERENTIAL RELEASE OF INTERLEUKINES 6, 8, AND 10 IN CEREBROSPINAL FLUID AND PLASMA AFTER TRAUMATIC BRAIN INJURY: *Shock*, *15*(6), 421-426. https://doi.org/10.1097/00024382-200115060-00002

Manley, G., Gardner, A. J., Schneider, K. J., Guskiewicz, K. M., Bailes, J., Cantu, R. C., Castellani, R. J., Turner, M., Jordan, B. D., Randolph, C., Dvořák, J., Hayden, K. A., Tator, C. H., McCrory, P., & Iverson, G. L. (2017). A systematic review of potential long-term effects of sport-related concussion. *British Journal of Sports Medicine*, *51*(12), 969-977. https://doi.org/10.1136/bjsports-2017-097791

Marín, O., & Rubenstein, J. L. R. (2003). Cell migration in the forebrain. *Annual Review of Neuroscience*, *26*(1), 441-483. https://doi.org/10.1146/annurev.neuro.26.041002.131058

Marklund, N., Bakshi, A., Castelbuono, D., Conte, V., & McIntosh, T. (2006). Evaluation of Pharmacological Treatment Strategies in Traumatic Brain Injury. *Current Pharmaceutical Design*, *12*(13), 1645-1680. https://doi.org/10.2174/138161206776843340

Markwardt, S. J., Wadiche, J. I., & Overstreet-Wadiche, L. S. (2009). Input-Specific GABAergic Signaling to Newborn Neurons in Adult Dentate Gyrus. *Journal of Neuroscience*, *29*(48), 15063-15072. https://doi.org/10.1523/JNEUROSCI.2727-09.2009

Martín-Suárez, S., Abiega, O., Ricobaraza, A., Hernandez-Alcoceba, R., & Encinas, J. M. (2020). Alterations of the Hippocampal Neurogenic Niche in a Mouse Model of Dravet Syndrome. *Frontiers in Cell and Developmental Biology*, *8*, 654. https://doi.org/10.3389/fcell.2020.00654

Martín-Suárez, S., & Encinas, J. M. (2021). The future belongs to those who prepare for it today. *Cell Stem Cell*, *28*(5), 783-785. https://doi.org/10.1016/j.stem.2021.04.014

Martín-Suárez, S., Valero, J., Muro-García, T., & Encinas, J. M. (2019a). Phenotypical and functional heterogeneity of neural stem cells in the aged hippocampus. *Aging Cell*, *18*(4). https://doi.org/10.1111/acel.12958

Martín-Suárez, S., Valero, J., Muro-García, T., & Encinas, J. M. (2019b). Phenotypical and functional heterogeneity of neural stem cells in the aged hippocampus. *Aging Cell*, *18*(4). https://doi.org/10.1111/acel.12958

Mathews, K. J., Allen, K. M., Boerrigter, D., Ball, H., Shannon Weickert, C., & Double, K. L. (2017). Evidence for reduced neurogenesis in the aging human hippocampus despite stable stem cell markers. *Aging Cell*, *16*(5), 1195-1199. https://doi.org/10.1111/acel.12641

Maxwell, W. L., Domleo, A., McColl, G., Jafari, S. S., & Graham, D. I. (2003). Post-Acute Alterations in the Axonal Cytoskeleton after Traumatic Axonal Injury. *Journal of Neurotrauma*, *20*(2), 151-168. https://doi.org/10.1089/08977150360547071

May, P. R. A. (1979). Woodpecker Drilling Behavior: An Endorsement of the Rotational Theory of Impact Brain Injury. *Archives of Neurology*, *36*(6), 370. https://doi.org/10.1001/archneur.1979.00500420080011

McAllister, T. W. (2011). Neurobiological consequences of traumatic brain injury. *Dialogues in Clinical Neuroscience*, *13*(3), 287-300. https://doi.org/10.31887/DCNS.2011.13.2/tmcallister

McCrea, M. A., Nelson, L. D., & Guskiewicz, K. (2017). Diagnosis and Management of Acute Concussion. *Physical Medicine and Rehabilitation Clinics of North America*, *28*(2), 271-286. https://doi.org/10.1016/j.pmr.2016.12.005

McDonald, S. (2013). Impairments in Social Cognition Following Severe Traumatic Brain Injury. *Journal of the International Neuropsychological Society*, *19*(3), 231-246. https://doi.org/10.1017/S1355617712001506

McGinn, M. J., & Povlishock, J. T. (2016). Pathophysiology of Traumatic Brain Injury. *Neurosurgery Clinics of North America*, *27*(4), 397-407. https://doi.org/10.1016/j.nec.2016.06.002

McKay, R. (1997). Stem Cells in the Central Nervous System. *Science*, *276*(5309), 66-71. https://doi.org/10.1126/science.276.5309.66

Mckee, A. C., Abdolmohammadi, B., & Stein, T. D. (2018). The neuropathology of chronic traumatic encephalopathy. En *Handbook of Clinical Neurology* (Vol. 158, pp. 297-307). Elsevier. https://doi.org/10.1016/B978-0-444-63954-7.00028-8

Mckee, A. C., & Daneshvar, D. H. (2015). The neuropathology of traumatic brain injury. En *Handbook of Clinical Neurology* (Vol. 127, pp. 45-66). Elsevier. https://doi.org/10.1016/B978-0-444-52892-6.00004-0

Meaney, D. F., Smith, D. H., Shreiber, D. I., Bain, A. C., Miller, R. T., Ross, D. T., & Gennarelli, T. A. (1995). Biomechanical Analysis of Experimental Diffuse Axonal Injury. *Journal of Neurotrauma*, *12*(4), 689-694. https://doi.org/10.1089/neu.1995.12.689

Menon, D. K., Schwab, K., Wright, D. W., & Maas, A. I. (2010). Position Statement: Definition of Traumatic Brain Injury. *Archives of Physical Medicine and Rehabilitation*, *91*(11), 1637-1640. https://doi.org/10.1016/j.apmr.2010.05.017

Merz, K., & Lie, D. C. (2013). Evidence that Doublecortin Is Dispensable for the Development of Adult Born Neurons in Mice. *PLoS ONE*, *8*(5), e62693. https://doi.org/10.1371/journal.pone.0062693

Messier, B., Leblond, C. P., & Smart, I. (1958). Presence of DNA synthesis and mitosis in the brain of young adult mice. *Experimental Cell Research*, *14*(1), 224-226. https://doi.org/10.1016/0014-4827(58)90235-0

Meythaler, J. M., Peduzzi, J. D., Eleftheriou, E., & Novack, T. A. (2001). Current concepts: Diffuse axonal injury–associated traumatic brain injury. *Archives of Physical Medicine and Rehabilitation*, *82*(10), 1461-1471. https://doi.org/10.1053/apmr.2001.25137

Michel, R. P., & Cruz-Orive, L. M. (1988). Application of the Cavalieri principle and vertical sections method to lung: Estimation of volume and pleural surface area. *Journal of Microscopy*, *150*(2), 117-136. https://doi.org/10.1111/j.1365-2818.1988.tb04603.x

Mierzwa, A. J., Marion, C. M., Sullivan, G. M., McDaniel, D. P., & Armstrong, R. C. (2015). Components of Myelin Damage and Repair in the Progression of White Matter Pathology After Mild Traumatic Brain Injury. *Journal of Neuropathology & Experimental Neurology*, *74*(3), 218-232. https://doi.org/10.1097/NEN.0000000000000165

Mignone, J. L., Kukekov, V., Chiang, A.-S., Steindler, D., & Enikolopov, G. (2004). Neural stem and progenitor cells in nestin-GFP transgenic mice. *The Journal of Comparative Neurology*, *469*(3), 311-324. https://doi.org/10.1002/cne.10964

Millen, J. E., Glauser, F. L., & Fairman, R. P. (1985). A comparison of physiological responses to percussive brain trauma in dogs and sheep. *Journal of Neurosurgery*, *62*(4), 587-591. https://doi.org/10.3171/jns.1985.62.4.0587

