Progressive hippocampal alterations in stress-induced major depression and novel therapeutic strategies

Anna Sancho Balsells

UNIVERSITAT DE BARCELONA

Progressive hippocampal alterations in stress-induced major depression and novel therapeutic strategies

Doctoral degree of Biomedicine in the Faculty of Medicine of the University of Barcelona

Dissertation submitted by:

Anna Sancho Balsells

This work was performed at the Department of Biomedicine in the Faculty of Medicine of the University of Barcelona under the supervision of Dr. Albert Giralt Torroella and Dr. Jordi Alberch

Anna Sancho Balsells	Albert Giralt Torroella Director	Jordi Alberch Vie Tutor

Programa de Doctorat en Biomedicina per la Universitat de Barcelona

RESUM

ANTECEDENTS

La depressió major (DM) o trastorn depressiu major és una malaltia mental greu, freqüent i recurrent que afecta milions de persones a tot el món (Proudman et al., 2021). Es caracteritza, entre d'altres, per la presencia d'una baixa autoestima, desesperança i pèrdua d'interès/plaer en activitats. Els símptomes de la DM cobreixen els dominis emocional, motivacionals, cognitius i fisiològics (Richards, 2011). La DM és un trastorn mental complex que té factors de risc tant genètics com ambientals. Un dels factors de risc ambiental més importants associat a la DM és l'estrès (Tian et al., 2022). En resposta a esdeveniments estressants aguts, l'organisme respon amb canvis adaptatius a molts nivells que ajuden a fer front a la situació (van Oort et al., 2017). En individus sans, aquesta resposta és limitada en el temps. Tanmateix, si aquesta resposta és prolongada i/o desproporcionada, pot provocar trastorns importants com ara ansietat i depressió (Sheng et al., 2021). Tot i que l'estrès i la depressió afecten diverses regions del cervell, l'hipocamp és una de les regions més crítiques. Les alteracions de l'hipocamp en la DM inclouen canvis volumètrics, alteracions en la neurogènesis i deteriorament cognitiu, entre d'altres (Pariante & Lightman, 2008; Tartt et al., 2022). A més, la presència de diferents subpoblacions cel·lulars dins la CA1 de l'hipocamp descrites recentment (Cembrowski, Bachman, et al., 2016; Valero & de la Prida, 2018) obre encara més preguntes, ja que es desconeix com aquestes subpoblacions podrien tenir un paper important en la gènesi del fenotip depressiu (Valero & de la Prida, 2018). Per tal d'identificar subpoblacions concretes implicades en una funció, els gens d'expressió primerenca, com ara *Egr1*, són essencials ja que ens permeten el marcatge de neurones activades en un moment específic (Minatohara et al., 2016).

La fisiopatologia de la DM és complexa ja que es veuen afectats múltiples mecanismes. A més, cap mecanisme pot explicar la malaltia per si mateix. Entre els mecanismes alterats, destaca la desregulació de l'eix hipotàlem-pituïtari-adrenal (HPA), la deficiència de BDNF, la disminució dels nivells de monoamines, les alteracions epigenètiques i la disbiosi intestinal (Kamran et al., 2022). Evidències actuals suggereixen que la microbiota intestinal i les interaccions microbiota-intestí-cervell podrien tenir un paper clau en la progressió i la gravetat de la depressió major (Amirkhanzadeh Barandouzi et al., 2020; L. Zhou & Foster, 2015). A més, és àmpliament acceptat que l'estrès crònic altera la microbiota intestinal (Winter et al., 2018). Diversos estudis proposen que les alteracions de la microbiota poden influir en el sistema nerviós central a través de diferents vies com el sistema immunitari, el nervi vague i la producció de metabòlits per part dels microorganismes que poden senyalitzar directament al cervell (Cryan et al., 2019).

A causa de la complexitat fisiopatològica de la DM, els medicaments actuals són ineficaços en el 30% dels pacients i sovint s'associen amb efectes secundaris importants (Berlim & Turecki, 2007). Per tant, el disseny d'estratègies terapèutiques més efectives és necessari. En aquest context, la fotobiomodulació (PBM, *photobiomodulation*) ha sorgit com una potencial estratègia terapèutica per millorar els símptomes depressius i modular la composició de la microbiota (Bicknell et al., 2019;

Cassano et al., 2016). Tot i que els mecanismes moleculars de la PBM no s'entenen completament, es creu que afecta la funció mitocondrial. A més, Sirt1, una histona desacetilasa (HDAC), s'ha proposat com una de les principals molècules implicades en els efectes de la PBM (Salehpour, Farajdokht, Mahmoudi, et al., 2019; Z. Zhang et al., 2020; Zhu et al., 2022). En aquesta tesi, ens vam plantejar si combinar la PBM en diferents teixits podria reforçar els eixos intestinals i cerebrals fent que els ratolins amb estrès crònic siguin més resistents al desenvolupament de símptomes depressius. D'alta banda, una altre diana terapèutica que s'ha estudiat àmpliament en els trastorns de l'estat d'ànim és GSK3β (R. S. Jope, 2011). Diferents estudis han demostrat la hiperactivitat de GSK3β a l'hipocamp de pacients amb DM i en models animals de DM (Oh et al., 2010; R. Silva et al., 2008). Així, s'ha demostrat que la inhibició de GSK3β és beneficiosa en el context de DM (R. Jope & Roh, 2012; X. Li & Jope, 2010). El liti ha estat l'inhibidor de GSK3β més utilitzat fins ara (De Sarno et al., 2002; X. Li et al., 2007), però el seu ús planteja algunes preocupacions com l'aparició d'efectes secundaris no desitjats (Del Ser et al., 2013; Dunn et al., 2005). Els productes naturals marins (PNM) són una font encara poc estudiada de compostos potencialment bioactius. Entre ells, les meridianines s'han proposat com a molècules prometedores amb accions bioactives com la inhibició *in vitro* de GSK3β (Llorach-Pares et al., 2017, 2020). En aquesta tesis, hem volgut utilitzar meridianines per reduir la hiperactivitat de GSK3β i rescatar el fenotip del tipus depressiu en el nostre model de ratolí.

Amb tota aquesta informació, en aquest treball pretenem dilucidar l'efecte de la durada de l'estrès en ratolins, determinar mecanismes patològics a l'hipocamp en el nostre model i modular-los mitjançant diferents estratègies, des del silenciament genètic fins a l'ús de la PBM i enfocaments farmacològics.

RESULTATS I DISCUSSIÓ

Per dur a terme el nostre primer objectiu i avaluar quins canvis ocorren durant els primers dies de la resposta a l'estrès vam utilitzar el protocol anomenat *chronic unpredictable mild stress* (CUMS). Aquest protocol es basa en presentar durant 28 dies diferents estímuls estressants al ratolí de manera aleatòria. Així doncs, vam dividir els animals en 3 grups: animals controls (CNT), animals estressats només durant 2 dies (STS, *short-term stressed*) i animals estressats durant 28 dies (LTS, *long-term stressed*). A nivell conductual, vàrem observar que els ratolins LTS presentaven alteracions importants, com ara alteracions en el pes o augment d'ansietat i indefensió apresa. A més, vam poder determinar que l'efecte de l'estrès sobre la conducta és progressiu.

Seguidament, vam voler esbrinar específicament quines subpoblacions cel·lulars juguen un paper fonamental en la resposta a l'estrès. De fet, hi ha un gran interès científic en esbrinar com l'estrès afecta diferents subtipus neuronals, però sorprenentment hi ha poca investigació sobre aquest tema, ja que la majoria dels estudis fins ara no distingeixen entre subpoblacions. Per dur a terme el nostre

objectiu, vam utilitzar el ratolí doble transgènic Egr1-CreER^{T2}xR26RCE. Aquest ratolí ens va permetre marcar cèl·lules activades dependents d'*Egr1* d'una manera controlada (només quan s'administra tamoxifè als animals) (Longueville et al., 2021). Així doncs, ratolins Egr1-CreER^{T2}xR26RCE es van dividir entre el grups CNT, STS i LTS. Cada grup va rebre una injecció de tamoxifè 30 minuts abans de la presentació d'un estímul estressant durant els dos últims dies consecutius del protocol CUMS. De manera interesant, a la CA1 vam observar canvis bidireccionals en el nombre de cèl·lules positives per GFP depenent de la cronicitat del protocol d'estrès. Concretament, les cèl·lules positives GFP van augmentar en el grup STS, però van disminuir en el grup LTS en comparació amb el grup CNT. Aquests resultats van en línia amb altres evidències científiques que suggereixen que *Egr1* respon de manera diferent en resposta a esdeveniments estressants aguts vs crònics (Cullinan et al., 1995; Duclot & Kabbaj, 2017; Melia et al., 1994). Seguidament, vàrem analitzar les espines dendrítiques d'aquestes neurones piramidals activades i vam trobar canvis en aquestes segons les dendrites analitzades (basals vs apicals) i la duració de l'estrès (STS vs LTS). Aquests resultats ens van portar a formular la hipòtesis de que la CA1 és molt sensible a la cronicitat de l'estrès. El següent pas va ser determinar la identitat d'aquestes cèl·lules activades. Primer vam demostrar que les cèl·lules activades eren d'identitat neuronal (MAP2+). Després, vam analitzar els nivells de calbindina (Calb1), un marcador que identifica neurones piramidals superficials. De manera interesant, vam veure que les cèl·lules activades al LTS eren majoritàriament Calb1-positives, mentre que en l'animal CNT la majoria eren Calb1-negatives. Aquests resultats van en acord amb el que s'ha descrit i suggereixen que les subpoblacions hipocampals juguen diferents rols tan en condicions basals con en condicions patològiques (Cembrowski, Bachman, et al., 2016; Valero & de la Prida, 2018). Per tal de demostrar el rol que cada subpoblació juga en l'estrès crònic, vam utilitzar diferents aproximacions. D'una banda, vam usar chemogenètica per tal d'activar les neurones profundes de l'hipocamp. Aquesta aproximació, però, no ens va donar cap millora en el fenotip final. De manera interesant, vam observar que la modulació de neurones superficials (Calb1-positives) *per se* tenia un impacte en la fisiopatologia de l'estrès, ja que la disminució dels nivells d'Egr1 només en aquesta subpoblació (mitjançant l'administració d'un shEgr1) va produir una millora en la indefensió apresa i en la pèrdua de memòria espacial. Donant suport a aquesta idea, s'ha demostrat que els nivells de Calb1 augmenten a les neurones piramidals CA1 amb l'estrès (Krugers et al., 1996) i que els nivells de Calb1 estan incrementats en animals susceptibles en comparació amb animals resilients a l'estrès (Kuga et al., 2023). En conclusió, aquests resultats assenyalen que Egr1 en neurones piramidals superficials pot tenir un paper crucial en les seqüeles induïdes per l'estrès crònic. Per entendre millors les alteracions hipocampals degudes a la cronicitat de l'estrès, vam realitzar un estudi proteòmic de l'hipocamp. Els resultats obtinguts van posar de relleu que Sirt1, una HDAC, estaven augmentats ens els ratolins LTS. Diversos estudis han demostrat la relació de Sirt1 amb depressió i estrès, tot i que hi ha inconsistències en si Sirt1 està augmentada o disminuïda en aquests context (Abe-Higuchi et al., 2016; Ferland & Schrader, 2011; Libert et al., 2011). Així doncs, les evidències científiques posicionen Sirt1 com una molècula complexa i interesant involucrada en la depressió causada per estrès crònic.

En aquest sentit, els resultats de la primera part de la tesis destaquen una progressió en les alteracions induïdes per l'estrès a diferents nivells: conductual, cel·lular i molecular. A més, proposem que les neurones superficials de l'hipocamp són crucials en la fisiopatologia de la DM. Finalment, suggerim que Sirt1 i Egr1 són molècules clau involucrades en la resposta a l'estrès a l'hipocamp.

En relació amb el segon objectiu de la tesis, vam volem utilitzar una nova potencial estratègia terapèutica, la PBM, per tal de millorar els efectes causats per l'estrès crònic. En aquest cas, volíem determinar si la PBM aplicada tant al cap (cervell) com a l'abdomen (intestí) podia tenir un efecte sinèrgic i revertir alteracions causades per l'estrès crònic. El primer pas va ser caracteritzar quines cèl·lules estaven regulades per la PBM. Per fer-ho, el doble ratolí transgènic Egr1-CreER^{T2}xR26RCE va ser sotmès a la PBM i les cèl·lules activades dependents d'Egr1 varen ser marcades i quantificades. En aquest experiment, teníem animals controls que no rebien la PBM (CNT), animals que rebien la estimulació al cap (*HEAD*) o a l'abdomen (*ABD*) o animals que rebien la doble PBM (*BOTH*). Vam veure que el nombre de cèl·lules activades a l'hipocamp, concretament a la CA1, era menor en tots els grups que rebien la PBM, suggerint que la PBM és capaç de modular l'activitat neural. Seguidament, vam voler estudiar l'efecte de la PBM combinada en les alteracions induïdes per estrès crònic. Per dur a terme aquest objectiu, vàrem combinar el protocol d'estrès crònic (CUMS) amb el tractament de PBM. Els resultats van demostrar una millora en tasques cognitives sobre tot en els animals estressats que rebien la doble PBM. Aquesta millora en tasques hipocampals, va anar acompanyada de canvis en les espines dendrítiques. Concretament, les espines dendrítiques de les cèl·lules piramidals van disminuir amb el CUMS, però es van restablir en aquells animals que rebien el CUMS i alhora la PBM o al cap (*HEAD*) o al cap i al abdomen (*BOTH*). A banda, vam analitzar els nivells de diferents metabòlits en l'hipocamp i vam determinar que tot i no tenir grans canvis, aquests es podien associar al rendiment en alguna tasca conductual. De fet, els nivells de nicotinamida estaven reduïts en els animals que rebien el CUMS i estaven parcialment recuperats en els animals que rebien el CUMS i alhora la PBM. Com que la nicotinamida es pot convertir en NAD^+ i s'ha demostrat que els nivells de NAD^+ poden regular tan l'expressió com l'activitat de Sirt1 (Braidy et al., 2011), vàrem analitzar els nivells d'aquesta HDAC en l'hipocamp d'aquests animals. De manera interesant, vam veure que els nivells augmentats de Sirt1 observats en animals després del CUMS, es recuperaven en els animals que rebien la PBM en l'abdomen o la doble PBM. Aquest resultat ens pot indicar que d'alguna manera l'estimulació intestinal està mediant els efectes beneficiosos de PBM pel que fa a l'expressió de Sirt1. Una possible explicació podria ser mitjançant l'alteració de la microbiota intestinal. Així doncs, vam analitzar el possible efecte de la PBM en la microbiota intestinal. Mitjançant la seqüenciació 16S de les femtes obtingudes dels animals, vàrem poder determinar que certes espècies alterades amb l'estrès es veien parcialment recuperades amb la PBM. Aquests resultats van suggerir que la PBM era capaç d'alterar la composició microbiana i aquests canvis podrien explicar, en part, les alteracions a nivell tan d'espines com de l'expressió de Sirt1.

Per últim, vam voler analitzar si la PBM tenia un efecte antiinflamatori en el cervell, tal com s'ha proposat en altres estudis (Hamblin, 2016). Per tal d'avaluar la inflamació, vam estudiar marcadors de microgliosis (Iba1) i astrogliosis (GFAP) en l'hipocamp d'aquests ratolins. Vàrem observar que el protocol CUMS induïa un increment de la expressió de GFAP i Iba1. D'altra banda, la PBM (independentment del lloc d'aplicació, cap o abdomen) induïa una baixada en l'expressió d'ambdós marcadors. Aquests resultats suggereixen que la PBM pot produir efectes beneficiosos en un context pro-inflamatori com és el que s'observa després de l'estrès crònic. En conjunt, en aquesta part de la Tesi, hem demostrat l'efecte beneficiós de la PBM combinada sobre els dèficits cognitius, les alteracions en espines dendrítiques, la inflamació i les modificacions metabòliques induïdes per l'estrès crònic. A més, hem demostrat evidències que PBM pot modular la microbiota intestinal i regular l'expressió de Sirt1.

Finalment, s'ha demostrat que en trastorns de l'estat d'ànim hi ha alteracions en GSK3β, una molècula molt important per l'homeòstasi cel·lular (R. Jope & Roh, 2012). De fet, diversos estudies han demostrat augment d'expressió o d'activitat en l'hipocamp (i d'altres regions) en models animals i en pacients de DM (Karege et al., 2007; Oh et al., 2010; Wilkinson et al., 2011). Primerament, vam confirmar estudis previs i vam observar que GSK3β estava hiperactivada en l'hipocamp de ratolins estressats. Seguidament, vam analitzar si l'administració intraventricular de les meridianines podia modular GSK3β. Confirmant estudis anteriors del grup (Rodríguez-Urgellés et al., 2022), vam poder determinar que les meridianines inhibien *in vivo* GSK3β. A més, vam poder analitzar molècules implicades en la senyalització de GSK3β i vàrem trobar que l'administració de meridinaines augmentava els substrats fosforilats de PKC i alhora els nivells de fosforilació de GluR1 en la Serina 831. Com que aquesta fosforilació està implicada en plasticitat sinàptica (H. K. Lee et al., 2003), vam estudiar si l'administració de les meridianines tenia algun efecte a nivell electrofisiològic. En aquest sentit, vam trobar un augment del rendiment sinàptic específicament a la CA1 després de l'administració de meridianines. Els resultats previs ens van portar a plantejar que l'administració de meridianines en un context d'estrès crònic podria ser beneficiosa. Així doncs, vam administrar de manera intraventricular (mitjançant bombes osmòtiques) meridianines durant el protocol CUMS. L'anàlisi conductual ens va posar de manifest que l'administració crònica de les meridianiens era beneficiosa i millorava alguna de els seqüeles induïdes pel CUMS com ara la pèrdua de pes, l'ansietat o les alteracions en la memòria espacial. Tenint en compte els resultats, en aquesta última part de la tesis hem proporcionat proves convincents dels efectes beneficiosos de l'administració de meridianines en ratolins amb estrès crònic via la inhibició de GSK3β.

En conclusió, els mecanismes patogènics de l'hipocamp demostrats en aquesta tesi proporcionen una nova comprensió de la fisiopatologia de la depressió major induïda per l'estrès. A més, proposem diverses estratègies d'avantguarda per intentar revertir les alteracions induïdes per l'estrès a diferents nivells: conductual, cel·lular i molecular.

ABBREVIATIONS

4-HT	4-Hidroxytamoxifen
5-HT	Serotonin
AAV	Adeno-associated virus
aCSF	Artificial cerebrospinal fluid
ACTH	Adrenocorticotropic hormone
AD	Antidepressants
ANS	Autonomic nervous system
ASV	Amplicon sequence variant
ATP	Adenosine triphosphate
AVP	Arginine-vasopressin
BAC	Bacterial artificial chromosome
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
CA	Cornus ammonis
Calb1	Calbindin 1
CCK	Cholecystokinin
CCO	Cytochrome C oxidase
cDNA	Complementary DNA
Chrna7	Cholinergic receptor nicotinic alpha 7 subunit
CNO	Clozapine-N-Oxide
CNT	Control
CRH	Corticotropin-releasing hormone
CUMS	Chronic unpredictable mild stress
CUS	Chronic unpredictable stress
CVS	Chronic variable stress
DA	Dopamine
DG	Dentate gyrus
DN	Default network
DNMT	DNA Methyltransferases
DREADDs	Designer Receptors Exclusively Activated by Designer Drugs technology

DSM-5	Diagnostic and statistical manual of mental disorders, 5th edition
EC	Entorhinal cortex
ECT	Electro-convulsive therapy
EEC	Enteroendocrine cell
EGFP	Enhanced green fluorescent protein
ENS	Enteric nervous system
ER	Estrogen receptor
FP	Frontoparietal network
FS	Forced Swim
GC	Glucocorticoids
GFAP	Glial fibrillary acidic protein
GH	Growth hormone
GI	Gastrointestinal
GLP-1	Glucagon-like peptide
GPC	Glycerophosphocholine
GR	Glucocorticoid receptor
GSK3β	Glycogen synthase kinase-3
GSH	Glutathione
GSSG	Glutathione disulfide
GWAS	Genome-wide association
HDAC	Histone deacetylase
HF	Hippocampal formation
HPA	Hypothalamus-pituitary-adrenal
IEGs	Immediate early genes
IL-1	Interleukin 1
IL-6	Interleukin 6
IMAO	Monoamine oxidase inhibitor
LFQ	Label-free quantification
LHb	Lateral habenula
LLLT	Low-level laser therapy
LPS	Lipopolysaccharides

LTD	Long-term depression
LTP	Long-term potentiation
LTS	Long-term stressed
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MAP2	Microtubule- associated protein 2
MDD	Major Depressive Disorder
MNP	Marine natural products
MR	Mineralocorticoid receptor
MRI	Magnetic resonance imaging
MTA	5'-Methylthioadenosine
MW	Molecular weight
NA	Noradrenaline
NAD^+	Nicotinamide adenine dinucleotide
NDRI	Norepinephrine-dopamine reuptake inhibitors
NDS	Normal donkey serum
NE	Norepinephrine
NGS	Normal goat serum
NIR	Near-infrared
NMDA	N-methyl-D-aspartate receptor
NO	Nitric oxide
NOLT	Novel object location test
NRI	Norepinephrine reuptake inhibitors
NT	Neurotransmitter
$P75^{NTR}$	P75 Neurotrophin receptor
PBM	Photobiomodulation
PBS	Phosphate buffer saline
PCA	Principal component analysis
PCR	Polymerase chain reaction
PD	Parkinson’s disease
PFA	Paraformaldehyde

PFC	Prefrontal cortex
PKA	Protein kinase A
PKC	Protein kinase C
PP2A	Protein phosphatase 2A
PP2B	Protein phosphatase 2B
PPI	Protein phosphatase 1
PPY	Peptide YY
PTMs	Post-transcriptional modifications
PTSD	Post-traumatic stress disorder
PV	Parvalbumin
PVN	Paraventricular nucleus
RCE	R26R CAG-boosted EGFP
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rTMS	Repetitive transcranial magnetic stimulation
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SAH	S-Adenosylhomocysteine
SAMe	S-adenosyl-L-Methionine
SCFA	Short-chain fatty acids
SEM	Standard error of the mean
Ser	Serine
SERT	Serotonin reuptake transporter
Sirt1	Sirtuin 1
SNP	Single nucleotide polymorphism
SNRI	Serotonin and norepinephrine reuptake inhibitors
SSRI	Selective serotonin reuptake inhibitors
STS	Short-term stressed
T3	Triiodothyronine
T4	Thyroxine
TCA	Tricyclic antidepressant
TF	Transcription factors

Thr	Threonine
TMP	Trimethoprim
TNF-α	Tumour necrosis factor-alpha
TRD	Treatment-resistant depression
Trkb	Tropomyosin receptor kinase
UPLC-ToF-MS	Time-of-Flight mass spectrometer
VNS	Vagus nerve stimulation
VTA	Ventral tegmental area
WHO	World Health Organization

TABLE OF CONTENT

INTRODUCTION

1. STRESS: GENERAL CONSIDERATIONS

1.1. The stress response

Stress exposure is one of the most familiar situations in our daily life. Stress can be defined as any challenge that induced physical, emotional, or psychological responses (Anisman & Merali, 1999; McEwen, 2007). The brain is one of the body's main organs involved in stress as it interprets situations as threatening or not and initiates a behavioural and physiological response to each experience. The stress response will vary depending on the type of stressor, its characteristics, the conditions under which the stressor is experienced, the host personality, and its previous life experiences (Anisman & Merali, 1999). Although stress can induce many responses, a hallmark of the stress response is the activation of the hypothalamus-pituitary-adrenal (HPA) axis (McEwen, 2007) (See Section 1.2.3). The HPA axis is a complex system composed of three different organs: the hypothalamus (a brain structure located below the thalamus), the pituitary gland (an endocrine structure located below the hypothalamus) and the adrenal gland (a small organ placed on top of the kidneys).

The HPA axis is crucial in the body's homeostasis and the body's response to stress (Figure 1). Under normal conditions, projections from the hippocampus inhibit the release of corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) from the paraventricular nucleus of the hypothalamus (S. M. Smith & Vale, 2006). On the contrary, under stressful conditions, the inhibitory regulation of the hippocampus is altered, which results in the release of CRH from the paraventricular nucleus (PVN) of the hypothalamus (Sheng et al., 2021). CRH goes to the anterior pituitary, where it stimulates the production of adrenocorticotropic hormone (ACTH). ACTH is delivered to the system's bloodstream, where it induces the release of glucocorticoids (cortisol in humans and corticosterone in rodents) from the adrenal cortex. Glucocorticoids (GC) then interact with their receptors (glucocorticoid receptor (GR) and mineralocorticoid receptor (MR)) found in diverse tissues, including the HPA axis itself (Pariante & Lightman, 2008). Increased cortisol levels lead to CRH and ACTH secretion inhibition by a negative feedback loop (Mikulska et al., 2021; Sheng et al., 2021). However, under chronic stress, the HPA is dysregulated because of an inhibition of the negative feedback loop, which leads to hyperactivity of the HPA axis. This increased activity induces a rise of CRH, ACTH, and glucocorticoids (Pariante & Lightman,

2008) and has been linked with different neuropsychiatric disorders, including major depression (Sheng et al., 2021).

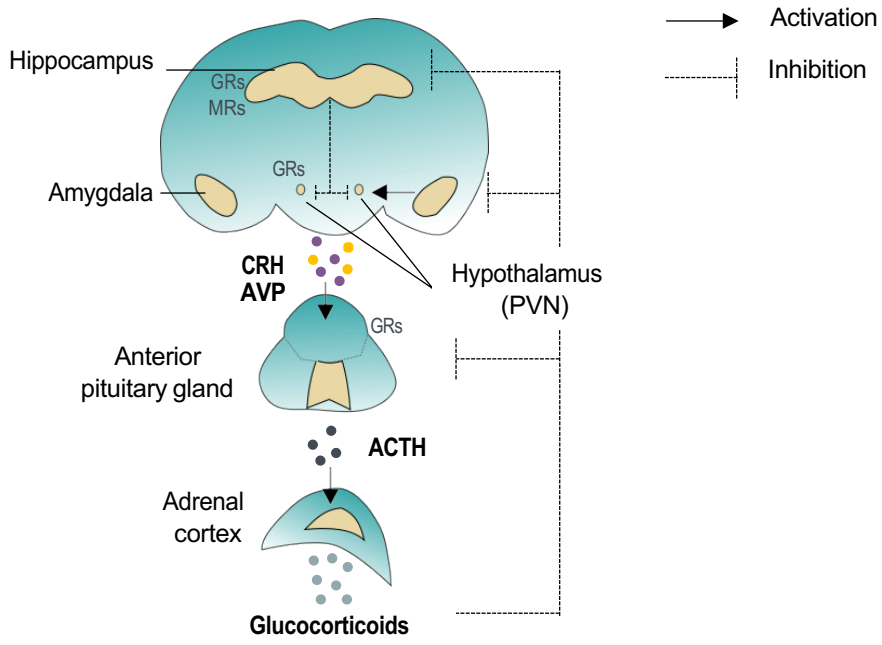


Figure 1: The hypothalamic-pituitary-adrenal (HPA) axis and the stress response. The paraventricular nucleus (PVN) located in the hypothalamus receives stress inputs from the brain stem and regions of the limbic system such as the hippocampus and the amygdala. The PVN projects to the pituitary gland where both corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are released. CRH binds to corticotropic cells in the anterior pituitary, which then release adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH then reaches the adrenal cortex where it causes the synthesis of cortisol (humans) or corticosterone (rodents). Glucocorticoids regulate their release by inhibiting the PVN and the pituitary gland via the glucocorticoid receptor (GR). Adapted from (Lupien et al., 2009).

1.1.1 Allostasis and allostatic load

Stress can be both protective and aversive. In response to acute stressful events, an organism responds with adaptive changes which help to cope with the situation (van Oort et al., 2017). In healthy individuals, this response is limited in time. However, if this response is prolonged in time and disproportionate in salience it leads to significant disorders such as anxiety and depression (McEwen et al., 2015). In line with this, two terms have become increasingly popular in stress research. Allostasis, introduced by Sterling and Eyer, is known as the active process of maintaining body stability or homeostasis (Sterling & Eyer, 1988). On the other hand, the allostatic load is referred to as the maladaptive response due to too much stress or inefficient management of the stress response (McEwen, 2006) (Figure 2). Some examples of allostatic response include chronic exposure to different types of stressors (top left); the failure to turn off each stress response efficiently (top right); the failure to habituate to

repeated stressors of the same type (bottom left) and the insufficient hormonal stress response, that induces the activity of other allostatic systems (bottom right).

In conclusion, effective coping implies that the stress response is activated rapidly when it is needed and is efficiently terminated afterwards (de Kloet et al., 2005). Thus, the distinction between stress being protective or damaging is related to the dynamics of its response (McEwen, 2013).

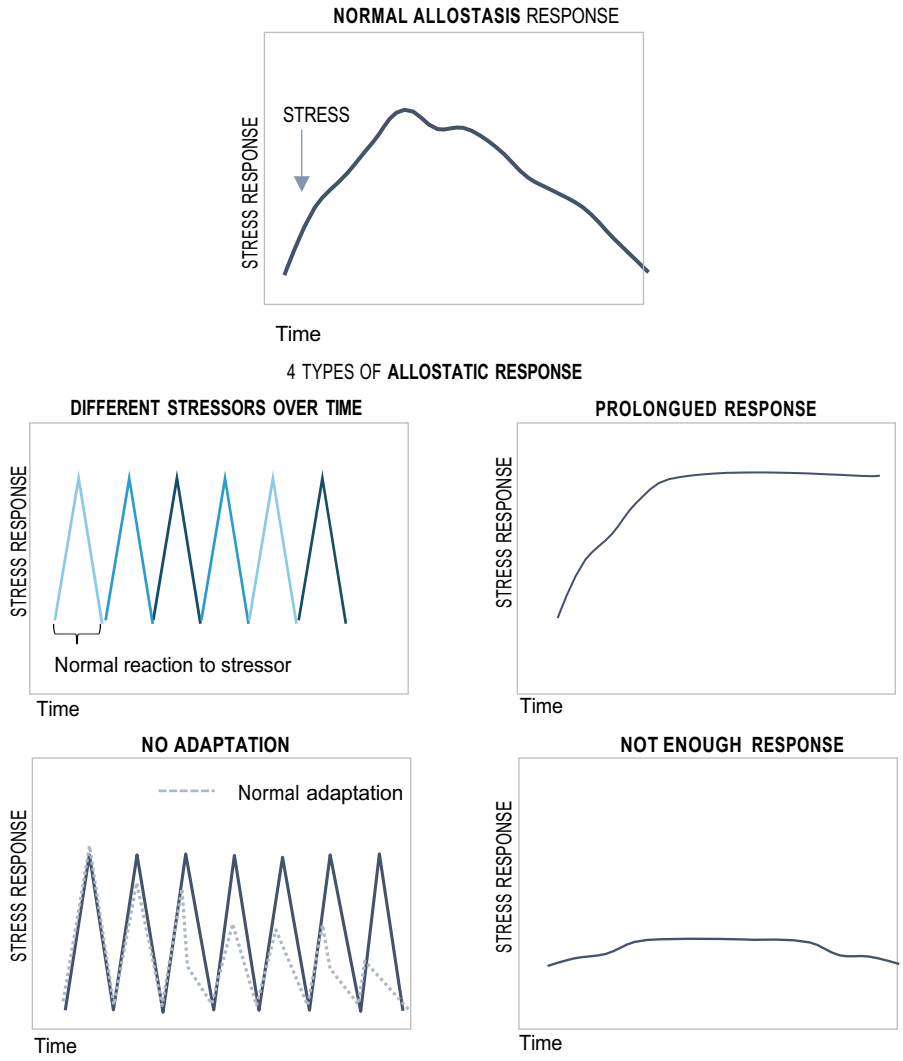


Figure 2. Allostasis and allostatic responses to stress. The top panel shows the normal allostatic response, in which a response is initiated by a stressor and after an appropriate interval of time, it is turned off. The other four panels represent 4 types of allostatic response: top left- repeated different stressors over time; top right- prolonged response caused by no proper termination of the stress response; bottom left – lack of adaptation to the stress; bottom right- inadequate response. Adapted from (McEwen, 2007)

1.1.2 Stress-related pathologies

Several health issues result, at least in part, from extended stress exposure and inadequate stress management. Stress has been associated with many pathologies, as it affects different systems of the body. Some of the conditions that are linked to stress include endocrine and metabolic disorders (i.e. diabetes and obesity)(Kivimäki et al., 2023), cardiovascular diseases (i.e. hypertension and vascular damage) (Whooley & Wong, 2013), skin abnormalities (i.e. hair loss, dry skin or acne)(Bin Saif et al., 2018), and cancer among others (Penninx et al., 2013). Stress is also a key trigger for some brain disorders. Focusing on the brain, we can define stress-related disorders as a group of psychiatric conditions whose causation, onset, or development is influenced by stress (Ising & Holsboer, 2006). The most common stress-related brain pathologies include post-traumatic stress disorder (PTSD), acute stress reaction, anxiety, adjustment disorder, and depression (Tian et al., 2022). In this Thesis, we are going to focus on stress-induced major depression.

1.2. Major depression disorder

Major depressive disorder (MDD) also called depression is a heterogeneous debilitating mood disorder that affects millions of people worldwide (American Psychiatric Association, 2022). The prevalence of MDD is extremely high and growing over time; in US adults the prevalence increased from 6.8% in 2005 to 7.1% in 2018 (Proudman et al., 2021). Moreover, with the influence of the COVID-19 pandemic, the prevalence of MDD is going to increase even more in the future years. MDD negatively impacts on daily life activities, quality of life, normal cognitive function, and work productivity. Thus, it is considered one of the most burdensome illnesses worldwide. It is difficult to assess the economic burden of MDD because it varies depending on the country, but recent US studies estimate that in 2020, 326.2 billion dollars were destinated to adults with MDD (Greenberg et al., 2021). In fact, depression is accepted by the World Health Organization (WHO) as the major contributor to the overall global disease burden (World Health Organization [WHO], 2020).

1.2.1 Clinical features

The diagnosis of depression is based on symptomatic criteria described in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5-TR) (American Psychiatric Association, 2022). To be diagnosed with MDD, a person must show at least five of the following symptoms almost every day for a period of 2 weeks. The clinical symptoms used

to diagnose MDD are depressed mood, decreased interest in almost all activities, alterations in body weight, insomnia or hypersomnia, feeling worthless or guilty, psychomotor agitation or retardation, fatigue or loss of energy, trouble concentrating on activities and recurrent thoughts of death or suicidal ideation. The first two symptoms of the list need to be mandatory present and evident for being diagnosed with MDD.

Once the diagnosis is made, more information can be added to the primary diagnosis in the form of specifiers, which are tags that allow for more specific information about a person's condition (Table 1). Thus, specifiers provide subcategories within mood disorder diagnoses. (Otte et al., 2016; Parikh et al., 2016).

Table 1: Depressive disorder subtypes/specifiers. Subtypes of MDD and its main characteristics.

Depressive disorder specifier	Main characteristics
Mixed features	Patients that have subthreshold bipolar features during the depressive episode but do not meet the criteria for full-fledged bipolar disorders.
Anxious distress	Presence of at least two out of five anxiety symptoms during most of the days of a MDD episode.
Atypical features	People who display "unusual" symptoms, including mood reactivity or leaden paralysis.
Persistent/chronic	Disorder that is normally less severe than MDD but that last much longer, around two years.
Melancholic	Subtypes in which the person does not feel better when something good happens.
Seasonal	Disorder that goes with the seasons, usually starting in the late fall or early winter.
Prenatal and postpartum	Disorder that a woman suffers during pregnancy or after birth.

Additionally, different studies have shown that the relative risk of various pathologies is increased in those patients suffering from MDD (Penninx et al., 2013). The mechanisms underlying this high risk to other pathologies are not completely understood but include unhealthy lifestyle, bad self-care adherence, undesired effects of the medication, and shared pathophysiology. Some of the pathologies with increased risk in patients suffering from

MDD are cancer, diabetes mellitus, heart disease, obesity, cognitive impairment, and anxiety (Otte et al., 2016; Penninx et al., 2013).

Among them, one of the most frequent problems in MDD patients is the presence of cognitive disorders, which include deficits in attention, executive function, and memory (Perini et al., 2019). Some of the cognitive alterations persist even when there is a remission of the depressive symptoms and are considered a fundamental characteristic of MDD as they are one of the main causes of functional impairment. Therefore, new treatments should also assess these affectations in MDD patients (Miskowiak et al., 2016; Perini et al., 2019).

Nevertheless, the strongest comorbidity of MDD is with anxiety disorders (T. Kaiser et al., 2021). It is estimated that around 51% of people diagnosed with MDD are also diagnosed with an anxiety disorder (Olfson et al., 2017). Furthermore, MDD patients with comorbid anxiety disorders present increased antidepressant resistance (L.-C. Chen et al., 2020) and higher rates of early termination psychotherapy (T. Kaiser et al., 2021; J. F. Young et al., 2006).

1.2.2 Risk factors

1.2.2.1. Genetic risk factors

Identifying genetic risk variants associated with MDD is crucial to improve the development of new and more effective therapeutical strategies. Family and twin studies have provided strong evidence of the contribution of genetic factors to the risk of depression (Shadrina et al., 2018). Thus, the heritability of MDD is around 37% and the odds ratio of developing MDD when a first-degree relative is affected increases at 2.84 (Flint & Kendler, 2014). Since the first study trying to identify candidate genes was published in 1978 (Beckman et al., 1978) a lot of effort has been put to find either single nucleotide polymorphism (SNP) or candidate genes that could be explaining MDD pathology.

Genome-wide association (GWAS) studies

GWAS tries to find common variations in the genome, usually with a higher frequency of 5%. This has been particularly difficult in the context of MDD for two main reasons: 1) these studies require a huge sample size to obtain adequate power to detect small effect variants; 2) technology limitations. One of the biggest studies published in 2019, describes a meta-analysis of the three largest depression cohorts (total n = 807.553, with 246.363 cases and 561.190 controls). They showed 102 independent variants, 269 genes, and 15 genesets associated with MDD including gene pathways linked to the synaptic structure and

neurotransmission (Howard et al., 2019). The results found in all the GWAS done to date should be taken into caution because the results derive from the analysis of patients that have been diagnosed by minimal phenotyping, most of the patients analysed also present other disorders, and there is a strong relation between sample size and the number of detected loci (Flint, 2023; Ormel et al., 2019).

Candidate genes studies

There are several studies showing important genes for MDD but there is not a general agreement between them (Flint & Kendler, 2014). This could be explained because MDD is a polygenic and very heterogeneous disorder that involved many genes with small effects (Hyman, 2014). A recent study shows that depression candidate genes were no more associated with MDD phenotype than non-candidate genes (Border et al., 2019). This study suggests that previous literature about historical candidate genes (i.e., *BNDF, 5HTTP/SLC6A4; APOE; DRD4; MAOA; HTR2A; SLC6AE*) in depression is full of false positives.

1.2.2.2. Environmental risk factors

Besides the role of genetics, environmental risk factors play an important part in the development of MDD (Bruce, 2002; de Kloet et al., 2005; M Li et al., 2016; C. Park et al., 2019). The most significant and well-known environmental risk factors are shown in Table 2. It is thought that repeated exposure to some of these factors, combined with a genetic predisposition, will produce an increased susceptibility to stress that can eventually lead to MDD (Ursin, 2014). Among them, the most characterized and accepted risk factor is chronic stress (Slavich & Irwin, 2014). Indeed, many animal models of depression are based on this risk factor (See Section 1.3.2). Because of that, in this thesis we will focus on the study of chronic stress and its impact on MDD.

Table 2. Environmental risk factors associated with MDD. Name and characteristics of the most common risk factors linked to MDD.

Category	Examples
Early-life factors	Prenatal trauma, parental loss, maltreatment
Demographic factors	Age, sex (women) and ethnicity
Socioeconomic status	Unemployment, poverty, low education status
Neighbourhood factors	Violence at home, inadequate housing, overcrowding

Socioenvironmental events	War, natural disasters, migration, trauma
Lifestyle factors	Drug abuse, high-sugar diet, physical inactivity
Stress	Puberty stress, chronic stress

1.2.2.3. Epigenetics (gene-environment interactions)

How environmental stressors can modify gene expression leading to behavioural alterations is not completely understood. However, there is growing evidence suggesting that epigenetic changes are key mechanisms through which stress leads to the development of depressive symptoms. Thus, epigenetics could act as a bridge between genes and the environment (H.-S. Park et al., 2021). Epigenetics is defined as sequence-independent DNA modifications that are heritable but reversible. The two main epigenetic modifications that have been related to depression are DNA methylation (mediated by DNA methyltransferases (DNMTs)) and histone acetylation/deacetylation (Han et al., 2018; Lin & Tsai, 2019). Indeed, Sirt1, a histone deacetylase (HDAC) has been related to depression both in patients and in clinical studies (Abe-Higuchi et al., 2016; H.-D. Kim et al., 2016; Kishi et al., 2010). In this line, different HDAC inhibitors have been proposed as novel therapeutic options (H.-S. Park et al., 2021). Diverse studies have shown epigenetic changes both in post-mortem patients and animal models of MDD and other stress disorders (Hobara et al., 2010; Jakobsson et al., 2008; McGowan et al., 2009; Seo et al., 2016). These epigenetic alterations are found in genes involved in the stress response (Turecki & Meaney, 2016; Vukojevic et al., 2014) but also in genes involved in neurotransmission (E. A. Duman & Canli, 2015) and neurotrophic genes (Zheng et al., 2016) among others. Although epigenetics open new promising therapeutic opportunities, epigenetic changes are usually less than 10%, which is a small percentage if we compared MDD with other pathologies such as cancer with bigger epigenetic footprint (Otte et al., 2016).

1.2.3 Pathophysiology

MDD is a complex disorder that presents a wide variety of altered mechanisms. Different theories have been postulated claiming the pathophysiology of depression.

The monoamine hypothesis

This hypothesis postulates that depressive symptoms are a consequence of decreased levels of monoamine neurotransmitters as serotonin (5-HT) (Cowen, 2008), noradrenaline (NA) (Delgado & Moreno, 2000) and/or dopamine (DA) (Klimek et al., 2002). This is the oldest

hypothesis and it is based on the multiple evidence that antidepressants (ADs) induce an increase in these neurotransmitters (Hamon & Blier, 2013). Despite being one of the most popular hypotheses, it presents some important limitations. First, it does not explain all the symptoms found in MDD. Secondly, it cannot unravel the delayed response to ADs, as even if they cause a rapid increase in monoamines, their benefits are only observed after weeks (Racagni & Popoli, 2008). Furthermore, assuming that monoamines are the only biologically active substance altered in MDD is oversimplistic, as many studies suggest other substances are involved in MDD etiopathogenesis (Boku et al., 2018). What is believed is that ADs produce an increase in synaptic monoamines that leads to long-term neuroplastic changes that have a transcriptional effect, mediating molecular and cellular plasticity.

The neuroendocrine hypothesis

This hypothesis is also named the stress-induced hypothesis of MDD as it postulates that MDD could be a cause of chronic stress and the subsequent malfunctioning of the HPA axis (See section 1.1). Besides HPA dysregulation, other abnormalities related to the endocrine system have been related to MDD aetiology, including alterations in the growth hormone (GH) levels (Sakkas et al., 1998), and abnormalities in thyroid hormones levels, namely triiodothyronine (T3) and thyroxine (T4) (Hage & Azar, 2012; Kamran et al., 2022). The hyperactivity of the HPA axis and the dysregulation of some hormones induces alterations in different body organs, including adrenal hypertrophy and thymic atrophy (Nemeroff et al., 1992; Ulrich-Lai et al., 2006). Around 40-60% of MDD patients present a rise in cortisol levels and/or HPA dysregulation (Mikulska et al., 2021) that can contribute to cognitive dysfunction and low mood. A question that remains unsolved is whether goes first; if the HPA axis dysregulation is a consequence of MDD or the HPA hyperactivity predisposes individuals to suffer MDD (Pariante & Lightman, 2008).

The neuroplasticity hypothesis

This hypothesis claims that a deficiency in neurotrophic support may be causing functional and structural alterations in the MDD brain. This theory has been focused on the study of the brain-derived neurotrophic factor (BDNF) and its possible implications in adult neurogenesis. Several studies have shown altered BDNF, pro-BDNF and/or its receptors (TrkB and p75) in MDD patients and MDD animal models (Castrén & Monteggia, 2021; Fuchikami et al., 2011; Karege et al., 2002; Nibuya et al., 1995). Moreover, it has been proved that some ADs can increase BDNF deficiency, although this does not occur uniformly for all ADs and all patients (Arumugam et al., 2017). Although neurogenesis by itself is

insufficient to explain the etiopathogenesis of MDD, it may contribute, in part, to the development of the behavioural and cognitive impairments that characterize depression.

The inflammatory-cytokine hypothesis

In the last decades, the functional activity of the immune system has been related to MDD pathogenesis (Medina-Rodriguez et al., 2018). The first evidence suggesting the role of the immune system in MDD dates back to the early 90s when clinicians showed that depressed patients present increased blood levels of inflammatory biomarkers, such as cytokines and other proteins (R. S. Smith, 1991). Since then, different clinical and animal studies have reported that proinflammatory cytokines could be related to the development of MDD. Indeed, tumour necrosis factor-alpha (TNF-alfa), interleukin-1 β (IL-1β), interleukin-6 (IL-6), and C-reactive protein, have been found raised in the plasma of patients suffering MDD when compared with healthy individuals (Azar & Mercer, 2013; Miller et al., 2009; Thomas et al., 2005). Furthermore, depressive disorders frequently occur in patients that are also suffering from disorders with a huge inflammatory component (Noto et al., 2014). Last, it has been demonstrated that some ADs present an important anti-inflammatory activity, further supporting this hypothesis (Noto et al., 2014; Tynan et al., 2012).