Miller, J. A., Nathanson, J., Franjic, D., Shim, S., Dalley, R. A., Shapouri, S., Smith, K. A., Sunkin, S. M., Bernard, A., Bennett, J. L., Lee, C.-K., Hawrylycz, M. J., Jones, A. R., Amaral, D. G., Sestan, N., Gage, F. H., & Lein, E. S. (2013). Conserved molecular signatures of neurogenesis in the hippocampal subgranular zone of rodents and primates. *Development*, *140*(22), 4633-4644. https://doi.org/10.1242/dev.097212

Milner, B. (1963). Effects of Different Brain Lesions on Card Sorting: The Role of the Frontal Lobes. *Archives of Neurology*, *9*(1), 90. https://doi.org/10.1001/archneur.1963.00460070100010

Mira, H., Andreu, Z., Suh, H., Lie, D. C., Jessberger, S., Consiglio, A., San Emeterio, J., Hortigüela, R., Marqués-Torrejón, M. Á., Nakashima, K., Colak, D., Götz, M., Fariñas, I., & Gage, F. H. (2010). Signaling through BMPR-IA Regulates Quiescence and Long-Term Activity of Neural Stem Cells in the Adult Hippocampus. *Cell Stem Cell*, *7*(1), 78-89. https://doi.org/10.1016/j.stem.2010.04.016

Mira, R. G., Lira, M., & Cerpa, W. (2021). Traumatic Brain Injury: Mechanisms of Glial Response. *Frontiers in Physiology*, *12*, 740939. https://doi.org/10.3389/fphys.2021.740939

Miyazaki, S., Katayama, Y., Lyeth, B. G., Jenkins, L. W., DeWitt, D. S., Goldberg, S. J., Newlon, P. G., & Hayes, R. L. (1992). Enduring suppression of hippocampal long-term potentiation following traumatic brain injury in rat. *Brain Research*, *585*(1-2), 335-339. https://doi.org/10.1016/0006-8993(92)91232-4

Moe, M. C., Varghese, M., Danilov, A. I., Westerlund, U., Ramm-Pettersen, J., Brundin, L., Svensson, M., Berg-Johnsen, J., & Langmoen, I. A. (2005). Multipotent progenitor cells

from the adult human brain: Neurophysiological differentiation to mature neurons. *Brain*, *128*(9), 2189-2199. https://doi.org/10.1093/brain/awh574

Mollayeva, T., Mollayeva, S., & Colantonio, A. (2018). Traumatic brain injury: Sex, gender and intersecting vulnerabilities. *Nature Reviews Neurology*, *14*(12), 711-722. https://doi.org/10.1038/s41582-018-0091-y

Moreno-Jiménez, E. P., Flor-García, M., Terreros-Roncal, J., Rábano, A., Cafini, F., Pallas-Bazarra, N., Ávila, J., & Llorens-Martín, M. (2019). Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nature Medicine*, *25*(4), 554-560. https://doi.org/10.1038/s41591-019-0375-9

Morganti-Kossman, M. C., Lenzlinger, P. M., Hans, V., Stahel, P., Csuka, E., Ammann, E., Stocker, R., Trentz, O., & Kossmann, T. (1997). Production of cytokines following brain injury: Beneficial and deleterious for the damaged tissue. *Molecular Psychiatry*, *2*(2), 133-136. https://doi.org/10.1038/sj.mp.4000227

Morris, R. G. M., Garrud, P., Rawlins, J. N. P., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681-683. https://doi.org/10.1038/297681a0

Morrison, B., Cater, H. L., Benham, C. D., & Sundstrom, L. E. (2006). An in vitro model of traumatic brain injury utilising two-dimensional stretch of organotypic hippocampal slice cultures. *Journal of Neuroscience Methods*, *150*(2), 192-201. https://doi.org/10.1016/j.jneumeth.2005.06.014

Morrison, B., Elkin, B. S., Dollé, J.-P., & Yarmush, M. L. (2011). In Vitro Models of Traumatic Brain Injury. *Annual Review of Biomedical Engineering*, *13*(1), 91-126. https://doi.org/10.1146/annurev-bioeng-071910-124706

Morrison III, B., Meaney, D. F., & McIntosh, T. K. (1998). Mechanical Characterization of an In Vitro Device Designed to Quantitatively Injure Living Brain Tissue. *Annals of Biomedical Engineering*, *26*(3), 381-390. https://doi.org/10.1114/1.61

Morrow, C. S., Porter, T. J., Xu, N., Arndt, Z. P., Ako-Asare, K., Heo, H. J., Thompson, E. A. N., & Moore, D. L. (2020). Vimentin Coordinates Protein Turnover at the Aggresome during Neural Stem Cell Quiescence Exit. *Cell Stem Cell*, *26*(4), 558-568.e9. https://doi.org/10.1016/j.stem.2020.01.018

Moser, E., Moser, M., & Andersen, P. (1993). Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *The Journal of Neuroscience*, *13*(9), 3916-3925. https://doi.org/10.1523/JNEUROSCI.13-09-03916.1993

Moser, M.-B., & Moser, E. I. (1998). Functional differentiation in the hippocampus. *Hippocampus*, *8*(6), 608-619. https://doi.org/10.1002/(SICI)1098-1063(1998)8:6<608::AID-HIPO3>3.0.CO;2-7

Moskowitz, P., & Oblinger, M. (1995). Sensory neurons selectively upregulate synthesis and transport of the beta III-tubulin protein during axonal regeneration. *The Journal of Neuroscience*, *15*(2), 1545-1555. https://doi.org/10.1523/JNEUROSCI.15-02-01545.1995

Mouzon, B. C., Bachmeier, C., Ferro, A., Ojo, J.-O., Crynen, G., Acker, C. M., Davies, P., Mullan, M., Stewart, W., & Crawford, F. (2014). Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model: Chronic Effects of r-mTBI. *Annals of Neurology*, *75*(2), 241-254. https://doi.org/10.1002/ana.24064

Mtchedlishvili, Z., Lepsveridze, E., Xu, H., Kharlamov, E. A., Lu, B., & Kelly, K. M. (2010). Increase of GABAA receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury. *Neurobiology of Disease*, *38*(3), 464-475. https://doi.org/10.1016/j.nbd.2010.03.012

N.

Nadel, L. (1968). Dorsal and ventral hippocampal lesions and behavior. *Physiology & Behavior*, *3*(6), 891-900. https://doi.org/10.1016/0031-9384(68)90174-1

Nakajima, K., & Kohsaka, S. (2004). Microglia:Neuroprotective and Neurotrophic Cells in the Central Nervous System. *Current Drug Target -Cardiovascular & Hematological Disorders*, *4*(1), 65-84. https://doi.org/10.2174/1568006043481284

Nakamura, K., Yamashita, Y., Tamamaki, N., Katoh, H., Kaneko, T., & Negishi, M. (2006). In vivo function of Rnd2 in the development of neocortical pyramidal neurons. *Neuroscience Research*, *54*(2), 149-153. https://doi.org/10.1016/j.neures.2005.10.008

Nakashiba, T., Cushman, J. D., Pelkey, K. A., Renaudineau, S., Buhl, D. L., McHugh, T. J., Barrera, V. R., Chittajallu, R., Iwamoto, K. S., McBain, C. J., Fanselow, M. S., & Tonegawa, S. (2012). Young Dentate Granule Cells Mediate Pattern Separation, whereas Old Granule Cells Facilitate Pattern Completion. *Cell*, *149*(1), 188-201. https://doi.org/10.1016/j.cell.2012.01.046

Neuberger, E. J., Swietek, B., Corrubia, L., Prasanna, A., & Santhakumar, V. (2017). Enhanced Dentate Neurogenesis after Brain Injury Undermines Long-Term Neurogenic Potential and Promotes Seizure Susceptibility. *Stem Cell Reports*, *9*(3), 972-984. https://doi.org/10.1016/j.stemcr.2017.07.015

Nguyen, R., Fiest, K. M., McChesney, J., Kwon, C.-S., Jette, N., Frolkis, A. D., Atta, C., Mah, S., Dhaliwal, H., Reid, A., Pringsheim, T., Dykeman, J., & Gallagher, C. (2016). The International Incidence of Traumatic Brain Injury: A Systematic Review and Meta-Analysis. *Canadian Journal of Neurological Sciences / Journal Canadien Des Sciences Neurologiques*, *43*(6), 774-785. https://doi.org/10.1017/cjn.2016.290

Ngwenya, L. B., Heyworth, N. C., Shwe, Y., Moore, T. L., & Rosene, D. L. (2015). Age-related changes in dentate gyrus cell numbers, neurogenesis, and associations with cognitive impairments in the rhesus monkey. *Frontiers in Systems Neuroscience*, *9*. https://doi.org/10.3389/fnsys.2015.00102

Nilsson, M., Perfilieva, E., Johansson, U., Orwar, O., & Eriksson, P. S. (1999). Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *Journal of Neurobiology*, *39*(4), 569-578. https://doi.org/10.1002/(SICI)1097-4695(19990615)39:4<569::AID-NEU10>3.0.CO;2-F

Nobes, C. D., Lauritzen, I., Mattei, M.-G., Paris, S., Hall, A., & Chardin, P. (1998). A New Member of the Rho Family, Rnd1, Promotes Disassembly of Actin Filament Structures and Loss of Cell Adhesion. *Journal of Cell Biology*, *141*(1), 187-197. https://doi.org/10.1083/jcb.141.1.187

Norris, C. M., & Scheff, S. W. (2009). Recovery of Afferent Function and Synaptic Strength in Hippocampal CA1 following Traumatic Brain Injury. *Journal of Neurotrauma*, *26*(12), 2269-2278. https://doi.org/10.1089/neu.2009.1029

O.

Obernier, K., & Alvarez-Buylla, A. (2019). Neural stem cells: Origin, heterogeneity and regulation in the adult mammalian brain. *Development*, *146*(4), dev156059. https://doi.org/10.1242/dev.156059

O'Keefe, J., & Nadel, L. (1978). *The hippocampus as a cognitive map*. Clarendon Press ; Oxford University Press.

P.

Pacary, E., Azzarelli, R., & Guillemot, F. (2013). Rnd3 coordinates early steps of cortical neurogenesis through actin-dependent and -independent mechanisms. *Nature Communications*, *4*(1), 1635. https://doi.org/10.1038/ncomms2614

Pacary, E., Heng, J., Azzarelli, R., Riou, P., Castro, D., Lebel-Potter, M., Parras, C., Bell, D. M., Ridley, A. J., Parsons, M., & Guillemot, F. (2011). Proneural Transcription Factors Regulate Different Steps of Cortical Neuron Migration through Rnd-Mediated Inhibition

of RhoA Signaling. *Neuron*, *69*(6), 1069-1084. https://doi.org/10.1016/j.neuron.2011.02.018

Palmer, A. M., Marion, D. W., Botscheller, M. L., Swedlow, P. E., Styren, S. D., & DeKosky, S. T. (1993). Traumatic Brain Injury-Induced Excitotoxicity Assessed in a Controlled Cortical Impact Model. *Journal of Neurochemistry*, *61*(6), 2015-2024. https://doi.org/10.1111/j.1471-4159.1993.tb07437.x

Palmer, T. D., Schwartz, P. H., Taupin, P., Kaspar, B., Stein, S. A., & Gage, F. H. (2001). Progenitor cells from human brain after death. *Nature*, *411*(6833), 42-43. https://doi.org/10.1038/35075141

Pavlovic, D., Pekic, S., Stojanovic, M., & Popovic, V. (2019). Traumatic brain injury: Neuropathological, neurocognitive and neurobehavioral sequelae. *Pituitary*, *22*(3), 270-282. https://doi.org/10.1007/s11102-019-00957-9

Perry, D. C., Sturm, V. E., Peterson, M. J., Pieper, C. F., Bullock, T., Boeve, B. F., Miller, B. L., Guskiewicz, K. M., Berger, M. S., Kramer, J. H., & Welsh-Bohmer, K. A. (2016). Association of traumatic brain injury with subsequent neurological and psychiatric disease: A meta-analysis. *Journal of Neurosurgery*, *124*(2), 511-526. https://doi.org/10.3171/2015.2.JNS14503

Peterson, A. B., Xu, L., Daughterty, J., & Braiding, M. J. (2019). *Surveillance Report of Traumatic Brain Injury-related Emergency Department Visits, Hospitalizations, and Deaths* (p. 24). National Center for Injury Prevention and Control.

Pilz, G.-A., Bottes, S., Betizeau, M., Jörg, D. J., Carta, S., Simons, B. D., Helmchen, F., & Jessberger, S. (2018). Live imaging of neurogenesis in the adult mouse hippocampus. *Science*, *359*(6376), 658-662. https://doi.org/10.1126/science.aao5056

Pineda, J. R., Daynac, M., Chicheportiche, A., Cebrian-Silla, A., Sii Felice, K., Garcia-Verdugo, J. M., Boussin, F. D., & Mouthon, M. (2013). Vascular-derived TGF-β increases in the stem cell niche and perturbs neurogenesis during aging and following irradiation in the adult mouse brain. *EMBO Molecular Medicine*, *5*(4), 548-562. https://doi.org/10.1002/emmm.201202197

Piolino, P., Desgranges, B., Manning, L., North, P., Jokic, C., & Eustache, F. (2007). Autobiographical Memory, the Sense of Recollection and Executive Functions After Severe Traumatic Brain Injury. *Cortex*, *43*(2), 176-195. https://doi.org/10.1016/S0010-9452(08)70474-X

Pitt, J., & Leung, Y. (2015). Clinical and Cellular Aspects of Traumatic Brain Injury. En *Handbook of Toxicology of Chemical Warfare Agents* (pp. 691-708). Elsevier. https://doi.org/10.1016/B978-0-12-800159-2.00047-6

Ponsford, J., Draper, K., & Schönberger, M. (2008). Functional outcome 10 years after traumatic brain injury: Its relationship with demographic, injury severity, and cognitive and emotional status. *Journal of the International Neuropsychological Society*, *14*(02). https://doi.org/10.1017/S1355617708080272

Povlishock, J. T., & Katz, D. I. (2005). Update of Neuropathology and Neurological Recovery After Traumatic Brain Injury: *Journal of Head Trauma Rehabilitation*, *20*(1), 76-94. https://doi.org/10.1097/00001199-200501000-00008

Prager, E. M., & Johnson, L. R. (2009). Stress at the Synapse: Signal Transduction Mechanisms of Adrenal Steroids at Neuronal Membranes. *Science Signaling*, *2*(86). https://doi.org/10.1126/scisignal.286re5

Prins, M. L., & Hovda, D. A. (1998). Traumatic Brain Injury in the Developing Rat: Effects of Maturation on Morris Water Maze Acquisition. *Journal of Neurotrauma*, *15*(10), 799-811. https://doi.org/10.1089/neu.1998.15.799

Pudenz, R. H., & Shelden, C. H. (1946). The Lucite Calvarium—A Method for Direct Observation of the Brain: II. Cranial Trauma and Brain Movement. *Journal of Neurosurgery*, *3*(6), 487-505. https://doi.org/10.3171/jns.1946.3.6.0487

R.

Rafaels, K., "Dale" Bass, C. R., Salzar, R. S., Panzer, M. B., Woods, W., Feldman, S., Cummings, T., & Capehart, B. (2011). Survival Risk Assessment for Primary Blast Exposures to the Head. *Journal of Neurotrauma*, *28*(11), 2319-2328. https://doi.org/10.1089/neu.2009.1207

Rakic, P. (1985). Limits of Neurogenesis in Primates. *Science*, *227*(4690), 1054-1056. https://doi.org/10.1126/science.3975601

Ramanathan, D. M., McWilliams, N., Schatz, P., & Hillary, F. G. (2012). Epidemiological Shifts in Elderly Traumatic Brain Injury: 18-Year Trends in Pennsylvania. *Journal of Neurotrauma*, *29*(7), 1371-1378. https://doi.org/10.1089/neu.2011.2197

Randolph, C. (2018). Chronic traumatic encephalopathy is not a real disease. *Archives of Clinical Neuropsychology*, *33*(5), 644-648. https://doi.org/10.1093/arclin/acy063

Rao, M. S., & Shetty, A. K. (2004). Efficacy of doublecortin as a marker to analyse the absolute number anddendritic growth of newly generated neurons in the adult dentate gyrus: Doublecortin and adult dentate gyrus. *European Journal of Neuroscience*, *19*(2), 234-246. https://doi.org/10.1111/j.0953-816X.2003.03123.x

Redell, J. B., Maynard, M. E., Underwood, E. L., Vita, S. M., Dash, P. K., & Kobori, N. (2020). Traumatic brain injury and hippocampal neurogenesis: Functional implications.