Other hypotheses

Other postulated theories include altered neurotransmitter (NT) balance and circadian rhythm abnormalities (Zaki et al., 2018). Briefly, the NT imbalance theory is based on the dysregulation of some neurotransmitters (besides monoamines), including glutamate, GABA, and acetylcholine. There is enormous evidence indicating decreased GABA in MDD patients (Fogaça & Duman, 2019; Luscher et al., 2011) and altered glutamate transmission (R. S. Duman et al., 2019; Onaolapo & Onaolapo, 2021; Sanacora et al., 2012). Therefore, drugs targeting this system have emerged in recent years (See Section 1.2.6). The circadian hypothesis postulates that daily rhythms are disrupted in MDD patients. Although the molecular link between MDD and circadian rhythms is not clearly understood, evidence supports the bidirectional link between sleep alterations and depression (Bunney et al., 2015; Zaki et al., 2018). Specifically, insomnia has been recognized as a risk factor for developing MDD, and MDD itself can alter sleep cycles in different ways (Nutt et al., 2008). Hence, melatonin, a major regulator of the circadian clock as well as metabolite obtained from 5-HT, has been identified as a target for novel antidepressant therapies (Tonon et al., 2021).

In summary, a variety of neurotransmitters, hormones, and other chemicals may contribute to the development of MDD. However, none of the theories outlining how depression develops are all-inclusive because none of them can cover all the pathophysiology.

1.2.4 Gut-brain axis in MDD

Another hypothesis that has gained attention in recent years is the dysregulation of the gut-brain axis. Science is beginning to understand how microbes affect the brain by producing several components in the gut that ultimately affect the neurological system. In this line, many of the pathophysiological alterations found in MDD patients (i.e., altered inflammation, increased cortisol, altered epigenetic regulation) could be influenced by the gut microbiome. In recent years, the gut microbiome has been related to psychiatric illnesses including MDD (Amirkhanzadeh Barandouzi et al., 2020; L. Liu et al., 2023; L. Zhou & Foster, 2015).

The human microbiota is defined as the range of microorganisms that live inside us and it is considered a critical factor in maintaining body homeostasis (Cryan et al., 2019). Certainly, the human body contains more microbial cells than humans cells, and >99% of our genes are microbial (Cryan et al., 2019). Most of the microbes live in our gut and although they include a diverse population (yeast, archaea, viruses, among others), the bacterial population is the most characterized. Dysregulation/malfunctioning of the micrbobiota-gut-brain axis has been linked to different pathologies, ranging from neurodegenerative (H. Zhang et al., 2022), neurodevelopmental (Iliodromiti et al., 2023), and psychiatric disorders (Simpson et al., 2021).

1.2.4.1. Microbiota-gut-brain axis: routes of communication

There are several pathways involved in the bidirectional communication between the gut microbiota and the brain. The main routes of communication are illustrated in Figure 3.

Neural pathways

The most studied and fastest route of communication between the gut and the brain is the vagus nerve; a cranial nerve that innervates the gastrointestinal (GI) tract (H.-R. Berthoud & Neuhuber, 2000). Afferent fibres of the vagus nerve respond to a variety of GI signals that can be mechanical, chemical, and hormonal. Contrary, efferent fibres propagate information from the brain to the GI (H. R. Berthoud et al., 2004).

The enteric nervous system (ENS), a part of the autonomic nervous system (ANS), also plays a crucial role in this axis communication (Morais et al., 2021). The ENS controls diverse

functions of the GI tract, including motility and fluid circulation (Furness, 2012). The afferent fibres of the ENS sense factors derived from the gut lumen (bacterial chemical, components of the microbes, among others) and influence both the gut and the CNS function.

Immune system

The GI tract contains the higher concentration of immune cells in the body and, as a result, both the CNS and the gut microbiota directly affect and are affected by the immune system (Cryan et al., 2019). Gut microbiota can activate the immune system through different mechanisms leading to the release of cytokines, chemokines, and other messengers that in turn will affect brain function (Duerkop et al., 2009; Vaishnava et al., 2008).

Chemical signalling

Several chemical molecules are produced by the microbiome and influence the CNS. Short-chain fatty acids (SCFA) are lipids produced by gut microbes via the fermentation of dietary fibre. SCFA can act on the CNS and affect important brain functions including synaptic plasticity, gene expression (Y. P. Silva et al., 2020), and the immune system (Erny et al., 2017). The gut microbiome can also regulate the levels of NT, as they can synthesize the NT themselves or induce its production in their hosts (Shishov et al., 2009; D. K. Smith et al., 1992; Strandwitz et al., 2019). Gut microorganisms can also regulate the enteroendocrine system. Enteroendocrine cells (EECs) are located in the GI and can produce signalling molecules such as cholecystokinin (CCK), glucagon-like peptide (GLP-1), and peptide YY (PPY) (Aresti Sanz & El Aidy, 2019; Buckley et al., 2020). Bacteria can regulate these signalling from the EECs and, in turn, alter the host's appetite and feeding behaviour.

In summary, there are many pathways (not all of them mentioned here) that allow gut-brain communication. These pathways are complex as involve connections of multiple biological systems and are not completely understood.

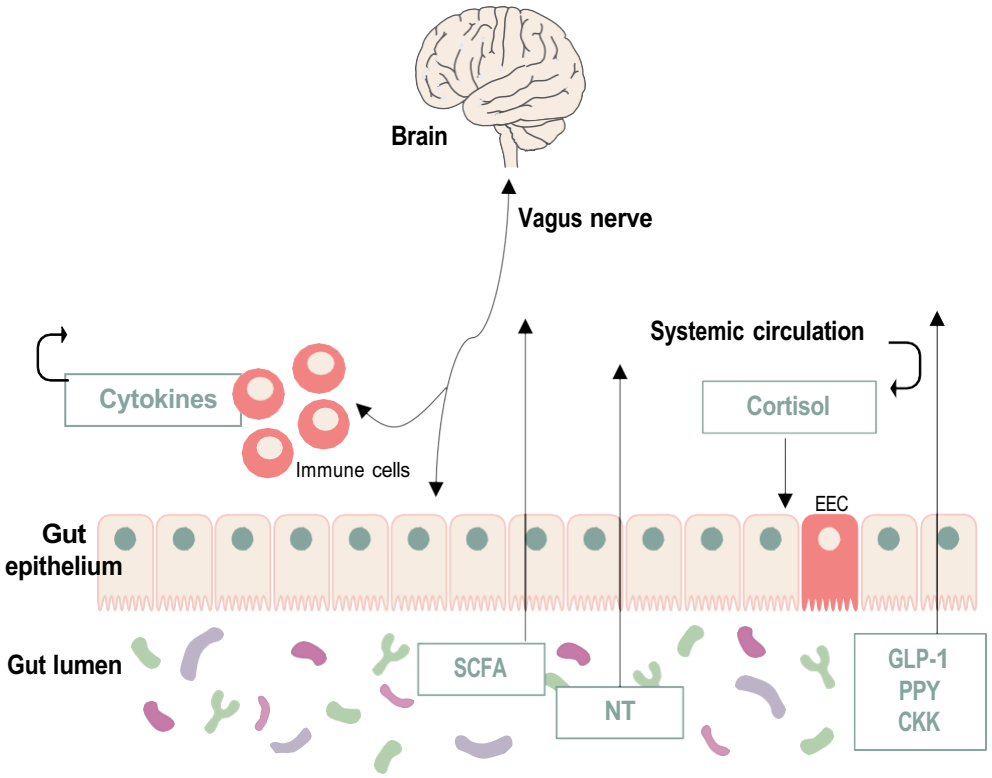


Figure 3. Bidirectional communication between the gut microbiota and the brain. Schematic illustration outlining the different bidirectional pathways of communication between the gut microbiota and the brain, including immune, metabolic, enteroendocrine signals and neuronal innervation among others. CCK: cholecystokinin; GLP-1: glucagon-like peptide-1; IL: interleukin; PYY: peptide YY; ECC: enteroendocrine cell; NT: Neurotransmitter; SCFA: short-chain fatty acids.

1.2.4.2. Microbiota alterations in stress and MDD

Cumulative evidence shows microbiota dysregulation in several neuropsychiatric conditions including MDD. Most of the data that we currently have comes from studies using germ-free mice, faecal microbiota transplantation or antibiotics.

One of the main mechanisms proposed to underly the effect of the microbiota in MDD is the concept of a "leaky gut" (Maes et al., 2009). It is thought that the epithelial barrier of the GI is compromised in the context of excessive stress, and this leads to increased intestinal permeability that, in turn, allows the translocation of bacteria outside the gut. This enhances a strong immune response (Foster et al., 2017).

Moreover, it has been shown in many studies that there is an imbalance between some genera/taxa in animal models of MDD and in MDD patients (Winter et al., 2018). In a simplified view, it has been found an increase in proinflammatory species (including *Enterobacteriaceae*, *Verrucomicrobiaceae* and *Streptococcus*) and reduced levels of anti-inflammatory and/or bacteria-producing SCFA (mainly *Faecalibacterium, Clostidria* and *Firmicutes*) (L. Liu et

al., 2023; Simpson et al., 2021). In this line, some ADs alter gut composition and function (Ait Chait et al., 2020; R. Jiang et al., 2022) and a study suggests that microbiota changes can be even used as treatment outcomes (S. M. Lee et al., 2022).

In general, the microbiota changes induced by a depressive state are subtitle (increase or decrease of an existing population) rather than a complete elimination or colonization of a population. As microbiota populations are dynamic and respond to external stimuli, they have been proposed as therapeutic intervention opportunities. In fact, treatments targeting the gut microbiota with probiotics and prebiotics appear to improve anxiety and depression in both human and animal models (Cryan et al., 2019; Desbonnet et al., 2010; Messaoudi et al., 2011).

1.2.5 Neuroanatomical changes

Despite all the pathophysiological alterations described in the aetiology of MDD, the mechanisms implicated in the long-term effects of stress on brain structure and function are not completely understood. Chronic stress induces structural and functional changes that are evident at different anatomical levels: from molecular and cellular alterations (See Section 2.4) to circuit changes and neuroarchitecture modifications, that ultimately lead to network dysregulation and disintegration, preventing normal brain function.

The circuit-level alterations induced by chronic stress include increased/decreased activation of specific neurons within concrete brain regions. This has been extensively studied by applying optogenetics and chemogenetics to rodent models of MDD (Spellman & Liston, 2020). The main circuit alterations that have been described in MDD are the hyperactivation of the infralimbic prefrontal cortex (PFC) that finally leads to an inhibition in the ventral tegmental area (VTA)(Belujon & Grace, 2017; Sanacora et al., 2022) and the hyperactivity of the lateral habenula (LHb)(Cerniauskas et al., 2019; Hu et al., 2020).

The changes in the neuroarchitecture observed after chronic stress affect different brain areas. Magnetic resonance imaging (MRI) studies have found a reduced volume of the hippocampus in MDD patients (Schmaal et al., 2016) (See Section 2.3), suggesting that this decrease could be explaining the cognitive deficits found in MDD patients. Moreover, some studies suggest that this decrease in the hippocampus could be specific to MDD when compared with other psychiatric disorders such as obsessive-compulsive disorder or bipolar disorder (Goodkind et al., 2015). It has also been reported dendritic atrophy in the PFC and hippocampus (R. S. Duman et al., 2016; H. Qiao et al., 2016) whereas there is an increased branching in the basolateral amygdala (H. Qiao et al., 2016). Moreover, the retraction of

dendrites is sometimes accompanied by decreased spine density (H. Qiao et al., 2016). Therefore, hippocampal alterations in function/structure have been extensively described in the context of MDD, placing the hippocampus as one of the main regions associated with this pathology.

The last level of complexity is the large-scale brain networks, that have been studied using MRI. These studies have found an abnormal activation/deactivation among different networks in MDD, including the frontoparietal network (FP), engaged in the regulation of attention and emotions; the default network (DN), involved in internally oriented attention; and the salience network, that plays a critical role in motivating behaviour. Research indicates that the FP is hypoconnected in MDD (R. H. Kaiser et al., 2015), causing deficits in goal-directed attention. On the contrary, the DN is hyperconnected in MDD (Dutta et al., 2014; Sheline et al., 2009), leading to increase levels of rumination (Figure 4B). Last, one of the most reported findings is the increased activity and connectivity of the amygdala, whereas the opposite result is found in the ventral striatum and other reward-related regions (J. P. Hamilton et al., 2012; Sanacora et al., 2022). Overall, these changes lead to a dysregulation of emotion processing that causes some of the MDD symptomatology.

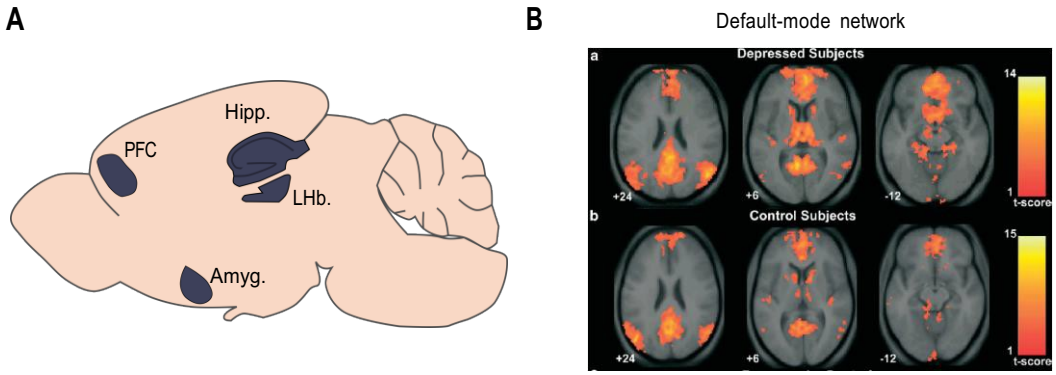


Figure 4. The stressed brain. Chronic stress affects the brain at different levels. (**A**) Schematic representation of some important brain regions altered after chronic stress. These regions can be hyperactivated, hypoactivated and/or present alterations in their structure/function. Moreover, these regions are interconnected and the connectivity between them is also impaired after chronic stress. (**B**) One of the most affected large-scale brain networks in depression, the so-called default-mode (DF) network. This network is hyperactivated in patient suffering MDD. Adapted from (Savitz et al., 2013) PFC: Prefrontal cortex; Amyg.: Amygdala; Hipp: Hippocampus; LHb: Lateral habenula..

1.2.6 Treatment

Treatment main strategies for MDD patients are a combination of psychotherapy and pharmacotherapy; although new therapeutical strategies are becoming increasingly popular

(See Emerging pharmacological opportunities). The main objectives of current treatments are to maintain remission, prevent relapses and manage adverse effects from medications.

1.2.6.1. Psychotherapy

There are different forms of psychotherapy for MDD and each one uses different mechanisms and prescribe techniques that differ in their focus and methods. Several control and randomized trials have shown the beneficial effect of psychotherapy (Cuijpers et al., 2008, 2013), although no big differences have been found between different types of them (Cuijpers et al., 2021). Among them, we can highlight the cognitive-behavioural therapy, based on teaching patients to identify the negative pattern that contributes to MDD and giving skills to overcome these negative thoughts (Gautam et al., 2020); the behavioural activation therapy, which makes patients increase their engagement with activities that give them a positive feeling or pleasure (Cuijpers et al., 2007; Kanter et al., 2010); and the psychodynamic therapy, that involves understanding how a person's past experience can have a negative effect in their present situation (Fonagy, 2015).

1.2.6.2. Classical pharmacotherapy

Monoamine Oxidase Inhibitors (MAOI)

Monoamine Oxidase (MAO) is the enzyme that catalyses the oxidative deamination of monoamines. Thus, pharmacological inhibition of MAO with MAOI increases the availability of monoamines neurotransmitters (NA, DA, and 5-HT) in the synapse by blocking their metabolism (Ban, 2001). MAOs have been widely used for atypical depression but also for other neurodegenerative disorders such as Parkinson's disease (PD) (Riederer & Laux, 2011). At present, they are not used for MDD as they can have fatal reactions with fermented foods that are enriched in tyramine, potentially leading to heart attack and stroke (Gerhard et al., 2016).

Tricyclic and Tetracyclic Antidepressants (TCA)

TCAs were first introduced in the pharma market in 1970 and were the main pharmacotherapy option until the use of SSRIs in the 80s and 90s. TCA affect both the serotonergic and the noradrenergic system, as they block the serotonin and norepinephrine transporters, producing an increase in both neurotransmitters in the synaptic cleft (Karrouri et al., 2021). TCA also block histamine H1 receptors, muscarinic acetylcholine receptors, and α1 adrenergic receptors (Gillman, 2007). Because of the lack of receptor specificity, they have diverse undesired effects such as weight gain, drowsiness, constipation, blurred vision, dry

mouth, decreased blood pressure, and cognitive alterations among others (Cassano & Fava, 2004).

Selective Serotonin Reuptake Inhibitors (SSRIs)

SSRIs are, at present, the first-line treatment for major depression (Karrouri et al., 2021). The mechanism of action of SSRIs is via the inhibition of the serotonin reuptake transporter (SERT). SSRIs are well tolerated but they have a therapeutic time lag (around 2 weeks) and important side effects including nausea, sexual dysfunction, weight gain, and sleep abnormalities (Amick et al., 2015; Sharpley & Cowen, 1995).

Other monoamine inhibitors

Other monoamines (NA and DA) reuptake inhibitors emerged in the 90s to overcome some side effects of SSRIs. Thus, norepinephrine-dopamine reuptake inhibitors (NDRI), norepinephrine reuptake inhibitors (NRI), and Serotonin and Norepinephrine Reuptake Inhibitors (SNRI) are also used to treat MDD. Their mechanism of action is to inhibit the reuptake of different monoamine neurotransmitters causing an increase of these molecules in the synaptic space (Fasipe, 2018). They are commonly prescribed when patients are not responding to SSRIs.

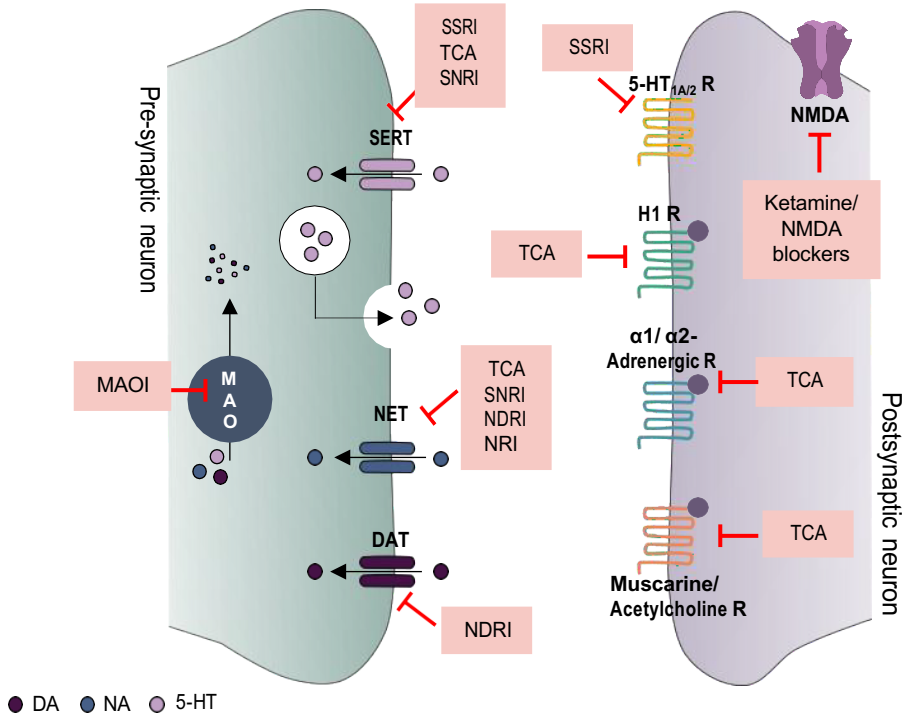


Figure 5. The mechanisms of action of antidepressant drugs. Schematic illustration showing the target molecules of classical antidepressants (TCA, SSRI, IMAO), other monoamine reuptake inhibitors (NRI, SNRI, NDRI) and new pharmacological approaches (ketamine). Each AD targets different molecules of the synapse

(both pre-synaptic and post-synaptic neurons). IMAO: Monoamine oxidase inhibitors; TCA: tricyclic antidepressants; SSRI: selective serotonin reuptake inhibitors; NRI: norepinephrine reuptake inhibitors; SNRI: serotonin-norepinephrine reuptake inhibitors; NDRI: norepinephrine - dopamine reuptake inhibitor; SER: serotonin reuptake transporter; NET: norepinephrine reuptake transporter; DAT: dopamine reuptake transporter; DA: dopamine; NA: noradrenaline/norepinephrine; 5-HT: serotonin. Adapted from (Otte et al., 2016).

In summary, most of the classical pharmacotherapy used to treat MDD patients regulates the pre and postsynaptic receptors or neurotransmitter transporters (Figure 5). Despite being effective in many of the cases, there are still between 25-30% of MDD patients not responding to those treatments and suffering what is known as treatment-resistant depression (TRD) (Berlim & Turecki, 2007; Fava & Davidson, 1996).

1.2.6.3. Emerging pharmacological opportunities

In the last decades, efforts have been made to create ADs medications that are not monoamine-based, free of some of their undesirable side effects, and capable of inducing faster clinical changes. New research on drug development has changed as a result of our new knowledge of depression as a condition of synaptic impairments. Treatments are no longer created to specifically raise monoamine levels; instead, they aim to boost synaptic plasticity and counteract the molecular effects of stress and depression (Gerhard et al., 2016; Murrough et al., 2017).

A new pharmacological approach that revolutionized the field of MDD in the early 2000s was the discovery of Berman and colleagues (Berman et al., 2000). They found that intravenous administration of a sub-anaesthetic dose of ketamine, an N-methyl-D-aspartate receptor (NMDA) antagonist, has a rapid and sustained AD effect. Ketamine produced an increase in glutamate in the synaptic space that originates a rapid cascade of molecular and cellular events that underlie enduring effects in the synapse. Despite being a promising medication, ketamine presents important limitations. First, due to its abuse potential and addiction, it cannot be prescribed in daily clinical practice (Gerhard et al., 2016). Secondly, it has undesired side effects including dizziness, neurotoxicity, cognitive dysfunction, blurred vision, psychosis, dissociation, and others (Yu Liu et al., 2016). Last, the most effective dose and administration route is still not completely explored (Kryst et al., 2020). Since the discovery of ketamine, other drugs regulating glutamatergic transmission have been proposed. Examples include memantine, a non-competitive NMDA receptor antagonist; traxoprodil, which is an NR2B subunit-specific NDMA receptor antagonist, and D-cycloserine, which acts as a functional NMDA glycine site modulator (Henter et al., 2021).

Other treatment options that are currently being developed include neurokinin antagonists (Ratti et al., 2013), anti-inflammatory drugs (Noto et al., 2014), opioid tone modulators and opioid antagonists (Ehrich et al., 2015), and hippocampal neurogenesis-stimulating treatments (Fava et al., 2016), among others.

1.2.6.4. Somatic therapy

Another therapeutic option, especially for those patients suffering from TRD is using somatic treatments. Somatic therapy focuses on using device-based techniques to modulate deep brain structures. The main used somatic therapy strategies are the following:

- **Electro-convulsive therapy (ECT):** is the most well-known treatment for TRD and it has been proposed as a safe and effective therapeutic option (UK ECT Review Group, 2003). It consists of sending small electrical currents through the brain that trigger a brief convulsion. Although being highly effective, it is not the first therapeutic option because the stigma surrounding ECT as well as its lack of specific targets (Ming Li et al., 2020).
- **Repetitive transcranial magnetic stimulation (rTMS):** TMS is a non-invasive approach since it works by applying a magnetic current to a metal coil placed on the patient's head to cause depolarization of cortical neurons with a maximum depth of 1.5-2.5 cm from scalp (Cusin & Dougherty, 2012).
- **Vagus nerve stimulation (VNS):** VSN mechanism of action is to implant a device under the collarbone that is attached to an electrode surrounding the vagus nerve. VNS results in the activation of various subcortical brain structures and the stimulation of hippocampal neurogenesis (Rizvi et al., 2011). Although it was approved by the FDA in 2005, it is not very commonly used (Karrouri et al., 2021).
- **Photobiomodulation (PBM) therapy:** PBM uses photons of light to repair, modulate and improve the function of the target tissue (Montazeri et al., 2022). This therapeutic strategy has been further studied in this Thesis (See Section 3).

1.3. Animal models of depression

Animal models of psychiatric diseased are procedures applied to laboratory animals to produce behavioural alterations that are meant to be analogous to features of psychiatric diseases patients (Paul Willner, 2017). Historically, rodent models of major depression have been based on the manipulation of already known risk factors to inducing symptoms in animals that can recapitulate somehow those find in humans (Planchez et al., 2019). This has

been particularly difficult in MDD due to its multifactorial aetiology, which makes models not always useful in the context of drug discovery (Gururajan et al., 2019; Planchez et al., 2019).

As stated previously, chronic stress is one of the main risk factors for MDD. Thus, many animal models of MDD rely on the application of stressors to mice (See Section 1.3.2). However, other models target biological substrates that are altered in MDD patients (See Section 1.3.1).

1.3.1 Biological models

As already mentioned, many mechanisms are known to be altered in MDD. These include altered network activity, increased neuroinflammation, hyperactivity of the HPA axis leading to increase cortisol levels, decreased levels of some monoamines, and/or polymorphisms in specific genes (M. Becker et al., 2021; Belzung et al., 2015). Biological models are established on the idea that these changes cause MDD and that, inducing these changes in rodents, would model MDD. Some of the most used biological models are summarized in Figure 6 and include optogenetics which is used to modulate different brain regions in order to alter brain connectivity and mimic what is found in MDD; the modulation of specific genes involved in MDD; and the administration of hormones involved in the stress response, among others.

1.3.2 Stress-based mouse models

These models are based on stress exposure either during early life or adulthood (Figure 6). One of the most popular paradigms used to model aspects of MDD is chronic social defeat stress (Gururajan et al., 2019). Briefly, this paradigm is based on placing mice with an aggressive resident mouse for 10 min daily for 10 days. This is considered a highly translational approach because social stress is something commonly experienced by humans (Krishnan et al., 2007; Pryce & Fuchs, 2017). Another commonly used approach is the learned helplessness model, which was first described in 1967 (Overmier & Seligman, 1967). This paradigm is used to investigate the impact of erratic and uncontrollable stress (i.e. food shock) applied in rodents, who further develop an incapacity to cope with unpleasant but avoidable circumstances (M. Becker et al., 2021). One of the main advantages of this model is that can be also used as a readout for helplessness behaviour induced by other stress-based models (Vollmayr & Gass, 2013). Additionally, it has been commonly used to determine the effect of both slow-acting and fast-acting ADs (Holanda et al., 2018; Roy et al., 2018). This last feature makes learned helplessness unique, as other tests need chronic AD treatment to

see an improvement of the phenotype (Gururajan et al., 2019). Other protocols used to induce MDD phenotype based on stress application are predator stress, chronic restrain, or social instability stress (Demin et al., 2019; Gururajan et al., 2019; Lupien et al., 2009; Planchez et al., 2019).

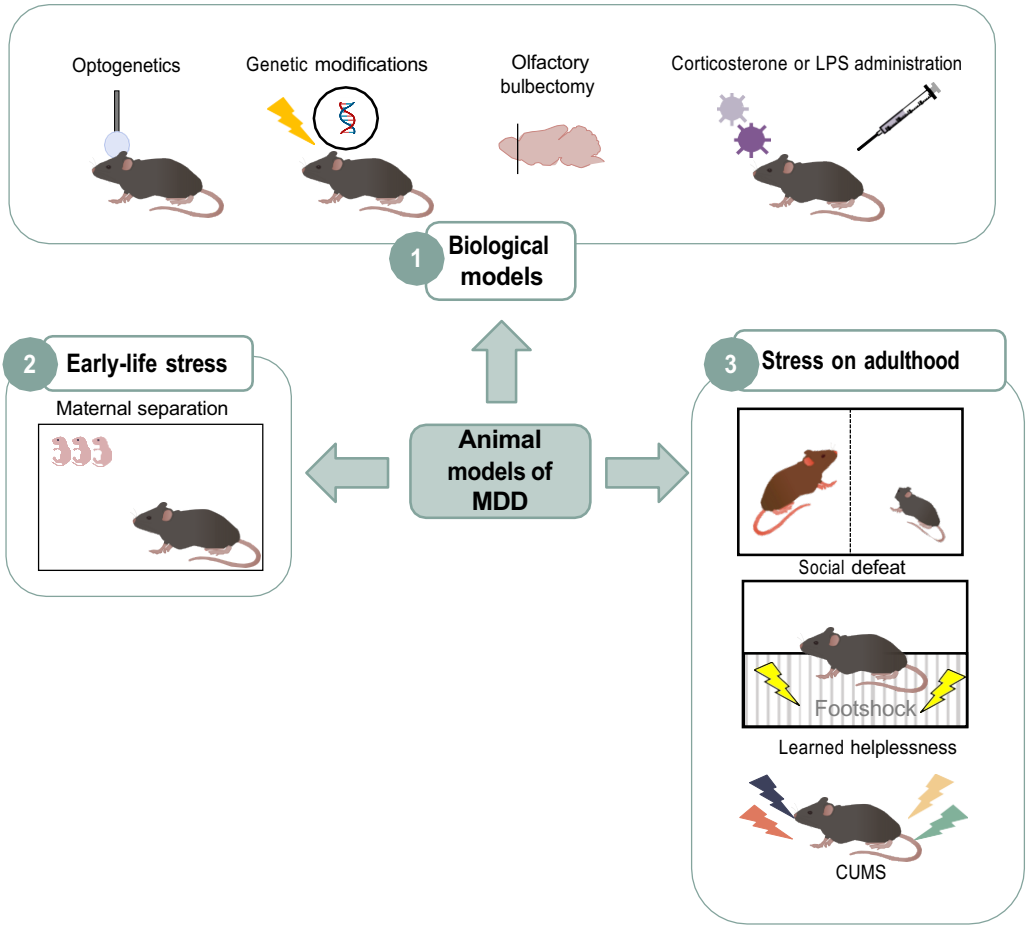


Figure 6. Animal models of depression. Animal models used to imitate aspects of depression in rodents. Different preclinical approaches are used to mimic MDD-like phenotype in mice, from biological models to early-life stress or application of stress during adulthood. LPS: Lipopolysaccharide. CUMS: chronic unpredictable mild stress.

Last, one of the most popular and validated procedures is the chronic unpredictable mild stress (CUMS) protocol, also called chronic mild stress (CMS) or chronic unpredictable stress (CUS), first described by Willner (P Willner et al., 1987, 1992). In this paradigm, mice are exposed to a series of mild stressors that are presented randomly during a 3–6-week period. Mice exposed to CUMS present multiple behavioural symptoms similar to those found in MDD patients, including anhedonia, anxiety, altered locomotion, helplessness behaviour and cognitive impairment, among others (Nollet, 2021; Sequeira-Cordero et al., 2019; Strekalova & Steinbusch, 2010; P Willner, 2005). Moreover, this model also causes a myriad of

neurobiological alterations such as HPA axis dysregulation, changes in the serotoninergic, noradrenergic and dopaminergic systems; decreased BDNF levels and changes in the expression of certain proteins in the brain that are known to also change in MDD patients (R. S. Duman & Monteggia, 2006; Hill et al., 2012; Lupien et al., 2009) (See Section 2.3). This model presents great face validity, as mice recapitulate the diseased phenotype; construct validity, as the pathophysiological process (chronic stress), is similar to those that cause the disease in humans; and predictive validity, as it can be reversed with AD chronic treatment (Sequeira-Cordero et al., 2019). However, this model also presents limitations as it is difficult to reproduce among different (and even in the same) laboratory: the applied stressors are distinct in each research facility resulting in changes in the stress severity on mice (P Willner, 2005; Paul Willner, 2017) and the behavioural outcomes can vary between strains, species and sexes (Pothion et al., 2004; Paul Willner & Belzung, 2015).

The CUMS model is the one that has been used throughout this Thesis to induce a depressive-like phenotype in mice.

2. HIPPOCAMPUS AND STRESS

The hippocampus is a seahorse-shaped region of the brain that belongs to the limbic system (Bartsch & Wulff, 2015). It plays an important role in the consolidation of information from short-term to long-term memory (Alonso et al., 2002; Izquierdo et al., 1999), spatial memory (Bird & Burgess, 2008; Hartley et al., 2007), and mood regulation (Campbell & Macqueen, 2004; Tartt et al., 2022). The hippocampus is a key region affected in severe disorders such as Alzheimer's disease (Fjell et al., 2014; Mu & Gage, 2011), schizophrenia (Lieberman et al., 2018; Wegrzyn et al., 2022) and MDD (Campbell & Macqueen, 2004; de Kloet et al., 2005; Schmaal et al., 2016).

2.1. The hippocampal formation

In rodents, the hippocampal formation (HF) is a C-shaped structure located in the posterior half of the hemisphere. The HF is formed of the dentate gyrus (DG), the *cornus ammonis* (CA) divided into CA1, CA2, and CA3, and the subiculum. The CA regions are also structured in clearly defined strata (or layers), as shown in Figure 7A (Andersen et al., 2006). The more superficial layer is the alveus, which contains the axons of the pyramidal neurons located in the *stratum pyramidale,* and it is considered one of the major outputs of the hippocampus.

Then, we can find the *stratum oriens* in which the basal dendrites of pyramidal cells and the cell bodies of inhibitory basket cells are placed. Next, we have the *stratum pydamidale* which contains the cell bodies of the pyramidal neurons of the hippocampus. Last, we have the *stratum radiatum*, which holds fibres from the CA3 (Schaffer collateral fibres) and some interneurons; and the stratum *laucosum-moleculare*, in which Schaffer collateral fibres and fibres coming from the cortex are located. In the DG, we can distinguish the hilus, the *stratum granulosum,* and the *stratum moleculare*. In the hilus, we can find interneurons and the axons of granule cells, which cell body is in the *stratum granulosum*. In the *stratum moleculare*, different fibres from the contralateral DG, the medial septum, and the perforant path are found. It is important to highlight that 90% of hippocampal neurons are glutamatergic excitatory cells (pyramidal and granule neurons), whereas 10% are GABA-producing inhibitory interneurons (Fritschy et al., 1998).

Classically and in a simplified view (Figure 7B) (Zemla & Basu, 2017), neurons in the entorhinal cortex (EC) received cortical information and project it to the molecular layer of DG, through the perforant pathway. Granule cells, in turn, project mossy fibres that reach the CA3. CA3 neurons send their axons, known as Schaffer collaterals, to the CA1. To close the loop, CA1 pyramidal cells give rise to projections that end in the subiculum and deep layers of the EC (IV, V and VI), which send projections to other cortical areas. Additionally, other neurons project from the EC (layer III) directly to the apical dendrites of CA1 pyramidal neurons. This is called the trisynaptic loop pathway (Van Hoesen & Pandya, 1975). New data suggests that the hippocampus connectivity is much more complex than this trisynaptic loop and proposes that there is a regionalization and cell-type specificity that was ignored so far (Valero & de la Prida, 2018).

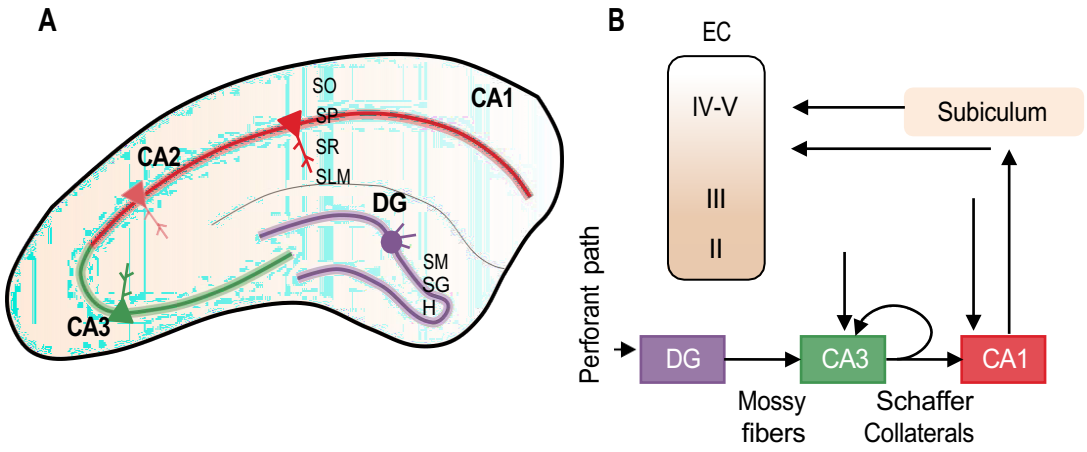


Figure 7. Hippocampal trisynaptic circuit in the mouse brain. The picture shows the different hippocampal subregions and stratums. The flux of projections connecting the EC and the hippocampus are represented with arrows. SO: *stratum oriens*; SP: *stratum pyramidale*; SR: *stratum radiatum*; SLM: *stratum laucosum-moleculare*; SM: *stratum moleculare*; SG: *stratum granulare*; H: hilus; EC: entorhinal cortex; DG: dentate gyrus; CA: *cornus ammonis.*

2.2. Hippocampal cell diversity

In recent years, a heterogeneity inside CA1 pyramidal neurons has been suggested. This heterogeneity was first described during electrophysiological studies (Mizuseki et al., 2011; Senior et al., 2008). After that, *in vivo* cell recordings have identified two subpopulations in the CA1, named deep (calbindin-negative) and superficial (calbindin-positive) pyramidal neurons, which present different features (Cembrowski, Bachman, et al., 2016). First, they are differentially positioned in the radial axis due to the time in which they are born (Figure 8A). Likely related to their different birthdates, they also present differences in their genetic programs; molecular, structural, and physiological properties and in their projections (Cembrowski, Bachman, et al., 2016; Soltesz & Losonczy, 2018; Valero & de la Prida, 2018). Diverse studies have shown different marker genes for superficial and deep cells; being *Calb1*(calbindin) enriched in superficial cells and *Chrna7 (*cholinergic receptor nicotinic alpha 7 subunit) highly expressed in deep neurons (Cembrowski, Wang, et al., 2016). They also show gene-expression differences, although these are not as clear as the ones found in the dorsoventral axis (Cembrowski, Bachman, et al., 2016). Thus, different transcriptional profiles can be found in each subpopulation, correlating with their different functions inside the hippocampus. Furthermore, they exhibit morphological differences, as deep cells present a larger soma and higher complexity of basal dendrites when compared with superficial cells. In contrast, superficial neurons have more complex apical dendritic arborization (Masurkar et al., 2017). The differences are also present in their electrophysiological properties. Briefly, superficial cells show a more depolarized somatic resting membrane potential and a larger somatic h-current (Mizuseki et al., 2011). Last, the presence of these two subpopulations has changed the classical view of the hippocampal system to a cell-type specific connectivity and function of the entorhinal–hippocampal circuit (Figure 8B).

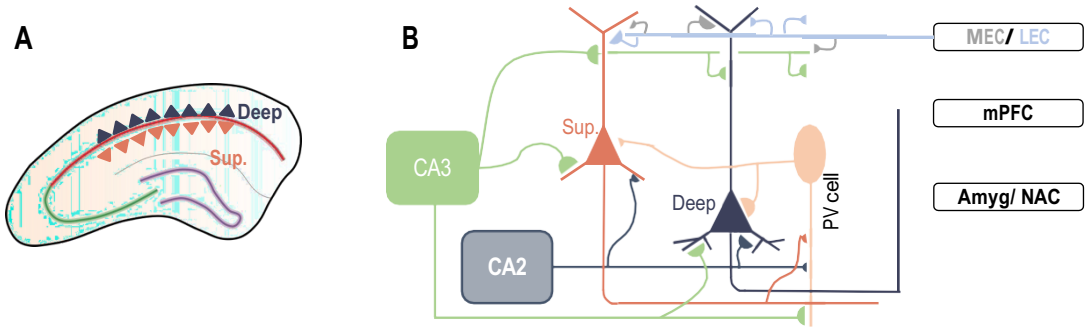


Figure 8. Hippocampal microcircuits and connectivity of superficial and deep CA1 pyramidal cells. (**A**) Schematic representation of the hippocampus showing the organization of deep and superficial PC. (**B**) Schematic illustration showing the complex connectivity within the hippocampus and with other brain regions. Deep PC receive stronger excitation from MEC and from CA2, whereas superficial PC receive stronger excitation from LEC. CA3 mainly projects to superficial PC. PV cells inhibit mostly deep PC and receive stronger excitation from superficial PC. Both superficial and deep PC project to cortical and subcortical areas. Stronger activation/inhibition is represented with bigger contact sites. Sup.: superficial; MEC: medial entorhinal cortex; LEC: lateral entorhinal cortex; Amyg: Amygdala; NAc: Nucleus Accumbens; PV: parvalbumin cell; PC: pyramidal cell. Adapted from (Soltesz & Losonczy, 2018).

The concept of different hippocampal CA1 subpopulations opens many questions: Does each subpopulation store information differently? Are there parallel subcircuits inside the CA1? Is each subpopulation controlling different behaviours? In summary, all this information points out that both subtypes could play different roles in the hippocampus in both, normal and stressful conditions. How stress affects each subpopulation will be further studied in this Thesis.

2.3. The hippocampus in stress

The hippocampus is a critical brain region in stress and MDD. First, it has an important role in the HPA axis regulation and it is very vulnerable to allostatic load as it presents high levels of GR (Pariante & Lightman, 2008; Q. Wang et al., 2013). Second, it is crucial for mood regulation as it is connected with other important brain regions such as the amygdala and the cingulate cortex (Tartt et al., 2022). Third, is one of the few regions able to generate new neurons during adulthood (Boldrini et al., 2018; Tartt et al., 2022). Hippocampal alterations in MDD include volumetric changes, impaired neurogenesis, and memory impairment among others.

It has been extensively described that there is a significant reduction of the hippocampal volume in depressed patients (Chan et al., 2016; Frodl et al., 2002; Nifosì et al., 2010). These volume changes could be due to dendrites retraction, decreased neurogenesis and/or loss of

glial cells. The mechanisms underlying this phenomenon are not completely understood but include alterations in glutamate signalling, excessive glucocorticoids levels, impaired neurogenesis, upregulation of pro-apoptotic pathways, and decreased BDNF expression (Tartt et al., 2022). However, the severity of depression is not correlated with the hippocampus volumetric changes (Nifosì et al., 2010) and different studies sometimes suggest confusing results.

Another hippocampal alteration that can be crucial for MDD pathogenesis is the impairment in adult neurogenesis (W. Liu et al., 2017). The rate of adult neurogenesis decreases with age, but also in some brain disorders including MDD (Eisch & Petrik, 2012). In fact, the neurogenic hypothesis of MDD suggests that adult newborn neurons in the adult brain are essential for mood regulation (Filatova et al., 2021). In line with this, several studies both in humans and in rodents have demonstrated that some ADs can enhance adult hippocampal neurogenesis leading to an improved phenotype (Anacker et al., 2011; Malberg & Schechter, 2005). However, insufficient evidence supports the idea that increased neurogenesis is required for ADs efficacy.

Hippocampal synaptic plasticity is also affected by chronic stress. First, stress can alter synaptic proteins that are essential for proper synaptic transmission (Sanacora et al., 2022). Moreover, stress can reduce long-term potentiation (LTP) in the CA3 while can facilitate long-term depression (LTD) in CA1 (W. Liu et al., 2017). All these synaptic changes can promote the cognitive impairment found in MDD. The cognitive alterations affect executive function, memory, and attention. Memory deficits are one of the most consistently reported cognitive deficit found in MDD patients (Bearden et al., 2009; Perini et al., 2019). Specifically, deficits in spatial (Cornwell et al., 2010; Finkelmeyer et al., 2016) and declarative memory (Deuschle A; Niemann, H; Erb-Bies, N; Colla, M; Hamann, B; Heuser, I, 2004; Iorio et al., 2022) among others have been described. These cognitive alterations can persist even when the depressive episode is over and are considered one of the main alterations affecting patients' quality of life (Perini et al., 2019). Therefore, cognitive symptoms should be considered an important dimension of MDD and should be specifically targeted with treatment strategies.

It is clear that other limbic areas, including the amygdala and the prefrontal cortex, are also essential for stress disorders, but in this Thesis, we will mostly focus on the role of the hippocampus in stress-induced MDD.

2.4. Molecular changes induced by CUMS

Many of the hippocampal changes that have been previously described in MDD patients are also found in mouse models of MDD, including the CUMS model. Although different mechanisms are known to be altered in mice after the CUMS protocol, here we will highlight those molecular mechanisms that are being further explored in this Thesis. Thus, the role of Egr1, Sirt1 and GSK3β in stress-induced MDD are explained.

2.4.1 Egr1

Memories can be stored as spatiotemporal representations within a specific neuronal network. Immediate Early Genes (IEGs), such as *Egr1*, are commonly used to label those memory traces and measure neuronal activity (Minatohara et al., 2016). Despite being typically used, their function in biological processes is still unexplored. IEGs encode transcription factors (TF) that regulate neuronal physiology by controlling the expression of late-response genes (Herdegen & Leah, 1998; Knapska & Kaczmarek, 2004).

2.4.1.1. Egr1 function

Egr1, also known as *Zif-268*, is a member of the zinc finger family of transcription factors (Figure 9A). *Egr1* is expressed by normal neuronal activity in different brain areas including the hippocampus (Beckmann et al., 1997; Wisden et al., 1990). Nevertheless, its expression can be upregulated by different signals, including injury, stress, neurotransmitters, and growth factors among others (Clements & Wainwright, 2010; Davis et al., 2003; Gallo et al., 2018). Thus, Egr1 first promotes a rapid and dynamic response to neural activity followed by a second transcriptional response that will promote lasting synaptic changes.

Egr1 is essential for learning and memory formation as different studies downregulating Egr1 in mice showed alterations in long-term, but not short-term memory (Jones et al., 2001). These studies suggest that Egr1 is crucial for memory consolidation, but the molecular mechanisms responsible for that are unclear. Egr1 is a highly regulated TF with many known target genes (probably a lot more to discover) that controls the expression of genes involved in every aspect of synaptic plasticity, including neurotransmitter release and transport through vesicles, synaptic architecture, endocytosis, and protein degradation (Duclot & Kabbaj, 2017). Moreover, Egr1 can also interact with other TF such as Fos or c/EBP (Knapska & Kaczmarek, 2004; Levkovitz & Baraban, 2002; F. Zhang et al., 2003) which increases the potential targets and biological processes regulated by this TF (Figure 9B).