Experimental Neurology, *331*, 113372. https://doi.org/10.1016/j.expneurol.2020.113372

Reeves, T. M., Kao, C.-Q., Phillips, L. L., Bullock, M. R., & Povlishock, J. T. (2000). Presynaptic excitability changes following traumatic brain injury in the rat. *Journal of Neuroscience Research*, *60*(3), 370-379. https://doi.org/10.1002/(SICI)1097-4547(20000501)60:3<370::AID-JNR12>3.0.CO;2-B

Reeves, T. M., Lyeth, B. G., Phillips, L. L., Hamm, R. J., & Povlishock, J. T. (1997). The effects of traumatic brain injury on inhibition in the hippocampus and dentate gyrus. *Brain Research*, *757*(1), 119-132. https://doi.org/10.1016/S0006-8993(97)00170-4

Revest, J.-M., Dupret, D., Koehl, M., Funk-Reiter, C., Grosjean, N., Piazza, P.-V., & Abrous, D. N. (2009). Adult hippocampal neurogenesis is involved in anxiety-related behaviors. *Molecular Psychiatry*, *14*(10), 959-967. https://doi.org/10.1038/mp.2009.15

Ridley, A. J. (2001a). Rho GTPases and cell migration. *Journal of Cell Science*, *114*(15), 2713-2722. https://doi.org/10.1242/jcs.114.15.2713

Ridley, A. J. (2001b). Rho GTPases and cell migration. *Journal of Cell Science*, *114*(15), 2713-2722. https://doi.org/10.1242/jcs.114.15.2713

Riou, P., Villalonga, P., & Ridley, A. J. (2010). Rnd proteins: Multifunctional regulators of the cytoskeleton and cell cycle progression. *BioEssays*, *32*(11), 986-992. https://doi.org/10.1002/bies.201000060

Roberts, P. J., Mitin, N., Keller, P. J., Chenette, E. J., Madigan, J. P., Currin, R. O., Cox, A. D., Wilson, O., Kirschmeier, P., & Der, C. J. (2008). Rho Family GTPase Modification and Dependence on CAAX Motif-signaled Posttranslational Modification. *Journal of Biological Chemistry*, *283*(37), 25150-25163. https://doi.org/10.1074/jbc.M800882200

Robinson, C., Apgar, C., & Shapiro, L. A. (2016). Astrocyte Hypertrophy Contributes to Aberrant Neurogenesis after Traumatic Brain Injury. *Neural Plasticity*, *2016*, 1-10. https://doi.org/10.1155/2016/1347987

Rochat, L., Beni, C., Annoni, J.-M., Vuadens, P., & Van der Linden, M. (2013). How Inhibition Relates to Impulsivity after Moderate to Severe Traumatic Brain Injury. *Journal of the International Neuropsychological Society*, *19*(8), 890-898. https://doi.org/10.1017/S1355617713000672

Rola, R., Mizumatsu, S., Otsuka, S., Morhardt, D. R., Noble-Haeusslein, L. J., Fishman, K., Potts, M. B., & Fike, J. R. (2006). Alterations in hippocampal neurogenesis following traumatic brain injury in mice. *Experimental Neurology*, *202*(1), 189-199. https://doi.org/10.1016/j.expneurol.2006.05.034

Romine, J., Gao, X., & Chen, J. (2014). Controlled Cortical Impact Model for Traumatic Brain Injury. *Journal of Visualized Experiments*, *90*, 51781. https://doi.org/10.3791/51781

Royo, N. C., Wahl, F., & Stutzmann, J.-M. (1999). Kinetics of polymorphonuclear neutrophil infiltration after a traumatic brain injury in rat: *NeuroReport*, *10*(6), 1363-1367. https://doi.org/10.1097/00001756-199904260-00038

Russell, W. R., & Smith, A. (1961). Post-Traumatic Amnesia in Closed Head Injury. *Archives of Neurology*, *5*(1), 4-17. https://doi.org/10.1001/archneur.1961.00450130006002

S.

Saatman, K. E., Duhaime, A.-C., Bullock, R., Maas, A. I. R., Valadka, A., & Manley, G. T. (2008). Classification of Traumatic Brain Injury for Targeted Therapies. *Journal of Neurotrauma*, *25*(7), 719-738. https://doi.org/10.1089/neu.2008.0586

Sahay, A., Scobie, K. N., Hill, A. S., O'Carroll, C. M., Kheirbek, M. A., Burghardt, N. S., Fenton, A. A., Dranovsky, A., & Hen, R. (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature*, *472*(7344), 466-470. https://doi.org/10.1038/nature09817

Saing, T., Dick, M., Nelson, P. T., Kim, R. C., Cribbs, D. H., & Head, E. (2012). Frontal Cortex Neuropathology in Dementia Pugilistica. *Journal of Neurotrauma*, *29*(6), 1054-1070. https://doi.org/10.1089/neu.2011.1957

Sánchez-Huerta, K., García-Martínez, Y., Vergara, P., Segovia, J., & Pacheco-Rosado, J. (2016). Thyroid hormones are essential to preserve non-proliferative cells of adult neurogenesis of the dentate gyrus. *Molecular and Cellular Neuroscience*, *76*, 1-10. https://doi.org/10.1016/j.mcn.2016.08.001

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., & Hen, R. (2003). Requirement of Hippocampal Neurogenesis for the Behavioral Effects of Antidepressants. *Science*, *301*(5634), 805-809. https://doi.org/10.1126/science.1083328

Santhakumar, V., Bender, R., Frotscher, M., Ross, S. T., Hollrigel, G. S., Toth, Z., & Soltesz, I. (2000). Granule cell hyperexcitability in the early post-traumatic rat dentate gyrus: The 'irritable mossy cell' hypothesis. *The Journal of Physiology*, *524*(1), 117-134. https://doi.org/10.1111/j.1469-7793.2000.00117.x

Santhakumar, V., Ratzliff, A. D. H., Jeng, J., Toth, Z., & Soltesz, I. (2001). Long-term hyperexcitability in the hippocampus after experimental head trauma. *Annals of Neurology*, *50*(6), 708-717. https://doi.org/10.1002/ana.1230

Savitsky, B., Givon, A., Rozenfeld, M., Radomislensky, I., & Peleg, K. (2016). Traumatic brain injury: It is all about definition. *Brain Injury*, *30*(10), 1194-1200. https://doi.org/10.1080/02699052.2016.1187290

Saxe, M. D., Battaglia, F., Wang, J.-W., Malleret, G., David, D. J., Monckton, J. E., Garcia, A. D. R., Sofroniew, M. V., Kandel, E. R., Santarelli, L., Hen, R., & Drew, M. R. (2006). Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proceedings of the National Academy of Sciences*, *103*(46), 17501-17506. https://doi.org/10.1073/pnas.0607207103

Scafidi, S., O'Brien, J., Hopkins, I., Robertson, C., Fiskum, G., & McKenna, M. (2009). Delayed cerebral oxidative glucose metabolism after traumatic brain injury in young rats. *Journal of Neurochemistry*, *109*, 189-197. https://doi.org/10.1111/j.1471-4159.2009.05896.x

Schaks, M., Giannone, G., & Rottner, K. (2019). Actin dynamics in cell migration. *Essays in Biochemistry*, *63*(5), 483-495. https://doi.org/10.1042/EBC20190015

Scharfman, H. E., & Myers, C. E. (2013). Hilar mossy cells of the dentate gyrus: A historical perspective. *Frontiers in Neural Circuits*, *6*. https://doi.org/10.3389/fncir.2012.00106

Schiefermeier, N., Teis, D., & Huber, L. A. (2011). Endosomal signaling and cell migration. *Current Opinion in Cell Biology*, *23*(5), 615-620. https://doi.org/10.1016/j.ceb.2011.04.001

Schmidt-Hieber, C., Jonas, P., & Bischofberger, J. (2004). *Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus*. *429*, 4.