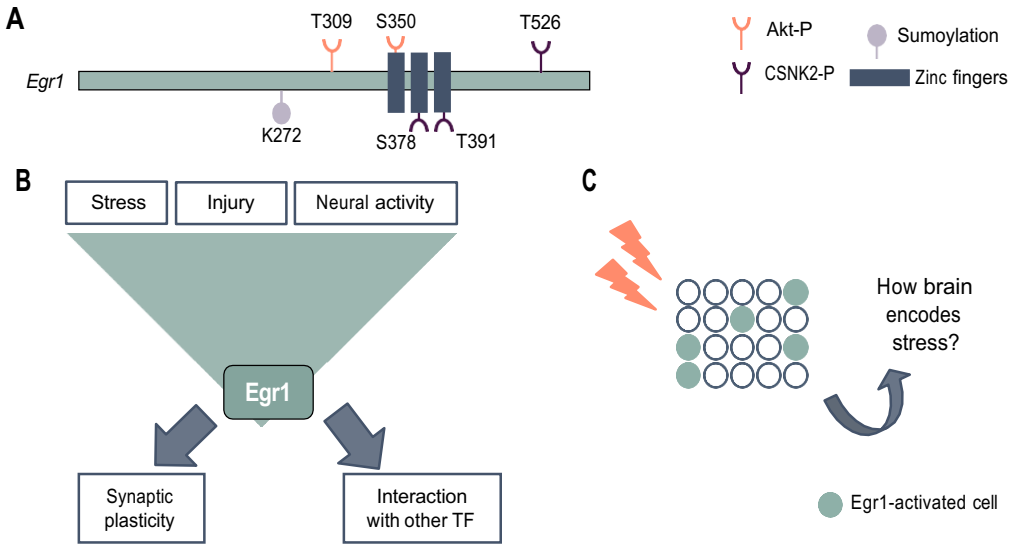


Figure 9. Schematic representation of Egr1 protein structure, regulation, and function. (**A**) Protein structure of Egr1 with the Zinc fingers domains (represented with blue squares) and the main post-translational modifications (phosphorylation and sumoylation). P: Phosphorylation; T: Threonine; S: Serine; K: Lysine. (**B**) In response to stress, injury, or neural activity/learning Egr1 transcription is induced. Egr1 activation directly regulates different biological functions related to synaptic plasticity. Moreover, Egr1 will interact with other TF to regulate gene expression. (**C**) Use of Egr1 as a marker for neural activity. Upon a specific stimulus (lightning icon), some cells will activate Egr1. The use of Egr1 (and other early genes) can help understand how different stressors are encoded in the brain and how different brain regions respond to stress.

2.4.1.2. Egr1 in CUMS

Egr1 has been found downregulated in the mPFC of MDD patients refractory to the treatment (Covington et al., 2010). Studies in rodents support the idea that Egr1 expression can vary depending on the nature, duration, and intensity of the stress presented (Knapska & Kaczmarek, 2004). In this line, acute stressors (including restrain or forced swim) increase Egr1 expression in different brain areas including the hippocampus (Cullinan et al., 1995; Schreiber et al., 1991) but this is blocked when the same stressor is presented repeatedly (Girotti et al., 2006; Melia et al., 1994). In agreement with these results, diverse studies using different chronic stress paradigms have found decreased expression of Egr1 in the mPFC and the hippocampus (Covington et al., 2010; Y. Xu et al., 2015). Moreover, Egr1 is upregulated in the hippocampus after a single dose of tricyclic ADs (Dahmen et al., 1997; Slattery et al., 2005) and following chronic ADs treatment (Gąska et al., 2012).

How changes in the expression of Egr1 can be a readout of how the brain encodes stress and its role in stress-induced MDD will be assessed in this Thesis (Figure 9C).

2.4.2 Sirt1

As mentioned before, mounting research indicates that epigenetic modifications are important processes through which stress promotes the emergence of depressive symptoms (H.-S. Park et al., 2021). Among the epigenetic mechanisms, the most investigated for their effects on depression are DNA methylation mediated by DNMTs and histone post-transcriptional modifications (PTMs), including acetylation/deacetylation.

2.4.2.1. Sirt1 function

Sirtuin 1 (Sirt1) which is encoded by the *Sirt1* gene is a nicotinamide adenine dinucleotide (NAD^+) dependent histone deacetylase (Figure 10A). To date, seven sirtuins have been identified in mammals, designated as Sirt1 to Sirt7. Sirt1 is expressed in many parts of the body, including the brain. Besides the deacetylation of histones, Sirt1 main molecular targets are the TFs p53 and Foxo1, and co-factors like peroxisome proliferator-activated receptor gamma (PPAR), and coactivator 1-alpha (PGC-1). Thus, it is involved in many physiological processes, such as apoptosis, cell differentiation and survival, inflammation, autophagy, mitochondrial biogenesis, and circadian rhythms (de Mello et al., 2019; Mazucanti et al., 2015; J. Xu et al., 2018) among others (Figure 10B). Therefore, dysregulation of Sirt1 expression and/or its activity has been linked to different pathologies, including neurodegenerative disorders (Kumar et al., 2013; R. Wang et al., 2013), drug addiction (Ferguson et al., 2013) and other psychiatric disorders including MDD (W. Li et al., 2019; Libert et al., 2011; Lu et al., 2018).

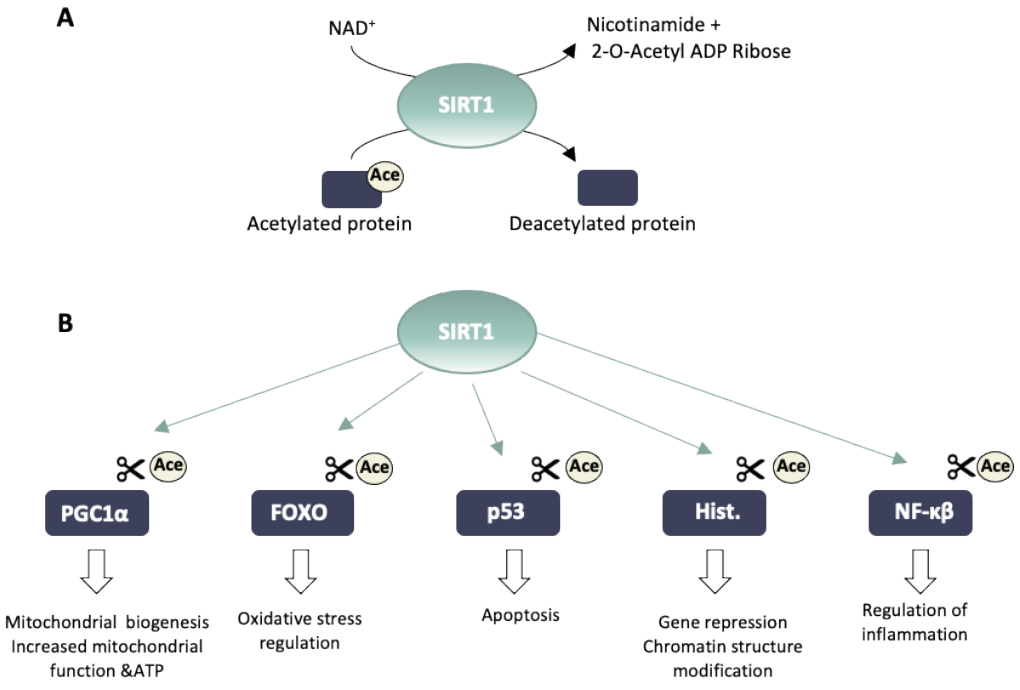


Figure 10. Regulation of Sirt1 and its roles in brain function. (**A**) NAD^+-dependent SIRT1 deacetylase reaction. (**B**) Sirt1 removes the acetyl (Ace) group from their main subcellular targets. This reaction induces different cellular functions associated with Sirt1 activity. The scissors illustrate how Sirt1 removes the Ace group of its substrates.

2.4.2.2. Sirt1 in CUMS

Compelling evidence has associated Sirt1 with the pathophysiology of MDD. Genetic studies have proposed a loci in chromosome 10 (P52.53310210), near the *Sirt1* gene, that contributed to the risk of MDD (Cai et al., 2015). Another study suggests that a tag SNP (rs10997875) in the *Sirt1* gene could play a role in MDD pathophysiology (Kishi et al., 2010). Moreover, Sirt1 levels are decreased in peripheral blood cells from MDD patients when compared with healthy subjects. Not only Sirt1 has been studied in MDD patients but also in animal models of MDD. In this sense, controversial results have been found. Some studies suggest that after chronic stress (and other stress paradigms) Sirt1 activity and expression are downregulated in important brain regions such as the hippocampus (Abe-Higuchi et al., 2016) and the Nacc (H.-D. Kim et al., 2016; Morató et al., 2022). In this line, resveratrol, a Sirt1 activator, has been shown to reduce anxiety and depressive-like phenotype induced by repeated corticosterone (Ali et al., 2015) and LPS (Ge et al., 2015) administration in mice. In contrast, another study knocking down Sirt1 in the brain showed decreased anxiety and resilience to depression induced by social defeat (Libert et al., 2011). Similarly, Ferland et al. found that protein levels and activity of Sirt1 in the hippocampus were elevated after chronic stress in rats (Ferland & Schrader, 2011). The same results were found in the amygdala (Guo et al., 2021).

The discrepancies observed between the results could be explained by the mice's different genetic backgrounds and the modifications between the stress protocols used. Thus, Sirt1-mediated control of depression and anxiety may depend on the cell type, genetic background, and brain region (Abe-Higuchi et al., 2016; Lu et al., 2018). Thereby, more studies are needed to clarify the role of Sirt1 and its potential as therapeutic target in stress-induced MDD.

All this information places Sirt1 as an important protein involved in stress disorders such as MDD and for this reason it will be further studied in this Thesis.

2.4.3 GSK3β

Glycogen synthase kinase-3 (GSK3β) is a highly specific serine (Ser)/threonine (Thr) kinase involved in the transfer of a phosphate group from adenosine triphosphate (ATP) to Ser and

Thr acid residues of target substrates. It was first described in 1980 and it is now considered one of the main regulators of cell function, with more than 100 known substrates (Embi et al., 1980). There are two isoforms of GSK3 (α and β), and although they are both expressed in the mouse brain, GSK3β is the most predominant isoform in the human brain (H.-B. Yao et al., 2002).

2.4.3.1. GSK3β function

GSK3β is crucial for multiple cellular signalling pathways as it has a wide spectrum of substrates, including TFs, glycolytic enzymes, factors controlling apoptosis, mitochondrial channels, membrane receptors, and proteins that regulate the cytoskeleton (Beurel et al., 2015). Thus, GSK3β is a key player in cell homeostasis maintenance (Duda et al., 2018). It is a highly regulated enzyme, which activity can be controlled by different kinases, phosphatases, and proteases. GSK3β is constitutively active and it is inhibited, rather than activated, in response to stimulation (Ding et al., 2000). The main regulators of GSK3β are protein kinase B or Akt, protein kinase A (PKA) and protein kinase C (PKC), which phosphorylate the N-terminal Ser9 of the enzyme, leading to its inhibition (Beurel et al., 2015). Contrarily, the dephosphorylation of Ser9 by different phosphatases (protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A) and 2B (PP2B) induce GSK3β activity (Hernández et al., 2010) (Figure 11).

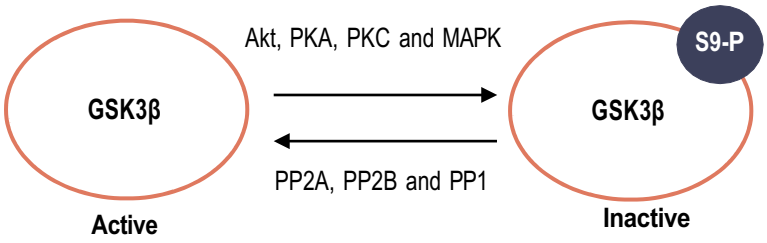


Figure 11. GSK3β regulation. Schematic illustration of the proteins regulating GSK3β activity. Several major kinases (Akt, PKA, PKC and MAPK) phosphorylate GSK3β in the Serine-9 residue. This phosphorylation inhibits GSK3β activity. Contrary, different major phosphatases such as PP2A, PP2B and PP1 remove the phosphorylation inducing GSK3β activation. S9-P: Serine-9 phosphorylation.

2.4.3.2. GSK3β in CUMS

The finding that lithium, a mood stabilizer, directly inhibits GSK3β suggests that GSK3β could be involved in stress-induced major depression. It has been shown that GSK3β activity

is enhanced in the PFC of depressed patients, although the total levels are unaltered (Karege et al., 2007, 2012). In addition, GSK3β increased activity has been reported in the platelets of depressed patients (Diniz et al., 2011; Pláteník et al., 2014). In rodent models of depression (including the CUMS but also others), various studies have found increased levels of GSK3β in the PFC, Nacc, and the hippocampus (Peng et al., 2018; Shen et al., 2019; R. Silva et al., 2008; Wilkinson et al., 2011). Last, different GSK3β haplotypes seem to be associated with MDD (Duda et al., 2020). SNPs in the *GSK3* gene (named rs6782799, rs334555 and rs334558) have been associated with susceptibility, age of onset of MDD and remission upon AD treatment respectively (Inkster et al., 2009; Levchenko et al., 2018; Saus et al., 2010; K. Zhang et al., 2010). In line with this, many ADs influence GSK3β pathway, usually increasing Akt activity or enhancing GSK3β inhibition (Chuang et al., 2020; Duda et al., 2020). Despite all that evidence, a critical goal is to identify the substrates phosphorylated by GSK3β that could mediate its role in mood regulation. Some candidate proteins or pathways have been proposed, including CREB (Grimes & Jope, 2001), β-catenin-destruction complex (Kaidanovich-Beilin et al., 2004), serotonin receptors (X. Li & Polter, 2011), and/ or neurotrophic factors (such as BDNF)(R. S. Duman & Voleti, 2012).

In summary, different molecules/pathways are affected by chronic stress. In this Thesis, we aim to greater explore how Egr1, Sirt1 and GSK3β play a role in the pathogenesis of MDD. To do so, we first aim to characterize the function of these molecules in mice after the stress protocol. Secondly, we plan to modulate this altered mechanism using different approaches such as genetic manipulation, non-invasive tissue-combined photobiomodulation and pharmacotherapy. All in all, our goal is to improve depressive sequelae using diverse strategies that are going to be explained in the following sections.

3. PHOTOBIOMODULATION

As already mentioned, MDD medication sometimes takes weeks or months to produce a beneficial effect and is not effective in all patients. Therefore, new treatment methods are needed. In this line, photobiomodulation (PBM) has raised as a promising opportunity. PBM therapy (also known as LLLT: Low-Level Laser Therapy) is defined as the use of light to repair, modulate and improve the function of the target tissue in a non-invasive way. PBM differs from other light treatments as it will not induce changes in the tissue structure nor

cause a significant increase in tissue temperature (Caruso-Davis et al., 2011). The effectiveness of PBM may vary depending on the parameters used such as emission source, wavelength, light pulse, and duration of the light application (de Freitas & Hamblin, 2016; Hashmi et al., 2010).

3.1. Mechanism of action

Several parameters need to be considered when using PBM. The first one is the wavelength. Different light wavelengths will produce different penetration and diffusion into the tissue. The most used wavelengths for PBM therapy are wavelengths in the red (600-700 nm) and near-infrared (NIR) (780-1100nm)(Figure 12)(de Freitas & Hamblin, 2016). NIR light has been described as the one with higher penetration into the skull (Jagdeo et al., 2012; Lapchak et al., 2015) with a penetration in the human brain of approximately 40-50 mm of depth (Meza et al., 2015). Secondly, we must consider the power density (mW/cm^2), which takes into account the area of the tissue that is irradiated (Hennessy & Hamblin, 2017). Last, treatment duration is something important as some studies suggest that light in pulse mode has a better effect than continuous light (Ando et al., 2011; Hashmi et al., 2010).

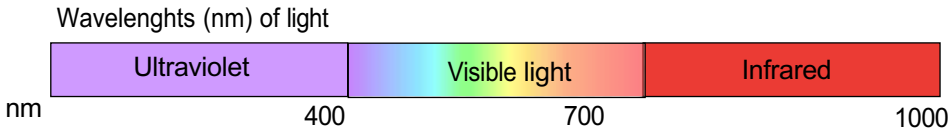


Figure 12. Scheme of the different wavelengths of light (nm)

Although the molecular mechanisms underlying PBM's beneficial effects are not completely understood, several studies have shown that PBM mainly acts on cytochrome c oxidase (CCO), a photoacceptor located in the mitochondria (Dompe et al., 2020). CCO is an enzyme of the mitochondrial respiratory chain responsible for the final reduction of oxygen that will ultimately cause ATP production. The light absorption by CCO induces a cascade of intracellular effects including an increase in the mitochondrial membrane potential (Pan et al., 2022); an increase in the production of ATP (Hennessy & Hamblin, 2017); a rise in nitric oxide (NO) production, that will act as a second messenger (Sharma et al., 2011); and an increase in the generation of reactive oxygen species (ROS), that will play a role in signalling (Figure 13) (de Freitas & Hamblin, 2016). Moreover, other light-sensitive ion channels can

contribute to PBM's effects. The activation of those channels will produce an increase in intracellular Ca^{+2} leading to different intracellular effects.

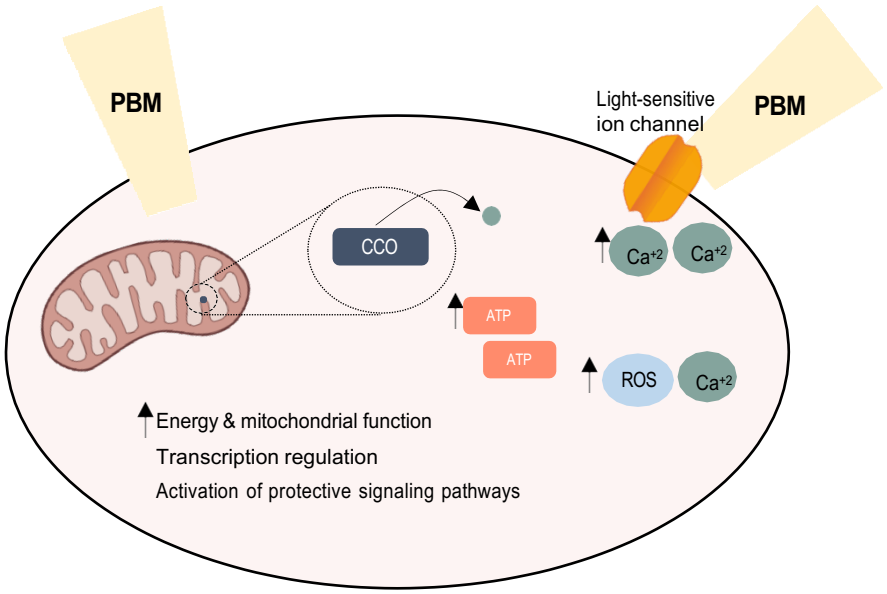


Figure 13. Mechanism of action of photobiomodulation. Schematic representation of the cellular effects of photobiomodulation. The light is mainly absorbed by the cytochrome c oxidase (CCO) located in the mitochondria. The light absorption induces nitric oxide (NO) dissociation from CCO, leading to an increased H^+ gradient that causes a rise in ATP and an elevation of 2on messenger molecules as ROS and Ca^{+2}. These molecules will have long-lasting actions such as an increase in energy production and mitochondrial function and regulation of the gene transcription. These mechanisms will activate protective signaling pathways. Other structures of the cell, such as light-sensitive ion channels can also absorb light and induce an increase in Ca^{+2}. levels.

3.2. PBM for stress-related disorders

PBM has been used for several medical applications as it has shown general analgesic, anti-inflammatory and healing properties (Dompe et al., 2020). Regarding the use of PBM for treating brain disorders, it was first described in acute stroke (Leung et al., 2002). Since then, PBM has been used to improve the sequelae induced by neurodegenerative disorders but also for the treatment of psychiatric disorders including MDD (Hamblin, 2016).

In the literature, different *in vivo* studies have reported beneficial effects of PBM in mice models of MDD. The main effects include improved body weight gain, decreased immobility in the forced swim test (FST), increased ATP and mitochondrial function, and decreased blood cortisol (Eshaghi et al., 2019; Mohammed, 2016; Salehpour, Farajdokht, Cassano, et al., 2019; X. Wu et al., 2012). Human studies have also reported beneficial effects of PBM in depressed patients, including remission of depression rate (37-83%) and improvement of sleep quality (Cassano et al., 2015, 2018; Maiello et al., 2019; Schiffer et al., 2009).

PBM can mediate its beneficial effects on MDD patients and MDD animal models through different mechanisms, although they are not clearly understood. These include restoration of brain metabolism via mitochondria regulation (Salehpour, Farajdokht, Cassano, et al., 2019; X. Zhang et al., 2022), controlling the inflammatory response and oxidative stress levels (Eshaghi et al., 2019), increasing neurogenesis (Hennessy & Hamblin, 2017; Xuan et al., 2014) and inhibiting GSK3β (Liang et al., 2012; L. Zhang et al., 2010). Moreover, independent groups have proposed Sirt1 as a key regulator involved in PBM effects (Salehpour, Farajdokht, Mahmoudi, et al., 2019; Z. Zhang et al., 2020; Zhu et al., 2022). This could be important for pathologies in which Sirt1 has been found dysregulated as MDD (See Section 2.4.2). Not only PBM can exert its effect on the brain but also on the gut (C. Hamilton et al., 2022; M. Wang et al., 2020). Interestingly, it has been proposed that PBM in the abdomen can alter gut composition (Bicknell et al., 2019). This is something that needs further exploration as it is unknown if the light can be absorbed by the microbiota itself or by the host cells surrounding the microbiome (Liebert et al., 2019) but proposes a new therapeutic window for diseases associated with gut dysbiosis such as MDD ( ).

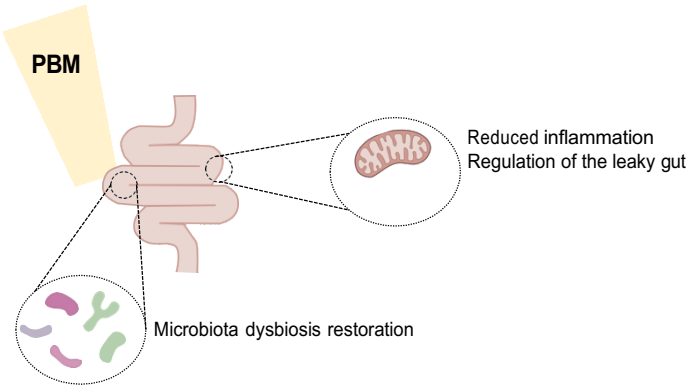


Figure 14. Possible effects of gut photobiomodulation. Schematic illustration showing possible mechanisms of PBM. Light can be absorved by the mitocondria of the host cells and produce benefical effects. Light coul be also be absorved by the gut microbiome inducing microbiota restoration.

In summary, PBM arises as an attractive therapeutic option for stress-induced MDD. First, it is a non-invasive approach that is already in the market. Second, it can modulate altered pathological mechanisms such as Sirt1. Last, it can improve gut dysbiosis. In this Thesis, we aim to use combined PBM in the brain and in the gut to first decipher its underlying mechanisms and, secondly, to improve depressive sequelae induced by CUMS.

4. MERIDIANINS: GSK3β REGULATORS

4.1. Meridianins definition

Marine natural products (MNPs) comprise a huge variety of chemical structures and are an incredible source of new molecules with potentially bioactive activity (Carroll et al., 2022; Molinski et al., 2009). Among them, Antarctic MNPs are the less understood, due to the difficult challenges associated with their collection, identification, and laboratory synthesis (Angulo-Preckler et al., 2020; Avila & Angulo-Preckler, 2021; Soldatou & Baker, 2017). Meridianins are a family of marine-derived indole alkaloids obtained from Antarctic tunicates, particularly they are isolated from *Aplidium ascidians* (Laura Núñez-Pons et al., 2012) (Figure 15A); but it is not clear whether tunicates are the true producers of meridianins or the associated microbes may play a role in their synthesis and chemical ecology (L. Núñez-Pons & Avila, 2015). Their molecular structure consists of a brominated and/or hydroxylated indole framework linked to an aminopyrimidine ring (Franco et al., 1998; Gompel et al., 2004; Laura Núñez-Pons et al., 2012; Seldes et al., 2007; G. Zhang et al., 2021) (Figure 15B). Meridianins possess extensive pharmacological activities such as inhibition of protein kinases (Gompel et al., 2004), antimalarial properties (Lebar et al., 2011) and antitubercular features (Yadav et al., 2015), among others.

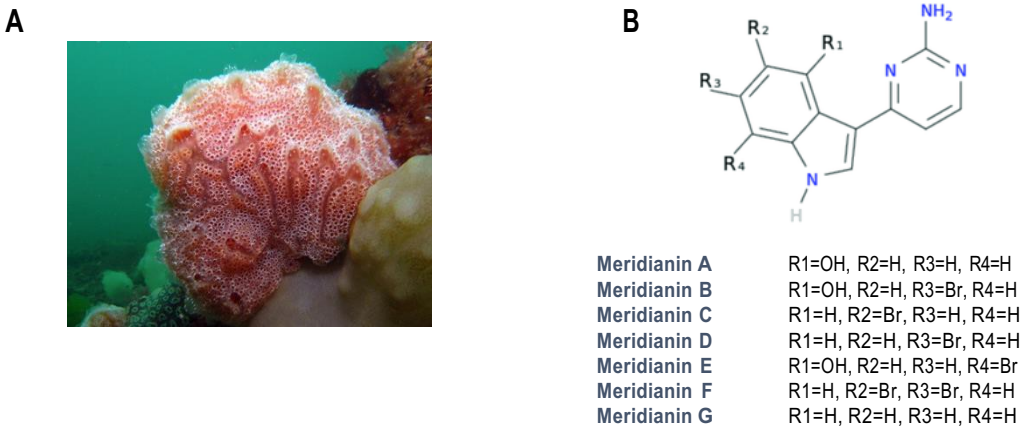


Figure 15. Structure of meridianins. (**A**) Image of the Antarctic tunicate from which meridianins are extracted. (**B**) Molecular structure of meridianins. OH: Hydroxyl; H: Hydrogen; Br: Bromine. Adapted from (Llorach-Pares et al., 2020).

4.2. Meridianins as GSK3β modulators

Mainly, GSK3 inhibitors can be classified into three categories: non-ATP-competitive, ATP-competitive, and substrate-competitive inhibitors (Eldar-Finkelman & Martinez, 2011). It has been recently published by our group that meridianins can act as ATP and non-ATP competitive inhibitors of GSK3 both *in vitro* (Llorach-Pares et al., 2020) and *in vivo* (Rodríguez-Urgellés et al., 2022) without side effects. Moreover, *in vivo* administration of meridianins in an Alzheimer's disease mouse model improves some of its cognitive alterations concomitant with a rescue of its loss of dendritic spine density (Rodríguez-Urgellés et al., 2022). Not only meridianins could be useful for the treatment of neurodegenerative disorders but also in mood disorders that present GSK3β dysregulation such as MDD (See Section 2.4.3) (R. Jope & Roh, 2012; Polter et al., 2010).

Thus, in this Thesis we aim to *in vivo* modulate GSK3β in mice suffering stress-induced MDD.

AIMS

The main objectives of this Thesis are the followings:

1. **To identify progressive behavioural, cellular, and molecular alterations induced by chronic stress.**
   1.1. To determine the effect of short and long-term stress on mice behaviour.
   1.2. To describe and characterize subpopulations of activated cells in the forebrain during short and long-term stress.
   1.3. To assess the role of these subpopulations in the MDD-like pathogenesis induced by chronic stress.
   1.4. To study hippocampal molecular alterations induced by chronic stress.

2. **To use tissue-combined photobiomodulation to try to restore the deficits induced by chronic stress.**
   2.1. To characterise the effects of tissue-combined photobiomodulation in the brain.
   2.2. To use tissue-combined photobiomodulation during chronic stress to try to restore the behavioural, molecular and microbiota alterations induced by CUMS.

3. **To rescue the deficits induced by chronic stress modulating GSK3β.**
   3.1. To assess *in vivo* the role of meridianins in GSK3β regulation.
   3.2. To evaluate if meridianins administration can rescue the sequelae induced by CUMS.

METHODS

1. ANIMALS

1.1. Egr1-CreER^{T2}

In order to study activated cells we used Egr1-CreER^{T2} mice (Longueville et al., 2021). These mice express the Cre recombinase fused to modified estrogen receptor (ER^{T2}) only activated by 4-hydroxytamoxifen (4-HT), under the control of the *Egr1* promoter.

1.2. Egr1-CreER^{T2} x R26RCE

Egr1-CreER^{T2} mice were crossed with R26^{RCE} mice (Gt(ROSA)26Sor$^{tm1.1(CAG-EGFP)Fsh}$/Mmjax, Strain 004077, The Jackson Laboratory. RRID:IMSR_JAX:004077), which harbour the R26R CAG-boosted EGFP (RCE) reporter allele with a loxP-flanked STOP cassette upstream of the enhanced green fluorescent protein (EGFP) gene, to create the double heterozygous mutant Egr1-CreER^{T2} x R26RCE mice (Figure 16A). In these mice, when stimuli are present (i.e., stressor), *Egr1* (an active-dependent early gene) will become active and transcribe Cre ER^{T2}. Only when those mice are injected with 4-HT, Cre will translocate to the nucleus, removing LoxP-flanked STOP signals and inducing permanent GFP expression (Figure 16B).

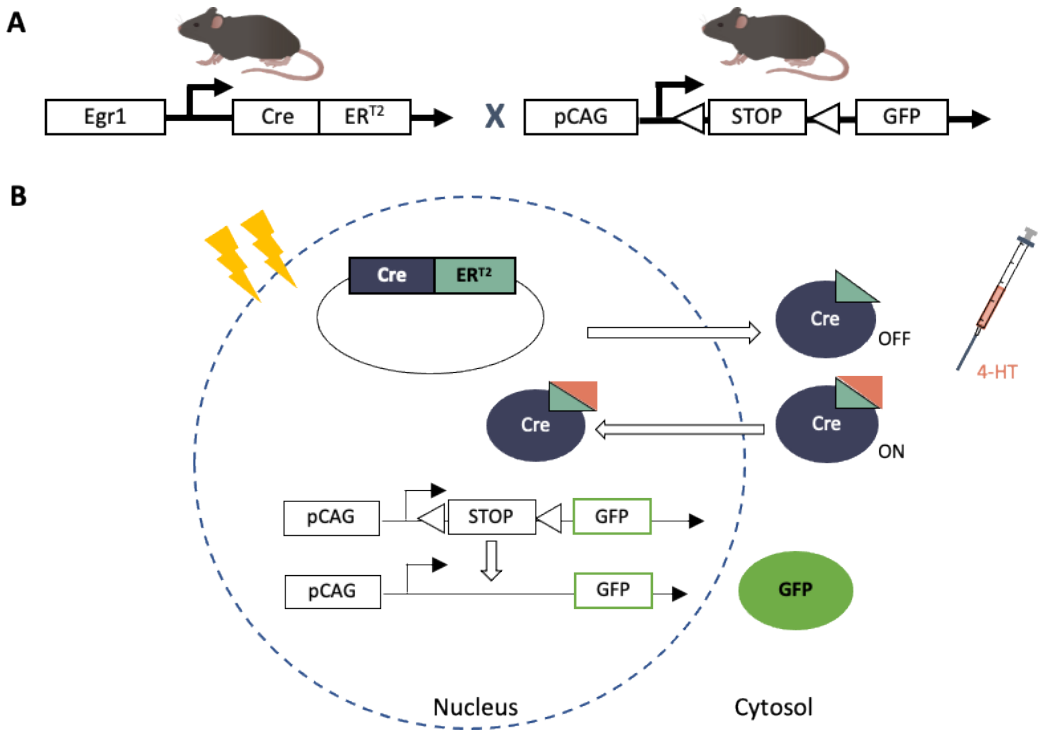


Figure 16. Schematic representation of Egr1-CreER^{T2} x R26RCE GFP mutant mice. (**A**) Illustration of the double heterozygous mutant mice. (**B**) Mechanism of action of the mutant mice co-expressing CreER^{T2} under the Egr1 promotor. Only those Egr1-dependent activated cells will express the Cre, but only when 4-HT is administered, the Cre will become active. Once active, it will cut the LoxP sequence and remove the STOP codon, allowing GFP expression.

1.3. Calbindin-cre and Chrna7-cre

To determine the role of deep and superficial pyramidal neurons, two different mice strains were used: Chrna7-cre mice (Tg(Chrna7-cre)NP348Gsat/Mmucd, GENSAT) and a Calb1-cre mice (Calb1-T2A-dgCre-D, Jackson Labs, #023531. RRID:IMSR_JAX:023531). Those mice express the Cre-recombinase under the cholinergic receptor nicotinic alpha 7 subunit (Chrna7) promoter, enriched in deep pyramidal neurons (Figure 17B) or the calbindin (Calb1) promoter, enriched in superficial pyramidal neurons (Figure 17C).

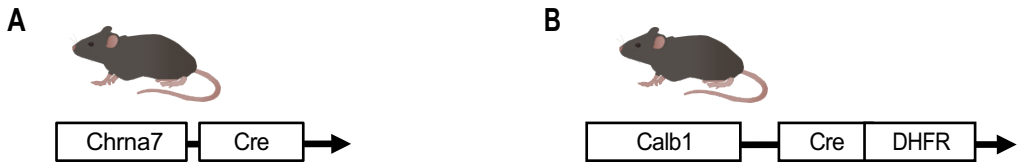


Figure 17. Schematic illustration of the mice models used. (**A**) Chrna7-cre mice, expressing the Cre under a Chrna7 promoter. (**B**) Calbindin-cre mice, expressing the Cre under Calbindin promoter. DHFR: Dihydrofolate reductase.

For other experiments, C57BL/6JOlaHsd mice from Envigo were used.

All mice used in the experiments were males. All mice were genotyped from a tail biopsy by PCR analysis. Mice were housed together in numerical birth order in groups of mixed genotypes (3–5 mice per cage) until starting the experiment. The animals were housed with access to food and water ad libitum in a colony room kept at 19–22ºC and 40–60% humidity, under an inverted 12:12h light/dark cycle (from 08:00 to 20:00). All animal procedures were approved by local committees [Universitat de Barcelona, CEEA (10141) and Generalitat de Catalunya (DAAM 315/18)], in accordance with the European Communities Council Directive (86/609/EU).

2. DRUGS

To induce recombination in Egr1-CreER^{T2} and in Egr1-CreER^{T2} x R26RCE transgenic mice models, mice were injected with 4-hydroxytamoxifen (4-HT) (H6278, Sigma-Aldrich). Briefly, 4-HT was dissolved in 1 mL ethanol (100%). Corn oil (C8267, Sigma-Aldrich) was then added, and ethanol was evaporated by incubating the open tubes at 50°C overnight in the dark. The volume of corn oil was adjusted for injecting the dose of the active drug at 50mg/kg.

Clozapine N-oxide (CNO) (BML-NS105-0025, Enzo) was used as a selective ligand for the experiments with Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). CNO was administered via drinking water as previously described (Zhan et al., 2019). Briefly, stock solution was prepared in saline solution at 5mg/ml. Then, required CNO stock solution was then added to 1% sucrose water to have a final concentration of 1mg/kg/day. The water was changed 3 times per week and was protected from light.

Trimethoprim (TMP) (Santa Cruz, sc-237332) was used to stabilize Cre expression in Calb1-cre mice. TMP was reconstituted in water at a concentration of 30 mg/ 100 ml. After surgery, all mice received TMP in drinking water for one week to induce Cre recombinase activity.

3. STEREOTAXIC SURGERY

3.1. AAV injection

Mice were stereotaxically injected with one of the following adeno-associated viruses (AAVs): AAV5-CAG-FLEX-GFP (UNC, Vector Core), pAAV5-hSyn-DIO-hM3D(Gq)-mCherry (Addgene, #44361) and AAV5-CAG/EF1a-DIO-mCherry-mEGR1-shRNAmir (Vector Biolabs, #258146). Following anaesthesia with isoflurane (2% induction, 1.5% maintenance) and 2% oxygen, mice were bilaterally injected with AAVs (3 x 10^{12} GS per injection) in the CA1 of the hippocampus. We used the following coordinates (millimetres) from bregma: antero-posterior, -2.0; lateral, ±1.5; and dorso-ventral, -1.3. AAV injection was carried out in 4 min. The needle was left in place for 2 min for complete virus diffusion. After recovery, mice were returned to their home cage for 3 weeks.

3.2. Intraventricular meridianins administration

For single meridianins intraventricular administration, C57BL/6JOlaHsd mice were deeply anaesthetized with isoflurane (2% induction, 1.5% maintenance) and 2% oxygen, and placed in a stereotaxic apparatus for injection into the third ventricle with 3 µl of 500 nM meridianins and were sacrificed 20 min, 1h or 3 h after administration. The following coordinates (millimetres) from bregma were used: antero-posterior, 0.1; lateral, ±0.8; and dorso-ventral, -2.5.

3.3. Osmotic minipumps implantation

For chronic meridianins treatment, C57BL/6JOlaHsd mice were deeply anaesthetized with isoflurane (2% induction, 1.5% maintenance) and 2% oxygen, and placed in a stereotaxic apparatus for osmotic minipump implantation (model 1,002; Alzet, Palo Alto, CA, United States). The brain infusion kit (#0008663) was also used to deliver into the lateral (left) ventricle 0.11 µl per hour of the vehicle or meridianins 500 nM (same coordinates as before). Cannulas were fixed on the skull with the Loctite 454 (from Alzet). Minipumps, previously equilibrated overnight at 37°C in PBS, were implanted subcutaneously in the back of the animal.

4. CHRONIC UNPREDICTABLE MILD STRESS

To induce a depressive-like phenotype, the chronic unpredictable mild stress (CUMS) model was applied to adult male mice. This protocol was adapted from previous work (Montalban et al., 2019; Sancho-Balsells et al., 2023). A total of seven stressors were used (Table 3 and Figure 18), which were applied randomly and one each day (Table 4).

Table 3. Stressors used during the CUMS protocol. Name and description of each of the stressors used during the CUMS protocol.

STRESSOR	DESCRIPTION
Food deprivation	Food was removed from each cage for 24 hours.
Water deprivation	Water was removed from each cage for 24 hours.

Forced swim	Mice were placed in a water tank for 5 minutes.
Restrain	Mice were confined in a 50 ml ventilated Falcon for 1 hour.
Home cage inclination	All sawdust and paper were removed from the cage. Cages were inclined 45º for 4 hours.
Light-dark cycle alteration	Light was turned on during the dark cycle, so mice have 24 hours of light.
Rat sawdust	A bunch of rat sawdust was placed in the mice cage for 4 hours.

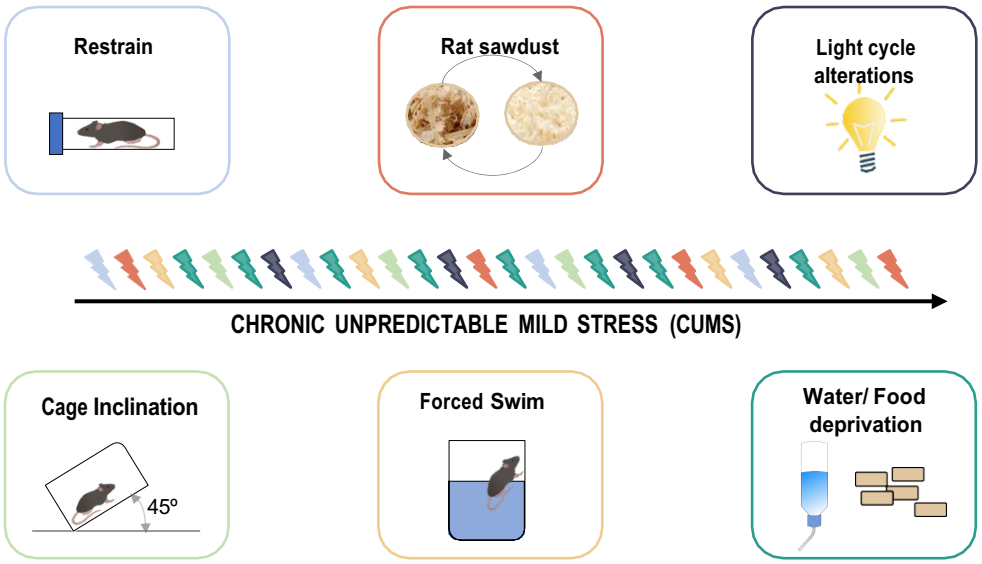


Figure 18. Schematic illustration of the CUMS protocol. Seven stressors were presented randomly and daily for 28 days. Each colour represents a different stressor.

Table 4. Unpredictable chronic mild stress (CUMS) weekly schedule. Schedule example of the CUMS protocol.

Monday	Tuesday	Wednesd.	Thursday	Friday	Saturday	Sunday
Light-dark cycle alterations	Forced swim	Rat sawdust	Home cage inclination	Restrain	Food deprivation	Water deprivation
Forced swim	Restrain	Rat sawdust	Home cage inclination	Home cage inclination	Water deprivation	Food deprivation
Restrain	Water deprivation	Rat sawdust	Home cage inclination	Forced swim	Light-dark cycle alterations	Food deprivation

Forced swim	Rat sawdust	Food deprivation	Home cage inclination	Restrain	Light-dark cycle alterations	Water deprivation

5. BEHAVIOUR

5.1. Open field and NOLT

For the novel object location test (NOLT), an open-top arena (40 × 40 × 40 cm) with a visual cue placed on one wall of the apparatus was used. Mice were first habituated to the arena (1 day, 15 min). We considered this first exposition to the open arena as an open field paradigm. We monitored total travelled distance (in cm) as a measure of locomotor activity, time in the centre as a measure of anxiety, parallel index as a measure of navigation strategy, and supported rearings as a measure of exploratory activity. On day 2, two identical objects were placed on one side of the arena and mice were allowed to explore them for 10 min. Exploration was considered when the mouse was in contact with the object and sniffed it. 24 hours later (day 3), one object was moved from its original location to the diagonally opposite corner and mice were allowed to explore the arena and the objects for 7 min (Figure 19). At the end of each trial, defecations were removed, and the apparatus was cleaned with 70% ethanol. Animal tracking and recording were performed using the automated SMART junior 3.0 software (Panlab, Spain).

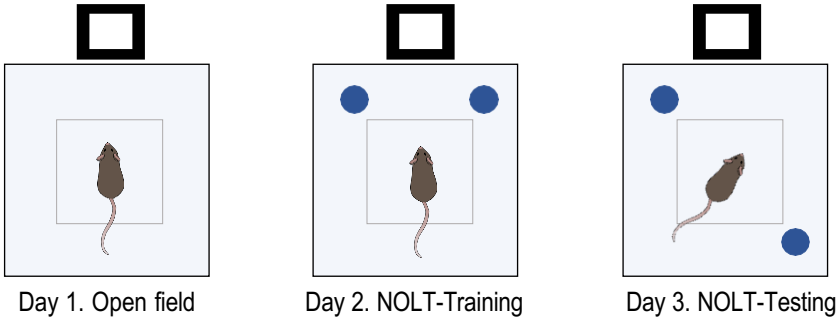


Figure 19. Schematic representation of the open field and NOLT test. Mice were placed in the open field apparatus with a visual cue in one of the walls for 3 consecutive days. On the second day, two identical objects were presented to the mice. On the third day, one of the objects was placed in a new location.

5.2. Y- MAZE

The spontaneous alternation in a Y-maze was used to determine spatial working memory. In this test, we evaluated whether mice continuously alternate (normal behaviour) or, instead, they show repetitive explorative behaviour. Briefly, mice were placed in the Y-shaped maze with three clear plastic arms at 120º from each other for 8 minutes (Figure 20). Around the clear Y-maze, different spatial cues were located. The total number of entries and the number of triads (% alternation) were analysed. The % of alternation was calculated as: number of triads/number of possible triads (number of arm entries -2) *100. Arm entries were only counted if the mice placed all four limbs inside the arm.

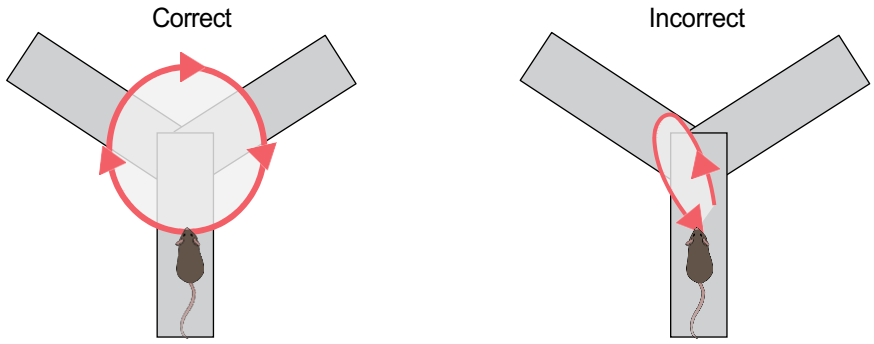


Figure 20. Schematic representation of the Y-maze. Schematic representation of a correct exploration behaviour and an incorrect/repetitive exploration behaviour**.**

5.3. Forced swim

The forced swim test was used to evaluate behavioural despair. Animals were subjected to a 6 min trial during which they were forced to swim in acrylic glass (35 cm height x 20 cm diameter) filled with water (23-25°C), from which they cannot escape. The time that the mice spent in the cylinder without making any movements except those required to keep its head above water was measured and considered as “resting time”. The rest of the time was considered as time swimming/struggling (Figure 21).

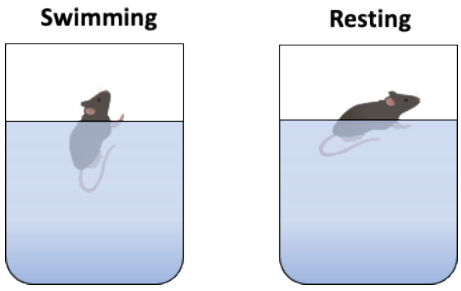


Figure 21. Forced swim test. Schematic representation of the forced swim test in which mice either swim/struggle to try to escape from the water (left) or present floating/resting behaviour (right).

5.4. Dark-light test

The dark-light test was used to assess anxiety-like behaviour. The apparatus consists of two connected compartments, one brightly illuminated and the other one completely in the darkness. The distribution is one-third for the dark compartment and two-thirds for the light compartment (Figure 22). Mice were placed in the dark chamber and the latency to cross to the light chamber was analysed. The more stressed mice are, the more time they need to cross.

Figure 22. Schematic illustration of the dark-light apparatus. The latency to cross from the dark compartment (right) to the light compartment (left) was measured.

All the tests were conducted during the light cycle and all mice were randomized throughout the day. Only one test was conducted per day.

5.5. Z-scoring

Z-score normalization was used to compare observations obtained at different behavioural tasks. As previously described (Guilloux et al., 2011), z-scores are standardized scores that

represent how many standard deviations (σ) an observation (X) is above or below the mean of a control group (μ).

$$\frac{X-\mu}{\sigma}$$

In short, Z-score was obtained by subtracting the average of observations in control mice from an individual raw value and then dividing this difference by the CNT standard deviation. The parameters integrated into the z-score are defined in each results section. In this work, an increase in Z-score was associated with a worse performance in the behavioural tasks, meaning mice are more affected by chronic stress.

6. IMMUNOHISTOCHEMISTRY

Animals were deeply anaesthetized and subsequently intracardially perfused with 4% (weight/vol) paraformaldehyde (PFA) in 0.1M phosphate buffer. Brains were dissected out and kept for 48h in 4% PFA 0.1M phosphate buffer in agitation. Alternatively, mice were sacrificed by cervical dislocation, and half of the brain was maintained in PFA 0.1 M for 48h hours. After fixation, free-floating coronal sections (30 or 40 µm) were obtained using a vibratome (Leica, VT1000).

For immunofluorescence, sections were first washed twice in PBS and incubated in 50mM NH_4Cl, for 30 minutes to reduce tissue autofluorescence. Blocking and permeabilization were performed for 1h in PBS-T with 0.02% azide, 3% NGS or NDS, and 0.2% BSA. Primary antibodies (Table 5) were diluted in blocking solution and sections were incubated overnight at 4ºC in agitation. Secondary antibodies (Table 6) were diluted in blocking solution and tissues were incubated for 2h at room temperature. Nuclei were stained for 10 min with 4′,6-diamidino-2- phenylindole (DAPI; catalogue #D9542, Sigma-Aldrich). The sections were mounted onto slides and cover-slipped with Mowiol. Tissue sections were examined using an Widefield AF6000 Monochroma Camera Leica or with a Leica Confocal SP5 (Leica Microsystems Heidelberg, Manheim, Germany) at different magnifications.

For Sirt1 immunohistochemistry, antigen retrieval was needed. Thus, incubation with citrate buffer 10mM at 80º for 30 min was performed at the beginning of the protocol. After this step, the immunohistochemistry was done as previously described.

Table 5. Primary antibodies used for immunohistochemistry. Information about the host specie, the dilution, the source, and the reference are provided.

ANTIGEN	SPECIE	DILUTION	SOURCE	REFERENCE
Calbindin D-28k	Rabbit	1:5000	Swant	CB38
cFos	Rabbit	1:150	Santa Cruz	Sc-52
Egr1	Rabbit	1:1000	Cell Signaling	4154S
GFAP	Rabbit	1:500	Dako	z0334
GFP	Chicken	1:500	Synaptic Systems	132006
GFP FITC-conjugated	Goat	1:500	Abcam	Ab6662
Iba1	Goat	1:500	Abcam	Ab5076
MAP2	Mouse	1:500	Sigma	M1406
Parva	Rabbit	1:1250	Swant	PV27
Sirt1	Rabbit	1:100	Cell Signaling	2028

Table 6. Secondary antibodies used for immunohistochemistry. Information about the antibody, the dilution, the source, and the reference are provided.

SECONDARY ANTIBODY	DILUTION	SOURCE	REFERENCE
Alexa Fluor 488 AffiniPure Donkey Anti-mouse IgG	1:200	Jackson Immunoresearch	715-545-150
Alexa Fluor 488 AffiniPure Donkey Anti-rabbit IgG	1:200	Jackson Immunoresearch	711-545-152
Alexa Fluor ™ 488 Goat anti-chicken IgY	1:200	Invitrogen	A11039
Alexa Fluor ™ 647 Goat anti-mouse IgG	1:200	Invitrogen	A21236
Cy™3 AffiniPure Donkey anti-goat IgG	1:200	Jackson Immunoresearch	705-165-003
Cy™3 AffiniPure Donkey anti-rabbit IgG	1:200	Jackson Immunoresearch	711-165-152

7. IMAGING ACQUISITION AND ANALYSIS

7.1. GFP-positive cells counting in different brain areas

For the results obtained in Figure 29 and Figure 30, images (512x512) were obtained in a mosaic format with a Leica confocal SP5 with an x20 objective and standard (1 Airy disc) pinhole (1 AU) and frame averaging (3 frames). To determine the neural subpopulation densities, the area of the striatum, septum, paraventricular nucleus, cingulate cortex, amygdala, CA3, DG, and CA1 was delimited by comparing the original images with the Gaidi atlas. Then, the number of GFP-positive cells in a specific area was counted using the ImageJ software. The number of GFP-positive cells was relativized per area (500 μm2).

For the results obtained in Figure 48 and Figure 49, images were obtained in a mosaic format with the Widefield AF6000 Monochroma Camera Leica microscope with a 5x objective. The number of activated cells/area was measured as described above.

7.2. Subpopulation analysis

To determine the identity of the GFP-positive cells, the percentage (%) of GFP-positive cells colocalizing with different markers (MAP2, GFAP, Parvalbumin, and Calb1) were estimated per area as described above. For double-positive cell quantification, qualitative criteria were used. Briefly, cells clearly presenting the expression of both markers were counted as double-positive cells.

7.3. Spine density analysis in GFP-positive cells

Spine density counting was performed using the ImageJ software. GFP-labelled pyramidal neurons from CA1 of the dorsal hippocampus were imaged using a Leica Confocal SP5 with a ×63 oil-immersion objective. Conditions such as pinhole size (1 AU) and frame averaging (4 frames per z-step) were held constant throughout the study. Confocal z-stacks were taken with a digital zoom of 5, a z-step of 0.2 μm, and at 1024×1024-pixel resolution, yielding an image with pixel dimensions of 49.25 × 49.25 μm. We examine second-order dendrites.

Thus, ramifications from the apical dendrite were analyzed. On average, 30 µm ±2 of length were evaluated per dendrite and the ratio was performed as the number of dendrites/total length analyzed. 17-27 dendrites from 7 different mice per group were quantified.

7.4. GFAP and Iba1 staining in mice after photobiomodulation

To analyse inflammatory markers in those mice receiving photobiomodulation, the Leica confocal SP5 was used with a 40x oil-immersion objective and a digital zoom of 2. Conditions such as pinhole size (1 AU), laser intensity, smart offset and smart gain were held constant throughout the conditions. Confocal z-stacks were taken with a z-step of 1 µm and a pixel resolution of 512x512.

7.5. Sirt1 analysis in PBM mice

To determine Sirt1 levels in the hippocampus the Leica confocal SP5 was used with a x40 oil immersion objective. Conditions such as pinhole size (1 AU), laser intensity, smart offset and smart gain were held constant throughout the conditions confocal z-stacks were taken with a z-step of 5um and a pixel resolution of 1024x1024. To do the image analysis, ImageJ software was used. Briefly, the ROI of the CA1 or the DG was delimited with DAPI and the mean intensity of Sirt1 was calculated in the corresponding channel.

8. GOLGI STAINING

Animals were sacrificed by cervical dislocation and brains were obtained and kept in Golgi-Cox solution as described previously (Giralt et al., 2017). After 48 hours, the Golgi-Cox solution (1% potassium dichromate, 1% mercury chloride and 0,8% potassium chromate) was renewed, and the brains were kept in the fresh solution for 3 weeks. Then, brains were washed 3 times for 2 minutes each in distilled water and then with ethanol 90º for 30 minutes. Next, 200 µm slices were obtained using a vibratome (Leica VT 1000S) and kept in 70% ethanol. After that, brain slices were washed in distilled water and reduced in 16% ammonia solution for 1 hour. After two more washes in water, slices were fixed with 1% sodium

thiosulfate for 7 minutes and then rinsed again with distilled water. Last, sections were mounted in SuperFrost coverslips and dehydrated for 3 minutes in 50%, 70%, 80% and 100% ethanol. Thereafter, slices were incubated 2 times for 5 minutes each in a mixed solution of isopropanol and ethanol (2:1) and then 1 time for 5 minutes with only isopropanol. Last, slices were washed 2 times for 5 minutes with xylol and mounted with mounting media (DPX, Merk).

8.1. Golgi staining analysis

Secondary dendrites from CA1 pyramidal neurons were photographed using a Widefield AF6000 Monochroma Camera Leica microscope. A maximum of 3 dendrites from the same neuron were taken. Z-stacks from 0.2 µm were obtained in a bright field and 63x oil objective. Dendritic segments (average 30 µm) were traced with ImageJ software, and spine density was quantified. Around 50 dendrites were analysed per condition. From each condition, 6-7 mice were quantified.

9. PHOTOBIOMODULATION

The photobiomodulation device (RGn530) (kindly provided by REGENLIFE©, Montpellier, France) was used to do all PBM experiments (Figure 23A). This apparatus is composed of 2 modules, one located at the top which exposes the head, and the other located at the bottom which exposes the abdomen. The “head module” is fixed because the position of the head does not vary according to the morphology of the mouse, whereas the “abdomen module” is adjustable to adapt to the morphology of the mouse. Each module contains a mixed emission source: lasers and LEDs (Figure 23B). Lasers (coherent lights) present great tissue penetration whereas LEDs (incoherent lights) present better diffusion. The device has two different types of LEDs: infrared and polychromatic lights. Additionally, the device also incorporated a static magnetic field. Thus, the device includes a near-infrared laser diode (850nm), a near-infrared LED (850nm), a red LED (625nm), and a ring magnet with a magnetic field of 200mT. The parameters used were emission in pulsed mode at 10 Hz with a total irradiance at the contact surface of mice of 28 mW/cm^2. The total fluence was 8.4 J/cm^2 for a 10 min exposure. To treat the animals, mice were placed in a plastic clear

restrainer which was then introduced into the apparatus in a way that the head of the mice was located under the "head module" and the abdomen was located above the "abdomen module".

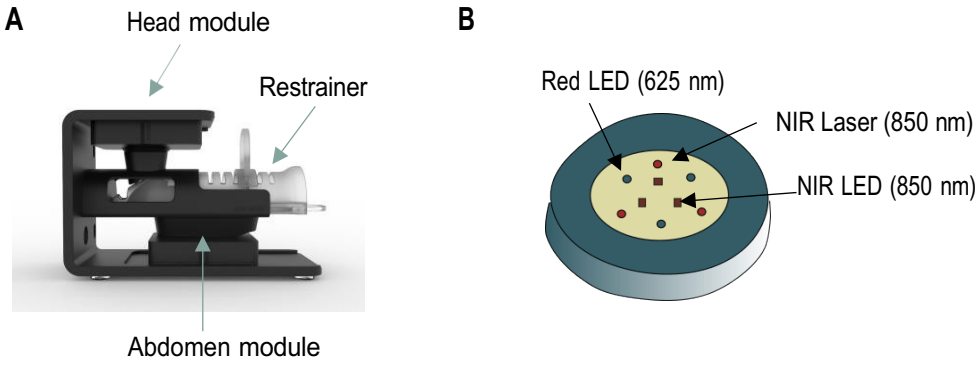


Figure 23. Photobiomodulation device. (**A**) Picture showing the device used for all the photobiomodulation experiments. Mice were restrained in the plastic restrainer during the 6 minutes of the treatment. (**B**) Scheme of the emission module with 3 near-infrared lasers, 3 near-infrared LEDs, 3 red LEDS and a ring magnet.

10. METABOLOMIC ANALYSIS

Metabolomic analysis of the hippocampal homogenates was done as previously described (Clos-Garcia et al., 2019). Hippocampus samples were homogenized in 500 µL of ice-cold extraction liquid with a tissue homogenizer (FastPrep) in 40 seconds cycle at 6000 rpm. Subsequently, 400 µL of the homogenate plus 400uL of chloroform was transferred to a new aliquot and shaken at 1400 rpm for 60 minutes at 4 °C. Next the aliquots were centrifuged for 30' at 13000 rpm at 4 °C. 300µL of the aqueous phase was transferred to a fresh aliquot and placed at -80 °C for 20'. The chilled supernatants were evaporated with a speedbag in approximately 2h. The resulting pellets were resuspended in 150 µL water/acetonitrile (MeCN)/formic acid (40/60/ v/v/).

Samples were measured with an ultra-performance liquid chromatographic system (Acquity, Waters Inc., Manchester, UK) coupled to a Time-of-Flight mass spectrometer (SYNAPT G2, Waters Inc.). A 2.1 × 100 mm, 1.7-µm BEH amide column (Waters Inc.), kept at 40 °C, was used to separate the analytes before the MS. The MS was operated in positive electrospray ionisation full scan mode. Extracted ion traces for relevant analytes were obtained in a 20 mDa window in their expected m/z-channels. These traces were subsequently smoothed and peak areas were integrated with TargetLynx software (Waters,

Manchester, UK). These calculated raw signals were adjusted by median fold-change (MFC) adjustment as previously described (Dieterle et al., 2006; Veselkov et al., 2011).

11. MICROBIOME SEQUENCING

Faecal samples were kept at -20ºC in RNA latter buffer until analysis. Total genomic DNA was extracted using ZymoBIOMICS DNA Miniprep (Zymo Research, Irvine, Canada) following manufactured indications. All samples were homogenized and lysate during 30 minutes through Vortex Genie. Qubit 4 Fluorometer (Invitrogen) was used for DNA quantification. DNA amplicon libraries were generated targeting the 16S rRNA gene, V3-V4 regions (341F/R805) and the sequencing was performed using Illumina MiSeq PE300 following the recommendations of Illumina Inc. Sequencing was performed in Genome Québec Inc. (Centre d'expertise et de services Génome Québec, Montréal (Québec), Canada). The raw sequencing data were processed using QIIME (Version 22021.2) and pair-end reads were merged using fastq-join. Chimeric sequences were detected and deleted, meanwhile, Amplicon Sequence Variant (ASV) assignment was completed using the dada2 plugin. Taxonomy was assigned at a 99% similarity level using the q2-feature-classifier plugin with the SILVA 132 database (version 2019.10.0)

R software (Version 1.4.1717) was used for statistical analysis and plots generations. Ecological analyses were performed for alpha and beta-diversities combining *vegan, phyloseq* and *microeco* packages. For alpha and beta diversity, the Shannon and Weighted Unifrac indexes were respectively calculated. Multivariate statistical significance analysis was carried out using permutational multivariate analysis of variance (PERMANOVA) (Adonis2) and pairwise PERMANOVA (pairwise Adonis2) with 999 permutations ($p < 0.05$). Taxonomical distribution was represented at phylum, family, and genus level. Differential abundance analysis was performed at genus level. Differential genera were identified using ANOVA ($p < 0.05$). Correlation analysis was carried out using the genus relative abundance and Z score values of each mouse with Pearson correlation method. All samples were processed together and by treatments with no post-hoc test.

12. MULTI-ELECTRODE ARRAY

Brain coronal sections of 20-week-old male mice were obtained at 350 m thickness on a vibratome (Microm HM 650 V, Thermo Scientific, Waltham, MA, USA) in an oxygenated

(95% O2, 5% CO2) ice-cold artificial cerebrospinal fluid (aCSF). The sections were transferred to an oxygenated 32ºC recovery solution for 15 min, as previously described (A. Kim et al., 2020) and then to oxygenated aCSF, where they were incubated at room temperature for at least 1 h before electrophysiological field recording. After recovery, the slices were transferred into 60MEA200/30iR-ITO MEA recording dishes and fully submerged in oxygenated aCSF at 37ºC. For the recording of spontaneous activity, the hippocampal formation slice surface was placed on MEA 60 planar electrodes arranged in an 8x8 array with the assistance of a digital camera. Raw traces were sampled at 5 kHz and recorded for 5 min from 58 electrodes simultaneously. To determine the effects of meridianins in the hippocampal spontaneous activity, after a 3 min baseline recording, the slices were incubated with 500 nM meridianins in aCSF for 20 min. Then, spontaneous activity was recorded for an additional 3 min.

Spikes were identified and quantified as previously described (Pérez-Sisqués et al., 2021). In brief, we applied a high-pass filter with a 200 Hz Butterworth 2nd-order filter. We then assessed the noise level by using the signal standard deviation on each electrode and identified the spikes as currents with slope values between 0.2 and 1 and a negative amplitude larger than -20 mV. We applied the max interval method (Legendy & Salcman, 1985) to quantify the burst activity with the following parameter values: maximum ISI beginning and end, 200 ms and 200 ms, respectively; minimum burst duration 20 ms; minimum number of spikes in a burst, 5; and minimum interburst interval, 20 ms. We used MC Rack from Multi Channel Systems software for recording and signal processing. We selected the electrodes specifically positioned on different fields of the hippocampal formation by the image taken with a digital camera (Figure 24).

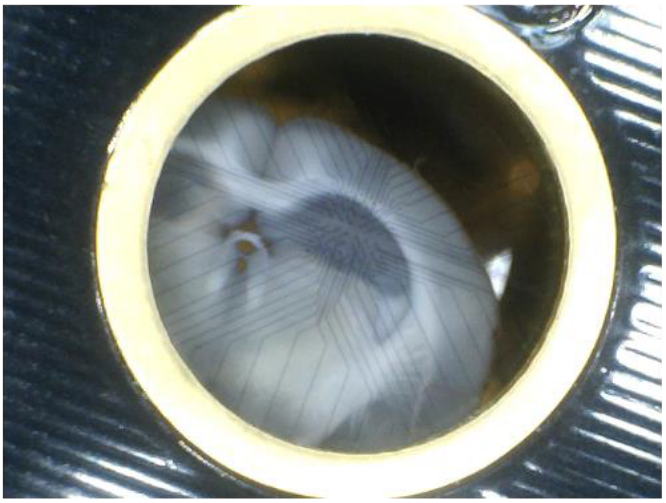


Figure 24. Picture of the hippocampus placed in the MEA device. Photograph of the hippocampus obtained with a digital camera. The electrodes corresponding to specific regions of interest were selected using this kind of images.

13. MOLECULAR BIOLOGY METHODS

13.1. Protein extraction

Mice were sacrificed by cervical dislocation and the hippocampus was quickly dissected and frozen on dry ice. Hippocampus were kept at -80 until use. Mouse frozen tissues were homogenized by sonication in ice-cold lysis buffer containing: 50mM Tris-HCl (pH 7.5), 10% Glycerol, 1% Triton X-100, 150mM NaCl, 100 mM NaF, 5 uM $ZnCl_2$ and 10 mM EDTA. Lysis buffer was supplemented with proteases and phosphatase inhibitors: 2mM PMSF, 10 ug/ml Aprotinin, 1ug/ml Leupeptin, 2mM Na_3VO_4 and protease inhibitor cocktail Sigma (Sigma-Aldrich, St.Louis, MO, USA). Samples were centrifuged at 16.000 g for 30 minutes at 4º and the supernatant was collected into a new tube and kept at -80º until use.

13.2. Protein quantification

To determine the protein concentration of the brain extracts, the Detergent Compatible Protein Assay Kit (Bio-Rad, ref. 500-0114, 500-0113, and 500-0115) was used following the manufacturer's instructions. This method is based on the colorimetric Lowry protein assay. Briefly, protein extracts were added into a 96 well-plate and incubated with the kit's reagents for 10 minutes. Bovine Serum Albumin (BSA, Sigma #A9647) was used to prepare the

standard curve and each sample was read in duplicate. Absorbance was read at 650-750 nm in a Synergy 2 Multi-Mode Multiplate reader (BioTek), which was then used to calculate protein concentration.

13.3. Western blot

Protein extracts (usually 20 μg) were denatured in 62.5 mM Tris-HCl (pH 6.8), 2% Sodium dodecyl sulfate (SDS), 10% glycerol, 140 mM β-mercaptoethanol and 0.1% bromophenol blue and heated at 100ºC for 5 min. Protein extracts were loaded into the wells of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) for around 1h30 min at 100V. Polyacrylamide concentration was variable depending on the molecular weight of the protein of interest. The Precision Plus ProteinTM Dual Colour ladder (Bio-Rad) was loaded along with the protein samples to ensure the correct size of the proteins of study. Afterwards, proteins were transferred to a nitrocellulose membrane (Whatman Schleicher & Schuell, Keene, NH, USA) for ± 1.5 hours at 90 V at 4ºC. Non-specific protein binding sites were blocked during 1 h incubation at RT in blocking solution containing 10% non-fat powdered milk and 10% BSA in TBS-T (50 mM Tris-HCl, 150 mM NaCl, pH 7.4, 0.05% Tween 20). Membranes were rinsed 3 times during 10 min in TBS-T and immunoblotted overnight at 4°C with the primary antibody (Table 7). Membranes were rinsed three times with TBS-T and incubated with the corresponding horseradish peroxidase-conjugated secondary antibody for 1h at room temperature. The secondary antibodies used were anti-mouse IgG 1:2000 Promega (Madison, WI, USA) and anti-rabbit IgG 1:2000 Promega (Madison, WI, USA).

After three more washes at RT, immunoreactive bands were developed using Western Blot Luminol Reagent (Santa Cruz, Cat. #sc-2048). The loading control was performed by incubating the membranes with loading control antibodies (Table 7). Loading control antibodies were incubated for 25 min at RT and then washed and developed as previously described. Image Lab software (BioRad) was used to measure the different densitometry immunoreactive bands relative to the intensity of the loading control band.

Table 7. Primary antibodies used for Western blot. Information about the specie, the molecular weight (MW), the dilution, the source and the ID of the antibodies used are provided.

ANTIGEN	SPECIE	Mw	Dilution	SOURCE	ID
Actin	Mouse	42	1:100000	Sigma Aldrich	A3854
Akt (pan)	Rabbit	60	1:1000	Cell Signaling	4691
Calbindin	Rabbit	28	1:2000	Swant	CB38
CREB	Rabbit	43	1:1000	Cell Signaling	9197S
GAPDH	Mouse	38	1:1000	Millipore	MAB374
GluR1	Rabbit	110	1:1000	Millipore	ABN241
GSK3β	Rabbit	46	1:1000	Cell Signaling	9315
Phospho-(Ser) PKC substrates	Rabbit		1:1000	Cell Signaling	2261
Phospho-Akt Ser (473)	Rabbit	60	1:1000	Cell Signaling	4060S
Phospho-CREB (Ser 133)	Rabbit	43	1:1000	Millipore	06-519
Phospho-GluR1 (Ser 831)	Rabbit	110	1:1000	Millipore	04-823
Phospho-GSK3β (Ser9)	Rabbit	46	1:1000	Cell Signaling	9336
Phospho-PKA substrates	Rabbit		1:1000	Cell Signaling	9624
Tubulin	Mouse	50	1:50000	Sigma Aldrich	T9026

13.4. Mass spectrometry

Hippocampal lysates were analysed via mass spectrometry by the Proteomic facility at the Max Planck Institute of Biochemistry, Martinsried, Germany using label-free quantitation (LFQ) (MaxQuant run). For analysis of the data derived from mass spectrometry, the LFQ-Analyst software was used (Shah et al., 2020). Briefly, all data were filtered using a Log_2 fold change of 0.5 and an adjusted p-value of 0,05. The MinProB imputation method was used.

13.5. RNA extraction

RNA from the hippocampus was extracted using the RNeasy Lipid Tissue Mini Kit (Qiagen, ref. 74804), following the instructions of the manufacturer. Briefly, the frozen tissue was placed in 1mL QIAzol and homogenized using a 25G syringe. After a 5-minute incubation, chloroform was added, and the sample was centrifuged at 12,000 x g for 15 minutes at 4ºC. The upper aqueous phase containing RNA was then transferred onto a RNeasy Mini Spin Column and subjected to sequential washing with buffers provided by the kit. The purified RNA was eluted with 40 µL nuclease-free H2O and the concentration was measured using the Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific). Quality was assessed by determining A260/280 and A260/230 ratios. Samples were stored at -80ºC until use.

13.6. Reverse transcription and real-time qPCR

For gene expression analysis, 500 ng of total RNA were retrotranscribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, ref. 4368814) according to manufacturer's instructions. cDNA synthesis was performed in a total volume of 20 µL setting the thermocycler to the following program: 25ºC for 10 min, 37ºC for 120 min and a final step at 85ºC for 5 min. The resulting cDNA was previously diluted 1/10 and analysed by qPCR using the primer-probe sets listed in Table 8 (PrimeTime qPCR Assays, Integrated DNA Technologies (IDT), Inc.) The qPCR reaction was performed on 96-well plates in a final volume of 12 µL using the Premix Ex Taq Probe based qPCR assay (Takara Biotechnology, ref. RR390A). All reactions were run in duplicate on a StepOnePlus Real-Time PCR System (Applied Biosystems) set to the following cycling program: 1 cycle 95ºC for 30 sec; 40 cycles 95ºC for 5 sec; 60ºC for 20 sec. To provide negative control and exclude contamination the PrimeScript RT enzyme was omitted in the cDNA synthesis step and samples were subjected to the PCR reaction in the same manner as each probe.

Relative enrichment was calculated using the ΔΔCt method, with Actinß (mouse) expression serving as a housekeeping gene.

Table 8. Probes used for gene expression assays. For each gene, information about the reference assay and source is shown.

GENE	ASSAY	SOURCE
Actin	Mm.PT.39a.22214843.g	Integrated DNA Technologies
Adra1d	Mm.PT.58.28987824	Integrated DNA Technologies
Arc	Mm.PT.56a.16160059	Integrated DNA Technologies
Dlgap2	Mm.PT.58.6919970	Integrated DNA Technologies
Egr1	Mm.PT.58.29064929	Integrated DNA Technologies
Icam5	Mm.PT.58.11718142	Integrated DNA Technologies
Sik1	Mm.PT.58.16880137	Integrated DNA Technologies

14. STATISTICAL ANALYSIS

All results were analysed using GraphPad Prism software version 8.0. Data were represented as mean ± standard error of the mean (SEM). Outliers were identified with the ROUT method. Data distribution was analysed by applying the Shapiro-Wilk normality test. Statistical analysis was performed using Student's t-test for comparison between two groups presenting normal distribution. For multi-component variables comparison, Kruskal-Wallis (for non-parametric data), One-way ANOVA followed by the post-hoc Dunn's test or Tukey's test (parametric data) or Two-way ANOVA were applied. A 95 % confidence interval was used and a p-value <0.05 was considered significant. The number of samples (n) and *p* values are specified in each figure legend.

RESULTS

1. BEHAVIOURAL, CELLULAR AND MOLECULAR ALTERATIONS ASSOCIATED WITH STRESS PROGRESSION

1.1. Characterization of the effects provoked by short- and long-term stress on mice behaviour

To find which are the predominant changes that transform a stressful situation from adaptative to pathologic is mandatory to understand the basis of stress-induced major depression. Many studies have focused on understanding acute stress's impact on mice behaviour (Chaoui et al., 2022; Musazzi et al., 2017; von Ziegler et al., 2022). On the other hand, many other groups have studied the impact of chronic stress on mice (Alqurashi et al., 2022; Planchez et al., 2019). However, it remains unclear the initial changes occurring during the first days of chronic stress and if these initial changes could be important for the final phenotype. In this context, the aim of this study was to determine the effects of stress duration on mice's behaviour.

To assess whether the stress duration influences the severity of the stress-induced sequelae, we divided our mice into 3 groups: control non-stressed (CNT), short-term stress mice (STS), and long-term stress mice (LTS). STS mice received only two days of stress whereas LTS mice underwent the chronic unpredictable mild stress protocol (CUMS) for 28 days. After the last day of stress, all groups were submitted to a battery of behavioural tasks (Figure 25).

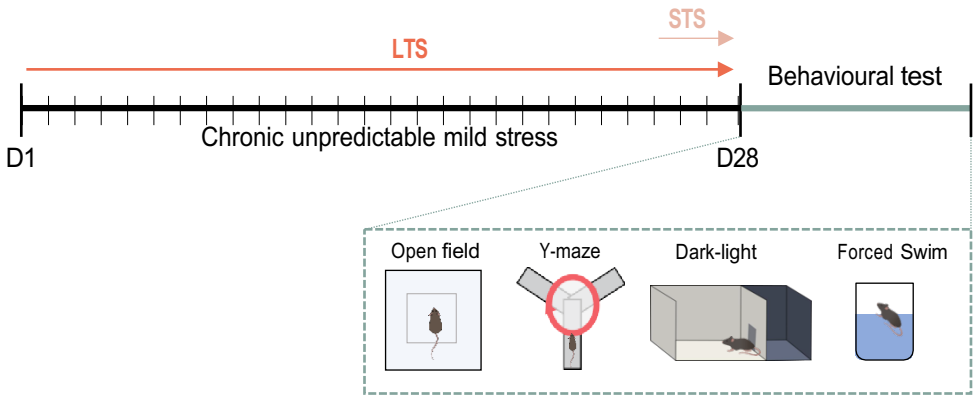


Figure 25. Schematic representation of the experimental design used. Mice received no stress (CNT), two days of stress (STS), or the entire CUMS protocol for 28 days (LTS). 24 hours after the last stressors, mice were submitted to a battery of behavioural tasks, including the open field, the Y-maze, the dark-light test, and the forced swim test. D: Day.

1.1.1 Chronic stress alters body weight, locomotion, anxiety and spatial working memory

One of the clearest changes that occur in stressed animals and humans are the changes in body weight (Paul Willner, 2017). After the last day of the stress protocol, mice were weighed, and we found that LTS, but not STS, mice present decreased body weight (Figure 26A) when compared with CNT mice. This goes in line with other studies that suggest that CUMS protocol induces a decrease in body weight (Strekalova et al., 2011). We then wanted to evaluate spontaneous locomotor activity in the open field test. Only LTS mice presented increased locomotor activity when compared with CNT mice (Figure 26B). This could suggest a hyperactive state and/or agitation of the mice after chronic stress. In line with this, LTS mice presented a rise in the number of supported rears, which is also associated with distance/activity (Figure 26C). We then investigated the navigation strategy using the parallel index (1.0 means walking straight). LTS mice presented a rise in the parallel index when compared with the other groups, revealing an altered locomotor navigation strategy (Figure 26D). We then measured anxiety levels by analysing the time mice spend in the centre of the open field arena. As expected, LTS mice spent less time in the centre when compared with CNT and STS (Figure 26E and Figure 26G-I), suggesting that chronic stress induces a depressive-like phenotype that is accompanied by increased anxiety levels. Last, we assessed spatial working memory by evaluating the number of alternations performed in a translucid Y-maze (Figure 26F). We first observed that all groups performed a number of alternations above the change levels (50%, dashed line). Then, we use one-way ANOVA to analyse the % of alternation between groups. As described by other (Alqurashi et al., 2022), we found a tendency to decrease number of alternations in the LTS, suggesting alterations in spatial working memory.

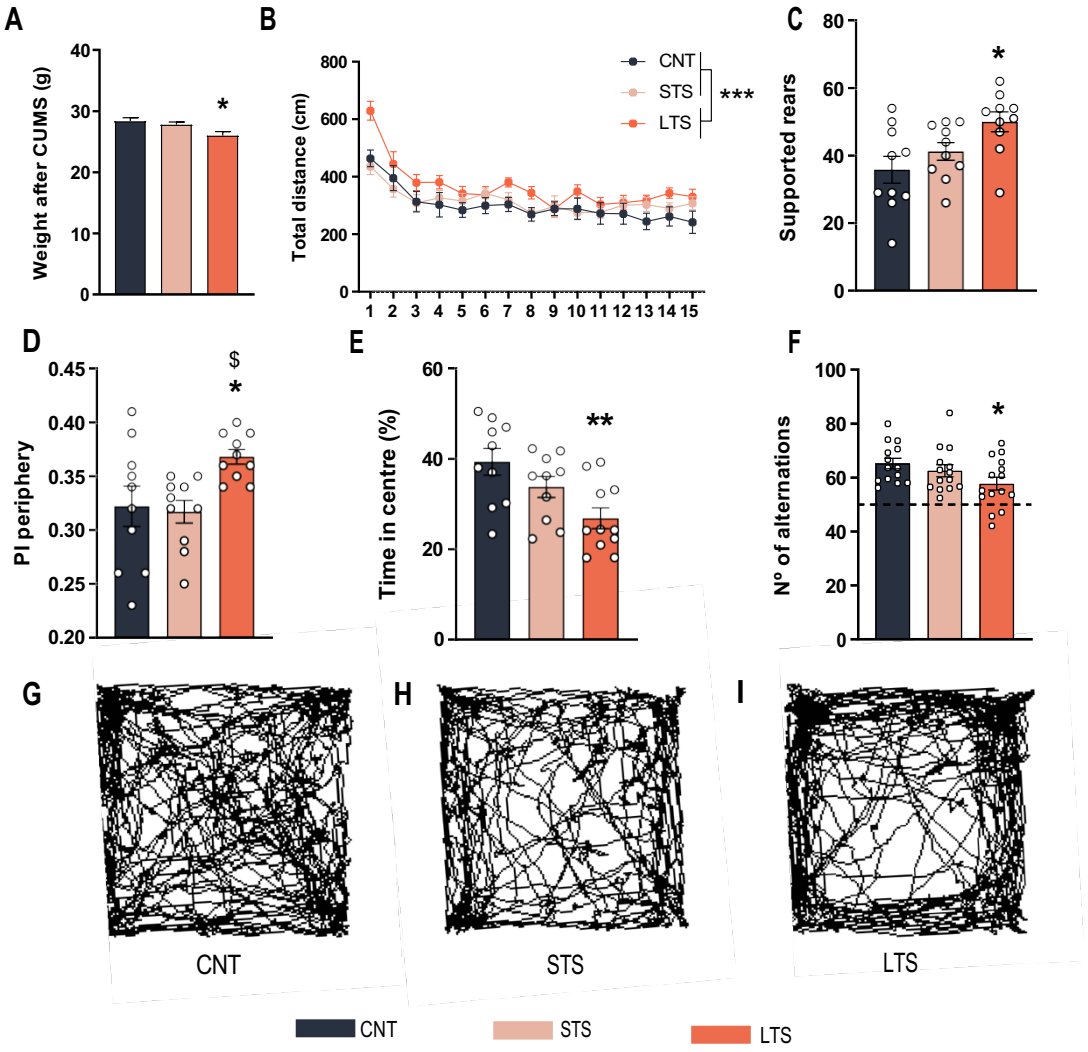


Figure 26. Effect of short- and long-term stress in mice weight, in the open field and in the Y-maze. (**A**) Weight after the stress protocol. One-way ANOVA, F(2,14)=4.659, P=0.0281. In the open field, total distance travelled for 15 minutes (**B**), supported rears (**C**), parallel index (**D**) and time in the centre (**E**) were analysed. In B, two-way ANOVA, time effect F(14, 420)=13.00, P<0.0001, group effect F(2, 420)= 2.75, P<0.0001. In C, one-way ANOVA F(2, 27)=4.966, P=0.0146. In D, one-way ANOVA F (2, 27) = 4,666, P=0,0182. In E, one way-ANOVA F(2, 28)=6.209, P=0.0059. (**F**) In the Y-maze, the number of alternations was calculated. One-way ANOVA, F(2,40)=2.987, P=0.0618. T-test between CNT and LTS, t-test=0,0231. From **G-I**, representative traces of mouse locomotor activity in the open field. Black lines represent mouse path. Tukey's test as a post hoc analysis was used. All values are mean ± SEM. N=10-11 mice per group**.** *P<0.05, **P<0.01 and ***P<0.001 compared to CNT mice. $P<0.05 compared to STS.

1.1.2 Chronic and short-term stress affect behavioural despair

We next aimed to investigate the effects of stress on behavioural despair, a feature that has been extensively linked to the depressive-like phenotype (M. Becker et al., 2021). To do so, the forced swim test was used. In this test, swimming and floating duration were measured, with the higher floating duration being associated with a depressive-like phenotype. Results showed that both STS and LTS mice present decreased swimming levels and increase floating

levels when compared with CNT mice (Figure 27A and B). These results point out that both stress paradigms (STS and LTS) are sufficient to induce behavioural despair in mice. Last, we used the dark-light box test to evaluate anxiety-like behaviour. The latency to cross to the light compartment was analysed (Figure 27C). Although no significant differences were found, a tendency to increase the time to cross was found in LTS when compared with the other two groups. Finally, a Z-score was performed to summarize the global effect of stress duration in all the behavioural tasks mentioned. This graph allowed us to integrate parameters obtained in multiple (but complementary) behavioural test, as previously described (Guilloux et al., 2011). As observed in Figure 27D, there was a progressive increase in the Z-score concomitant with stress duration.

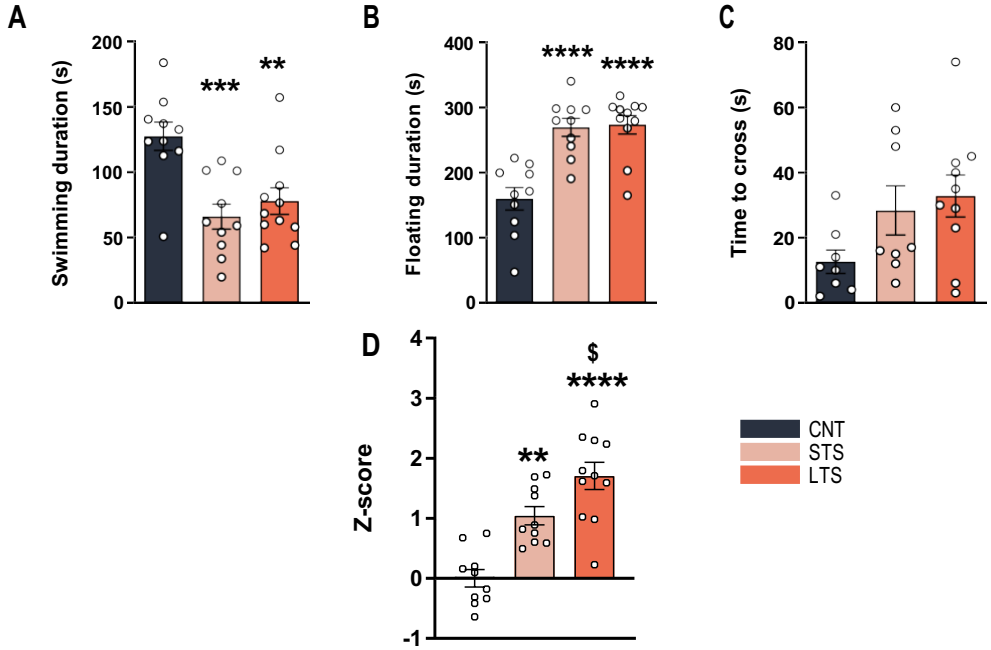


Figure 27. Effect of short- and long-term stress in the forced swim and dark-light test. In the forced swimming test, the swimming duration (**A**) and the floating duration (**B**) were recorded. Swimming duration, one-way ANOVA, $F(2, 28)=9.967$, $P=0.0005$. Floating duration, one-way ANOVA $F(2, 28)=17.89$, $P<0.0001$. In the dark-light test (**C**), time to cross to the light area was analysed. One-way ANOVA $F(2,23)=2.879$, $P=0.0766$. (**D**) Integrated z-score. One-way ANOVA $F(2,28)=22.30$, $P<0.0001$. Tukey's test as a post hoc analysis was used. All values are mean ± SEM. N=10-11 mice per group. **P<0.01 and ***P<0.001 compared to CNT. $P<0.05 compared to STS.

In summary, these results indicate that LTS induces a more severe depressive-like symptoms than STS as demonstrated in different behavioural tasks.

1.2. Identification of subpopulations playing a role in stress-induced major depression

The specific systems and/or cells involved in stress-induced MDD are poorly understood. Thus, identifying specific neural subtypes playing crucial roles in the underlying processes is mandatory to understand the pathophysiology of MDD and to design more effective therapeutic approaches. In this line, the expression of Immediate Early Genes (IEGs), such as Egr1, are upregulated in neuronal subpopulations when a stimulus is present. Changes in the expression of Egr1 can be a readout of how the brain encodes external situations and in which brain regions this information is stored.

1.2.1. Egr1-dependent neural activation in hippocampal CA1 depends on the chronicity of the stress

It is widely accepted that several brain regions such as the amygdala, cingulate cortex, septum and hippocampus among others play a role in chronic stress (Fee et al., 2020; Laine et al., 2017; Musazzi et al., 2017). Here, we mapped the forebrain by counting the density of GFP-positive cells in brain regions highly affected by chronic stress. To identify which neural cells were activated/deactivated in short-term vs long-term stress conditions, we used the Egr1-CreER^{T2}xR26RCE double transgenic mice (Figure 28A and Figure 16). Thus, we could permanently label Egr1-dependent activated cells in a time-dependent manner (only when tamoxifen was administered). We then subjected Egr1-CreER^{T2}xR26RCE double transgenic mice to the CUMS protocol. Mice were separated into three groups: control non-stressed (CNT group), short-term stressed (2 days, STS group), and long-term stressed (28 days, LTS group). Each group received one injection of 4-HT 30 minutes before the presentation of a stressful stimulus during the last two consecutive days of the CUMS protocol (Figure 28B). After one week of the last stressor, mice were sacrificed, and the brain was obtained. Serial coronal tissue sections were cut and stained for GFP-positive cell counting.

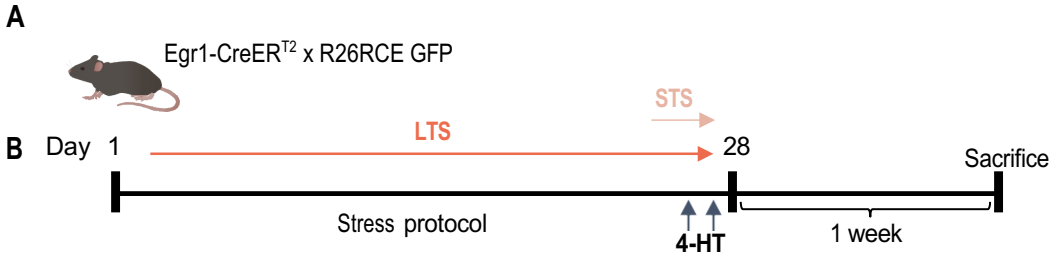
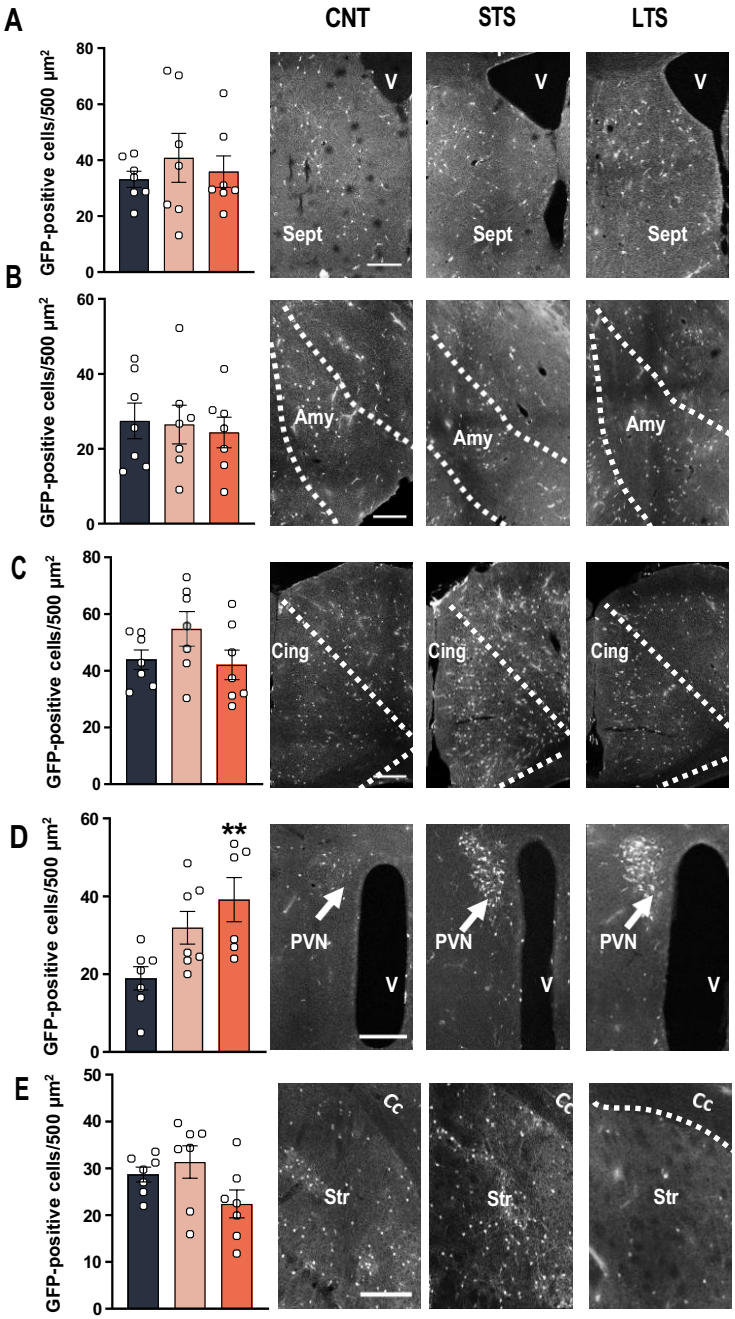


Figure 28. Schematic representation of the experimental design used. (**A**) Scheme of the double heterozygous mutant Egr1-CreER^{T2} x R26RCE GFP mice. (**B**) Egr1-CreER^{T2} x R26RCE mice were subjected to 0 (CNT), 2 (STS) or 28 (LTS) days of CUMS. In the last two days of the protocol, all mice received 50 mg/kg of 4-hydroxytamoxifen (4-HT, i.p) and one week later they were sacrificed.