Seaberg, R. M., & van der Kooy, D. (2003). Stem and progenitor cells: The premature desertion of rigorous definitions. *Trends in Neurosciences*, *26*(3), 125-131. https://doi.org/10.1016/S0166-2236(03)00031-6

Segi-Nishida, E., Warner-Schmidt, J. L., & Duman, R. S. (2008). Electroconvulsive seizure and VEGF increase the proliferation of neural stem-like cells in rat hippocampus. *Proceedings of the National Academy of Sciences*, *105*(32), 11352-11357. https://doi.org/10.1073/pnas.0710858105

Seki, T., & Arai, Y. (1993). Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat. *The Journal of Neuroscience*, *13*(6), 2351-2358. https://doi.org/10.1523/JNEUROSCI.13-06-02351.1993

Semerci, F., Choi, W. T.-S., Bajic, A., Thakkar, A., Encinas, J. M., Depreux, F., Segil, N., Groves, A. K., & Maletic-Savatic, M. (2017). Lunatic fringe-mediated Notch signaling regulates adult hippocampal neural stem cell maintenance. *ELife*, *6*, e24660. https://doi.org/10.7554/eLife.24660

Seri, B., García-Verdugo, J. M., McEwen, B. S., & Alvarez-Buylla, A. (2001). Astrocytes Give Rise to New Neurons in the Adult Mammalian Hippocampus. *The Journal of Neuroscience*, *21*(18), 7153-7160. https://doi.org/10.1523/JNEUROSCI.21-18-07153.2001

Serra-Grabulosa, J. M. (2005). Cerebral correlates of declarative memory dysfunctions in early traumatic brain injury. *Journal of Neurology, Neurosurgery & Psychiatry*, *76*(1), 129-131. https://doi.org/10.1136/jnnp.2004.027631

Shapiro, L. A. (2017). Altered Hippocampal Neurogenesis during the First 7 Days after a Fluid Percussion Traumatic Brain Injury. *Cell Transplantation*, *26*(7), 1314-1318. https://doi.org/10.1177/0963689717714099

Shors, T. J., Miesegaes, G., Beylin, A., Zhao, M., Rydel, T., & Gould, E. (2001). Neurogenesis in the adult is involved in the formation of trace memories. *Nature*, *410*(6826), 372-376. https://doi.org/10.1038/35066584

Shultz, S. R., McDonald, S. J., Vonder Haar, C., Meconi, A., Vink, R., van Donkelaar, P., Taneja, C., Iverson, G. L., & Christie, B. R. (2017). The potential for animal models to provide insight into mild traumatic brain injury: Translational challenges and strategies. *Neuroscience & Biobehavioral Reviews*, *76*, 396-414. https://doi.org/10.1016/j.neubiorev.2016.09.014

Sieg, F., Wahle, P., & Pape, H.-C. (1999). Cellular Reactivity to Mechanical Axonal Injury in an Organotypic *in Vitro* Model of Neurotrauma. *Journal of Neurotrauma*, *16*(12), 1197-1213. https://doi.org/10.1089/neu.1999.16.1197

Siegel, A., & Tassoni, J. P. (1971). Differential Efferent Projections from the Ventral and Dorsal Hippocampus of the Cat. *Brain, Behavior and Evolution*, *4*(3), 185-200. https://doi.org/10.1159/000125433

Sierra, A., Encinas, J. M., Deudero, J. J. P., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., Tsirka, S. E., & Maletic-Savatic, M. (2010). Microglia Shape Adult Hippocampal Neurogenesis through Apoptosis-Coupled Phagocytosis. *Cell Stem Cell*, *7*(4), 483-495. https://doi.org/10.1016/j.stem.2010.08.014

Sierra, A., Martín-Suárez, S., Valcárcel-Martín, R., Pascual-Brazo, J., Aelvoet, S.-A., Abiega, O., Deudero, J. J., Brewster, A. L., Bernales, I., Anderson, A. E., Baekelandt, V., Maletić-Savatić, M., & Encinas, J. M. (2015). Neuronal Hyperactivity Accelerates Depletion of Neural Stem Cells and Impairs Hippocampal Neurogenesis. *Cell Stem Cell*, *16*(5), 488-503. https://doi.org/10.1016/j.stem.2015.04.003

Silva, D. dos S., Brito, J. N. P. de O., Ibiapina, J. O., Lima, M. F. M. B., Medeiros, A. R. G. de V., Queiroz, B. H. C. e, Paiva, A. L. C., & Guedes, V. O. de M. (2012). Closed head injury in rats: Histopathological aspects in an experimental weight drop model. *Acta Cirurgica Brasileira*, *27*(4), 290-294. https://doi.org/10.1590/S0102-86502012000400002

Silva, R. F. M., Falcão, A. S., Fernandes, A., Gordo, A. C., Brito, M. A., & Brites, D. (2006). Dissociated primary nerve cell cultures as models for assessment of neurotoxicity. *Toxicology Letters*, *163*(1), 1-9. https://doi.org/10.1016/j.toxlet.2005.09.033

Singh, I. N., Sullivan, P. G., Deng, Y., Mbye, L. H., & Hall, E. D. (2006). Time Course of Post-Traumatic Mitochondrial Oxidative Damage and Dysfunction in a Mouse Model of Focal Traumatic Brain Injury: Implications for Neuroprotective Therapy. *Journal of Cerebral Blood Flow & Metabolism*, *26*(11), 1407-1418. https://doi.org/10.1038/sj.jcbfm.9600297

Singleton, R. H., Zhu, J., Stone, J. R., & Povlishock, J. T. (2002). Traumatically Induced Axotomy Adjacent to the Soma Does Not Result in Acute Neuronal Death. *The Journal of Neuroscience*, *22*(3), 791-802. https://doi.org/10.1523/JNEUROSCI.22-03-00791.2002

Skandsen, T., Kvistad, K. A., Solheim, O., Strand, I. H., Folvik, M., & Vik, A. (2010). Prevalence and impact of diffuse axonal injury in patients with moderate and severe head injury: A cohort study of early magnetic resonance imaging findings and 1-year outcome: Clinical article. *Journal of Neurosurgery*, *113*(3), 556-563. https://doi.org/10.3171/2009.9.JNS09626

Snyder, J. S., Soumier, A., Brewer, M., Pickel, J., & Cameron, H. A. (2011). Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature*, *476*(7361), 458-461. https://doi.org/10.1038/nature10287

Sofroniew, M. V. (2005). Reactive Astrocytes in Neural Repair and Protection. *The Neuroscientist*, *11*(5), 400-407. https://doi.org/10.1177/1073858405278321

Sofroniew, M. V., & Vinters, H. V. (2010). Astrocytes: Biology and pathology. *Acta Neuropathologica*, *119*(1), 7-35. https://doi.org/10.1007/s00401-009-0619-8

Song, J., Zhong, C., Bonaguidi, M. A., Sun, G. J., Hsu, D., Gu, Y., Meletis, K., Huang, Z. J., Ge, S., Enikolopov, G., Deisseroth, K., Luscher, B., Christian, K. M., Ming, G., & Song, H. (2012). Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. *Nature*, *489*(7414), 150-154. https://doi.org/10.1038/nature11306

Sorrells, S. F., Munhoz, C. D., Manley, N. C., Yen, S., & Sapolsky, R. M. (2014). Glucocorticoids Increase Excitotoxic Injury and Inflammation in the Hippocampus of Adult Male Rats. *Neuroendocrinology*, *100*(2-3), 129-140. https://doi.org/10.1159/000367849

Sorrells, S. F., Paredes, M. F., Cebrian-Silla, A., Sandoval, K., Qi, D., Kelley, K. W., James, D., Mayer, S., Chang, J., Auguste, K. I., Chang, E. F., Gutierrez, A. J., Kriegstein, A. R., Mathern, G. W., Oldham, M. C., Huang, E. J., Garcia-Verdugo, J. M., Yang, Z., & Alvarez-Buylla, A. (2018). Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature*, *555*(7696), 377-381. https://doi.org/10.1038/nature25975

Spalding, K. L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H. B., Boström, E., Westerlund, I., Vial, C., Buchholz, B. A., Possnert, G., Mash, D. C., Druid, H., & Frisén, J. (2013). Dynamics of Hippocampal Neurogenesis in Adult Humans. *Cell*, *153*(6), 1219-1227. https://doi.org/10.1016/j.cell.2013.05.002

Sporns, O. (2011). *Networks of the brain*. Massachusetts institute of technology.