First, we observed that the density of GFP-positive cells in the septum (Figure 29A), the amygdala (Figure 29B) and the cingulate cortex (Figure 29C) was similar between groups. The LTS group showed an increase of GFP-positive cells in the paraventricular nucleus compared to the CNT and STS groups (Figure 29D). In the striatum, we could not detect significant differences between any condition (Figure 29E). In the hippocampal CA3 region (Figure 29F), GFP-positive cells were reduced in the LTS group compared with the CNT group.



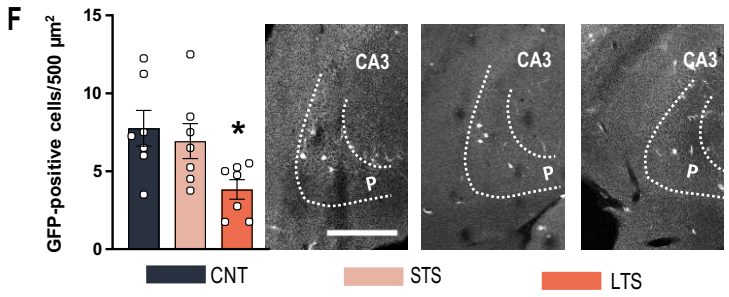


Figure 29. Identification of activated cells in different brain regions. Representative images and quantification of Egr1-dependent activated cells (Estimated number of GFP-positive cells/area of 500 µm2) per region in: (**A**) The septum, one-way ANOVA, group effect: F(2, 18) = 0.3858, P=0.6854; (**B**)The amygdala, group effect: F(2, 18) = 0.1160, P=0.8911; (**C**) The cingulate cortex, group effect, F(2,18) = 1.946, P = 0.1718; (**D**) The paraventricular nucleus (PVN), group effect, F(2, 17) = 5.686, P=0.0129; The striatum (**E**), group effect, F(2, 18)= 2.716, P=0.0931; The CA3 (**F**) group effect: F(2, 18) = 4.322, P=0.0293. Dunnett's as a post hoc analysis was used. All values are mean ± SEM. N = 7 mice per condition. *P<0.05, **P<0.005 compared with CNT. V, ventricle; CC, *corpus callosum*; P, *stratum pyrdamidale*. Scale bar: 150 µm.

1.2.2. The CA1 is highly sensitive to stress duration

In the CA1 we observed interesting bidirectional changes in the number of GFP-positive cells depending on the chronicity of the stress protocol (Figure 30A). Concretely, GFP-positive cells increased in the STS group but decreased in the LTS group compared to the CNT group. From the latter result, we hypothesize that the CA1 could be highly sensitive to the chronicity of stress. Finally, we performed immunofluorescence to validate that GFP-positive cells also expressed Egr1 (Figure 30B).

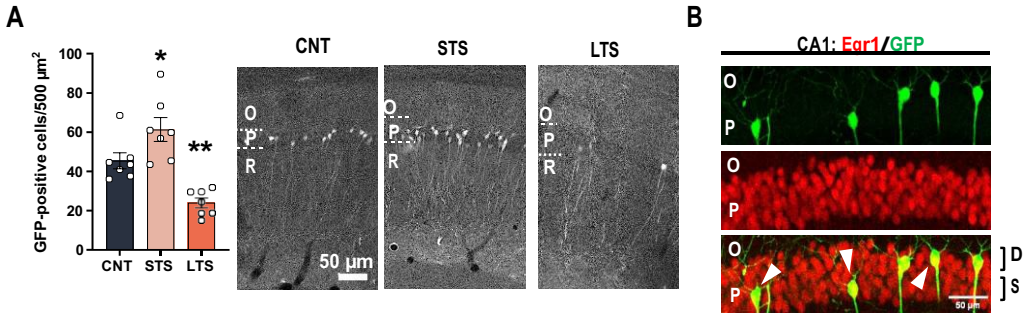


Figure 30. Identification of activated cells in the CA1 of the hippocampus. (**A**) Representative images and quantification of Egr1-dependent activated cells (Estimated number of GFP-positive cells/area of 500 µm2) in the CA1. One-way ANOVA, group effect: F(2, 18) = 17.63, P<0.0001. Dunnett's as a post hoc analysis was used. The values are expressed as the mean ± SEM. N = 7 mice per condition. *P<0.05, **P<0.005 compared with CNT. (**B**) Representative images of the CA1 of the hippocampus showing GFP- activated cells (green) and Egr1-positive cells (red). White arrows indicate double Egr1-positive and GFP-positive cells; O: *Stratum oriens*; P: *Stratum pyramidale;* R: *Stratum radiatum*.

1.2.3. Specific spine density alterations in Egr1-positive activated cells

Since we observed that stress induces opposite changes in the activation of Egr1-dependent neural ensembles in the CA1 depending on its duration, we hypothesized that such changes could be accompanied by alterations in synapse numbers specifically in these cells. To explore this, we used Egr1-CreER^{T2} mice (Figure 31A) injected in the dorsal CA1 with AAV-CaMKII-FLEX-EGFP to allow the quantification of dendritic spines specifically in the Egr1-positive ensembles (Figure 31B). Again, we used a control group (CNT), a group of mice subjected to stress for 2 days (STS), and a group of mice subjected to stress for 28 days (LTS) (Figure 31C).

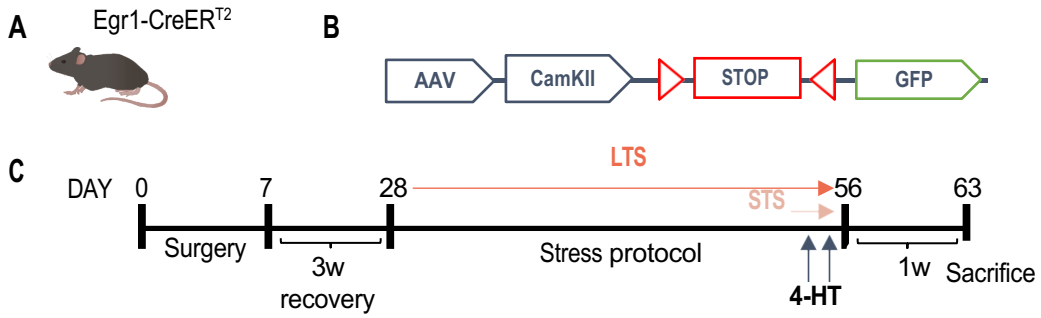


Figure 31: Schematic representation of the experimental design used. (**A**) Scheme of the heterozygous mutant mice named Egr1-CreER^{T2} mouse. (**B**) AAV used in which GFP is expressed under the control of a STOP floxed codon. (**C**) Egr1-CreER^{T2} mice received surgery and after recovery were subjected to 0 (CNT), 2 (STS) or 28 (LTS) days of stress. The last two days of the protocol, all mice received 50 mg/kg of 4-hydroxytamoxifen (4-HT, i.p) and one week later they were sacrificed. W: week.

Both, apical and basal dendrites from CA1 pyramidal neurons were analysed in the three groups (Figure 32A). We observed that spine density in apical dendrites was significantly decreased in the LTS group whereas it was unchanged in the STS mice (Figure 32B and C). In contrast, we found a significant increase in spine density in the basal dendrites only in the STS whereas no changes were observed in these dendrites in the LTS mice (Figure 32D and E). Taken together, the results highlight that short vs long-term stress result in opposite changes in spine density from activated pyramidal neurons of the CA1.

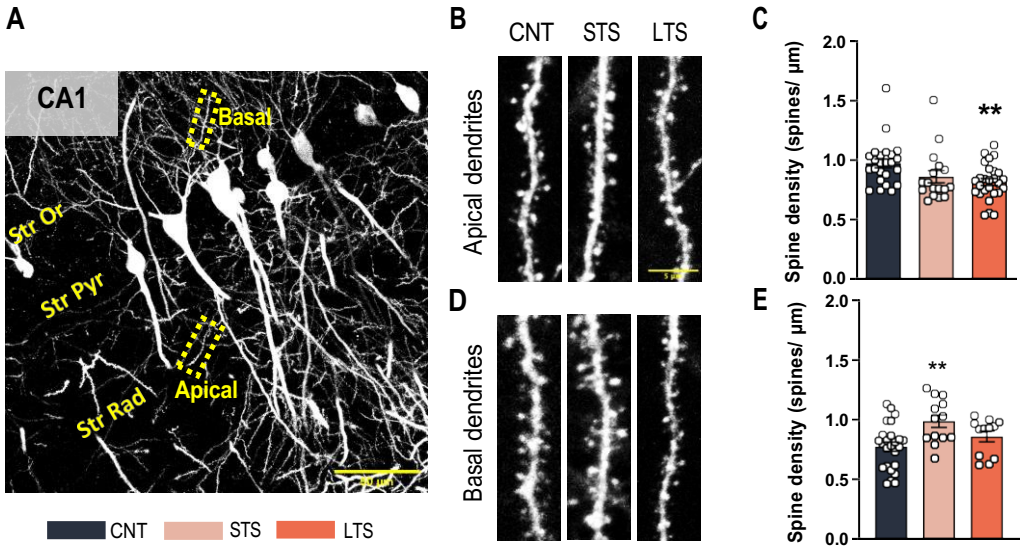


Figure 32. CUMS specifically alters dendritic spine density in Egr1-activated pyramidal neurons. (**A**) Representative image showing labelled neurons of the CA1 region of one STS mouse. (**B**) Representative apical dendrites of CNT, STS and LTS mice. (**C**) Effect of stress on apical spine density. One-way ANOVA, group effect: $F(2, 47) = 5.86$, $P = 0.005$. (**D**) Representative apical dendrites of CNT, STS and LTS mice. (**E**) Effect of stress on basal spine density. One-way ANOVA, group effect: $F(2,47)=5.857$, $P=0.0054$. Tukey's test as a post hoc analysis was used. Values are mean ± SEM. N = 17-27 dendrites from 7 different mice per group were quantified. **P<0.005 compared with CNT group. Str Or: *Stratum oriens*; Str Pyr: *Stratum pyramidale*; Str Rad: *Stratum Radiatum*.

1.3. Different hippocampal subpopulations are activated depending on stress duration

1.3.1. Changes in the percentage of Calb1-positive/GFP-positive cells over stress duration

We observed that the CA1 displayed the highest contrast in neural activation depending on the duration of the stress protocol as indicated by Egr1-activity induction. Such activation was accompanied by changes in dendritic spine densities. This led us to hypothesize that a possible progressive cellular reorganization (in terms of Egr1-dependent activation) could take place in the CA1 during the progression of chronic stress. To characterize such potential cellular reorganization, we immunostained CA1 of Egr1-CreER^{T2}xR26RCE mice in the three conditions (CNT, STS and LTS) for different markers including GFAP, a marker of astrocytes, PV, a marker of parvalbumin interneurons and MAP2, a general marker for CA1 pyramidal cells. We first observed that neither GFAP (Figure 33A and Figure 34A) nor PV (Figure 33C and Figure 34B) colocalized with GFP (Egr1-dependent activated neural cells) in any group. In contrast, almost all the GFP-positive cells colocalized with MAP2-positive cells in the three groups (Figure 33B and Figure 34C). Therefore, we concluded that Egr1-

positive activated cells were pyramidal neurons of the CA1. We then immunostained hippocampal sections of the 3 groups of mice for Calb1 which identifies the superficial pyramidal neurons of CA1, whereas pyramidal neurons negative for Calb1 are considered to be deep pyramidal cells (Cembrowski, Bachman, et al., 2016; Valero & de la Prida, 2018).

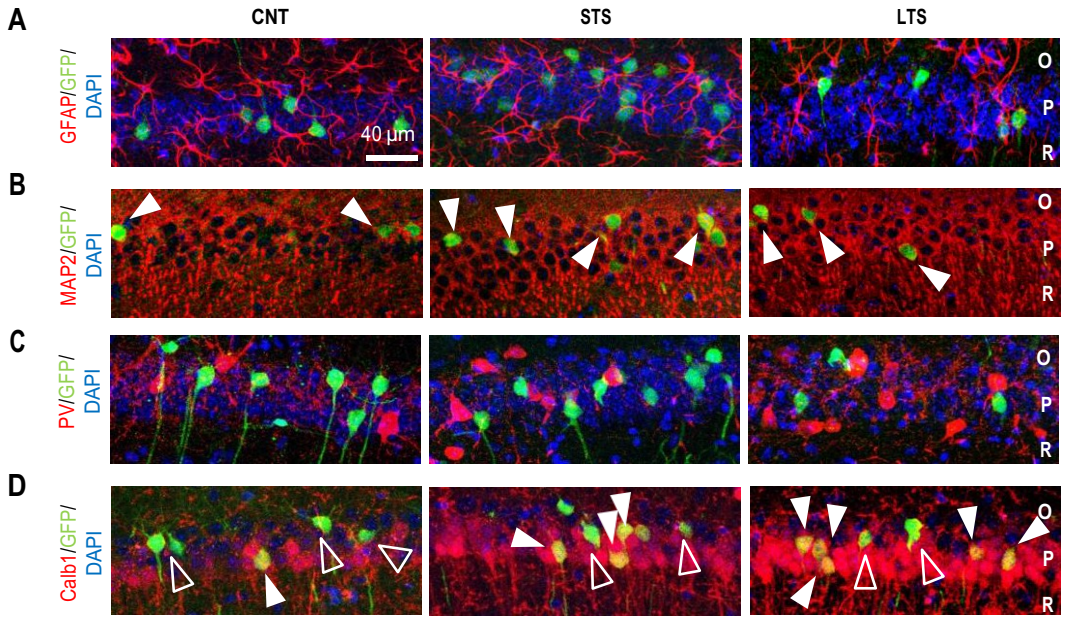


Figure 33. Characterization of the Egr1-dependent activated cells by short or long-term stress in the CA1. Representative images showing the identity of the activated cells (green) by co-labelling with specific cell markers (red). In all rows, GFP-positive cells are in green. (**A**) Representative images from all conditions (CNT, STS and LTS) showing labelling of astrocytes (red). (**B**) Labelling of MAP2 (red). White arrows point to GFP- and MAP2-double positive neurons. (**C**) Labelling of parvalbumin (PV, red). (**D**) Labelling of calbindin1 (Calb1, red). White arrows point to double-positive GFP- and calbindin double-positive neurons. Open arrows point to GFP-positive calbindin-negative cells. O, *Stratum oriens;* R, *Stratum radiatum*; P,*Stratum pyramidale*

Remarkably, our quantifications revealed that the percentage of GFP-positive CA1 pyramidal cells co-stained with Calb1 was different in the three groups (Figure 33D and Figure 34D). The percentage of double-stained Calb1-positive/GFP-positive cells was low in the CNT group, significantly increased in the STS and reached its highest level in the LTS group (Figure 34D). However, these percentages did not reflect the changes in the total number of GFP-positive cells depending on the duration of the CUMS protocol (Figure 30A). Therefore, we also analysed the total number of Calb1-positive/GFP-positive neurons, corresponding to activated superficial pyramidal neurons. This number was increased in the STS group whereas in the LTS group, the number of Calb1-positive/GFP-double-positive neurons was similar to the CNT group (Figure 34E). In contrast, the density of Calb1-negative/GFP-positive neurons (activated deep pyramidal neurons) were unchanged in the

STS group compared to the CNT group but significantly decreased in the LTS group (Figure 34F).

All these results taken together indicate that, although there is a progressive reduction of CA1 GFP-positive pyramidal neurons at the end of the CUMS protocol (Figure 30A), there is a transient and concomitant increase in the percentage of Egr1-dependent activated neurons which are in turn Calb1-positive neurons (superficial pyramidal neurons). In contrast, Calb1-negative neurons (deep pyramidal neurons) are severely and progressively deactivated (in terms of Egr1-dependent activation) at the end of the CUMS protocol.

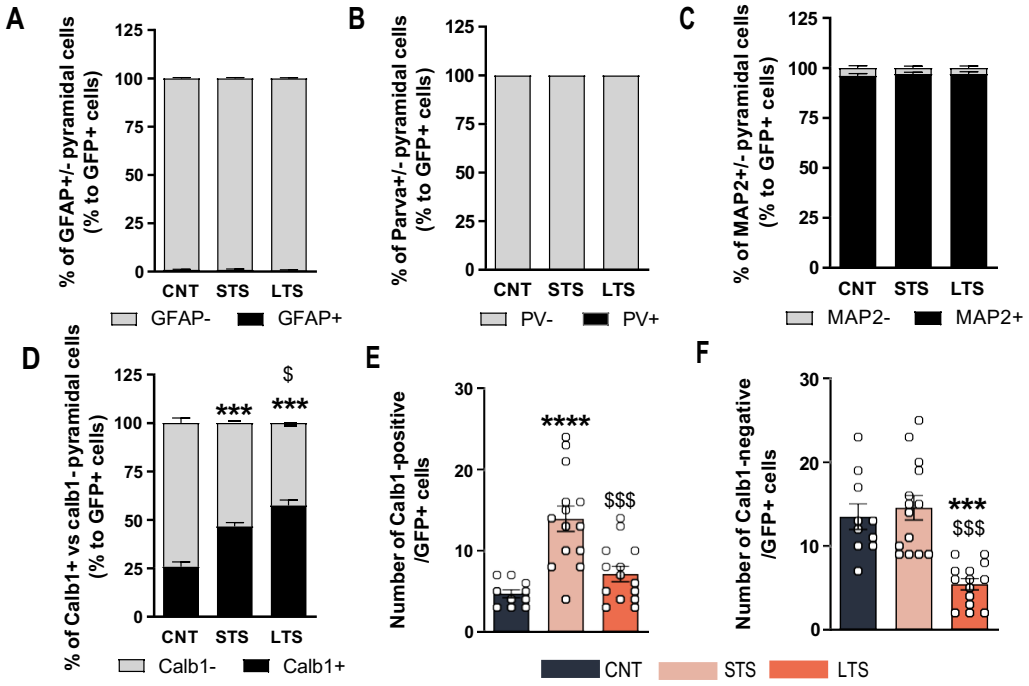


Figure 34. Quantification of the Egr1-dependent activated cells by short or long-term stress in the CA1. (**A**) Quantification of the percentages of double-positive cells in CA1 for GFP and GFAP. (**B**) Quantification of the percentages of double-positive cells in CA1 for GFP and PV. (**C**) Quantification of the percentages of double-positive cells in CA1 for GFP and MAP2. (**D**) Quantification of the percentages of double-positive cells in CA1 for GFP and Calb1. One- way ANOVA, group effect, $F(2,18) = 42.31$, $P<0.0001$. (E) Total number of GFP-positive cells per surface unit that are also calbindin1-positive or calbindin-negative (F) In E, group effect: $F(2,35)=15.91$, $P<0.001$. In F, group effect: $F(2,35)=17.40$, $P<0.0001$. Tukey's test as a post hoc analysis was used. Values are mean ± SEM. N = 7 mice group. ***$P<0.001$ compared to CNT and $\$<0.05$, $\$\$\$P<0.001$ compared to STS mice.

1.3.2. Calb1 total levels are unaltered after stress

To confirm that these alterations are not due to global changes in total Calb1 levels, we measured hippocampal Calb1 levels using Western Blot (Figure 35A) and immunofluorescence (Figure 35B). Our quantifications confirmed that total Calb1 levels are

unaltered by stress. Thus, the results obtained are specific to hippocampal subpopulations rather than a general effect.

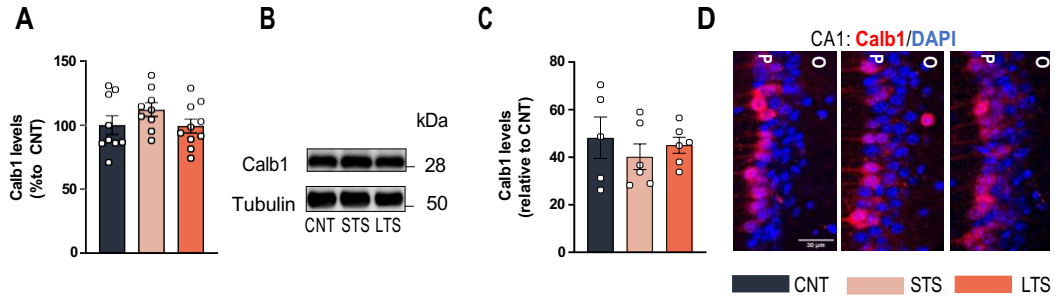


Figure 35. Analysis of total Calb1 levels in mice hippocampus after stress. (**A**) Densitometry quantification and representative immunoblots of Calbindin levels in the hippocampal lysates of CNT, STS or LTS mice. N=9-10 mice per group. (**B**) Quantification of Calbindin intensity in the CA1 and representative images of CNT, STS and LTS mice N= 5-6 mice per group. All values are mean ± SEM. O: *Stratum oriens*; R: *Stratum radiatum;* P: *Stratum pyramidale.* Molecular weight markers positions are indicated in kDa.

1.4. Chemogenetic activation of dorsal CA1 deep pyramidal cells does not prevent sequelae induced by CUMS

1.4.1. Verification of the chemogenetic activation of deep neurons in mouse hippocampus

We observed that chronic stress (LTS) induced a reduction in the number of Egr-1-dependent activated deep pyramidal cells in the CA1 (Figure 34F). To test whether such Egr1-dependent deactivation was relevant for the sequelae induced by chronic stress we aimed to activate them by using Designer Receptors Exclusively Activated by Designer Drugs technology (DREADDs). To selectively express hM3Dq DREADDs in deep pyramidal neurons we used the Chrna7-Cre mice (Figure 36A), which selectively express the Cre-recombinase mainly in deep pyramidal neurons. Chrna7-mice were injected in the dorsal hippocampus with an AAV vector construct containing a double-floxed inverted open-reading frame (DIO) sequence encoding hM3Dq-mCherry (Figure 36B). With such design, only those Chrna7-positive neurons (deep pyramidal neurons) expressing the Cre-recombinase would be able to recombine and express the DREADD in the correct orientation and only when CNO is given to the animal (in drinking water), those cells would become activated. We then subjected the mice to CUMS and divided them into 4 different groups: non-stressed treated with vehicle (CNT VEH group), non-stressed treated with CNO (CNT CNO), long-term (28 days) stressed treated with VEH (LTS VEH) and long-term stressed (28 days) treated with CNO (LTS CNO) (Figure 36C).

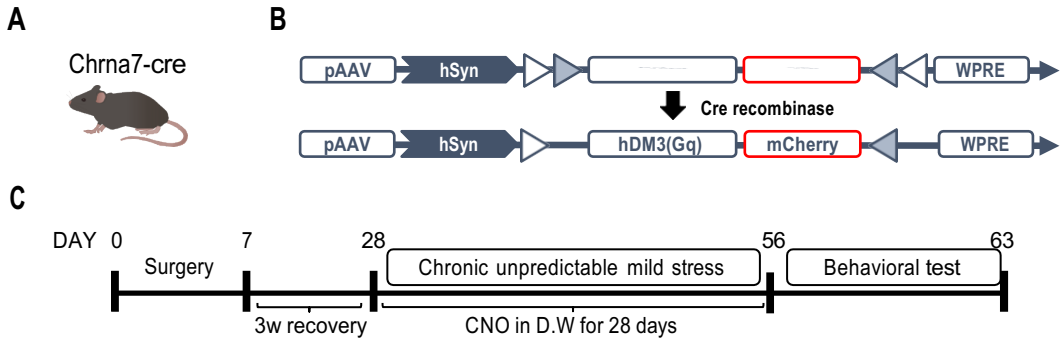


Figure 36. Schematic representation of the experimental design used. (**A**) Schematic illustration of Chrna7-Cre mouse model. (**B**) Scheme of the AAV used to express the activator DREADD (hDM3(Gq)) after Cre recombination. (**C**) Timeline of the experiment. Mice were injected with the vector shown in B and after recovery, they were subjected to the CUMS protocol. During the stress protocol, mice received either CNO (1 mg/kg) or vehicle (VEH) solution in drinking water (D. W.). Twenty-four hours after the last stressor, mice were subjected to a battery of behavioural tests.

In parallel, we used a small subgroup of mice treated with CNO in drinking water for 2 days but without behavioural manipulation to avoid unspecific changes due to extensive experimentation. In these mice, immunofluorescence staining confirmed that only deep pyramidal cells were transduced and that CNO in drinking water induced the activation of Chrna7-positive neurons (cFos+ cells) (Figure 37A-C). We then analysed the % of cFos+ cells out of those transduced and clearly observed that the treatment with CNO induced activation of those transduced neurons (Figure 37B).

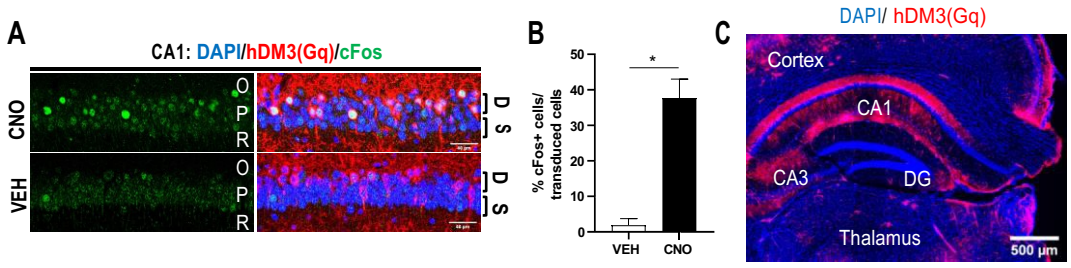


Figure 37. Validation of the DREADDs expression and function. (**A**) Representative image of CA1 showing cFos-positive cells (green) and viral transduction (mCherry, red) and cell nuclei (blue, DAPI). (**B**) Percentage of cFos+ cells (green) co-labelled with mCherry (red) in mice treated with VEH or CNO (unpaired t-test, t= 2.83, P = 0.01). (**C**) A representative global image of viral transduction specifically in the hippocampal CA1. O: *Stratum oriens;* R: *Stratum radiatum;* P: *Stratum pyramidale*; D: Deep neurons; S: Superficial neurons.

1.4.2. Chemogenetic activation of deep pyramidal neurons has no effect on mice behaviour

For the CUMS procedure we only used mice with the correct allocation of the viral transduction in the CA1 (Figure 37C). Interestingly, we first observed that both groups of

LTS mice presented increased locomotor activity in the open Field test (Figure 38A). Next, to assess cognitive sequelae after CUMS, we performed the novel object location test which is a sensitive task to detect cognitive impairment upon CUMS (Y. Xu et al., 2015). In this test, only CNT VEH and CNT CNO groups were capable to discriminate between the new and the old location of the object (Figure 38B). The ability to discriminate was lost in both LTS groups. Finally, we analysed the time struggling in the forced swim test. Both groups of LTS mice spent less time struggling specifically during the first minutes of the test compared to both groups of CNT mice (Figure 38C-D).

Altogether, our results indicate that the activation of Chrna7-positive (deep) neurons during the CUMS protocol has no effect on the CUMS-induced sequelae.

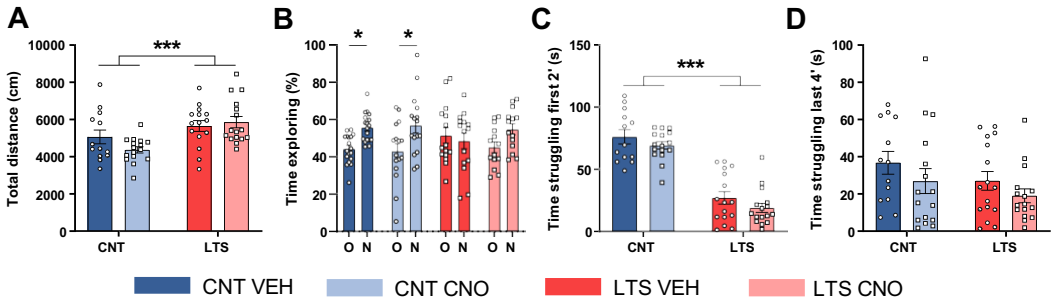


Figure 38. Effects of chemogenetic activation of CA1 deep pyramidal neurons during CUMS. (**A**) In the open field, locomotor activity was monitored for 15 minutes. Two-way ANOVA, stress effect: $F(1, 56) = 13.23$, $P=0.0006$. (**B**) In the novel object location test, spatial memory was evaluated 24 h after a training trial as the percentage of total time spent exploring either the object placed at a new location (N) or the object placed at the old location (O). Two-way ANOVA, new location effect, $F(1, 122) = 8.64$, $P=0.0039$, N = 13-17 mice per condition. (**C-D**) In the forced swimming test, the immobility time was evaluated during the first 2 minutes and the last 4 min of the 6 min trial in all groups. In the first 2 minutes, two-way ANOVA, group effect, $F(1, 57) = 128.6$, $P<0.0001$. Tukey's test as a post hoc analysis was used in A, C and D; Bonferroni's test as a post hoc analysis was used in B. All values are mean ± SEM. *P<0.05 and ***P<0.001.

1.4.3. Possible off-target effects of CNO

Several studies point that CNO can be metabolized to clozapine and exert some effects on mice behaviour (Gomez et al., 2017; Mahler & Aston-Jones, 2018). To exclude the possibility of unspecific or off-target effects of CNO *per se*, we repeated the same experimental design as in Figure 36C but without surgery. Hence, control mice received normal housing or the CUMS protocol while they were treated with VEH or CNO in drinking water. CNO did not produce any effect on the locomotion in the open field when comparing vehicle vs CNO conditions (Figure 39A). Similarly, we did not find any effect mediated by CNO either, in the novel object location task (Figure 39B) or in the forced swim test (Figure 39C-D). These

results ruled out unspecific off-target effects induced by CNO in the behavioural tasks performed.

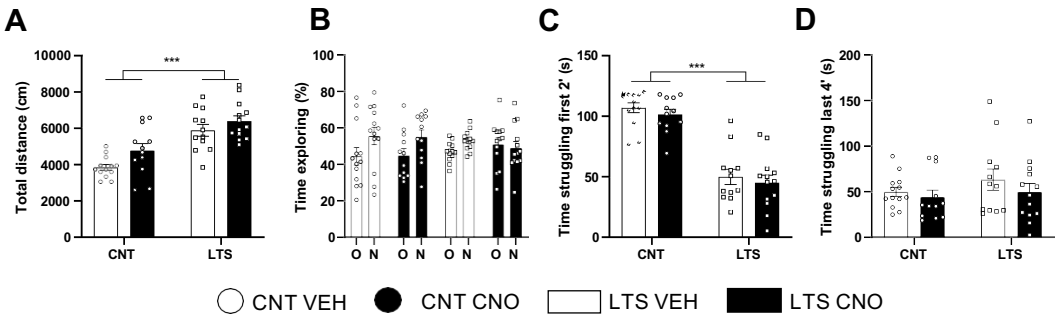


Figure 39. Effects of CNO administration in mice behaviour. An independent new cohort of WT mice underwent CUMS or not and was treated with vehicle or CNO to determine the undesired effects of CNO. We used the same experimental design as in Figure 36C but without surgery. (**A**) In the open field, locomotor activity was monitored for 15 minutes. Two-way ANOVA, stress effect: $F(1, 46) = 37.43$, $P<0.0001$. (**B**) Spatial memory was evaluated in the novel object location test. Two-way ANOVA, new location effect, $F(1,92)=6.1$, $P= 0.0152$. In the forced swim test, the immobility time was evaluated during the first 2 minutes (**C**) and the last 4 min (**D**) of the 6 min trial in all groups. In the first 2 minutes, two-way ANOVA, group effect, $F(1, 46) = 113$, $P<0.0001$. Tukey's test as a post hoc analysis was used in A, C and D; Bonferroni post hoc analysis was used in B. All values are mean ± SEM. N= 12-13 mice per condition. *P<0.05 and ***P<0.001.

1.5. Specific downregulation of Egr1 in CA1 superficial pyramidal cells during CUMS improves depressive sequelae

Trying to upregulate the activity of the deep pyramidal cells by using DREADDs was inefficient in preventing the sequelae induced by stress. Then, we decided to prevent the effects mediated by the early increase in Egr1-dependent activated cells in the superficial pyramidal cells (Figure 34E-D) by reducing their endogenous Egr1 levels. We hypothesized that suppressing the increase of Egr1 in superficial neurons found at the beginning of the stress response could prevent some alterations induced by CUMS.

1.5.1. Verification of Egr1 downregulation in the mouse hippocampus

To specifically modulate superficial neurons, we used the Calb1-cre mice (Figure 40A). We thus transduced only superficial CA1 pyramidal (Calb1-positive) with shRNA against Egr1 (Figure 40B) or with a GFP CNT virus. Mice were then divided into four different conditions: CNT mice injected with control AAV (AAV-CNT), CNT mice injected with an AAV-shRNA against Egr1 (AAV-shEGR1), chronically stressed mice injected with control AAV (AAV-CNT+CUMS) and chronically stressed mice injected with an AAV-shRNA against Egr1 (AAV-shEGR1+CUMS). After the CUMS protocol, mice were tested for

different behavioural tasks as illustrated in our experimental design (Figure 40C), and finally, they were sacrificed for viral transduction verification.

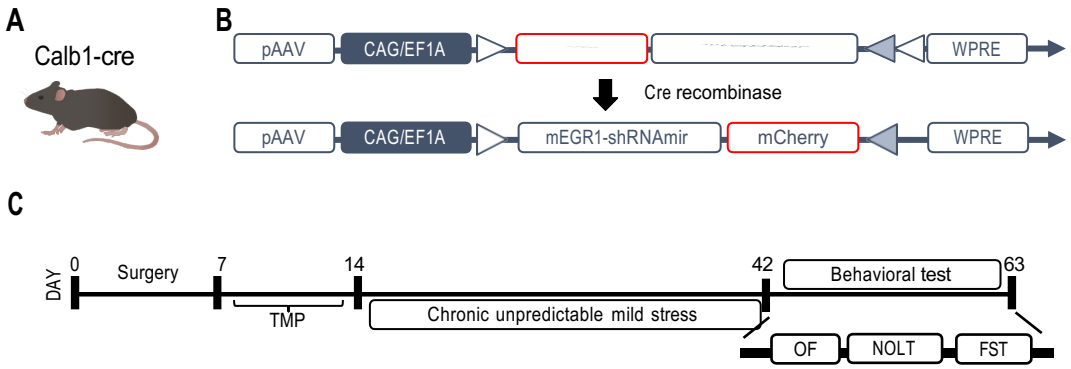


Figure 40. Schematic representation of the experimental design used. (**A**) Schematic representation of the calbindin-Cre mice. (**B**) Schematic diagram showing the AAV-mCherry-floxed-shEGR1 viral construct. (**C**) Timeline of the experiment. Mice were injected with the construct shown in B or with a control floxed construct and, after surgery, they received TMP to stabilize Cre expression. Three weeks after surgery, they were subjected to the CUMS protocol or stayed in their home cages.

We first showed widespread viral transduction in the CA1 superficial pyramidal neurons of the dorsal hippocampus (Figure 41A and C). Egr1 levels were specifically downregulated in those superficial pyramidal cells of the CA1 that were transduced with the SH-viral vector (Figure 41B).

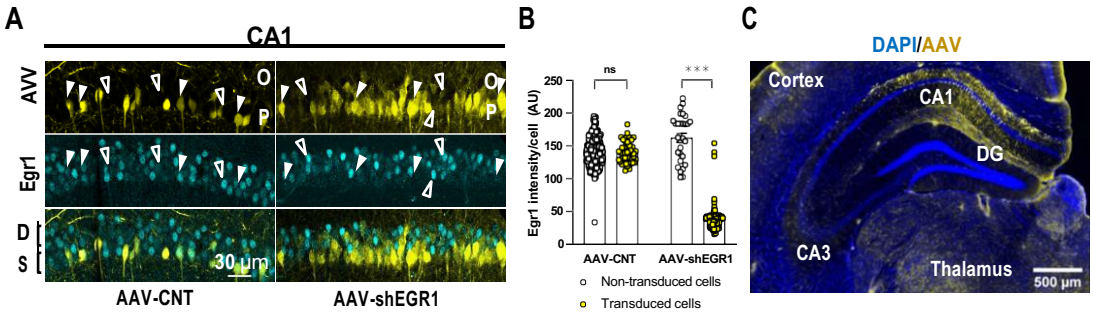


Figure 41. Validation of the Egr1 downregulation in the CA1 of the hippocampus. (**A**) Representative images in CA1 showing Egr1 levels (cyan) in mice injected with control floxed virus (AAV-CNT, left panel) or with the AAV containing the floxed shRNA against Egr1 (AAV-shEGR1). Viral expression is depicted in yellow. White arrows designate transduced neurons expressing the virus. Open arrows designate non-transduced neurons. (**B**) Quantification of Egr1 optical density (IOD) in non-transduced cells (white dots) and in transduced cells with the AAV (yellow dots) in each condition (AAV-CNT or AAV-shEGR1). T-test, $t = 56.77$, $P<0.0001$. (**C**) A representative global image of viral transduction specifically in the hippocampal CA1.D: Deep neurons; S: superficial neurons; DG: *Dentate gyrus*.

1.5.2. Downregulation of Egr1 during CUMS improves cognitive and emotional sequelae induced by stress

Once we proved that our system was working as expected, we submitted mice to a battery of behavioural tasks. In the open field test, both groups of mice subjected to the CUMS presented hyperlocomotion compared to the CNT groups (Figure 42A). We then used the NOLT to test spatial memory. AAV-CNT mice were able to distinguish between the old and the new object position. Conversely, AAV-shEGR1 mice could not differentiate the new location of the object. Interestingly, the deficits in spatial memory caused by CUMS (as observed in the AAV-CNT+CUMS mice) were rescued in the AAV-shEGR1+CUMS mice (Figure 42B). Finally, we subjected the mice to the forced swim test to evaluate behavioural despair. In the first two minutes of the test (Figure 42C), both groups of mice subjected to the CUMS significantly spent less time swimming compared to non-stressed mice. This was maintained for the last four minutes of the task (Figure 42D). Interestingly, in the last four minutes of the test, we observed that those stressed mice injected with SH- against Egr1 swam significantly more than those stressed mice injected with the control AAV (Figure 42D). In summary, inhibition of Egr1 expression in Calb1-positive (superficial) neurons corrected different sequelae caused by CUMS.

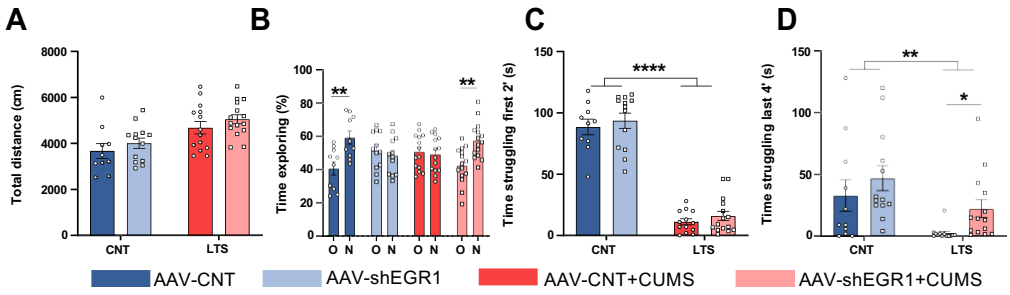


Figure 42. Effects of Egr1 shRNA delivery in CA1 superficial pyramidal neurons during CUMS. In the open field, locomotor activity (**A**), was monitored for 15 minutes. Locomotor activity, two-way ANOVA, Group effect: $F(1,48)= 16.36$, $P<0.0001$. In the novel object location test (**B**), spatial memory was evaluated 24h after a training trial as percentage of time exploring the object placed in a new location (N) versus the time exploring the object placed in an old location (O). Two-way ANOVA, object in a new location effect: $F(1,94) =10.33$, $P=0.0018$. Interaction effect: $F(3,95)= 5.878$, $p=0.001$. In the forced swimming test, the immobility time was evaluated during the first 2 minutes (**C**), and the last 4 min (**D**), of the 6 min trial in all groups. In (**C**), two way ANOVA, group effect: $F(1, 47)=257.7$, $P<0.0001$. In (**D**), two-way ANOVA, group effect: $F(1 47)=10.28$, $P=0.0024$. T-test between LTS mice ($t25 = 2.568$, $P=0.0166$). Tukey's test as a post hoc analysis was used. All values are mean ± SEM. N= 10-15 mice per condition. *P<0.05, **P<0.005, ****P<0.001.

1.5.3. Characterization of Egr1 downstream signalling

To try to understand which molecular mechanisms are underlying the beneficial effect of Egr1 downregulation in superficial neurons upon chronic stress, we made the following

experimental approach. RNA-seq data from previously published studies using chronic stress paradigms (Floriou-Servou et al., 2018; Kwon et al., 2019) was overlapped with available data of Egr1-regulated genes (Castro-Mondragon et al., 2022; Matys et al., 2006). Thus, we analysed the expression of genes that are known to be altered by stress but also controlled by Egr1 and expressed in the hippocampus. To do so, we performed qPCR of hippocampal tissue. We first measured total levels of Egr1 in our four conditions. As expected, we found decreased Egr1 levels in both groups expressing the shEgr1 (AAV-shEGR1 and AAV-shEGR1+CUMS). Then, we evaluated levels of Arc, another early gene involved in memory consolidation (Gallo et al., 2018; Minatohara et al., 2016), and Icam5, a cellular adhesion molecule involved in spine maturation (Ning et al., 2015). In both cases, we could not find significant differences between groups. Next, we analysed levels of Adra1d, a receptor for NA that is also a risk factor for unipolar and bipolar MDD (Bouayed et al., 2012; De Paermentier et al., 1997). We again could not find any difference between conditions. Last, we assessed the levels of Sik1, a Ser/Thr Kinase (Ying Liu et al., 2012) and Dlgap2, which may play a role in synapse organization (Rasmussen et al., 2017). No significant differences were observed between conditions. These results could suggest several things: first, any of the genes analysed were responsible for the changes observed after Egr1 manipulation; second, the behavioural manipulation performed on the mice masked the potential molecular changes; third, changes specifically in sup Calbindin-positive cells are not too heavy and, on top of that, they are diluted or overshadowed in our total hippocampal lysates.

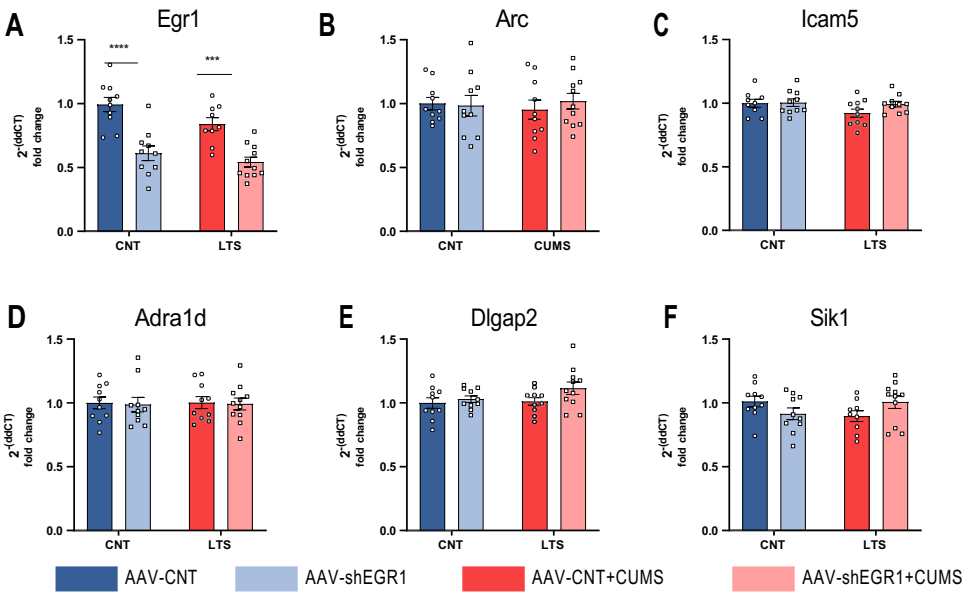


Figure 43. qPCR analysis of Egr1-regulated genes in the hippocampus of CNT and LTS mice. Quantitative RT-PCR analysis of Egr1 (**A**), Arc (**B**), Icam5 (**C**), Adra1d (**D**), Dlgap (**E**) and Sik1 (**F**). Histogram represents relative mRNA abundance expressed as fold change relative to CNT mice. Levels of mRNA were

normalized to Actinβ. In A, group effect F(1, 36)=4.851, P=0.0341. Virus effect F(1, 36)=44.35, P<0.0001. Bonferroni as a post hoc analysis was used. Values are mean ± SEM. N= 10-11 mice/group. ***P<0.0005, ****P<0.0001

1.6. Progressive hippocampal proteomic alterations induced by stress

From the previous results, we concluded that the hippocampus is essential for the stress response and that long periods of stress are needed to see a clear phenotype in mice behaviour. Then, we wanted to know how stress duration has also an effect on the hippocampal proteome. Several studies have already investigated the impact of stress on hippocampal proteins (Jung et al., 2017; Stankiewicz et al., 2015; von Ziegler et al., 2022). However, it is known that the stress protocol itself can have a huge impact on the data obtained. Indeed, the type of stressors used, the duration of those stressors, and the subregion of the hippocampus studied can hugely influence the final results (Floriou-Servou et al., 2018).

To better understand the hippocampal alterations induced by different stress protocol durations, we followed the same experimental design as described above in previous sections (See Section 1.1). Briefly, WT mice were divided into three groups: non-stressed control mice (CNT), mice stressed for two days (STS) and mice stressed for 28 days with the CUMS protocol (LTS). 24 hours after the last stressor, mice were sacrificed, and the hippocampus was obtained and analysed using Mass-spec technology. Using LFQ-Analyst software, we determined differently expressed proteins between conditions. First, we obtained a principal component analysis (PCA) plot that grouped our samples. As seen in Figure 44A, we could distinguish three big groups that correspond to our three experimental conditions (CNT, STS and LTS mice). As expected, LTS mice presented the highest variability within the group. Then we compared the hippocampal proteome between conditions using a p-value<0.05 and a log2 fold change >0.5 as a screening threshold. We first compared CNT and STS groups and observed that few proteins were significantly altered between groups (Figure 44B).

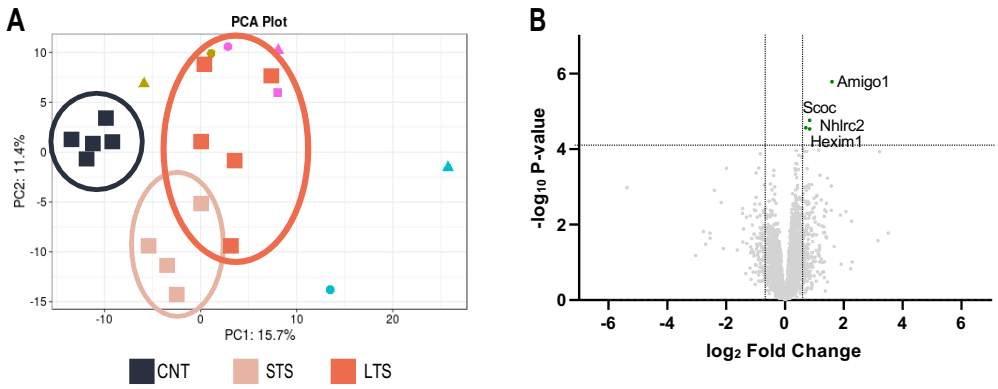


Figure 44. Proteomic analysis of mice hippocampus. (**A**) PCA plot from the hippocampal samples analysed. Circles surround samples within groups that were included in the analysis. (**B**) Volcano plot showing the differently expressed proteins between CNT and STS mice. Green dots represent upregulated proteins in CNT mice compared with STS mice. Grey dots represent no significant proteins. Names of representative proteins are indicated.

Next, we examined the differences between CNT and LTS mice. Here, we obtained around 80 proteins altered between conditions (Figure 45A). Some of the differently expressed proteins include Bclaf1, Bcl2l1 and several proteins involved in the transport of substances into, out of or within the mitochondrion such as Slc25a5, Timm9, Tmem14c, Atp5e, Mpc1 and Agt (Blake et al., 2021; H. Jiang et al., 2022; Kunji et al., 2020; Mayr et al., 2010; Stelzer et al., 2016; Yien et al., 2014).

These results indicate that hippocampal proteomic changes increase along the stress progression. This goes in line with the behavioural results obtained in Section 1.1. The next step was to explore whether these changes in hippocampal proteins were associated with specific pathways. To do so, differently expressed proteins between CNT and LTS mice were subjected to EnrichR to investigate underlying biological processes and signalling pathways (Kuleshov et al., 2016). We found that the Sirt1 pathway was the most altered in our data analysis (p-value=0.0044, odds ratio=22.11) (Figure 45B), as it is involved in the regulation of the expression of Bcl2l1 and Bclaf1 (Yu et al., 2022). This goes in line with studies suggesting that genes involved in survival could play a role in depressive-like behaviour (Kosten et al., 2008; Shishkina et al., 2012). These results suggest that Sirt1 is in part explaining the proteomic changes observed after chronic stress.

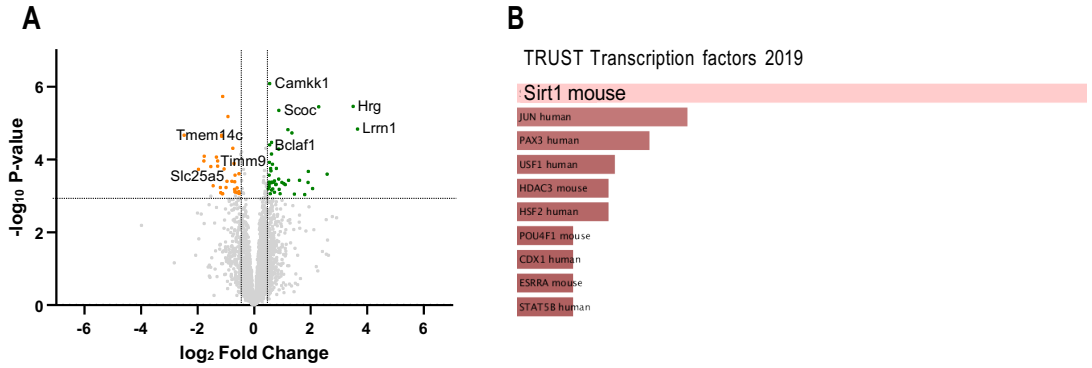


Figure 45. Proteomic analysis of LTS mice. (**A**) Volcano plot showing the differently expressed proteins between CNT and LTS mice. Green dots represent upregulated proteins in CNT mice, orange dots represent upregulated proteins in LTS mice and grey dots represent no significant proteins. Names of representative proteins are indicated. (**B**) Diagram of the TF mostly affected in the proteomic data. P-value= 0.0044, odds ratio=22.11

1.6.1. Sirt1 levels are increased in long-term stressed mice

To better understand how Sirt1 could be altered in our long-term stress mice, total levels of the protein were measured using immunostaining in mouse hippocampus. We first measured the mean intensity of Sirt1 in the CA1 and found increased levels in LTS when compared with CNT mice (Figure 46A-B). Then, we also determined Sirt1 levels in the DG and again found that LTS presented higher Sirt1 immunoreactivity (Figure 46C-D).

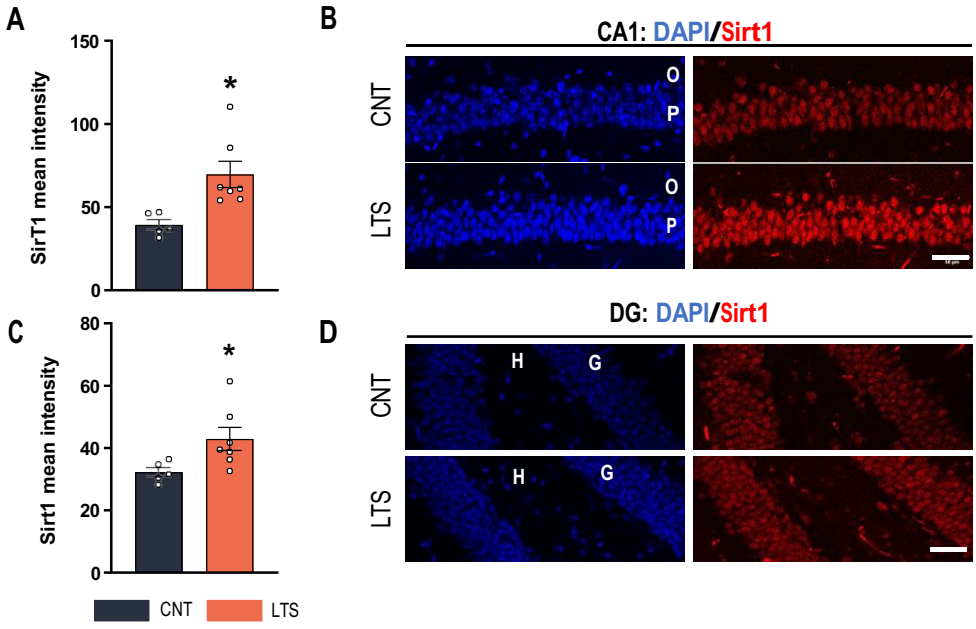


Figure 46. Sirt1 levels are upregulated after LTS in the hippocampus. Quantification of Sirt1 levels in the CA1 (**A**) and representative images (**B**). Unpaired t-test; p=0.0111, t=3.109, df=10. Quantification of Sirt1 levels in the DG (**C**) and representative images (**D**). Unpaired t-test, p=0.0422, t=2,328, df=10. Values are

mean ± SEM. N=5-7 mice/group. *P<0.05. O: *Stratum Oriens*; P: *stratum pyramidale*; H: *Hilus*; G: *Stratum granulare.*

Overall, these results support and strengthen the proteomic results and propose Sirt1 as a potential core molecule mediating the progressive hippocampal changes during chronic stress.

In summary, these results indicate that the deficits observed at the molecular, cellular and behavioural level are more prominent in LTS. Because of that, during this Thesis, we will focus on the LTS (also referred as CUMS or chronically stressed mice) as is the one showing a more robust effect.

2. TISSUE-COMBINED PHOTOBIOMODULATION AS A NON-INVASIVE THERAPY TO TREAT STRESS-INDUCED MDD

As mentioned in the introduction, there are many concerns associated with classical ADs treatment. For this reason, in the last years, research has focused on finding new molecular therapeutic targets and approaches. In this context, photobiomodulation (PBM) has emerged as a promising method. Different studies postulate PBM as a good option for MDD and it has already been used both in animal models and in MDD patients (Cassano et al., 2018). Not only the brain has been the target of PBM to treat MDD, but also the gut. Some studies suggest that PBM in the gut can improve the microbiome dysbiosis observed in stress-related pathologies such as MDD (Bicknell et al., 2019). Here, we wanted to determine if the PBM of both the head (brain) and the abdomen (gut) could have a synergic effect and improve the stress-induced sequelae that we observed after long periods of stress (CUMS). Additionally, it has been proposed that PBM can exert its functions by regulation of Sirt1 (Salehpour, Farajdokht, Mahmoudi, et al., 2019; Z. Zhang et al., 2020; Zhu et al., 2022). From the previous results, we obtained that LTS mice present, among others, alterations in Sirt1 expression. Thus, PBM could be a useful approach to try to reverse those alterations found both at behavioural and molecular levels.

2.1. Characterization of the effects mediated by tissue-combined photobiomodulation in the brain

2.1.1. Tissue-combined photobiomodulation decreases Egr1-activated cells in the mouse brain

Although PBM has been widely used in both animal models and patients, the mechanisms underlying its beneficial effects are not completely understood. In this line, studying which cells are being activated/deactivated after PBM treatment is mandatory to shed light on the molecular mechanisms involved. To do so, we used again the Egr1-CreER^{T2}xR26RCE double transgenic mice (Figure 47A) and subjected them to PBM treatment. Here, we had four experimental conditions; mice that were placed in the device but without light treatment (CNT), mice placed in the device and stimulated in the head (HEAD), mice placed in the device and stimulated in the abdomen (ABD) and mice placed in the device and stimulated in both head and abdomen (BOTH). The treatment consisted of 6 minutes of

photobiomodulation from Monday to Friday for 3 weeks (Figure 47B). On Monday of the last week, all mice received 4-HT i.p. 30 min prior to the PBM treatment. Thus, activated cells during the PBM will be labelled permanently. 2 hours after the last PBM administration, mice were perfused, serial coronal sections were obtained, and stained for GFP-positive cell counting.

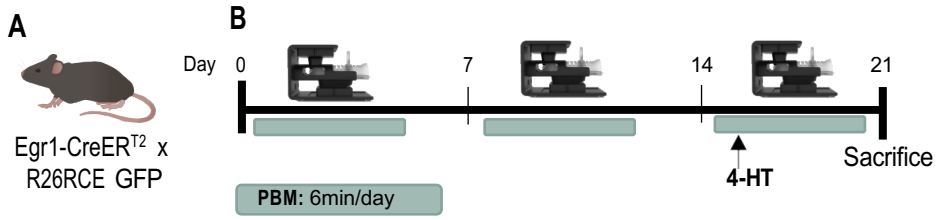
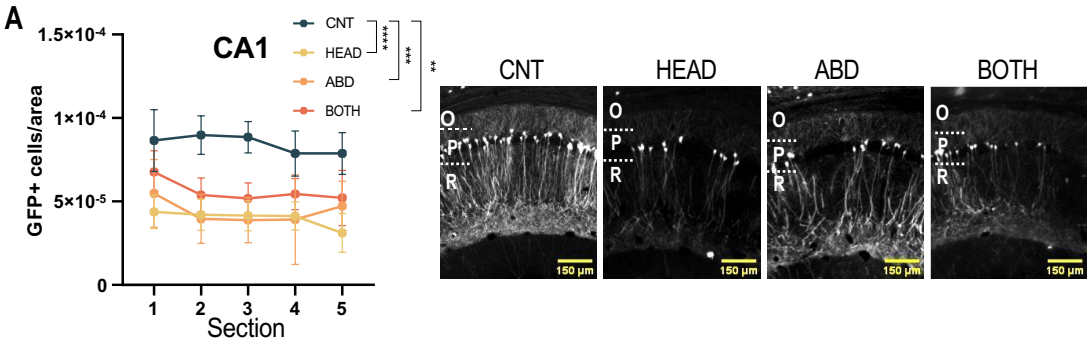


Figure 47. Schematic representation of the experimental design used. (**A**) Scheme of the double heterozygous mutant Egr1-CreER^{T2} x R26RCE GFP mice. (**B**) Egr1-CreER^{T2} x R26RCE mice were placed in the photobiomodulation device and stimulated for 6 minutes. This procedure was repeated for three weeks once per day from Monday to Friday. On the first day of the last week of photobiomodulation, all mice were treated with 4-HT, 50mg/kg, 30 minutes prior to the stimulation to induce recombination by CreER^{T2}.

We first counted GFP-positive cell density in the hippocampus, as it is one of the regions most affected by stress and MDD. In the CA1, we found a significant decrease in GFP-positive cells between CNT mice and all the other conditions (Figure 48A). Then, in the CA3 we found the same tendency although it was only significant when comparing CUMS HEAD with CNT mice (Figure 48B). We also studied the DG and found the same tendency as in the other regions, but this was not significant. In summary, we observed decreased number of Egr1-activated cells in the hippocampus after PBM administration.



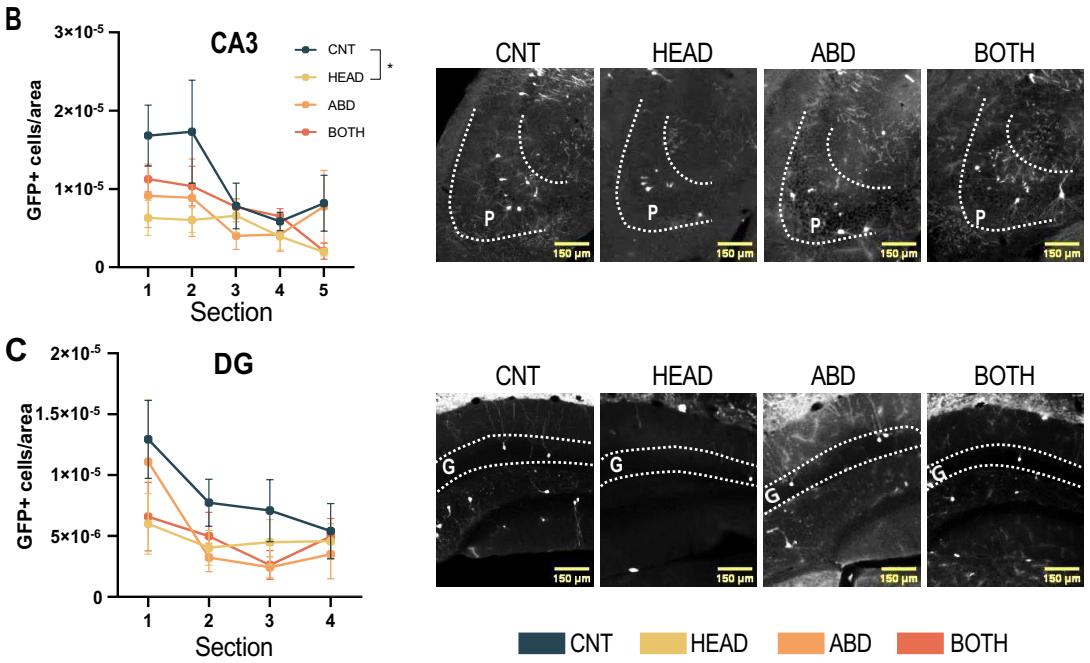


Figure 48. Identification of activated cells in different areas of the hippocampus. Representative images and quantification of Egr1-dependent activated cells (Number of GFP-positive cells/area of 500 µm2) per region in: the CA1 (**A**), the CA3 (**B**) and the DG (**C**). In A, group effect, $F(3,64)=9.441$, $P<0.001$. In B, group effect, $F(3,69)=3.039$, $P=0.0347$. Tukey's test as a post hoc analysis was used. Values are mean ± SEM. N=6-7 mice per group. P: *Stratum pyramidale*; G: *Stratum granulare*. In all graphs, numbers in the X-axis represent the sections analysed (from rostral 1- to caudal 5).

To assess whether this decrease was specific to the hippocampus or not, we counted the number of activated cells in other brain regions that are known to be altered in MDD. In the mPFC, we found the same trend although we could not find any difference probably due to high variability (data not shown). Interestingly, in the cingulate cortex, we found a decline in the number of activated cells in all conditions when compared to CNT mice (Figure 49). These results propose that PBM triggers a general reduction of Egr1-activated cells.

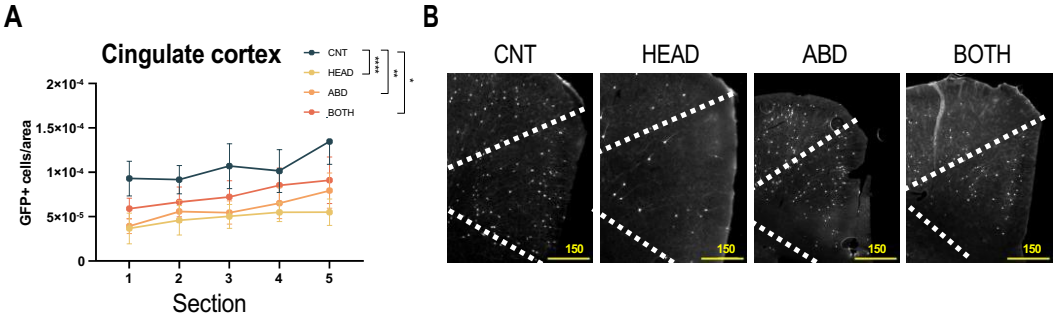


Figure 49. Identification of activated cells in the cingulate cortex. Representative images and quantification of Egr1-dependent activated cells (Number of GFP-positive cells/area of 500 µm2) per region. Two-way ANOVA, group effect: $F(3,82)=10.11$, $P<0.001$. Values are mean ± SEM. N=6-7 mice per group.

Tukey's test as a post hoc analysis was used. In A, numbers in the X-axis represent the sections analysed (from rostral 1- to caudal 5).

2.1.2. Tissue-combined photobiomodulation does not change the type of the activated neurons

We showed that tissue-combined photobiomodulation decreases the number of Egr1-activated cells. We next wanted to examine if PBM treatment was inducing changes not only in the density but also in the type of activated cell. To characterize this, we immunostained the CA1 of EgrCreER^{T2}xR26RCE mice from our four conditions (CNT, HEAD, ABD and BOTH) for different markers including MAP2, a marker for CA1 pyramidal neurons, PV, a marker of parvalbumin interneurons and Calb1, a marker of superficial neurons of the CA1 (Figure 50).

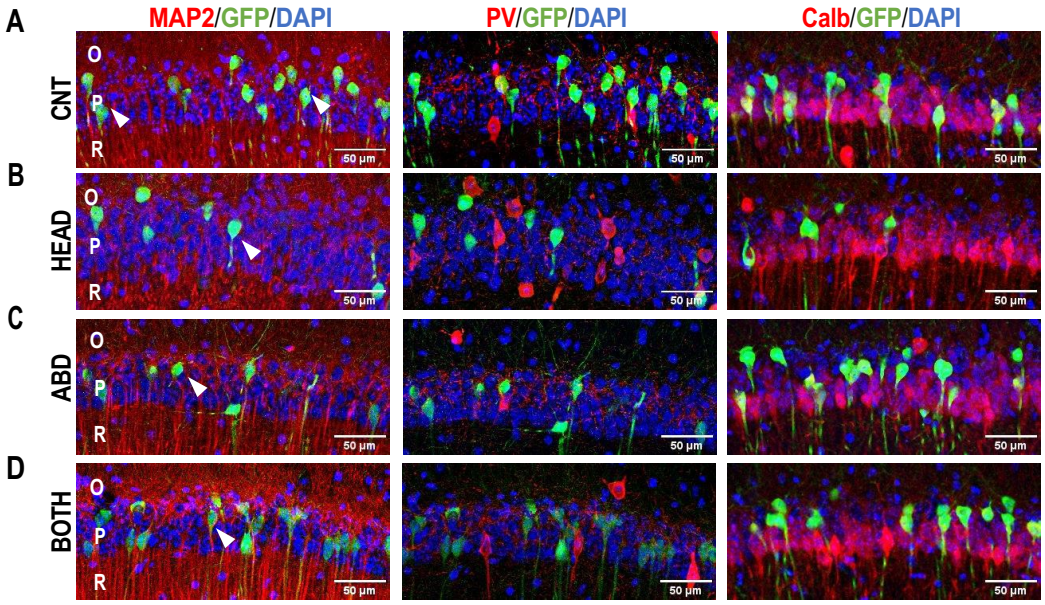


Figure 50. Characterization of the Egr1-dependent activated cells by photobiomodulation. Representative images showing the identity of the activated cells (green) by co-labelling with specific cell markers (red). In all rows, GFP-positive cells are in green. Representative images from CNT (**A**), HEAD (**B**), ABD (**C**) and BOTH (**D**) mice showing labelling of MAP2 (red) in the first column, PV (red) in the second column and Calb1 (red) in the last column. White arrows point to double-positive cells for each marker. O: *Stratum oriens*; R: *Stratum radiatum*; P:*Stratum pyramidale.*

We confirmed our previous data as the Egr1-positive cells were all pyramidal neurons (Figure 51A). No interneurons were found to co-localize with Egr1-positive cells (Figure 51B). Then, we checked for changes in the number of deep (calbindin-negative) or superficial (calbindin-positive) neurons (Figure 51C). We could not find any difference in this marker. Overall,

these results suggest that although PBM causes a reduction of Egr1-positive cells, it does not induce any cellular reorganization.

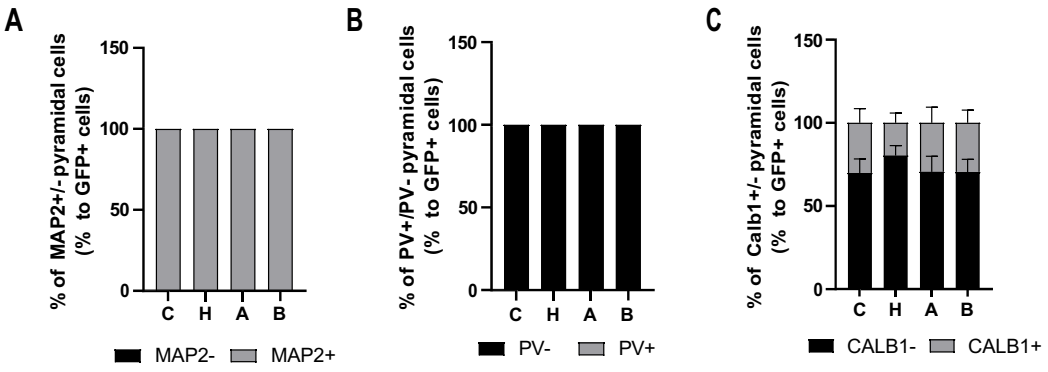


Figure 51. Quantification of the Egr1-dependent activated cells by different photobiomodulation approaches. (**A**) Quantification of the percentages of double-positive cells in CA1 for GFP and MAP2. (**B**) Quantification of the percentages of double-positive cells in CA1 for GFP and PV. (**C**) Quantification of the percentage of double-positive cells in the CA1 for GFP and Calb1. C, CNT mice; H, HEAD mice; A, ABD mice, and B, BOTH mice.

2.2. Tissue-combined photobiomodulation improves some deficits induced by chronic stress

With the previous results, we determined which cells are being modulated by PBM. Then, we wanted to apply tissue-combined PBM in mice that underwent the CUMS protocol.

2.2.1. Combined PBM improves cognitive but not emotional alterations induced by chronic stress

To assess whether tissue-combined PBM could modulate sequelae induced by chronic stress, the following experimental design was used (Figure 52A). Mice were subjected to the CUMS protocol for 28 days or not (CNT mice). The stress protocol was combined with the PBM treatment, which started in the second week of the experiment and was done daily (from Monday to Friday), 6 minutes per day. In this experimental design, 5 experimental groups were used (Figure 52B). A control group that did not receive any stress or PBM treatment (CNT), mice that underwent the CUMS protocol but did not receive any PBM treatment (CUMS), mice that underwent the CUMS protocol and received PBM treatment in the head (CUMS HEAD), mice that underwent the CUMS protocol and received PBM treatment in the abdomen (CUMS ABD) and mice that underwent the CUMS protocol and received PBM treatment both in the head and the abdomen (CUMS BOTH). 24 hours after the last stressor, faecal samples were recollected for posterior microbiota studies, and mice were subjected to

a battery of behavioural tasks. After all behavioural tasks, mice were sacrificed, and their brains were dissected out to do several analyses including, Golgi-cox staining, metabolomic analysis and immunostaining.

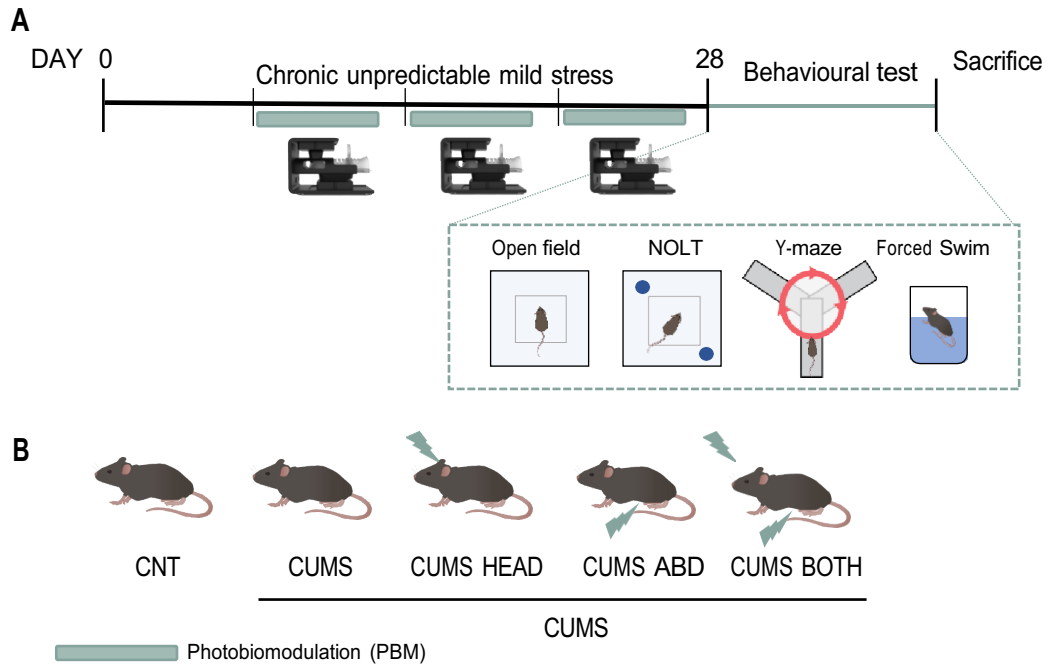


Figure 52. Schematic representation of the experimental design used. (**A**) Mice underwent chronic stress for 28 days (CUMS groups) or were kept in their homecages (CNT). Starting on day 8, all groups (except for CNT and CUMS) started the photobiomodulation treatment 6 min per day, from Monday to Friday. On day 29, mice started a battery of behavioural tests. 24h after the last test, mice were sacrificed. (**B**) Experimental groups used for this experiment. N=14-16 mice per group.

First, we measured the total distance in the open field and observed that all mice that received stress (CUMS) presented increased levels of locomotor activity when compared with CNT mice (Figure 53A). No effect of PBM treatment was observed in this parameter. We also measured anxiety by calculating the time mice spend in the centre of the arena. Although no significant differences were obtained, a tendency to spend less time in the centre was observed in CUMS mice when compared with CNT. This tendency was abolished with the PBM treatment in the head (CUMS HEAD) and in the double-stimulated mice (CUMS BOTH), which presented levels comparable to those found in CNT mice (Figure 53B). Then, we analysed behavioural despair using the forced swim test. CNT mice present increased swimming duration (Figure 53C) when compared with all the other stressed groups. Again, no effect of PBM treatment was observed.

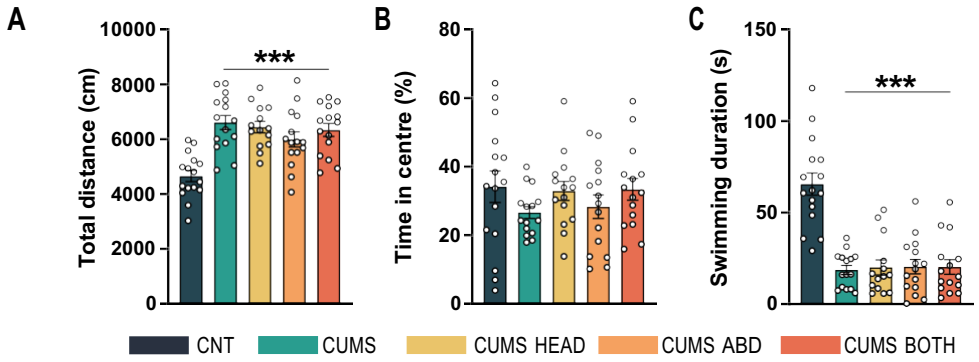


Figure 53. PBM treatment during chronic stress does not have an effect on emotional tasks. In the open field, total distance (**A**) and time in the centre (**B**) were measured for 15 minutes. In A, two-way ANOVA, group effect $F(4,70)=11.52$, $P<0.001$. (**C**) Swimming duration in the forced swim was used to measure behavioural despair. $F(4,70)=23.28$, $P<0.0001$. Tukey's test as a post hoc analysis was used. All values are mean ± SEM. N=14-16 mice per group. ***$P<0.0001$ compared to CNT.

As we did not find any clear result in emotional tasks, we wanted to see whether PBM could influence cognitive tasks such as the NOLT and the Y-maze. In the NOLT, we first observed that CNT mice were able to discriminate between the old location (O) and the new location (N) of the object. This ability to discriminate was lost in those mice that received the CUMS protocol (CUMS). Interestingly, those mice that received the double stimulation (CUMS BOTH) were also able to discriminate between objects (Figure 54A). This result indicates that combined PBM could be beneficial for the memory alterations induced by stress. To deepen on that, we then examined spatial working memory with the Y-maze. Briefly, mice were placed in a translucid Y-maze with surrounding spatial cues. The number of alternations between the three arms of the Y-maze was analysed. We observed that CNT mice performed a % of alterations above those expected by chance (dashed line). Regarding the stressed mice, the only group that was not able to perform a % of alterations above the chance level was the CUMS mice (Figure 54B). This result suggests that PBM, either in the head, the abdomen, or in both places, could be useful for the spatial working memory alterations induced by chronic stress.

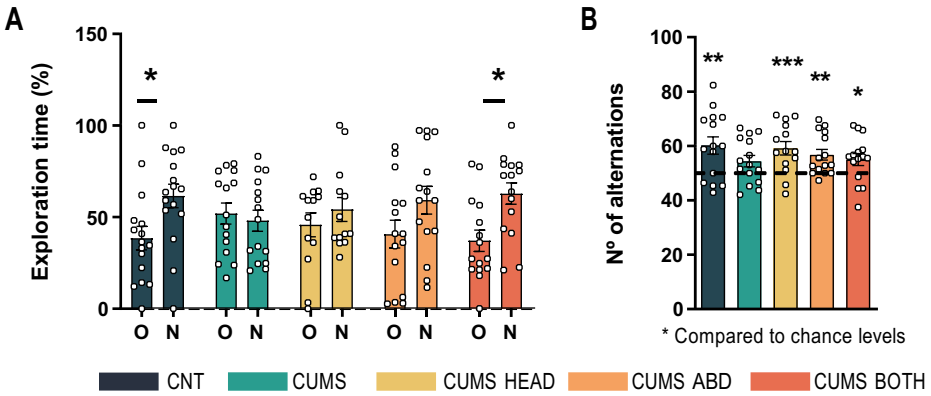


Figure 54. PBM treatment during chronic stress rescues cognitive alterations induced by CUMS. In the novel object location test (**A**), spatial memory was evaluated 24h after a training trial as the percentage of total time spent exploring either the object placed at a new location (N) or the object placed at the old location (O). The last 2 minutes of the test were analysed. Two-way ANOVA, new location effect: $F(1,138)=12,25$, $P=0.0006$. (**B**) Spatial working memory was studied using the Y-maze. The number of alternations (%) was calculated and compared with the chance level (50% -dashed line). T-test between % of alternation and 50%. In A, Bonferroni's as a post hoc analysis was used. All values are mean ± SEM. N=14-16 mice per group., *P<0.05, **P<0.005 and ***P<0.001.

Finally, a Z-score was conducted to summarize the global effect of stress duration and PBM treatment in mice behaviour. First, a general Z-score was performed, which include the data obtained in the mice weight, the total distance in the open field, the time exploring the old object in the NOLT and the number of alternations in the Y maze (Figure 55A). A significant increase in the Z-score was observed in all stressed groups, although the group with higher values (meaning worse behavioural performance) was the CUMS mice. Additionally, as we found a clearer effect of PBM on cognitive task, we made a cognitive Z-score which was calculated with the data obtained in the NOLT and in the Y-maze (Figure 55B). In this graph, we also observed a tendency to higher Z-score values in the CUMS mice, although this was not significant. However, we found statistically significant differences when comparing CUMS mice to the CUMS BOTH mice. These results illustrate the positive and beneficial effect of tissue-combined PBM in the cognitive alterations induced by CUMS.

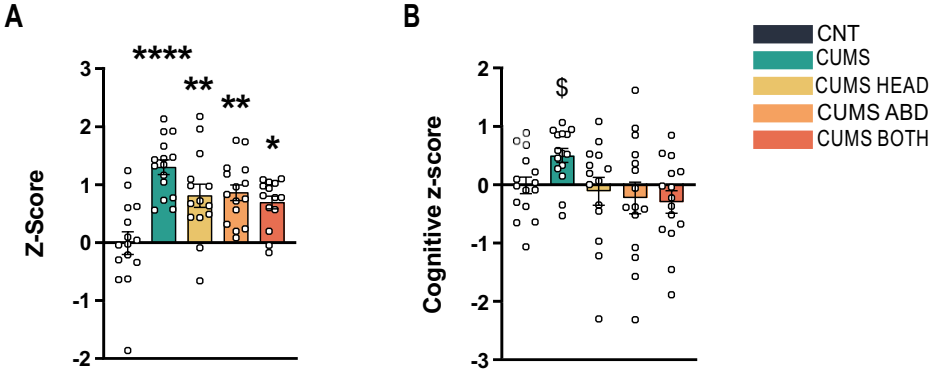


Figure 55. Global effects of PBM in behaviour. (**A**) Global Z-score. One-way ANOVA, group effect: F(4,68)=9.171, P<0.0001. (**B**) Cognitive Z-score. One-way ANOVA, group effect: F(4,69)=2.535, P<0.0479. Increased Z-score means worse performance in behavioural tasks. Tukey's test as a post hoc analysis was used. All values are mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 compared to CNT. $P<0.05 compared to CUMS BOTH.

2.2.2. Combined PBM restores CA1 spine density loss

As we observed an effect of PBM in cognitive tasks in which the hippocampus is highly engaged, we asked whether those alterations could be linked to changes in hippocampal spine density. Thus, spine density was analysed in pyramidal neurons of the CA1 of the hippocampus using the Golgi staining method. We observed that stressed mice with no PBM treatment (CUMS) presented decreased spine density when compared with CNT mice. Interestingly, this reduction was prevented in those mice receiving the light treatment in the head (CUMS HEAD) and in both the head and the abdomen (CUMS BOTH) (Figure 56A-B).

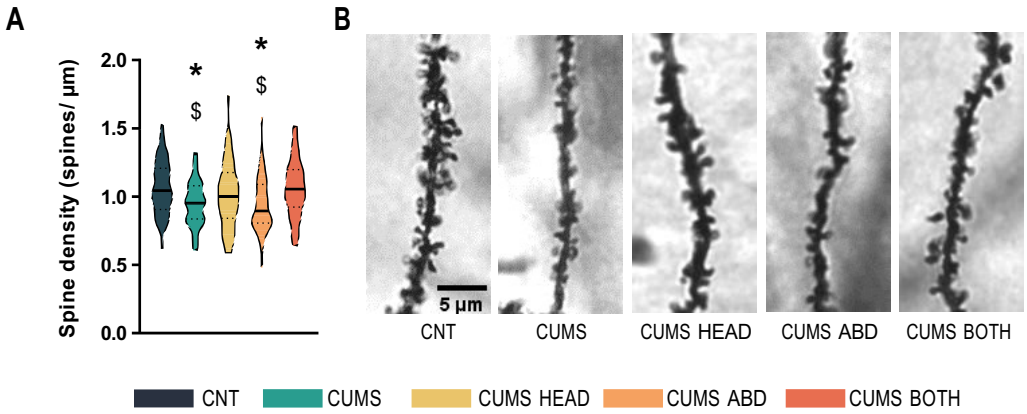


Figure 56. Effect of PBM on spine density in the hippocampus. (**A**) Quantification of CA1 spine density after chronic stress and PBM treatment. One-way ANOVA, F(4,255)=4.453, P=0.0017. Tukey's test as a post hoc analysis was used. N=50-55 dendrites from 6-7 mice per group were counted. *P<0.05 compared to CNT; $P<0.05 compared to CUMS BOTH. (**B**) Representative images of CA1 apical dendrites from each group.

2.2.3. PBM partially rescues some metabolites altered by stress

It has been found that PBM is able to modulate brain metabolites (dos Santos Cardoso et al., 2021). Therefore, we wanted to study how different metabolites could be altered by stress and PBM treatment. To do so, we focused on the hippocampus as we observed that PBM improves hippocampal-related deficits and prevents spine density loss. Thus, hippocampal samples were analysed with an ultra-performance liquid chromatographic system coupled to a Time-of-Flight mass spectrometer (UPLC-ToF-MS). The metabolites studied were

betaine, choline, cystathionine, GABA, glycerophosphocholine (GPC), glutathione (GSH), glutathione disulfide (GSSG), glutamate, glutamine, 5'-Methylthioadenosine (MTA), methionine, nicotinamide, S-Adenosylhomocysteine (SAH), S-adenosyl-L-Methionine (SAMe), spermidine, spermine and tryptophan. First, we found no significant changes using one-way ANOVA in any of the metabolites studied (data not shown). Then, a t-test between conditions was performed to see if some metabolites could be altered by chronic stress. We found that nicotinamide and spermine were significantly decreased in CUMS mice when compared with the CNT group (nicotinamide p-value= 0.038; spermine, p-value=0.033). Moreover, we found that GSH was decreased in CUMS mice when compared with CUMS HEAD and CUMS BOTH (compared with CUMS HEAD, p-value=0.021; compared with CUMS BOTH, p-value=0.046). To examine if these changes could be linked to mice behaviour, we conducted correlation analysis of the three metabolites (nicotinamide, spermine and GSH) with the Z-score and the cognitive Z-score (Z-cognitive). We could not observe any significant correlation between the metabolites and the behaviour performance in CNT, CUMS HEAD and CUMS BOTH groups (Figure 57A, C and E). Nevertheless, significant correlations between spermine, GSH and the Z-score were only found in the CUMS mice (Figure 57B). In both cases, increased levels of the metabolite were associated with better behavioural performance (less Z-score). Additionally, nicotinamide levels were correlated with Z-cognitive only in the CUMS mice. Again, increased nicotinamide was associated with better behavioural response. In the CUMS ABD, Z-score and GSH were significantly correlated (Figure 57D). These results suggest that although no big differences were detected when measuring total levels of the metabolites, small differences could be found when comparing specifically the levels of the metabolites with the global performance in behavioural tasks. Altogether suggests that PBM could be beneficial for stress-induced changes in the metabolomic hippocampal profile.

A

CONTROL	Z-score	Spermine	Nicotinamide	GSH	Z-cognitive
Z-score	1.00				
Spermine	0.02	1.00			
Nicotinamide	0.39	0.29	1.00		
GSH	0.40	-0.24	0.50	1.00	
Z-cognitive	0.16	-0.17	-0.17	-0.06	1.00

B

CUMS	Z-score	Spermine	Nicotinamide	GSH	Z-cognitive
Z-score	1.00				
Spermine	-0.71*	1.00			
Nicotinamide	-0.05	-0.25	1.00		
GSH	-0.79*	0.71	0.21	1.00	
Z-cognitive	-0.04	0.40	-0.63*	0.17	1.00

C

CUMS HEAD	Z-score	Spermine	Nicotinamide	GSH	Z-cognitive
Z-score	1.00				
Spermine	-0.50	1.00			
Nicotinamide	-0.61	0.63	1.00		
GSH	-0.04	0.48	0.50	1.00	
Z-cognitive	-0.11	0.16	-0.28	-0.45	1.00

D

CUMS ABD	Z-score	Spermine	Nicotinamide	GSH	Z-cognitive
Z-score	1.00				
Spermine	0.28	1.00			
Nicotinamide	0.16	0.04	1.00		
GSH	-0.06*	0.52	0.52	1.00	
Z-cognitive	0.54	-0.35	0.39	-0.05	1.00

E

CUMS BOTH	Z-score	Spermine	Nicotinamide	GSH	Z-cognitive
Z-score	1.00				
Spermine	0.45	1.00			
Nicotinamide	0.59	0.49	1.00		
GSH	0.22	0.78	0.29	1.00	
Z-cognitive	-0.04	0.21	0.46	0.47	1.00

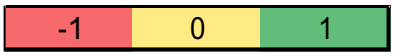


Figure 57. Metabolomic analysis of mice hippocampus after PBM. Heatmap tables showing the correlation values between the Z-Score and the cognitive Z-score (Z-cognitive) with three different metabolites in CNT (**A**), CUMS (**B**), CUMS HEAD (**C**), CUMS ABD (**D**) and CUMS BOTH (**E**) mice. The numbers shown in the table correspond to the Pearson R value. * Denotes significant correlations ($p<0.05$) between two variables. GSH: Glutathione

2.2.4. PBM regulates the levels of Sirt1

As we did not find a huge effect of PBM on hippocampal metabolome, we next asked whether other molecular mechanisms could be involved in the beneficial effects of PBM. As mentioned before, several studies have suggested that Sirt1 is one of the main molecules mediating PBM effects at a cellular level (Salehpour, Farajdokht, Mahmoudi, et al., 2019; Z. Zhang et al., 2020; Zhu et al., 2022). Thus, levels of Sirt1 were evaluated in the hippocampus of mice treated with PBM using immunohistochemistry. In line with our previous results, we found a significant rise in Sirt1 intensity in the CA1 of stressed mice (CUMS) when compared with the CNT group. This increase was prevented in those mice receiving the PBM in the abdomen (CUMS ABD) and the double stimulation (CUMS BOTH). These results indicate that targeting the gut (and/or the microbiome resident in the gut) with PBM induces a reduction of Sirt1 levels in the hippocampus.

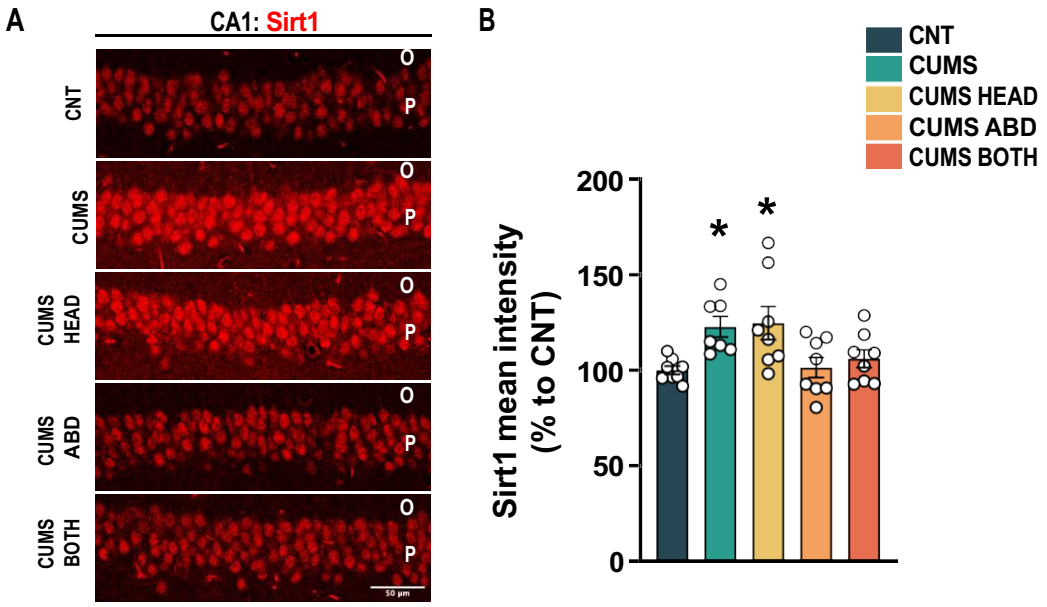


Figure 58. Sirt1 levels are normalised by PBM. (**A**) Representative images showing Sirt1 levels in the CA1 of the hippocampus. (**B**) Quantification of the intensity of Sirt1. Two-way ANOVA, F(4,34)=4.391, P=0.0057. Dunnett's as a post hoc analysis was used. Values are mean ± SEM. N=7-8 mice per group. *P<0.05 compared to CNT. O: *Stratum oriens*; P: *Stratum radiatum*.

2.2.5. Combined PBM restores some microbes altered by stress

To determine the global effect of stress and PBM treatment on mice microbiota, a general characterization of bacterial taxonomy was performed. As stated before, faecal samples were collected just after the last day of stress (See Figure 52). Thus, microbiota composition at the phylum level was analysed between groups. Overall, we could detect 12 phyla, 42 families

and 114 genera. The most dominant phyla in all samples were *Bacteroidetes* and *Firmicutes* (Figure 59). Despite inter-individual variability, *Verrucomicrobia* was significantly increased in all the stress groups. The most abundant families in the control group were *Muribaculaceae, Lachnospiraceae), Rikkenellaceae, Lactobacillaceae and Ruminococcaceae* (not shown).