Sporns, O. (2013). Structure and function of complex brain networks. *Dialogues in Clinical Neuroscience*, *15*(3), 247-262. https://doi.org/10.31887/DCNS.2013.15.3/osporns

Steiner, B., Klempin, F., Wang, L., Kott, M., Kettenmann, H., & Kempermann, G. (2006). Type-2 cells as link between glial and neuronal lineage in adult hippocampal neurogenesis. *Glia*, *54*(8), 805-814. https://doi.org/10.1002/glia.20407

Stevens, R., & Cowey, A. (1973). Effects of dorsal and ventral hippocampal lesions on spontaneous alternation, learned alternation and probability learning in rats. *Brain Research*, *52*, 203-224. https://doi.org/10.1016/0006-8993(73)90659-8

Strich, S. J. (1956). DIFFUSE DEGENERATION OF THE CEREBRAL WHITE MATTER IN SEVERE DEMENTIA FOLLOWING HEAD INJURY. *Journal of Neurology, Neurosurgery & Psychiatry*, *19*(3), 163-185. https://doi.org/10.1136/jnnp.19.3.163

Stuss, D. T. (2011). Traumatic brain injury: Relation to executive dysfunction and the frontal lobes. *Current Opinion in Neurology*, *24*(6), 584-589. https://doi.org/10.1097/WCO.0b013e32834c7eb9

Sullivan, P. G., Keller, J. N., Bussen, W. L., & Scheff, S. W. (2002). Cytochrome c release and caspase activation after traumatic brain injury. *Brain Research*, *949*(1-2), 88-96. https://doi.org/10.1016/S0006-8993(02)02968-2

Sun, D., Colello, R. J., Daugherty, W. P., Kwon, T. H., McGinn, M. J., Harvey, H. B., & Bullock, M. R. (2005). Cell Proliferation and Neuronal Differentiation in the Dentate Gyrus in Juvenile and Adult Rats following Traumatic Brain Injury. *Journal of Neurotrauma*, *22*(1), 95-105. https://doi.org/10.1089/neu.2005.22.95

Sun, D., Daniels, T. E., Rolfe, A., Waters, M., & Hamm, R. (2015). Inhibition of Injury-Induced Cell Proliferation in the Dentate Gyrus of the Hippocampus Impairs Spontaneous Cognitive Recovery after Traumatic Brain Injury. *Journal of Neurotrauma*, *32*(7), 495-505. https://doi.org/10.1089/neu.2014.3545

Sun, D., McGinn, M. J., Zhou, Z., Harvey, H. B., Bullock, M. R., & Colello, R. J. (2007). Anatomical integration of newly generated dentate granule neurons following traumatic brain injury in adult rats and its association to cognitive recovery. *Experimental Neurology*, *204*(1), 264-272. https://doi.org/10.1016/j.expneurol.2006.11.005

Sun, G. J., Zhou, Y., Stadel, R. P., Moss, J., Yong, J. H. A., Ito, S., Kawasaki, N. K., Phan, A. T., Oh, J. H., Modak, N., Reed, R. R., Toni, N., Song, H., & Ming, G. (2015). Tangential migration of neuronal precursors of glutamatergic neurons in the adult mammalian brain. *Proceedings of the National Academy of Sciences*, *112*(30), 9484-9489. https://doi.org/10.1073/pnas.1508545112

Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., Hen, R., & Belzung, C. (2008). Drug-Dependent Requirement of Hippocampal Neurogenesis in a Model of Depression and of Antidepressant Reversal. *Biological Psychiatry*, *64*(4), 293-301. https://doi.org/10.1016/j.biopsych.2008.02.022

T.

Tanaka, H., Fujita, H., Katoh, H., Mori, K., & Negishi, M. (2002). Vps4-A (vacuolar protein sorting 4-A) is a binding partner for a novel Rho family GTPase, Rnd2. *Biochemical Journal*, *365*(2), 349-353. https://doi.org/10.1042/bj20020062

Tanapat, P., Hastings, N. B., Rydel, T. A., Galea, L. A. M., & Gould, E. (2001). Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *The Journal of Comparative Neurology*, *437*(4), 496-504. https://doi.org/10.1002/cne.1297

Tang-Schomer, M. D., Patel, A. R., Baas, P. W., & Smith, D. H. (2010). Mechanical breaking of microtubules in axons during dynamic stretch injury underlies delayed elasticity, microtubule disassembly, and axon degeneration. *The FASEB Journal*, *24*(5), 1401-1410. https://doi.org/10.1096/fj.09-142844

Tapia, R. N., & Eapen, B. C. (2017). Rehabilitation of Persistent Symptoms After Concussion. *Physical Medicine and Rehabilitation Clinics of North America*, *28*(2), 287-299. https://doi.org/10.1016/j.pmr.2016.12.006

Tartt, A. N., Fulmore, C. A., Liu, Y., Rosoklija, G. B., Dwork, A. J., Arango, V., Hen, R., Mann, J. J., & Boldrini, M. (2018). Considerations for Assessing the Extent of Hippocampal Neurogenesis in the Adult and Aging Human Brain. *Cell Stem Cell*, *23*(6), 782-783. https://doi.org/10.1016/j.stem.2018.10.025

Tashiro, A., Sandler, V. M., Toni, N., Zhao, C., & Gage, F. H. (2006). NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature*, *442*(7105), 929-933. https://doi.org/10.1038/nature05028

Tate, D. F., & Bigler, E. D. (2000). Fornix and Hippocampal Atrophy in Traumatic Brain Injury. *Learning & Memory*, *7*(6), 442-446. https://doi.org/10.1101/lm.33000

Taverna, E., & Huttner, W. B. (2010). Neural Progenitor Nuclei IN Motion. *Neuron*, *67*(6), 906-914. https://doi.org/10.1016/j.neuron.2010.08.027

Terreros-Roncal, J., Moreno-Jiménez, E. P., Flor-García, M., Rodríguez-Moreno, C. B., Trinchero, M. F., Cafini, F., Rábano, A., & Llorens-Martín, M. (2021). Impact of neurodegenerative diseases on human adult hippocampal neurogenesis. *Science*, *374*(6571), 1106-1113. https://doi.org/10.1126/science.abl5163

Tharmaratnam, T., Iskandar, M. A., Tabobondung, T. C., Tobbia, I., Gopee-Ramanan, P., & Tabobondung, T. A. (2018). Chronic Traumatic Encephalopathy in Professional American Football Players: Where Are We Now? *Frontiers in Neurology*, *9*, 445. https://doi.org/10.3389/fneur.2018.00445

Thompson, H. J., Lifshitz, J., Marklund, N., Grady, M. S., Graham, D. I., Hovda, D. A., & McIntosh, T. K. (2005). Lateral Fluid Percussion Brain Injury: A 15-Year Review and Evaluation. *Journal of Neurotrauma*, *22*(1), 42-75. https://doi.org/10.1089/neu.2005.22.42