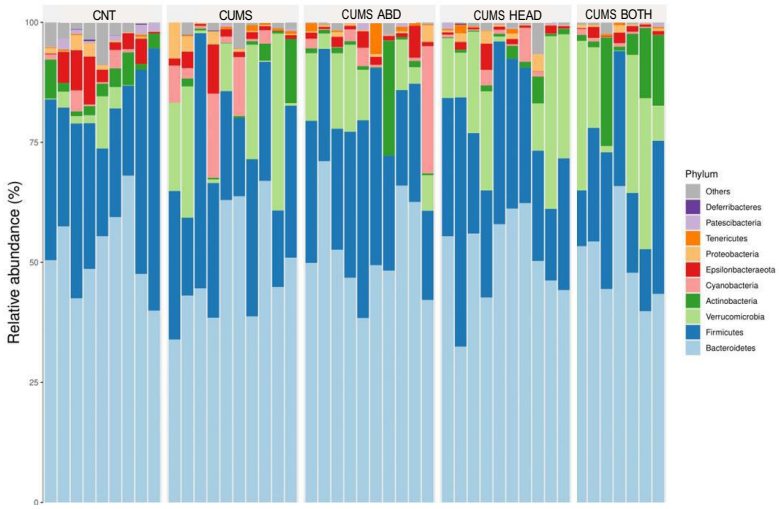
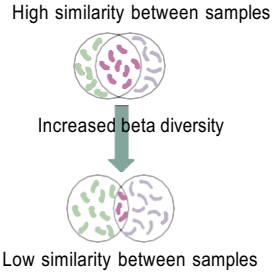


Figure 59. Microbiota composition at phylum level for all faeces samples, presented as a barplot in relative abundances. Treatments are presented grouped, from left to right, in the following order: CNT, CUMS, CUMS ABD, CUMS HEAD and CUMS_DOBLE. The top 10 most abundant phyla have been coloured as indicated in the legend.

Next, we calculated beta diversity to determine between-group differences in microbiota communities (Simpson et al., 2021) (Figure 60A). For analysing beta diversity, we used the Weighted UniFrac method. Results showed a more homogenous clustering in the control samples compared to the other groups (Figure 60C). Moreover, a significant statistical difference was only obtained between the control and CUMS groups (Figure 60B). This indicates that the microbiota composition of CUMS mice is the most different when compared with CNT mice. The other groups did not achieve statistically significant differences between them. Thus, the significant alterations in the gut bacterial communities after stress, reported in other similar studies (Y. Qiao et al., 2020), are reduced and become less evident when stress is combined with PBM. This suggests a beneficial effect of PBM in the microbiota dysbiosis induced by stress.

A



B

Beta diversity index	Pairs	P-value
Weighted Unifrac	CNT vs CUMS	0.04*
Weighted Unifrac	CNT vs CUMS ABD	0.45
Weighted Unifrac	CNT vs CUMS HEAD	0.5
Weighted Unifrac	CNT vs CUMS BOTH	0.7

C

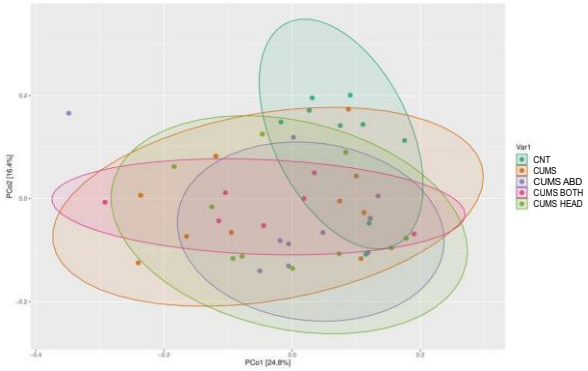


Figure 60. Beta diversity calculations for each treatment. (**A**) Schematic illustration of the concept of beta-diversity. (**B**) Beta diversity statistical analysis. Significant differences (p-value <0.05) between Unifrac values of sample types between treatments, calculated by pairwise PERMANOVA. (**C**) Scores plot for the PCoA performed on the weighted UNIFRAC beta-diversity index grouped by treatments. Samples are coloured and shaped accordingly to their type. Variance explained by each coordinate is indicated between parentheses in the corresponding axis. The differences between groups were calculated using PERMANOVA with 999 permutations.

Then, we moved to analyse our data at a genus level by measuring the relative abundance of specific genera. Among the genus analysed, interesting data was found in the *Enterorhabdus* genus. The results showed a tendency to have decreased levels of the *Enterorhabdus* genus after the stress protocol (CUMS), but a partial recovery of this genus was observed when mice were treated with combined PBM (CUMS BOTH).

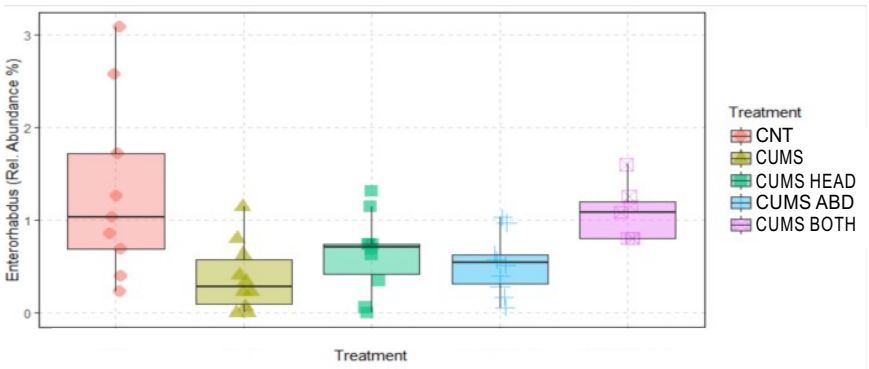


Figure 61. Differential abundance analysis representing genera with significantly different CLR-transformed counts between treatments. Significance was assessed with an ANOVA test (p-value<0.05). Boxplots represent the mean relative abundance and dispersion of each differential genera.

As we observed some interesting tendency in the *Enterorhabdus* genus (but also others) we decided to complete our study by comparing the levels of specific genera with the performance in the behavioural test (Z-score). Hence, the correlation between each genus abundance and the Z-score was calculated. Overall, we found that the genera *Roseburia*, *Enterorhabdus, Butyricicoccus* and *Prevotellaceae NK3B31* have a significative correlation with Z score values (Table 9).

Table 9. Correlation between genera and Z score. Significant correlations between the genera and the Z-score value from all groups. *P<0.05, **P<0.01

Taxa	Correlation	P-value
Roseburia	-0.44888	0.00175**
Enterorhabdus	-0.34979	0.011717*
Butyricicoccus	-0.33331	0.02359*
Prevotellaceae NK3B31	-0.30963	0.03626*

To further explore these correlations, the samples were split by condition to perform a more detailed analysis. Interestingly, we found that while each treatment followed a particular correlation trend, the CUMS BOTH correlation was the closest to the CNT group (Figure 62). This was mainly represented by the identical negative correlation of *Roseburia* relative abundance with the Z-score (black square).

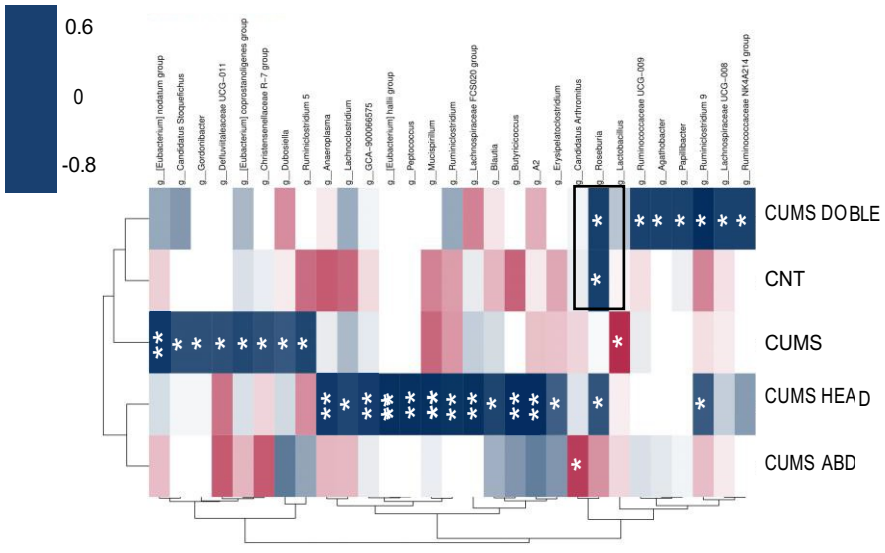


Figure 62. Heatmap of Pearson statistically significant correlations between Z score values and genus abundance, grouped the samples by treatment using auto clustering. *means statistically significant correlation. *P<0.05, **P<0.005. Black square highlight the Roseburia correlation with Z-score in the CNT and CUMS BOTH groups.

Overall, the results obtained are in line with previous literature showing that stress significantly impacts gut microbiota, mainly increasing bacteria associated with inflammation such as *Verrucomicrobia* (L. Liu et al., 2023). Moreover, similar abundance levels of *Enterorhabdus* between CNT and CUMS BOTH groups were found, suggesting that tissue-combined PBM could have a potential therapeutic impact on the microbiome.

2.2.6. PBM reduces hippocampal inflammation

Several studies have shown that gut microbiota is able to influence brain inflammation (Carlessi et al., 2021; Suda & Matsuda, 2022). Moreover, it has been also demonstrated that PBM could improve neuroinflammation (Blivet et al., 2018). Hence, we wanted to determine if the microbiota changes observed after PBM could be accompanied by changes in neuroinflammation. Thus, we evaluated microglial processes (Figure 63) and astrogliosis (Figure 64) in the the *stratum radiatium* of the CA1 of the hippocampus using immunohistochemistry. Interestingly, we found that CUMS mice presented higher levels of Iba1-immunoreactivity when compared with CNT mice, whereas this was prevented in those mice receiving PBM (Figure 63A and B).

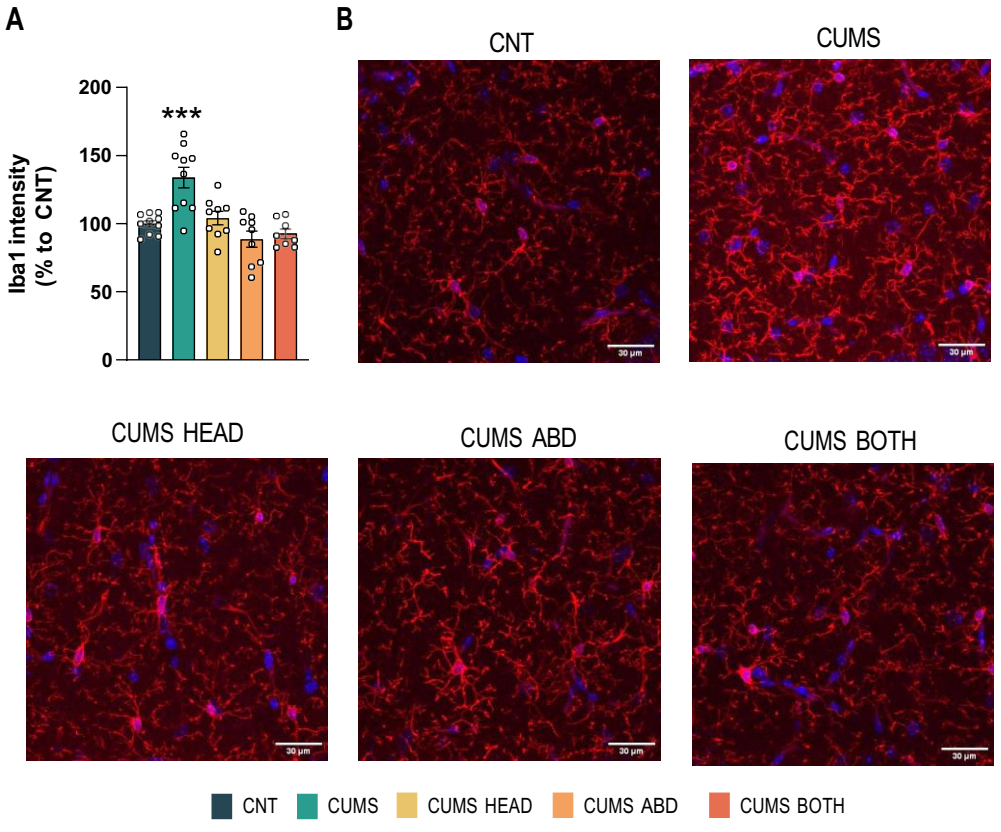


Figure 63. Effect of PBM on hippocampal microgliosis. (**A**) Quantification of Iba1 total intensity in the *stratum radiatum* of the hippocampus. One-way ANOVA, group factor, F(4,25)=5,316, P=0.0031. (**B**)

Representative images of Iba1 staining (red) in each condition. Dunnett's test as a post hoc analysis was used. All values are mean ± SEM. N=8-19 mice per group. **P<0.05 compared to CNT.

Last, we analysed astrogliosis by measuring GFAP intensity in those same mice. We again observed higher intensity of GFAP staining in the *stratum radiatum* of CUMS mice when compared with CNT mice. This increase was normalised with all the PBM treatments, independently of where the light was delivered (Figure 64).

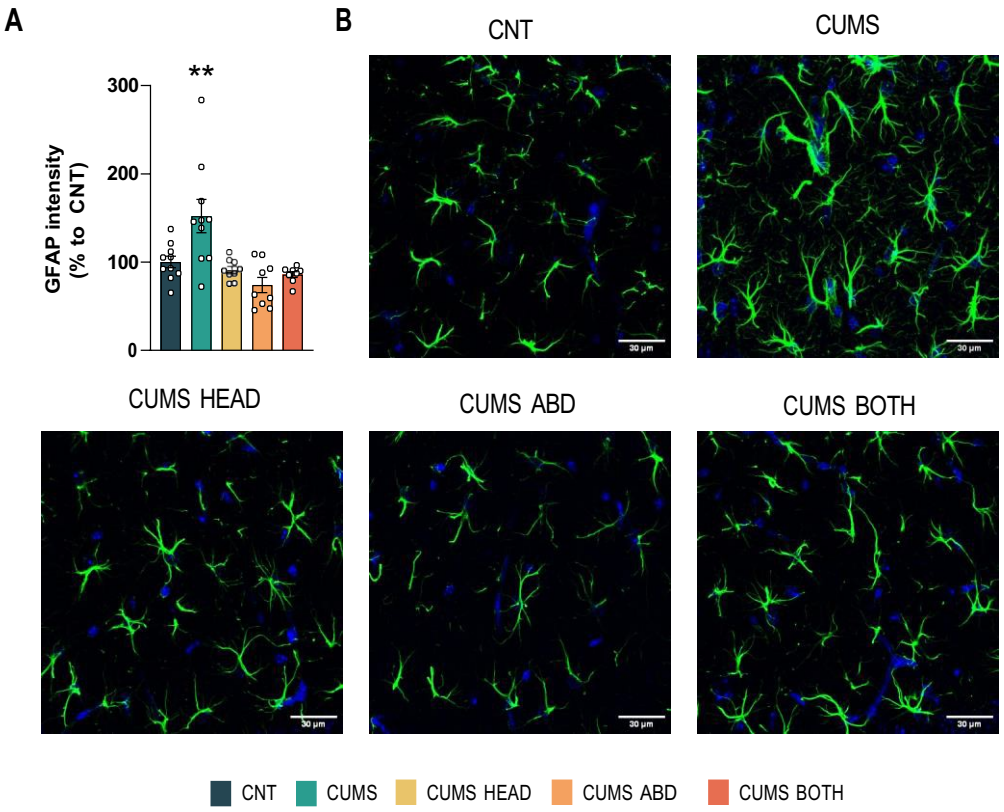


Figure 64. **Effect of PBM on hippocampal astrogliosis.** (**A**) Quantification of GFAP total intensity in the *stratum radiatum* of the hippocampus. One-way ANOVA, group factor: $F(4,41)=11.89$, $P<0.0001$. (**B**) Representative images of GFAP staining (green) in each condition. Dunnett's test as a post hoc analysis was used. All values are mean ± SEM. N=8-10 mice per group. **P<0.05 compared to CNT.

Collectively, all these findings support the idea that PBM could be beneficial for stress-induced MDD as it improves cognitive alterations, prevents spine density loss, modulates gut dysbiosis, restores Sirt1 expression and decreases the levels of neuroinflammation induced by chronic stress. Moreover, tissue-combined PBM (CUMS BOTH) is the group with the strongest and global effects suggesting that the simultaneous stimulation of the brain and the gut is inducing a moderate but synergic effect.

3. Meridianins inhibit GSK3β *in vivo* and improve behavioural alterations induced by Chronic Stress

Last, we wanted to determine if the pharmacological manipulation of the master molecule GSK3β could have a beneficial effect in stressed mice. It has been shown that GSK3β inhibition improves depressive-like phenotypes and that some ADs modulate GSK3β activity. Nevertheless, the use of GSK3β inhibitors presents important limitations as it is a molecule involved in many important cell processes. For this reason, new specific GSK3β inhibitors with fewer secondary effects are needed in the context of MDD and mood disorders.

3.1. In Vivo Inhibition of GSK3β by meridianins

Here, we first wanted to analyse GSK3β activity in chronically stressed mice. To this end, we subjected the mice to the chronic unpredictable mild stress protocol (CUMS) for 28 days (Figure 65A). Then, 24 h after the last stressor, mice were sacrificed to evaluate GSK3β activity. We analysed the Ser9 phosphorylation of GSK3β as a measure of inhibition. We found that the stressed mice (CUMS) presented decreased Ser9 phosphorylation levels when compared with CNT mice in the hippocampus (Figure 65B). We could also find this hyperactivity in other brain regions related to stress and MDD (data not shown). These results confirmed previous literature supporting that chronic stress induces the hyperactivity of GSK3β.

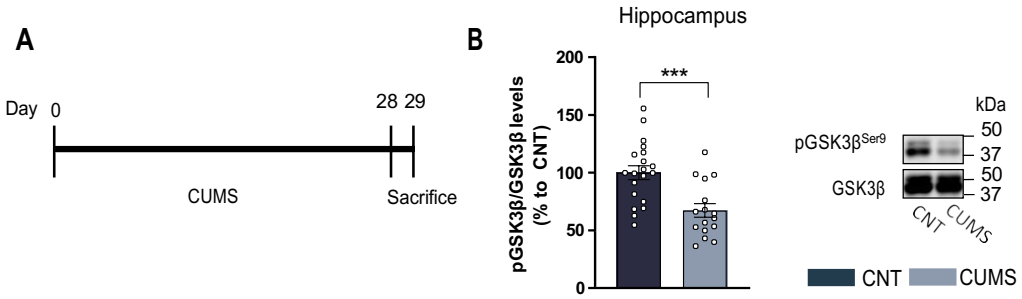


Figure 65. GSK3β phosphorylation levels are altered upon chronic stress. (**A**) Schematic representation of the experimental design. Ten-week-old C57BL6/J mice were subjected to the chronic unpredictable mild stress (CUMS) protocol for 28 days. One day after the last stressor, mice were sacrificed; (**B**) Densitometry quantification and representative immunoblot of phospho-GSK3β Ser9 levels in the hippocampus. Unpaired t-test: t =3.835, df = 43. All values are mean ± SEM. N=16-20 mice per group. *** P< 0.0005 compared with CNT.

Then, we wanted to test if meridianins could inhibit GS3Kβ *in vivo*. To this end, we injected vehicle solution or meridianins (500 nM) in the third ventricle of the brain of adult (3-month-old) mice (Figure 66A). This dose was selected based on previous work from our lab in which we observed that this was the most efficient dose (Llorach-Pares et al., 2020; Rodríguez-Urgellés et al., 2022). We then sacrificed the mice at different time points to evaluate GSK3β inhibition (Figure 66B). We found GSK3β inhibition at 20 min and 1 h after injection in the hippocampus (Figure 66C). These results confirm that the intraventricular injection of meridianins (500 nM) inhibits GSK3β in different brain areas, including the hippocampus 20 min and 1h after injection.

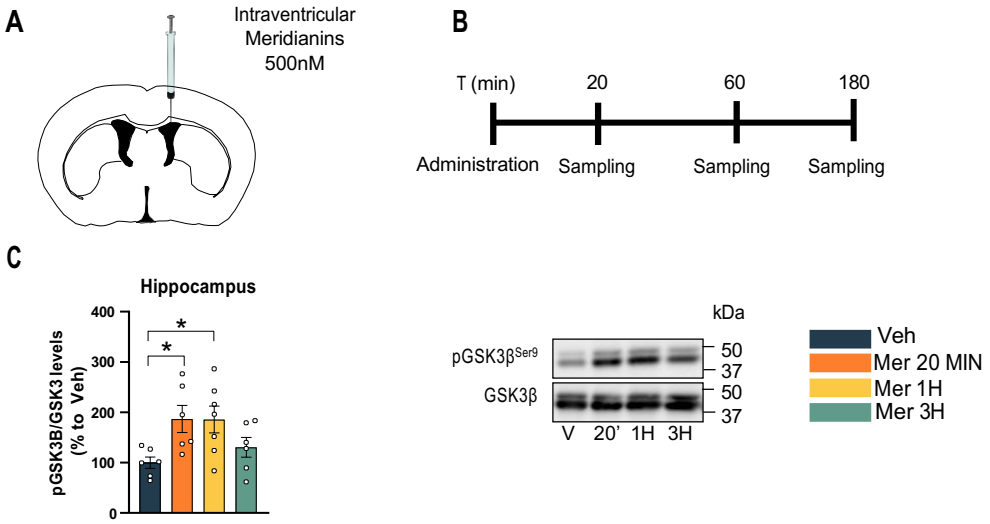


Figure 66. In vivo GSK3β inhibition in different brain regions. (**A**) Three-month-old C57BL6/J male mice were stereotaxically injected into the lateral (left) ventricle with 3 uL of 500 nM meridianins and were sacrificed 20 min, 1 h or 3 h after administration (**B**). (**C**) Densitometry quantification and representative immunoblots of phospho-GSK3β ^{Ser9} levels in the hippocampus. One-way ANOVA F(3,21)=3.568, p=0.0314. Dunnett's test as a post hoc analysis was used. All values are mean ± SEM. N= 5-7 mice per group. *P<0.05. Min: minutes ; V/Veh: Vehicle; 20': Mer 20 min; 1H: Mer 1H; 3H: Mer 3H. Mer: Meridianins.

3.2. Meridianins modulate molecular pathways involved with GSK3β signalling

Once we observed that meridianins could modulate GSK3β activity in the hippocampus we then aimed to know if this inhibition was accompanied by changes in other proteins that are known to be involved with GSK3β signalling. To do so, we performed Western blot experiments in lysates from hippocampus of those mice that were treated with meridianins for 20 min and their respective controls. First, we evaluated the levels of kinases that are

described as GSK3β modulators such as Akt, PKC and PKA (Beurel et al., 2015; R. S. Duman & Voleti, 2012). To check for Akt activity, we looked at phospho AktSer473 levels and found no differences between conditions (Figure 67A). Then, we analysed the levels of PKA activation. To this end, we used an anti-phospho-PKA substrate antibody that detects proteins containing phosphorylated Ser/Thr residue within the consensus sequence for PKA, giving us a readout of PKA activity. No significant differences were observed in this region (Figure 67B). Next, we looked for PKC activity by using an antibody that detects phosphorylation of proteins at phosphor-Ser PKC substrate motifs. We found an increased activity of PKC in the hippocampus in mice treated with meridianins with respect to mice treated with vehicle (Figure 67C). Last, we assessed the levels of a major phosphorylated substrate by PKC activity, the so-called glutamate receptor subunit GluR1. We evaluated GluR1 activity using an antibody that detects phosphorylation of the receptor at Ser 831. We found increased phospho-GluR1Ser831 levels in mice treated with meridianins compared with mice treated with vehicle specifically in the hippocampus (Figure 67D). Taken together, these findings point out that meridianins could modulate synaptic transmission in the hippocampus.

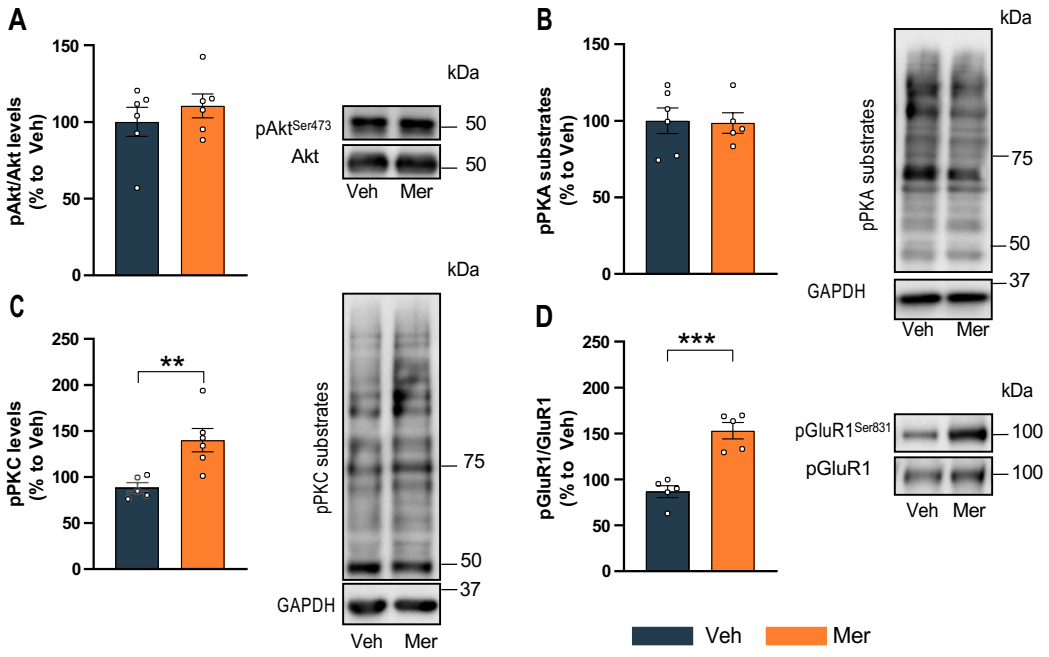


Figure 67. Biochemical assessment of mice treated with meriadins. Densitometry quantification and representative immunoblots of hippocampal lysates of (**A**) phosphorylated Akt at Ser 473 (t=0.08531, df=10. P=0.04), of (**B**) phosphorylated PKA substrates (t=0.1293, df=9, p=0.9), of (**C**) phosphorylated PKC substrates (t=3.596m df=9, p<0.05) and (**D**) phosphorylated GluR1 at Ser831 (t=6.028, df=8, p<0.05). Two-tailed Student t-test was used. Values are mean ± SEM. N= 5-7 mice/group. **P< 0.005; ***P<0.001 compared with Veh mice. Veh = vehicle and Mer = meridianins.

3.3. Meridianins increase spontaneous synaptic activity in the hippocampal CA1

Given the fact that meridianins increased the phosphorylation of the excitatory glutamate receptor subunit GluR1, we wonder if this was also accompanied by electrophysiological changes. Thus, we assessed whether meridianins modulate hippocampal synaptic transmission in brain slices. To this aim, we measured neuronal spikes rate and bursting in the pyramidal CA1 and CA3 and in the granular DG subfields using a multi-electrode array (MEA) (Figure 68B). Hippocampal slices were recorded before meridianins administration and 20 minutes after its addition (Figure 68A). We found a meridianin-dependent increase in the spikes rate of the pyramidal CA1 cells when compared with baseline (Figure 68C-D), but no meridianins-dependent effects were observed in the spike rating of pyramidal CA3 or granular DG layers (Figure 68C-D). We found no differences in the burst parameters (data not shown) in any of the three hippocampal areas analysed. These results support the idea that meridianins have a moderate but specific effect on synaptic transmission or neuronal excitability in the hippocampal CA1.

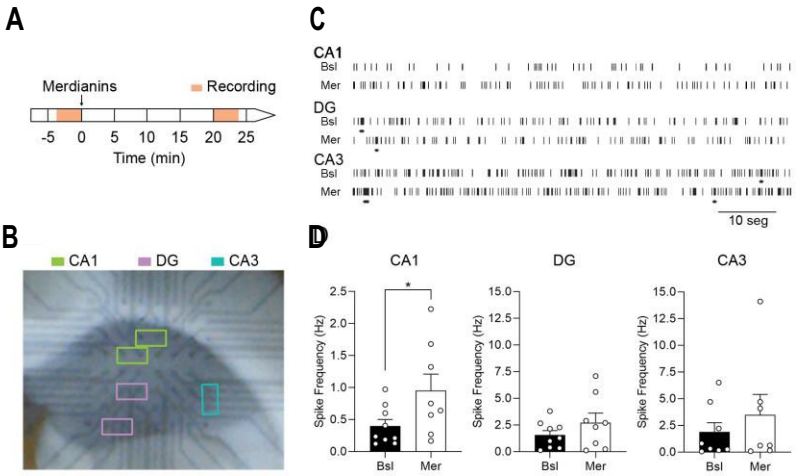


Figure 68. Meridianins specifically increase synaptic activity in pyramidal CA1 neurons. (**A**) Schematic diagram of the experimental multi-electrode array (MEA) recording timeline. (**B**) Illustrative image of a brain coronal slice on the MEA (magnification). Recordings from the selected electrodes located on CA1, DG and CA3 (in green, purple, and blue respectively) were analysed. (**C**) Illustrative timescale spike raster of CA1, DG and CA3 spontaneous activity in basal (Bsl) conditions and after incubation with 500 nM meridianins (Mer). The horizontal lines under each raster define bursts. (**D**) Graphs show quantification of spike frequency in basal conditions and after treatment with meridianins in CA1 (t=2.568, p=0.0371), DG (t=1.944, p=0.0930)

and CA3 (t=1.449, p=0.1975). Two-tailed Student t-test. All values are mean ± SEM. N = 7-8 mice/group. *P<0.05.

3.4. Meridianins improve emotional and cognitive deficits induced by CUMS

We demonstrate that GSK3β is hyperactivated in the hippocampus of chronically stressed mice. We also proved that intraventricular administration of meridianins could inhibit GSK3β in this region. The final experiment was to examine the possible role of GSK3β inhibition in an MDD-like mouse model. To do so, we implanted adult (10-week-old) mice with osmotic minipumps to deliver continuously meridianins or saline (VEH) in the lateral ventricle for 28 days (Figure 69A). After surgery, mice were randomly distributed to CNT or CUMS groups (Figure 69B).

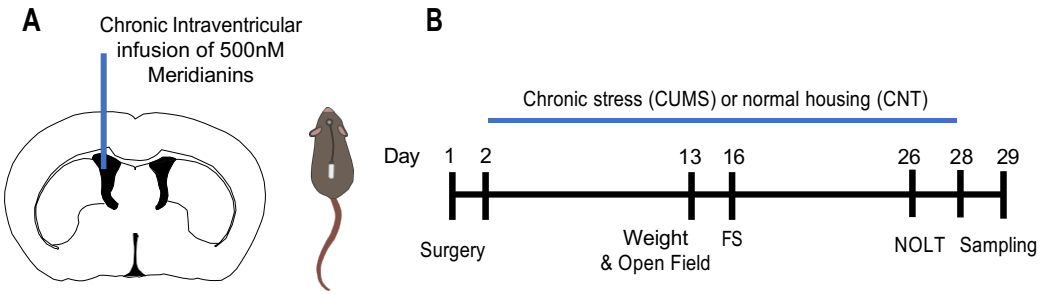


Figure 69. Schematic representation of the experimental design used. (**A**) An osmotic minipump which delivered meridianins chronically was implanted in mice. (**B**) Timeline and behavioural tasks performed.

During the CUMS, we analysed different behavioural task. First, we measured body weight in the middle of the CUMS protocol (day 13) and observed that only stressed mice without treatment presented decreased body weight compared with CNT VEH (Figure 70A). Then, we performed an open field test to measure locomotor activity and anxiety. Results showed that all groups presented similar levels of covered distance (Figure 70B). We then evaluated anxiety-like behaviour by measuring the time spent in the centre of the arena (Figure 70C). Interestingly, we found that CUMS VEH mice spent less time in the centre of the arena in comparison with CNT VEH mice, and this was totally recovered in those stressed mice treated with meridianins (CUMS MER). Moreover, we evaluated the parallel index (1.0 means walking straight) as a measure of navigation strategy in mice (Figure 70D). We found that CUMS VEH mice present alterations in navigation in comparison with control mice and this

was recovered in those stressed mice that received meridianins (CUMS MER). We then measured behavioural despair using the forced swim test. Results indicated that both groups of stressed mice spent more time floating when compared to CNT mice (Figure 70E). Finally, we performed the novel object location task (NOLT) to evaluate spatial memory. Results indicated that CNT mice were able to distinguish between the old and the new object position. Conversely, CUMS VEH mice could not differentiate the new location of the object. However, these deficits were totally rescued in those mice treated with meridianins (CUMS MER) (Figure 70F).

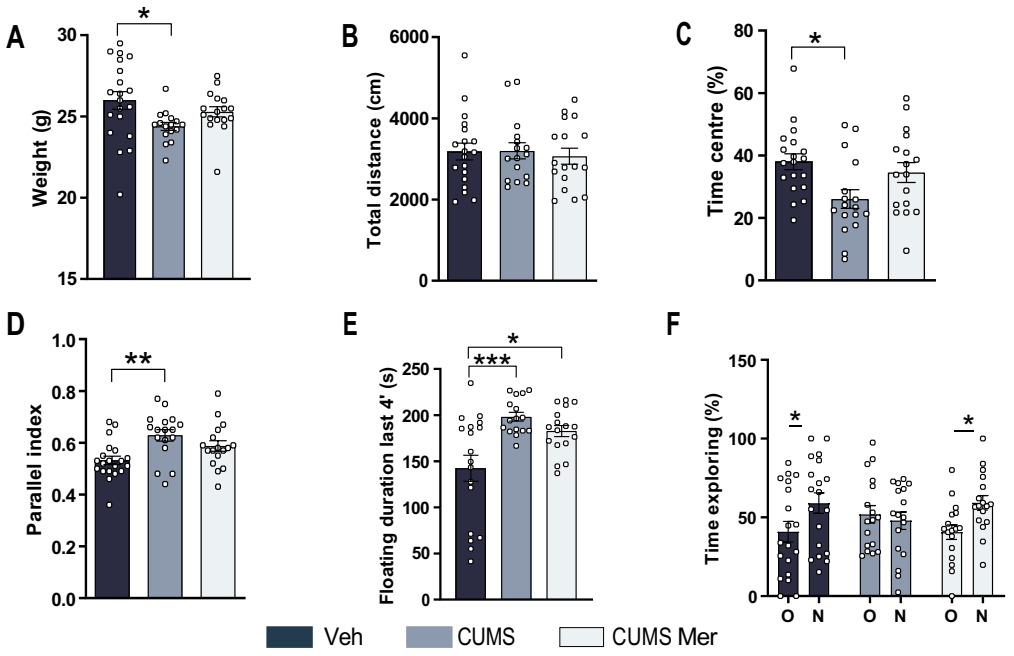


Figure 70. Meridianins administration during CUMS improves stress-induced sequelae. (**A**) Body weight changes induced by chronic stress. One-way ANOVA: F(2,50) = 3,716, p = 0.013. In the open field, locomotor activity (**B**), time spent in the centre (**C**), and parallel index (**D**) were monitored for 15 min. Locomotor activity, one-way ANOVA, F(2,49)=0.1206, p =0.8866. Time in centre, one-way ANOVA, F(2,50) =4.519, p=0.0157. Parallel index, F(2,51) = 6.441, p = 0.0032. In the forced swim test, floating duration (**E**) was analysed for the last 4 min of the test. One-way ANOVA, F(2,48)=9.128, p=0.0004. (**H**) In the novel object location task, spatial memory was evaluated 24 h after a training trial as the percentage of total time spent exploring either the object placed at a new location (N) or the object placed at the old location (O). Two-way ANOVA, new location effect, F(1,100) = 5.460, p = 0.0214. Tukey's test as a post hoc analysis was used in A-E, Bonferroni's test as a post hoc analysis was used in F. All values are mean ± SEM. N=17–20 mice per group. *P<0.05; ** P<0.005; ***P< 0.0005 compared to Veh. Veh= vehicle and Mer= meridianins.

Taken together, these results suggest that meridianins could improve some of the deficits induced by chronic stress such as body weight loss, anxiety, navigation, and spatial memory.

DISCUSSION

Stress is one of the buzzwords of modern societies commonly used in our daily life to describe a variety of situations, events, and emotions that triggers an overwhelming state. Stress exposure is recognized as the most important risk factor for the development of important disorders, including neuropsychiatric diseases (Kessler, 1997). Among them, major depression disorder (MDD) is considered the major contributor to the overall global disease burden with an enormous socioeconomic impact (Proudman et al., 2021). Intensive research into MDD pathophysiology has provided broad knowledge about numerous altered mechanisms. Although there are several brain areas affected in MDD, the hippocampus has been postulated as a key region in which several pathophysiological hallmarks have been described (Tartt et al., 2022). In this thesis, we aimed to greater explore how hippocampal alterations play a role in the pathogenesis of MDD. Specifically, we evaluated how Egr1, Sirt1 and GSK3β contribute to MDD. We then further investigated how the modulation of these molecules using different strategies could have an impact on mice's behaviour. In summary, in this thesis, we wanted to understand and ameliorate the impact of chronic stress using mouse models.

1. BEHAVIOURAL, CELLULAR AND MOLECULAR ALTERATIONS ASSOCIATED WITH STRESS PROGRESSION

Chronic stress is known to affect mice at different levels: from molecular to cellular and behavioural levels. Thus, understanding the key molecules, cells and systems that turn a stressful circumstance from adaptative to pathological is mandatory. In this thesis, we wanted to decipher those changes by studying the impact of short periods of stress versus long periods of stress, which are known to induce an MDD-like phenotype.

1.1. Characterization of the effects provoked by short- and long-term stress on mice behaviour

To induce an MDD-like phenotype in mice we used the widely accepted chronic unpredictable mild stress protocol (CUMS) (Paul Willner, 2017). Additionally, we tested the impact of a short period of stress (STS) to see if the effects of stress are accumulative. Using this approach, we could find that long-term stress (LTS) induces much more severe

depressive-like symptoms than STS (Figure 72). This goes in line with previous research supporting the idea that chronic stress is useful to induce a depressive-like phenotype in mice (P Willner, 2005; P Willner et al., 1992). Specifically, we found that only LTS produces a decrease in mice's body weight. This confirms previous findings suggesting that this could be related to a decrease in food intake (L. Yao et al., 2016) caused by a decline in the appetite and/or an increase in the metabolic rate (Kuti et al., 2022). We could also describe increased anxiety and hyperlocomotion in the open field. This is consistent with previous findings indicating that chronic stress leads to a rise in anxiety that can be assessed with the open field test (Belovicova et al., 2017). Regarding the results obtained in the locomotor activity, although most studies describe decreased locomotion after chronic stress (J. Wu et al., 2021; L. Yao et al., 2016) we observed an increase in this parameter that was persistent throughout all our experiments. This could be interpreted as agitation, a feature that occurs in psychiatric disorders such as depression (Avery & Silverman, 1984) and psychosis (Mohr et al., 2005). Supporting our results, other laboratories also evidenced hyperlocomotion in the open field after stress periods (Boulle et al., 2014; Pardon et al., 2000; Sequeira-Cordero et al., 2019). On the contrary, some studies suggest that this rise in locomotion could be affected by the testing conditions, such as excessive illumination (Strekalova et al., 2011; Strekalova & Steinbusch, 2010). With regards to the parallel index (PI), little is described on how stress could alter navigation strategy. We found an increase in the PI after LTS that could be associated with increased agitation. In fact, our group has already published a rise in the PI in a mouse model of epilepsy (Fernández-García et al., 2020). Thus, the increase in this parameter could be a readout of neuronal damage with implications in the ambulation pattern. Last, behavioural despair was found both in STS and LTS mice. This feature has been widely described after chronic stress (M. Becker et al., 2021). Regarding the results of the STS, little is known about the effect of short periods of stress on behavioural despair. It has been published that with only 10 days of stress, mice already present alterations in this task (Fang et al., 2021). Thus, this evidence suggests that despair is one phenotype observed at the beginning of the stress response. Nevertheless, this result should be taken into caution as the forced swim was used as a stressor itself in both STS and LTS protocols. Although many studies also use the forced swim during the CUMS protocol and then as a behavioural task (Alqurashi et al., 2022; Tianzhu et al., 2014; Y.-L. Wang et al., 2021), it has been demonstrated that the presentation of the forced swim during the stress can alter the results observed in this task as mice are already habituated to it (Cameron et al., 2021). To strengthen

and complement our results, future studies should evaluate behavioural despair using other approaches such as the tail-suspension test.

The global effects of stress on behaviour were summarised in the Z-score graph, which confirmed that there is a progression in the behavioural alterations induced by stress. It is important to highlight that we observed considerable variability in the behavioural tasks assessed, mostly in the LTS group. Regarding this situation, numerous works have shown a remarkable inter-individual variability in animals' responses to stress (Krishnan & Nestler, 2011). To try to overcome this problem, several studies separate resilient and stressed mice using the sucrose preference test (Strekalova et al., 2004; Strekalova & Steinbusch, 2010). Based on the results of this task, resilient animals are either excluded from further experiments or treated as a different cohort. However, the cut-off to identify susceptible mice vary between studies. Thus, it has been proposed that splitting mice based on this test could introduce bias by overestimating the effects of chronic stress (Berrio & Kalliokoski, 2023). Furthermore, in the "rescue" experiments shown throughout this thesis in which we wanted to ameliorate the depressive-like phenotype, doing this stratification would not allow us to distinguish between rescued and resilient mice. Hence, in this thesis, we worked with stressed mice as a whole group without making any subdivision.

Notably, an important issue is that the behaviour was only evaluated in male mice. In our laboratory, we already tried to apply the CUMS to female mice but, in our hands, they were more resistant to the effects of stress as demonstrated in their coat state, weight changes or in their anxiety levels. It is generally acknowledged that chronic stress differently affects male and female mice (Markov & Novosadova, 2022). This also happens in humans, as it is known that women are substantially more prone than males to experience the onset of depression because of stressful conditions (Sherrill et al., 1997). In neurobiology, it is commonly accepted that the lack of use of female mice is justified by the possible influence of hormonal fluctuations (Markov & Novosadova, 2022). Recent studies question this result, as they found that the variability obtained in females was not higher than the one observed in males (J. B. Becker et al., 2016; Beery, 2018; Smarr & Kriegsfeld, 2022). Hence, studying the impact of stress on female mice has become an urgent question in the research field. We suggest that not only is mandatory to find stressors that are sensitive to female mice but also to measure behaviour that is more present and relevant in female MDD patients.

1.2. Specific downregulation of Egr1 in CA1 superficial pyramidal cells during CUMS improves depressive sequelae

Once we described how stress duration affects behaviour, we then aimed to elucidate the specific neuronal subtypes playing pivotal roles in the stress responses. Indeed, there is great interest in how stress affects different neuronal subtypes but surprisingly there is little research on this topic, with most studies to date not distinguishing between neuronal subpopulations (Ramirez et al., 2015; T. R. Zhang et al., 2019).

To identify specific activated cells, we took advantage of Egr1, an active-dependent early gene that has been commonly used in the literature to label specific cells upon concrete events (Longueville et al., 2021; Minatohara et al., 2016). We identified changes in Egr1-dependent activated cells by CUMS in several brain regions, but the most exciting results were found in the CA1 of the hippocampus. In this area, we described that Egr1-dependent activated cells were of neuronal identity and differently affected by short- or long-term stress. Specifically, short-term stress induced an increase of Egr1-activated cells whereas the opposite was found in LTS. In this line, other studies have also shown dynamic changes in Egr1 using different stress paradigms including social defeat, exposure to predators and chronic mild stress (Duclot & Kabbaj, 2017; Gallo et al., 2018). Moreover, some groups have found increased levels of Egr1 in the hippocampus upon acute stress (Cullinan et al., 1995; Hall et al., 2001; Knapska & Kaczmarek, 2004; Melia et al., 1994; Rosen et al., 2005; Schreiber et al., 1991) whereas other groups found decreased Egr1 levels when the stress was continuous in time (Covington et al., 2010; Hodges et al., 2014; Matsumoto et al., 2012) but see (Ieraci et al., 2016; Northcutt & Lonstein, 2009; W.-J. Zhang et al., 2018). Thus, our work reinforces the idea that chronicity is a core characteristic of stress which determines if Egr1 is going to be up- or downregulated. Altogether indicates that Egr1 is very dynamic and sensitive to stress duration (Clements & Wainwright, 2010; Isingrini et al., 2010; W.-J. Zhang et al., 2018; Ziegler et al., 2021) and places the CA1 as a core brain region that differentially reacts depending on the stress chronicity.

Moreover, we could also describe specific alterations in dendritic spine density in the activated cells of the CA1. Although spine atrophy due to chronic stress is something widely described in the CA3 (Y. Chen et al., 2010; Conrad et al., 2012; Krzystyniak et al., 2019; H. Qiao et al., 2014; Qu et al., 2017; Sun et al., 2020) more confusing results have been described in the CA1. In this line, some authors have shown reduced spine density (Leem et al., 2019; Patel et al., 2018; H. Qiao et al., 2014; W.-J. Zhang et al., 2018) or unchanged spine density

(Donohue et al., 2006; Krzystyniak et al., 2019; Qu et al., 2017; Sun et al., 2020) or even increased spine density (Conrad et al., 2012; McLaughlin et al., 2010) in CA1 pyramidal neurons in different stress paradigms. Based on our results, we could hypothesise that changes in dendritic spines in CA1 neurons upon chronic stress could depend on the duration of the stress, the type of dendrites analysed (basal/apical) and be specific to neuronal subpopulation. A potential mechanism by which increased Egr1 activity may lead to decreased spine density in these neuronal ensembles could be by reducing PSD-95 expression and inducing AMPAR endocytosis as previously described (Qin et al., 2015; W.-J. Zhang et al., 2018). These results suggest that stress could be differently affecting the spine density of deep and superficial pyramidal neurons.

We also determined that the type of activated hippocampal neurons is different depending on the stress duration. Concretely, Egr1-dependent activated cells were preferentially deep CA1 pyramidal cells in control conditions whereas Egr1-dependent activated cells were preferentially CA1 superficial pyramidal cells in chronic stress conditions. Hence, we hypothesise that each subpopulation could play different roles in stress-induced MDD. This is consistent with recent studies indicating that both subpopulations could be involved in different processes both in the health and in the diseased hippocampus (Soltesz & Losonczy, 2018).