Tobin, M. K., Musaraca, K., Disouky, A., Shetti, A., Bheri, A., Honer, W. G., Kim, N., Dawe, R. J., Bennett, D. A., Arfanakis, K., & Lazarov, O. (2019). Human Hippocampal Neurogenesis Persists in Aged Adults and Alzheimer's Disease Patients. *Cell Stem Cell*, *24*(6), 974-982.e3. https://doi.org/10.1016/j.stem.2019.05.003

Todd, S., Lien, J., Marques, F., Stevick, P., & Ketten, D. (1996). Behavioural effects of exposure to underwater explosions in humpback whales (*Megaptera novaeangliae*). *Canadian Journal of Zoology*, *74*(9), 1661-1672. https://doi.org/10.1139/z96-184

Tomaiuolo, F. (2004). Gross morphology and morphometric sequelae in the hippocampus, fornix, and corpus callosum of patients with severe non-missile traumatic brain injury without macroscopically detectable lesions: A T1 weighted MRI study. *Journal of Neurology, Neurosurgery & Psychiatry*, *75*(9), 1314-1322. https://doi.org/10.1136/jnnp.2003.017046

Toni, N., Laplagne, D. A., Zhao, C., Lombardi, G., Ribak, C. E., Gage, F. H., & Schinder, A. F. (2008). Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nature Neuroscience*, *11*(8), 901-907. https://doi.org/10.1038/nn.2156

Toth, Z., Hollrigel, G. S., Gorcs, T., & Soltesz, I. (1997). Instantaneous Perturbation of Dentate Interneuronal Networks by a Pressure Wave-Transient Delivered to the Neocortex. *The Journal of Neuroscience*, *17*(21), 8106-8117. https://doi.org/10.1523/JNEUROSCI.17-21-08106.1997

Tran, L. D., Lifshitz, J., Witgen, B. M., Schwarzbach, E., Cohen, A. S., & Grady, M. S. (2006). Response of the Contralateral Hippocampus to Lateral Fluid Percussion Brain Injury. *Journal of Neurotrauma*, *23*(9), 1330-1342. https://doi.org/10.1089/neu.2006.23.1330

U.

Urbán, N., & Cheung, T. H. (2021). Stem cell quiescence: The challenging path to activation. *Development*, *148*(3), dev165084. https://doi.org/10.1242/dev.165084

V.

Vadodaria, K. C., Brakebusch, C., Suter, U., & Jessberger, S. (2013). Stage-Specific Functions of the Small Rho GTPases Cdc42 and Rac1 for Adult Hippocampal Neurogenesis. *Journal of Neuroscience*, *33*(3), 1179-1189. https://doi.org/10.1523/JNEUROSCI.2103-12.2013

Vadodaria, K. C., & Jessberger, S. (2013). Maturation and integration of adult born hippocampal neurons: Signal convergence onto small Rho GTPases. *Frontiers in Synaptic Neuroscience*, *5*. https://doi.org/10.3389/fnsyn.2013.00004

van Praag, H., Kempermann, G., & Gage, F. H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience*, *2*(3), 266-270. https://doi.org/10.1038/6368

Vile, A. R., & Atkinson, L. (2017). Chronic Traumatic Encephalopathy: The cellular sequela to repetitive brain injury. *Journal of Clinical Neuroscience*, *41*, 24-29. https://doi.org/10.1016/j.jocn.2017.03.035

Villasana, L. E., Kim, K. N., Westbrook, G. L., & Schnell, E. (2015). Functional Integration of Adult-Born Hippocampal Neurons after Traumatic Brain Injury. *Eneuro*, *2*(5), ENEURO.0056-15.2015. https://doi.org/10.1523/ENEURO.0056-15.2015

Villasana, L. E., Westbrook, G. L., & Schnell, E. (2014). Neurologic impairment following closed head injury predicts post-traumatic neurogenesis. *Experimental Neurology*, *261*, 156-162. https://doi.org/10.1016/j.expneurol.2014.05.016

Vivar, C., Potter, M. C., Choi, J., Lee, J., Stringer, T. P., Callaway, E. M., Gage, F. H., Suh, H., & van Praag, H. (2012). Monosynaptic inputs to new neurons in the dentate gyrus. *Nature Communications*, *3*(1), 1107. https://doi.org/10.1038/ncomms2101

W.

Wakita, Y., Kakimoto, T., Katoh, H., & Negishi, M. (2011). The F-BAR Protein Rapostlin Regulates Dendritic Spine Formation in Hippocampal Neurons. *Journal of Biological Chemistry*, *286*(37), 32672-32683. https://doi.org/10.1074/jbc.M111.236265

Walker, T. L., Overall, R. W., Vogler, S., Sykes, A. M., Ruhwald, S., Lasse, D., Ichwan, M., Fabel, K., & Kempermann, G. (2016). Lysophosphatidic Acid Receptor Is a Functional Marker of

Adult Hippocampal Precursor Cells. *Stem Cell Reports*, *6*(4), 552-565. https://doi.org/10.1016/j.stemcr.2016.03.002

Wang, S., Scott, B. W., & Wojtowicz, J. M. (2000). Heterogenous properties of dentate granule neurons in the adult rat. *Journal of Neurobiology*, *42*(2), 248-257. https://doi.org/10.1002/(SICI)1097-4695(20000205)42:2<248::AID-NEU8>3.0.CO;2-J

Wang, X., Gao, X., Michalski, S., Zhao, S., & Chen, J. (2016). Traumatic Brain Injury Severity Affects Neurogenesis in Adult Mouse Hippocampus. *Journal of Neurotrauma*, *33*(8), 721-733. https://doi.org/10.1089/neu.2015.4097

Warden, D. (2006). Military TBI During the Iraq and Afghanistan Wars: *Journal of Head Trauma Rehabilitation*, *21*(5), 398-402. https://doi.org/10.1097/00001199-200609000-00004

Watt, F. M., & Hogan, and B. L. M. (2000). Out of Eden: Stem Cells and Their Niches. *Science*, *287*(5457), 1427-1430. https://doi.org/10.1126/science.287.5457.1427

Weber, K., Thomaschewski, M., Benten, D., & Fehse, B. (2012). RGB marking with lentiviral vectors for multicolor clonal cell tracking. *Nature Protocols*, *7*(5), 839-849. https://doi.org/10.1038/nprot.2012.026

Wei, X.-E., Wang, D., Li, M.-H., Zhang, Y.-Z., Li, Y.-H., & Li, W.-B. (2011). A Useful Tool for the Initial Assessment of Blood–Brain Barrier Permeability After Traumatic Brain Injury in Rabbits: Dynamic Contrast-Enhanced Magnetic Resonance Imaging. *Journal of Trauma: Injury, Infection & Critical Care*, *71*(6), 1645-1651. https://doi.org/10.1097/TA.0b013e31823498eb

Weiss, K. H., Johanssen, C., Tielsch, A., Herz, J., Deller, T., Frotscher, M., & Förster, E. (2003). Malformation of the radial glial scaffold in the dentate gyrus of reeler mice, scrambler mice, and ApoER2/VLDLR-deficient mice. *The Journal of Comparative Neurology*, *460*(1), 56-65. https://doi.org/10.1002/cne.10644

Weiss, S., Reynolds, B. A., Vescovi, A. L., Morshead, C., Craig, C. G., & der Kooy, D. van. (1996). Is there a neural stem cell in the mammalian forebrain? *Trends in Neurosciences*, *19*(9), 387-393. https://doi.org/10.1016/S0166-2236(96)10035-7

Wennerberg, K., Forget, M.-A., Ellerbroek, S. M., Arthur, W. T., Burridge, K., Settleman, J., Der, C. J., & Hansen, S. H. (2003). Rnd Proteins Function as RhoA Antagonists by Activating p190 RhoGAP. *Current Biology*, *13*(13), 1106-1115. https://doi.org/10.1016/S0960-9822(03)00418-4

Whitney, G. (1970). Timidity and fearfulness of laboratory mice: An illustration of problems in animal temperament. *Behavior Genetics*, *1*(1), 77-85. https://doi.org/10.1007/BF01067373

Whyte, J., Grieb-Neff, P., Gantz, C., & Polansky, M. (2006). Measuring sustained attention after traumatic brain injury: Differences in key findings from the sustained attention to response task (SART). *Neuropsychologia*, *44*(10), 2007-2014. https://doi.org/10.1016/j.neuropsychologia.2006.02.012

Whyte, J., Polansky, M., Fleming, M., Coslett, H. B., & Cavallucci, C. (1995). Sustained arousal and attention after traumatic brain injury. *Neuropsychologia*, *33*(7), 797-813. https://doi.org/10.1016/0028-3932(95)00029-3

Williams, A. J., Wei, H. H., Dave, J. R., & Tortella, F. C. (2007). Acute and delayed neuroinflammatory response following experimental penetrating ballistic brain injury in the rat. *Journal of Neuroinflammation*, *4*(1), 17. https://doi.org/10.1186/1742-2094-4-17

Witcher, K. G., Eiferman, D. S., & Godbout, J. P. (2015). Priming the Inflammatory Pump of the CNS after Traumatic Brain Injury. *Trends in Neurosciences*, *38*(10), 609-620. https://doi.org/10.1016/j.tins.2015.08.002

Witgen, B. M., Lifshitz, J., Smith, M. L., Schwarzbach, E., Liang, S.-L., Grady, M. S., & Cohen, A. S. (2005). Regional hippocampal alteration associated with cognitive deficit following experimental brain injury: A systems, network and cellular evaluation. *Neuroscience*, *133*(1), 1-15. https://doi.org/10.1016/j.neuroscience.2005.01.052

Woitke, F., Ceanga, M., Rudolph, M., Niv, F., Witte, O. W., Redecker, C., Kunze, A., & Keiner, S. (2017). Adult hippocampal neurogenesis poststroke: More new granule cells but aberrant morphology and impaired spatial memory. *PLOS ONE*, *12*(9), e0183463. https://doi.org/10.1371/journal.pone.0183463

Wu, H.-M., Huang, S.-C., Hattori, N., Glenn, T. C., Vespa, P. M., Hovda, D. A., & Bergsneider, M. (2004). Subcortical White Matter Metabolic Changes Remote from Focal Hemorrhagic Lesions Suggest Diffuse Injury after Human Traumatic Brain Injury. *Neurosurgery*, *55*(6), 1306-1317. https://doi.org/10.1227/01.NEU.0000143028.08719.42

X.

Xiong, Y., Gu, Q., Peterson, P. L., Muizelaar, J. P., & Lee, C. P. (1997). Mitochondrial Dysfunction and Calcium Perturbation Induced by Traumatic Brain Injury. *Journal of Neurotrauma*, *14*(1), 23-34. https://doi.org/10.1089/neu.1997.14.23

Xiong, Y., Mahmood, A., & Chopp, M. (2013). Animal models of traumatic brain injury. *Nature Reviews Neuroscience*, *14*(2), 128-142. https://doi.org/10.1038/nrn3407

Xu, X., Jiang, R., Gong, P., Liu, Q., Chen, Y., Hou, S., Yuan, D., Shi, J., & Lan, Q. (2018). Up-regulation of FOS-like antigen 1 contributes to neuronal apoptosis in the cortex of rat

following traumatic brain injury. *Metabolic Brain Disease*, *33*(1), 115-125. https://doi.org/10.1007/s11011-017-0129-7

Y.

Yamaguchi, M., Saito, H., Suzuki, M., & Mori, K. (2000). Visualization of neurogenesis in the central nervous system using nestin promoter-GFP transgenic mice: *NeuroReport*, *11*(9), 1991-1996. https://doi.org/10.1097/00001756-200006260-00037

Yang, K., Taft, W. C., Dixon, C. E., Todaro, C. A., Yu, R. K., & Hayes, R. L. (1993). Alterations of Protein Kinase C in Rat Hippocampus Following Traumatic Brain Injury. *Journal of Neurotrauma*, *10*(3), 287-295. https://doi.org/10.1089/neu.1993.10.287

Yeh, C.-Y., Asrican, B., Moss, J., Quintanilla, L. J., He, T., Mao, X., Cassé, F., Gebara, E., Bao, H., Lu, W., Toni, N., & Song, J. (2018). Mossy Cells Control Adult Neural Stem Cell Quiescence and Maintenance through a Dynamic Balance between Direct and Indirect Pathways. *Neuron*, *99*(3), 493-510.e4. https://doi.org/10.1016/j.neuron.2018.07.010

Yoshimura, S., Teramoto, T., Whalen, M. J., Irizarry, M. C., Takagi, Y., Qiu, J., Harada, J., Waeber, C., Breakefield, X. O., & Moskowitz, M. A. (2003). FGF-2 regulates neurogenesis and degeneration in the dentate gyrus after traumatic brain injury in mice. *Journal of Clinical Investigation*, *112*(8), 1202-1210. https://doi.org/10.1172/JCI16618

Yu, T.-S., Washington, P. M., & Kernie, S. G. (2016). Injury-Induced Neurogenesis: Mechanisms and Relevance. *The Neuroscientist*, *22*(1), 61-71. https://doi.org/10.1177/1073858414563616

Yu, T.-S., Zhang, G., Liebl, D. J., & Kernie, S. G. (2008). Traumatic Brain Injury-Induced Hippocampal Neurogenesis Requires Activation of Early Nestin-Expressing Progenitors. *Journal of Neuroscience*, *28*(48), 12901-12912. https://doi.org/10.1523/JNEUROSCI.4629-08.2008

Yun, S., Reynolds, R. P., Masiulis, I., & Eisch, A. J. (2016). Re-evaluating the link between neuropsychiatric disorders and dysregulated adult neurogenesis. *Nature Medicine*, *22*(11), 1239-1247. https://doi.org/10.1038/nm.4218

Z.

Zhang, B., Chen, X., Lin, Y., Tan, T., Yang, Z., Dayao, C., Liu, L., Jiang, R., & Zhang, J. (2011). Impairment of synaptic plasticity in hippocampus is exacerbated by methylprednisolone in a rat model of traumatic brain injury. *Brain Research*, *1382*, 165-172. https://doi.org/10.1016/j.brainres.2011.01.065

Zhang, C.-L., Zou, Y., He, W., Gage, F. H., & Evans, R. M. (2008). A role for adult TLX-positive neural stem cells in learning and behaviour. *Nature*, *451*(7181), 1004-1007. https://doi.org/10.1038/nature06562

Zhang, D., Wang, X., & Lu, X.-Y. (2016). Adiponectin Exerts Neurotrophic Effects on Dendritic Arborization, Spinogenesis, and Neurogenesis of the Dentate Gyrus of Male Mice. *Endocrinology*, *157*(7), 2853-2869. https://doi.org/10.1210/en.2015-2078

Zhang, Z., Saraswati, M., Koehler, R. C., Robertson, C., & Kannan, S. (2015). A New Rabbit Model of Pediatric Traumatic Brain Injury. *Journal of Neurotrauma*, *32*(17), 1369-1379. https://doi.org/10.1089/neu.2014.3701

Zhao, C. (2006). Distinct Morphological Stages of Dentate Granule Neuron Maturation in the Adult Mouse Hippocampus. *Journal of Neuroscience*, *26*(1), 3-11. https://doi.org/10.1523/JNEUROSCI.3648-05.2006

Zhao, J., Hylin, M. J., Kobori, N., Hood, K. N., Moore, A. N., & Dash, P. K. (2018). Post-Injury Administration of Galantamine Reduces Traumatic Brain Injury Pathology and Improves Outcome. *Journal of Neurotrauma*, *35*(2), 362-374. https://doi.org/10.1089/neu.2017.5102

Ziino, C., & Ponsford, J. (2005). Measurement and prediction of subjective fatigue following traumatic brain injury. *Journal of the International Neuropsychological Society*, *11*(4), 416-425. https://doi.org/10.1017/S1355617705050472

Zimmer, J. (1971). Ipsilateral afferents to the commissural zone of the fascia dentata, demonstrated in decommissurated rats by silver impregnation. *The Journal of Comparative Neurology*, *142*(4), 393-416. https://doi.org/10.1002/cne.901420402