Indeed, we observed that the modulation of Calb1-positive (superficial) cells *per se* has an impact on the pathophysiology of stress since decreasing Egr1 levels only in this subpopulation produced an improvement in behavioural despair and spatial memory loss. Supporting this idea, it has been shown that Calb1 levels increase in CA1 pyramidal neurons upon stress (Krugers et al., 1996). In line with this, recent data demonstrate that Calb1 levels are increased in susceptible but not resilient mice and that downregulation of Calb1 levels in the ventral hippocampus makes mice more resilient to social defeat stress (Kuga et al., 2023). Furthermore, reducing Calb1 levels in the CA1 is enough to rescue memory loss in stress models (J. T. Li et al., 2017). With all this data, we speculate that with only a few days of stress, the molecular Egr1-dependent events in superficial pyramidal cells are initiated and that all the changes that we observed in 28 days of stress could be just a consequence triggered by those initial changes. This reinforces the idea that preventing this initial Egr1 increase in superficial cells (by hippocampal delivery of shRNA against Egr1) would prevent both, spatial learning deficits and behavioural despair induced by CUMS. Nevertheless, as Egr1 is a critical transcription factor for memory formation (Besnard et al., 2013; Davis et al., 2010; Penke et al., 2014) it is not surprising that its inhibition in basal conditions

(unstressed control mice) is deleterious for proper hippocampal function. All in all, our results place Egr1 as a critical regulator in the hippocampus involved in the behavioural deficits induced by chronic stress.

Lastly, we wanted to understand which molecular mechanisms were underlying the beneficial effects of Egr1 downregulation in superficial CA1 pyramidal neurons. Unfortunately, we could not elucidate the molecules involved, probably because we analysed the total lysated hippocampus without discriminating between neural subpopulations or subfields. Further studies should focus on identifying Egr1 upstream and downstream molecular mechanisms underlying stress-induced sequelae. Specifically, we should address the molecular changes occurring in deep and superficial neurons using cutting-edge technology such as laser capture RNA-seq and /or single-nucleus RNA-seq upon chronic stress. In this line, recent work in epilepsy-mice models already described specific and significant alterations in deep vs superficial cells in response to seizures (Cid et al., 2021). Moreover, we should not forget that both subpopulations are part of the complex hippocampal circuitry and that is not possible to modulate one subtype without influencing the other (Soltesz & Losonczy, 2018). Thus, it is feasible to postulate that Egr1 downregulation in superficial neurons is indeed impacting deep pyramidals.

Despite our results demonstrating that Egr1 downregulation in superficial neurons is beneficial for some sequelae induced by chronic stress, one question that is still unsolved is if the beneficial effects are due to Egr1 downregulation or due to the inhibition of a specific subpopulation. Using optogenetics in each subpopulation should answer this and many other currently unclear questions. Another issue to consider for future experiments is that endogenous Egr1 levels can vary during the oestrous cycle (Duclot & Kabbaj, 2017). Thus, understanding the dynamics of this transcription factor in female mice should give a broader picture of the possible implications of this early gene in the pathophysiology of MDD.

Overall, our work points out the hippocampal CA1 as a sensitive brain region to the duration of chronic stress and that Egr1 in the superficial CA1 pyramidal neurons may play a crucial role in stress-induced sequelae such as behavioural despair and impaired spatial learning (Figure 71). This work shed light on the heterogeneity inside the CA1 of the hippocampus and suggests that each subpopulation could have different types of response upon stressful events. Thus, there is a need for using modern tools to understand the genetic, molecular, structural, and functional differences between subpopulations in disease conditions to find specific therapeutical tools that could correct these dysfunctions.

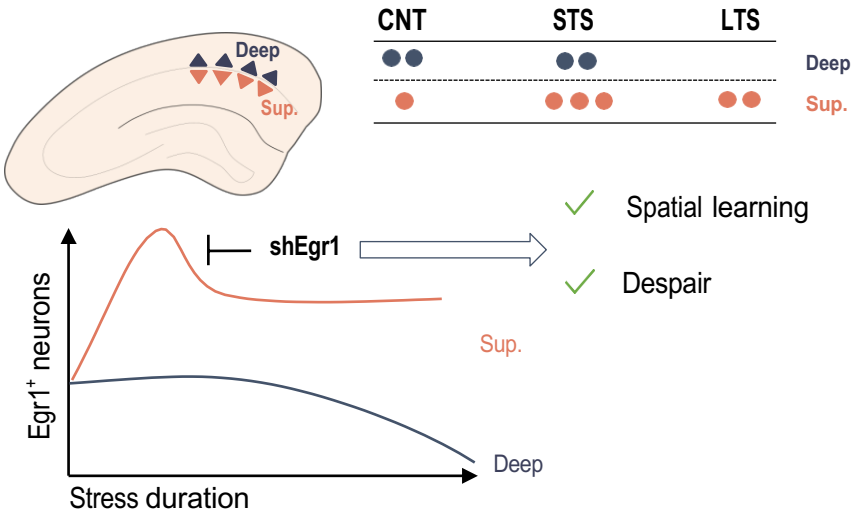


Figure 71. The hippocampus is sensitive to the stress duration and Egr1 in superficial CA1 neurons is important for stress-induce sequalae. In basal conditions (CNT mice) there are Egr1-dependent activated cells that are deep and superficial pyramidal neurons. After short-term stress, there is a global increase in the number of activated cells, both superficial and deep pyramidal neurons. After long-term stress, there is a general decrease in the number of activated cells, but the remaining activated neurons are mostly superficial pyramidal neurons. Thus, there is a change in the percentage of superficial activated cells over stress duration. Therefore, the inhibition of Egr1 in superficial cells using an shEgr1 is beneficial and improves spatial learning and behavioural despair.

1.3. Progressive hippocampal proteomic alterations induced by stress

As already mentioned, the hippocampus is hugely implicated in the stress response, and it is tremendously affected by stress-induced MDD (Tartt et al., 2022). Thus, understanding the proteomic changes occurring during stress progression could shed light on new molecular pathways specifically altered in this brain region.

In this thesis, we analysed total hippocampal lysates and found that LTS mice present much more hippocampal alterations at the proteomic level than STS mice (Figure 72). A more detailed analysis of the proteins altered after chronic stress revealed an important dysregulation of the Sirt1 pathway. Ulterior experiments revealed increased levels of this protein in the hippocampus after chronic stress. Sirt1 is an NAD^+- dependent histone deacetylase (HDAC) which regulates the levels of acetylation and deacetylation of its countless substrates. Thus, its activity (and in turn its dysregulation) hugely impacts cell homeostasis as it is involved in epigenetic modifications but also mitochondrial function and mitophagy, among other important cell functions (D'Angelo et al., 2021; Song et al., 2019). Previous literature has already described an implication of Sirt1 in stress pathologies.

Whereas some papers demonstrate that Sirt1 is raised in the hippocampus (but also in other brain regions) after stress (Ferland & Schrader, 2011; W. Li et al., 2019) other works suggest the opposite and described a fall of Sirt1 expression in stress disorders (Abe-Higuchi et al., 2016; Morató et al., 2022) which illustrates the complexity of this pathway regulation. Taken altogether evidence places Sirt1 as an interesting molecule involved in stress-induced MDD.

Despite we described increased levels of Sirt1, we did not address how these changes in expression lead to changes in Sirt1 activity. In this line, previous literature supports the idea that increased expressions does not always relate to higher activity (Elibol & Kilic, 2018). As Braidy and colleagues (Braidy et al., 2011) indicated, decreased Sirt1 activity was found in aged mice with higher levels of this protein. This could be understood as a compensatory mechanism and could advocate that the decline in the activity of Sirt1 may not be directly associated with its protein levels but also with its downstream or upstream molecules. Actually, studies support this hypothesis and suggest that NAD^+ levels are highly associated with Sirt1 activity (Ramsey et al., 2008). Besides, a recent study has shown that Sirt1 undergoes alternative splicing to generate a shorter isoform with minimal deacetylase activity (Shlomi et al., 2022). They show that this shorter isoform is increased in stressed female (but not male) mice and could be associated with cognitive alterations and with BDNF signalling. Thus, measuring Sirt1 activity or its specific isoforms rather than its total levels could strengthen our results and give us more robust findings.

With all this information, what seems clear is that Sirt1 dysregulation affects mood and could be related to MDD pathogenesis. One possible mechanism that could explain this effect is Sirt1 regulation of serotonin (5-HT). Previous evidence has shown that overexpression of Sirt1 in the brain leads to increased anxiety and decreased levels of 5-HT via the activation of monoamine oxidase (MAO) (Libert et al., 2011). Thus, it could be hypothesised that the increased levels of Sirt1 found after chronic stress are inducing a decrease in 5-HT and contributing to the depressive-like phenotype. Therefore, finding new Sirt1 targets affected by stress is crucial and could give insight into novel therapeutic opportunities.

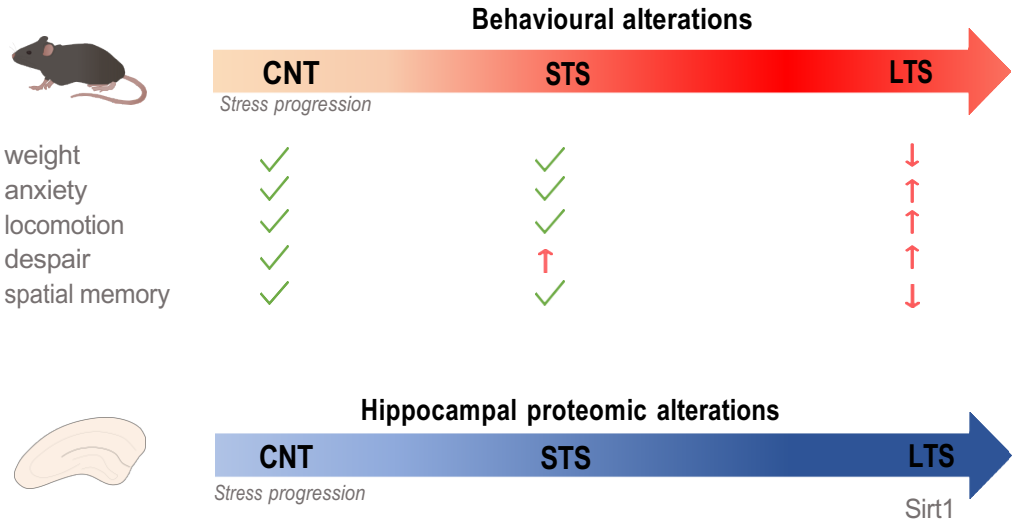


Figure 72. Progressive molecular and behavioural alterations induce by stress. After short-term stress, mice present behavioural despair. After long-term stress, mice present several behavioural alterations, including decreased body weight; increased anxiety, locomotion, and behavioural despair; and spatial memory deficits. At the proteomic level, the effects of stress are also accumulative as after short-term stress a few proteins are altered, whereas after long-term stress, many proteins are dysregulated. One of the main proteins altered is Sirt1, which expression is increased in LTS mice.

Taken together, the results from this first part of the Thesis indicate that there is a progression in the alterations induced by stress at the behavioural, cellular, and molecular levels. We also conclude the hippocampus is highly sensitive to stress and that superficial pyramidal neurons of the CA1 are crucial for the MDD pathophysiology. Lastly, we propose Egr1 and Sirt1 as important molecules involved in the stress response.

2. TISSUE-COMBINED PHOTOBIOMODULATION AS A NON-INVASIVE THERAPY TO TREAT STRESS-INDUCED MDD

Next, we focused on the gut-brain axis. In recent years the complex bidirectional communication between the microbiome-gut-brain axis has emerged as an important system affected in major depression (Long-Smith et al., 2020). How this axis works is not completely understood and a major question that needs to be addressed is the causality in the connection between the two of them, which will support the understanding of the role that the gut microbiota plays in depression (Cryan et al., 2019). In this line, mounting evidence suggests that targeting the gut microbiome could be an interesting therapeutic option for

MDD(Schaub et al., 2022). Not only this, but it has been advised that gut microbiome and its produced metabolites could serve as an indicator of good ADs response (Dong et al., 2022). In this thesis, we used for the first-time tissue-combined photobiomodulation (PBM) to target both the brain and the gut (and its microbiome) at the same time.

Despite PBM has already been used in patients, little is known about which brain cells are regulated by this approach. Hence, we first described which cells are being controlled by PBM by looking for Egr1-dependent activated neurons. Interestingly, we found fewer activated cells after PBM, suggesting that PBM modulates brain activity. As already explained in the introduction, PBM's main cellular effects are thought to occur via the mitochondria (de Freitas & Hamblin, 2016). Therefore, the regulation of mitochondrial function with PBM could explain the associated changes in neural activity. Moreover, our photobiomodulation device is surrounded and preceded by a ring magnet which creates a static magnetic field of 200mT. Thus, we cannot rule out the possibility that the downregulation of activated cells found after PBM could be due to this static magnetic field. Some studies have shown that static magnetic fields can induce effects on the human brain and interfere with neuronal activity (Hernando et al., 2020). This is not surprising as cells present electric properties that can be altered by magnetic fields. Although some articles suggest decreased neural activity after the application of a static magnetic field (Aguila et al., 2016; McLean et al., 2008), the biological explanation underlying this process is not clearly understood. Indeed, the fact that mice receiving stimulation only in the abdomen also present fewer Egr1-positive cells reinforces this idea. It is plausible thinking that the reduction of Egr1-activated neurons with PBM could be beneficial as we could be preventing the rise in Egr1 observed during the first days of stress. As discussed above, blocking Egr1 in superficial pyramidal cells is useful for some deficits induced by chronic stress. It could be interesting to study if PBM is decreasing Egr1 levels in both subpopulations and if superficial cells are more susceptible to PBM than deep neurons.

Then, we aimed to use PBM to modulate not only the depressive-like phenotype induced by CUMS but also the microbiome alterations observed after chronic stress. In emotional tasks, we could not find any improvement after PBM treatment. Contrary to the findings of other groups, we could not find a beneficial effect of PBM in either anxiety or behavioural despair (Eshaghi et al., 2019; Meynaghizadeh-Zargar et al., 2020; Salehpour, Farajdokht, Cassano, et al., 2019; D. Zhang et al., 2021). This could be explained by several reasons. First, the characteristics of the PBM procedure differ in each study (intensity, type of light and duration of the stimulation). Secondly, the use of different mice's ages, strains and sexes also affect

the behavioural outcomes. Third, each study uses a different stress protocol, which can modify mice's behaviour. Additionally, other groups have also shown no effect on anxiety and in the fear-conditioning test, further supporting our results (X. Zhang et al., 2022). On the other hand, we found that some cognitive symptoms induced by chronic stress were prevented or at least ameliorated by using this type of PBM that allowed us to simulate different tissues simultaneously. This result was accompanied by the prevention of spine density loss in the CA1 of the hippocampus. This result ties in well with previous studies wherein PBM was used to improve cognitive sequelae and increase PSD95 puncta in models of temporal lobe epilepsy and cognitive impairment (Hong et al., 2023; X. Zhang et al., 2022).

Despite the structural changes observed at the hippocampal CA1, we wanted to also determine if PBM could have an impact at the metabolomic level. Indeed, some studies claim that chronic PBM can restore some metabolites altered in aged mice (dos Santos Cardoso et al., 2021). Although we could not detect tremendous changes in the metabolites analysed (probably due to the huge variability within groups), we found interesting data when associating the levels of some metabolites with the behavioural outcome. GSH (the main endogenous antioxidant compound of the organisms (Zalachoras et al., 2020), and spermine (a polyamine involved in cellular metabolism) (Frühauf-Perez et al., 2018) were lower in those stressed mice not receiving PBM with the worst behavioural performance. This goes in line with literature supporting the idea that GSH is altered in stress pathologies and that GSH enhancers could be useful for the treatment of MDD (Zalachoras et al., 2020). A similar conclusion was obtained regarding spermine, as some studies support its implication in stress-related pathologies (Genedani et al., 2001; Zomkowski et al., 2002) and propose this metabolite as an interesting therapeutic target (Fiori & Turecki, 2008). Last, nicotinamide (a form of vitamin B3)(Song et al., 2019) levels were closely associated with the performance in the cognitive tasks in the stressed mice without PBM treatment. Specifically, nicotinamide levels were decreased in those stressed mice with important cognitive deficits. This metabolite has been already associated not only with stress exposure (Morató et al., 2022) but also with cognitive impairment and ageing (Lautrup et al., 2019). Interestingly, nicotinamide is rapidly converted inside the cell to NAD^+. As discussed above, NAD^+ levels regulate Sirt1 activity, which is heavily affected in our chronically stressed mice. In this line, several papers suggest that Sirt1 activity is tightly related to nicotinamide/NAD^+/NADH levels (Braidy et al., 2011; Song et al., 2019). With all this information, we postulate that the alterations in nicotinamide levels observed in chronically stressed mice without PBM could

be contributing to the changes in Sirt1 expression. We cannot rule out that changes in nicotinamide levels could be also affecting Sirt1 activity.

In this line, we next studied the levels of Sirt1 in the hippocampus of the stressed mice treated with PBM. Again, we could detect higher levels of this protein in the stressed hippocampus when compared with CNT conditions. Interestingly, this was prevented in those mice receiving stimulation in the abdomen or the double-stimulated mice. This outcome indicates somehow that gut stimulation is mediating the beneficial effects of PBM regarding Sirt1 expression. Further studies should decipher how the changes in Sirt1 expression observed after stress and recovered after PBM impact their final function. In addition, it could be interesting to explore by which mechanisms PBM in the abdomen can alter Sirt1 expression. A possible explanation could be via the alteration of gut microbiota, which is known to produce several metabolites such as butyrate and other short-chain fatty acids (SCFA) that can modulate HDACs function in the brain (Begum N, Mandhare A, Tryphena KP, Srivastava S, Shaikh MF, 2022).

To explore this possibility the gut composition was analysed. It is widely accepted that stress-induced MDD is associated with gut dysbiosis and that this could be an important mechanism for the progression, severity and ADs efficacy (Rathour et al., 2023). Moreover, it has been suggested that PBM could impact the gut microbiome and be useful for pathologies associated with gut alterations such as MDD. However, little is known about how PBM could alter the microbiome and research in this area is scarce. In this thesis, we confirmed previous research and found dysbiosis in mice that underwent chronic stress. We also described modest but exciting evidence when performing the analysis at the genus level. Specifically, we showed interesting data in the genus *Enterorhabdus* and *Roseburia*. Regarding *Enterorhabdus,* it has been established that MDD patients present fewer levels of this bacteria. Additionally, it has been proved that some genera of the *Coriobacteria* class (the class to which *Enterorhabdus* belongs) are important regulators of the effects of the AD ketamine. Indeed, it has been postulated that the antidepressant effects of ketamine may be partially mediated by the composition of the gut microbiota (Huang & Lin, 2020). Although little is described about *Enterorhabdus* specifically, the fact that members of the same class could modulate ketamine response suggests that *Enterorhabdus* could also be involved in this mechanism. Along the same line, a recent study performed on patients suffering from geriatric depression has shown that *Roseburia*, a butyrate-producing bacteria, could be involved in the remission of MDD upon specific AD treatment (S. M. Lee et al., 2022). It is important to remind that butyrate is a type of SCFA that can heavily impact brain function (Agus et al., 2021; Caspani

et al., 2019). Among the characteristics associated with SCFA and specifically butyrate, it is noteworthy its function as an epigenetic modulator (Begum N, Mandhare A, Tryphena KP, Srivastava S, Shaikh MF, 2022). In particular, butyrate is an important HDAC inhibitor (Schroeder et al., 2007). Thus, bacteria-producing butyrate impact on several HDACs, including Sirt1 (Begum N, Mandhare A, Tryphena KP, Srivastava S, Shaikh MF, 2022). This could in part explain the restoration of Sirt1 expression after PBM in the abdomen or in both the head and abdomen.

Moreover, it has been proposed that microbiota is essential for normal neuronal morphology and that bacteria dysbiosis can heavily disrupt appropriate synapse formation. In this line, studies using germ-free mice indicate that mice lacking microbiome present alterations in neuron branching and brain network connectivity (Aswendt et al., 2021; Luczynski et al., 2016). Hence, our results regarding the prevention of spine loss after stress when PBM is applied to the head or in both the head and abdomen could be, in part, associated with microbiome restoration. Although how microbiota changes can lead to alterations in brain ultrastructure is unclear, the activation of pro-inflammatory molecules such as cytokines has been proposed as a possible mechanism (J.-C. Zhang et al., 2017). To further elaborate on this hypothesis, general markers of inflammation were evaluated.

Indeed, several studies have proposed that the beneficial effects observed after PBM could be due to a reduction of inflammatory markers (Hamblin, 2017). Hence, we performed an analysis of the inflammation status in the hippocampus of mice after stress and PBM. We found an increase in GFAP immunoreactivity in stressed mice that were blocked after PBM stimulation. In this line, compelling evidence shows that astrocyte dysfunction and inflammation are associated with the pathogenesis of MDD (Cho et al., 2022; X. Zhou et al., 2019). However, the molecular processes controlling astrocyte-mediated neuroinflammation in depression remain elusive (Leng et al., 2018). In MDD post-mortem samples a decline in the number of astrocytes and changes in astrocyte morphology and volume have been described in different brain regions (O'Leary et al., 2021; Rajkowska & Stockmeier, 2013; Si et al., 2004; Webster et al., 2005). Contrary to these findings, not all MDD mouse models recapitulate this phenotype despite astrocytic alterations still being present (Aten et al., 2023; Naskar & Chattarji, 2019; Tynan et al., 2013). Here, we observed a rise in GFAP expression in the *stratum radiatum* of the hippocampal. Similarly, other groups also reported increased GFAP expression in the hippocampus, hypothalamus and spinal cord of stress mice models (Golovatscka et al., 2012; Lambert et al., 2000; Yunhui Liu et al., 2021; Marco et al., 2013). A possible explanation for this discrepancy might be the stress protocol used, the

hippocampal subregion analysed and the methodological approach for which parameters are used to analyse reactive astrocytes. Regarding the microglia results, we could confirm previous evidence and found increased levels of Iba1 after chronic stress. Interestingly, this was reduced in all PBM-treated mice. Supporting these results, changes in Iba1 expression and morphology have been reported in several stress animal models (Calcia et al., 2016; Troubat et al., 2021). Thus, it is widely accepted that stress exposure activates microglia although how microglia activation leads or contributes to the depressive phenotype is unclear. Additionally, some studies using PBM reported decreased Iba1 levels after the treatment (U.-J. Kim et al., 2022; X. Wang et al., 2021). The fact that all PBM treatments despite their site of action reduce both GFAP and Iba1 levels is curious. Encouraging our results, an interesting study in a Parkinson's disease mice model evaluated the hypothesis that PBM's beneficial effects are not only related to PBM targeting the brain but also its effects on the whole body. To test this theory, they compare delivering normal PBM to the head with delivering light to a mouse in which the head was covered with aluminium foil. They discovered that irradiation to the head had a positive influence on mice behaviour, but that there was also a statistically significant (although less clear effect), effect when the head was shielded from light (D M Johnstone et al., 2014; Daniel M Johnstone et al., 2015). Hence, we speculate that the brain will still benefit from the positive effects of PBM despite not being the specific target for treatment. This could explain why applying PBM to the abdomen can affect neuroinflammation in the brain. Another possibility to consider is that with the abdominal PBM we could be stimulating the vagus nerve terminals, and this could be affecting gut-brain communication and finally brain function. A reasonable method to validate this hypothesis would be the excision of the vagus nerve, which should give us more information about the importance (or not) of the vagus nerve and the gut microbiome in PBM's beneficial effects. If this axis is necessary, mice with disrupted vagus nerve will not induce any type of cognitive/neurological improvement.

One possible mechanism by which PBM could be reducing the inflammation in the hippocampus is through the regulation of NF-κB (Lim et al., 2013). It has been suggested that the light absorption by the mitochondria produces a rise in the ROS levels that finally inhibits NF-κB translocation to the nucleus, thus repressing the transcription of pro-inflammatory genes (Hennessy & Hamblin, 2017). Moreover, it has been shown that Sirt1 plays an important role in the inflammatory response also via the modulation of NF-κB

(Yunshu Yang et al., 2022; Yeung et al., 2004). Decreased activity of Sirt1 has been associated with the activation of NF-κB. With all this information considered, we could theorise that the dysfunction of Sirt1 expression observed after CUMS can be impacting the inflammatory phenotype and the restoration of Sirt1 levels after PBM could be associated with the improvement at the inflammatory level.

Despite several questions remain unanswered at present, our work suggests that tissue-combined PBM can be beneficial in the cognitive deficits, hippocampal spine alterations, inflammation and metabolic modifications induced by chronic stress. Moreover, we proved that PBM can modulate the gut microbiome and regulate Sirt1 expression (Figure 73). Future work should shed light on the specific molecular mechanisms responsible for the beneficial effects of PBM. In this line, it has been proposed that some beneficial effects of PBM occur through the inhibition of GSK3β. Specifically, PBM activates Akt which will, in turn, inhibit GSK3β (Liang et al., 2012). It could conceivably be hypothesised that GSK3β inhibition with PBM could play a role in the beneficial effects observed. In the next section, we will further discuss how GSK3β inhibition with a pharmacological approach is beneficial in the context of stress-induced major depression.

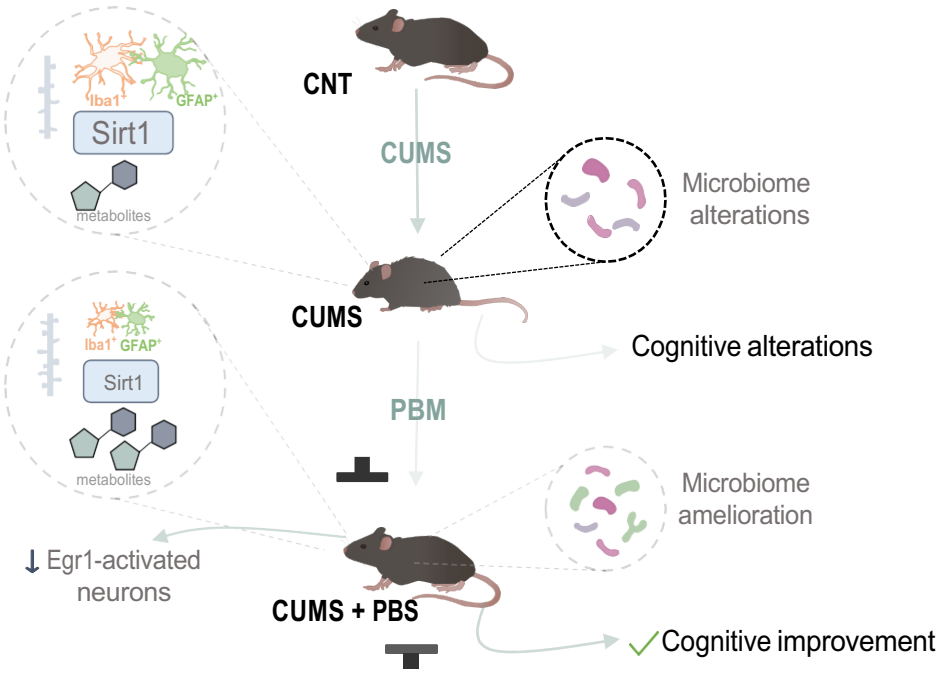


Figure 73. Tissue-combined photobiomodulation improves important alterations induced by chronic stress. After the CUMS protocol, mice present several hippocampal changes including a decrease in the CA1 spine density, a decline in the levels of different metabolites (i.e. NAD^+), an increased expression of Sirt1 and a rise in neuroinflammation. Chronic stress also induces microbiome dysbiosis, mostly by increasing the number of pro-inflammatory bacteria. All these changes are associated to cognitive alterations. When the CUMS protocol is combined with PBM both in the head and in the abdomen, several parameters are recovered. There is an increase in the spine density, a partially recovery of some metabolites, a restoration of Sirt1 levels

and a decrease in neuroinflammation. Moreover, there is also an amelioration of the microbiota. All these improvements lead to a better cognitive performance in different behavioural tasks.

3. MERIDIANINS INHIBIT GSK3β *IN VIVO* AND IMPROVE BEHAVIOURAL ALTERATIONS INDUCED BY CHRONIC STRESS

In the last part of this thesis, we intended to use a pharmacological approach to try to modulate a master molecule altered in mood disorders, GSK3β (R. S. Jope, 2011). Despite the importance of GSK3β as a potential therapeutic target, only a few of the many inhibitors that have been developed have reached clinical trials in human subjects (Arciniegas Ruiz & Eldar-Finkelman, 2022). Several inhibitors have failed at various preclinical and clinical stages, mainly due to a lack of effects or excessive toxicity (Bhat et al., 2018).

In this study, we first confirmed that chronic stress induces the hyperactivation of GSK3β in the hippocampus. Our results are consistent with previous research indicating that both the activity and the expression of GSK3β are altered in MDD patients (Karege et al., 2007; Oh et al., 2010) and in stress-based mice models (R. Silva et al., 2008; Wilkinson et al., 2011). Then, we aimed to modulate *in vivo* GSK3β with a novel approach, meridianins administration. Meridianins, a marine natural product obtained from a mollusc living in Antarctica, have been described as potent GSK3β inhibitors. Moreover, natural-marine products have gained popularity in recent years due to their ease of degradation, environmental friendliness, and unique mechanisms of action.

We first confirmed that intraventricular meridianins administration can inhibit GSK3β in different brain areas, including the hippocampus *in vivo*. Then, we further explored whether meridianins could modulate other proteins involved in GSK3β signalling. In our study, we found increased levels of PKC phosphorylated substrates in the hippocampus that were accompanied by increased phosphorylation of GluR1 at Ser831. This is in line with previous research that points out PKC as one of the main kinases phosphorylating GluR1 specifically at this site (Franco & Palermo, 2003; Tibiletti et al., 2010). This phosphorylation has been linked to enhanced synaptic plasticity and long-term potentiation (LTP) (Karpov et al., 2005). Moreover, studies using mice lacking this phosphorylation showed deficits in hippocampal tasks such as spatial learning (Echalier et al., 2008). This supports the idea that the GluR1

phosphorylation at specific Ser residues is crucial for memory formation and that meridianins can modulate the GluR1 activity. In this line, alterations in the levels and function of GluR1 have been already described in patients with mood disorders (Giraud et al., 2011) and in mouse models of depression (Mohan et al., 2018; W. Young et al., 2022).

To verify that meridianins could modulate synaptic activity, we performed electrophysiological recordings in acute hippocampal slices. Several studies reported reduced synaptic activity after CUMS (S. C. Liu et al., 2019) and in other mouse models of stress (Schnell et al., 2012; Yu Yang et al., 2018). These alterations are often associated with cognitive deficits and changes in dendritic spines' density (S. C. Liu et al., 2019). Here, we found an increase in synaptic activity specifically in the CA1 after meridianins administration that could be beneficial in a disease context such as MDD. In agreement with our findings, GSK3β activity has been associated with decreased excitability, whereas GSK3β inhibition is necessary for LTP induction, a process underlying new memory formation (Duda et al., 2020).

Last, we used the CUMS protocol to induce a depressive-like phenotype. We observed that meridianins administration during CUMS was able to ameliorate some of the important sequelae induced by chronic stress, such as the decrease in body weight and the increase in anxiety. We also described a clear effect of meridianins in cognition that could be associated with an enhancement of synaptic transmission. One interesting hypothesis of this improvement in cognitive tasks is that merdianins could increase the phosphorylation levels of GluR1, although we cannot rule out other possibilities (Kallarackal et al., 2013; Y. Xu et al., 2015). We could hypothesise that meridianins regulate PKC and that the activation of this kinase is leading to GSK3β inhibition and increase GluR1 phoshporylation. Another interesting hypothesis is that Sirt1 dysregulation could in turn affect GSK3β activity. In this line, it has been indicated that Sirt1 can deacetylase Akt, making Akt more active (Pillai et al., 2014). Akt activation will provoke an increase in the phosphorylation of GSK3β, thus causing its inhibition (Hermida et al., 2017). We could speculate that the dysregulation of Sirt1 found after chronic stress will cause less activation of Akt that consecutively will produce GSK3β hyperactivity. To test this hypothesis, further work should be focused on measuring GSK3β activity in those stressed mice that received PBM and in which we know that Sirt1 levels are restored. Alternatively, a feasible approach to test this possibility would be to measure the levels of Sirt1 in those mice treated with meridianins.

Moreover, although we demonstrated GSK3β inhibition by meridianins, we cannot exclude the possibility of alternative mechanisms of action that could be mediating the improvements observed in the stressed mice when treated with meridianins. In this line, different groups have shown that besides GSK3β, meridianins are able to inhibit CDK5, casein-kinase 1 delta, and dual-specificity kinases as dual specificity tyrosine phosphorylation regulated kinase 1, among others (Bharate et al., 2012; Llorach-Pares et al., 2022). Although little is known about the dysregulation of these kinases in depression, we cannot exclude the possibility that our effects are due to the modulation of these other kinases.

Finally, it is reasonable to wonder which GSK3β substrates could mediate its effect on mood regulation and how GSK3β inhibition can contribute to an improvement of the MDD phenotype. However, little is known about GSK3β specific substrates in the context of stress-related disorders (Duda et al., 2020). Furthermore, future work should clarify whether all the meridianins administered, or only some of them, or perhaps some of them acting synergistically, are the directly responsible molecules for the observed effects. These could be achieved by using synthesized molecules, although not all the meridianins have been synthesized so far (Echalier et al., 2008; Franco & Palermo, 2003; Karpov et al., 2005; Walker et al., 2014).

Altogether, in this last part of the Thesis, we have provided compelling evidence of the beneficial effects of meridianins administration in chronically stressed mice (Figure 74).We have demonstrated that meridianins inhibit GSK3β in vivo and we have deepened our understanding of the molecular pathways involved in such inhibition. We finally have shown that intraventricularly delivered meridianins ameliorate several sequelae induced by chronic stress.

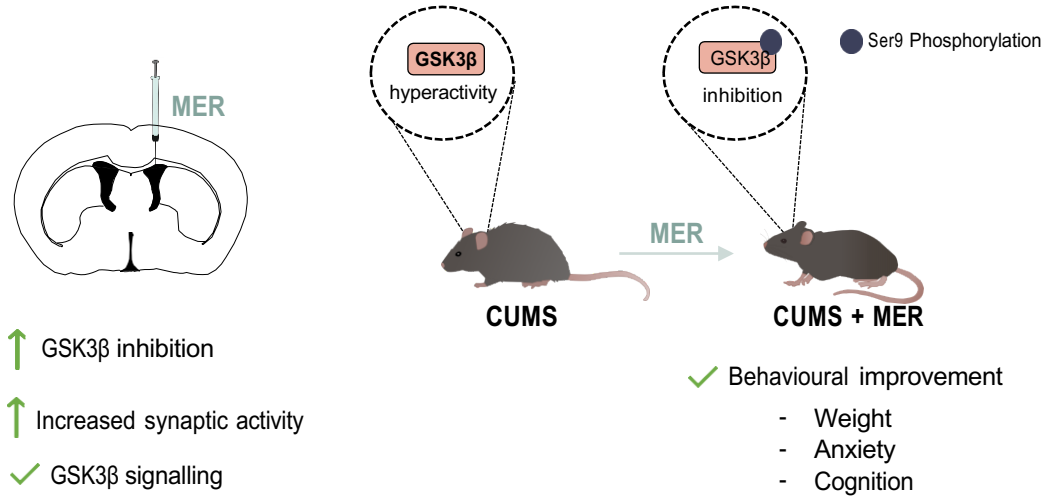


Figure 74. Meridianins improve behavioural deficits induced by CUMS. In basal conditions, intraventricular meridianins administration inhibits GSK3β in the hippocampus, increases spontaneous synaptic activity in the CA1 and regulates kinases involved in GSK3β signalling. In chronically stressed mice, GSK3β is hyperactivated, causing behavioural alterations in different tasks. When the CUMS protocol is combined with meridianins administration, GSK3β inhibition leads to a behavioural improvement.

In summary, the hippocampal pathogenic mechanisms demonstrated in this Thesis provide a great and novel insight into the pathophysiology of stress-induced major depression (Figure 73). Furthermore, we put forth a variety of cutting-edge strategies to attempt to reverse the alterations induced by stress at different levels: behavioural, cellular, and molecular. Altogether, we demonstrated that Egr1, Sirt1 and GSK3β dysregulation is occurring in the stressed hippocampus. Moreover, although in each section we tried to modulate one of these molecules, compelling evidence suggests that these proteins could be related. Indeed, Egr1 is known to regulate GSK3β and Sirt1(Rouillard et al., 2016). Thus, it could be speculated that the modulation of one of these molecules could affect the others. Hence, we could think that the different therapeutical strategies applied in this thesis could be modulating different aspects of the pathology of MDD by targeting different hippocampal altered pathways.

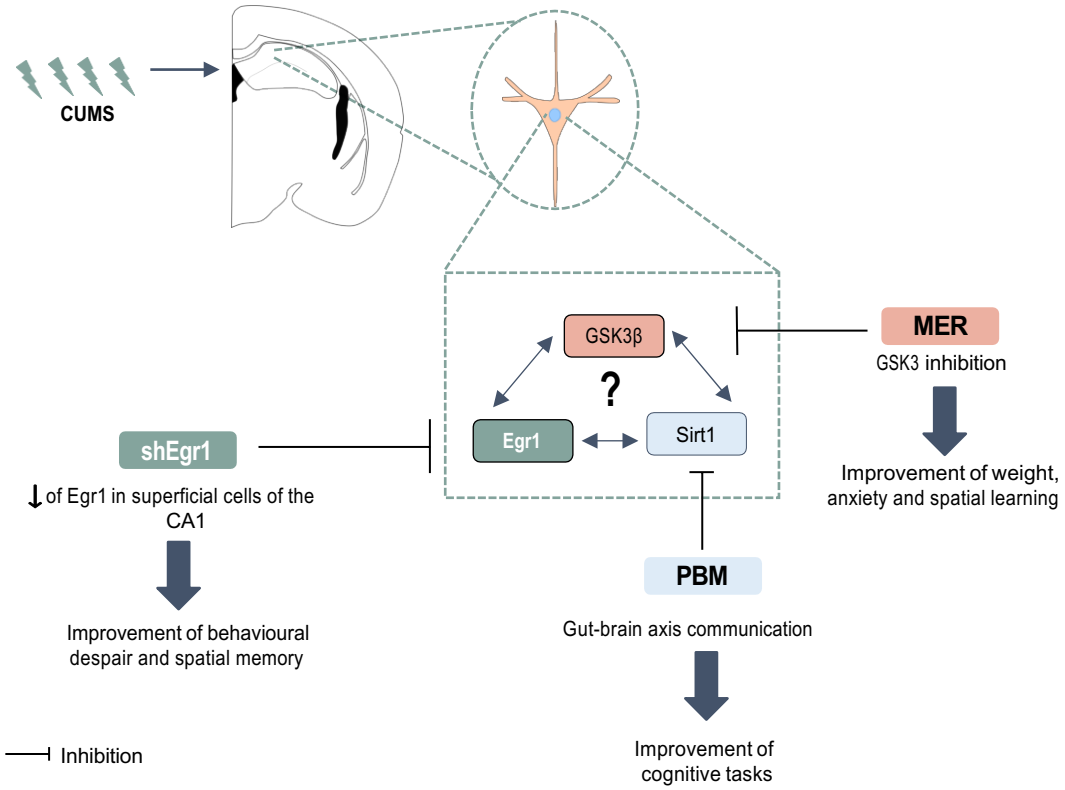


Figure 75. Chronic stress induces important hippocampal alterations leading to behavioural impairment. Schematic illustration of this thesis in which we first described the hippocampus as one of the main regions sensitive to stress. Moreover, we studied three different molecules/pathways altered in pyramidal neurons and provide new knowledge about its contribution in stress-induced major depression. Last, we applied different approaches to modulate each molecule and improve the sequelae induced by chronic stress.

CONCLUSIONS

The main conclusions extracted from the results presented in this Thesis are the following:

1. There is a progression in the depressive-like symptoms induced by stress since long, but not short-term stress, produces an increase in anxiety and agitation, a decrease in body weight and alterations in spatial working memory.

2. The hippocampal CA1 is a brain region very sensitive to the duration of stress. Short periods of stress increase the number of Egr1-activated neurons whereas long periods of stress produce a decrease in the number of Egr1-activated neurons.

3. There is an increase in the proportion of Egr1-dependent activated neurons which are in turn Calb1-positive neurons (superficial pyramidal neurons) concomitant with the stress duration.

4. The inhibition of Egr1 expression in superficial pyramidal neurons (Calb1+) of the CA1 improves some depressive-like phenotypes observed in chronically stressed mice such as behavioural despair and spatial learning.

5. Hippocampal proteomic alterations are pronounced after long-term stress and the Sirt1 pathway is the most affected. In fact, Sirt1 is overexpressed in the hippocampus of chronically stressed mice suggesting its involvement in stress-induced major depression.

6. Photobiomodulation produces a decrease in the number of Egr1-activated neurons in the hippocampus.

7. Tissue-combined photobiomodulation ameliorates cognitive but not emotional tasks induced by chronic stress, likely by the rescue of CA1 hippocampal spine loss induced by chronic stress.

8. Tissue-combined photobiomodulation induces changes in the microbiome, increases the levels of some important brain metabolites, decreases the expression of Sirt1 and diminishes neuroinflammation in the hippocampus of stressed mice.

9. GSK3β is hyperactivated in the hippocampus of chronically stressed mice and meridianins can inhibit this activation, probably through the modulation of PKC.

10. Chronic treatment with meridianins ameliorates several sequelae induced by chronic stress.

REFRENCES

Abe-Higuchi, N., Uchida, S., Yamagata, H., Higuchi, F., Hobara, T., Hara, K., Kobayashi, A., & Watanabe, Y. (2016). Hippocampal Sirtuin 1 Signaling Mediates Depression-like Behavior. *Biological Psychiatry*, *80*(11), 815–826. https://doi.org/https://doi.org/10.1016/j.biopsych.2016.01.009

Aguila, J., Cudeiro, J., & Rivadulla, C. (2016). Effects of Static Magnetic Fields on the Visual Cortex: reversible Visual Deficits and Reduction of Neuronal Activity. *Cerebral Cortex (New York, N.Y. : 1991)*, *26*(2), 628–638. https://doi.org/10.1093/cercor/bhu228

Agus, A., Clément, K., & Sokol, H. (2021). Gut microbiota-derived metabolites as central regulators in metabolic disorders. *Gut*, *70*(6), 1174–1182. https://doi.org/10.1136/gutjnl-2020-323071

Ait Chait, Y., Mottawea, W., Tompkins, T. A., & Hammami, R. (2020). Unravelling the antimicrobial action of antidepressants on gut commensal microbes. *Scientific Reports*, *10*(1), 17878. https://doi.org/10.1038/s41598-020-74934-9

Ali, S. H., Madhana, R. M., K V, A., Kasala, E. R., Bodduluru, L. N., Pitta, S., Mahareddy, J. R., & Lahkar, M. (2015). Resveratrol ameliorates depressive-like behavior in repeated corticosterone-induced depression in mice. *Steroids*, *101*, 37–42. https://doi.org/10.1016/j.steroids.2015.05.010

Alonso, M., Vianna, M. R. M., Depino, A. M., Mello e Souza, T., Pereira, P., Szapiro, G., Viola, H., Pitossi, F., Izquierdo, I., & Medina, J. H. (2002). BDNF–triggered events in the rat hippocampus are required for both short- and long-term memory formation. *Hippocampus*, *12*(4), 551–560. https://doi.org/https://doi.org/10.1002/hipo.10035

Alqurashi, G. K., Hindi, E. A., Zayed, M. A., Abd El-Aziz, G. S., Alturkistani, H. A., Ibrahim, R. F., Al-thepyani, M. A., Bakhlgi, R., Alzahrani, N. A., Ashraf, G. M., & Alghamdi, B. S. (2022). The Impact of Chronic Unpredictable Mild Stress-Induced Depression on Spatial, Recognition and Reference Memory Tasks in Mice: Behavioral and Histological Study. In *Behavioral Sciences* (Vol. 12, Issue 6). https://doi.org/10.3390/bs12060166

American Psychiatric Association. (2022). Diagnostic and Statistical Manual of Mental Disorders. In *Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Association Publishing. https://doi.org/10.1176/APPI.BOOKS.9780890425787

Amick, H. R., Gartlehner, G., Gaynes, B. N., Forneris, C., Asher, G. N., Morgan, L. C., Coker-Schwimmer, E., Boland, E., Lux, L. J., Gaylord, S., Bann, C., Pierl, C. B., & Lohr, K. N. (2015). Comparative benefits and harms of second generation antidepressants and cognitive behavioral therapies in initial treatment of major depressive disorder: systematic review and meta-analysis. *BMJ*, *351*, h6019. https://doi.org/10.1136/bmj.h6019

Amirkhanzadeh Barandouzi, Z., Starkweather, A. R., Henderson, W. A., Gyamfi, A., & Cong, X. S. (2020). Altered composition of gut microbiota in depression: A systematic review. *Frontiers in Psychiatry*, *11*(June), 1–10. https://doi.org/10.3389/fpsyt.2020.00541

Anacker, C., Zunszain, P. A., Cattaneo, A., Carvalho, L. A., Garabedian, M. J., Thuret, S., Price, J., & Pariante, C. M. (2011). Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. *Molecular Psychiatry*, *16*(7), 738–750. https://doi.org/10.1038/mp.2011.26

Andersen, P., Morris, R., Amaral, D., Bliss, T., & O'Keefe, J. (Eds.). (2006). *The Hippocampus Book*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780195100273.001.0001

Ando, T., Xuan, W., Xu, T., Dai, T., Sharma, S. K., Kharkwal, G. B., Huang, Y.-Y., Wu, Q., Whalen, M. J., Sato, S., Obara, M., & Hamblin, M. R. (2011). Comparison of therapeutic effects between pulsed and continuous wave 810-nm wavelength laser irradiation for traumatic brain injury in mice. *PloS One*, *6*(10), e26212. https://doi.org/10.1371/journal.pone.0026212

Angulo-Preckler, C., Castro-Fernandez, P. De, Martín-Martín, R., Figuerola, B., & Avila, C. (2020). Chemical ecology in the Southern Ocean. In *Life in Extreme Environments* (pp. 251–278). Cambridge University Press. https://doi.org/10.1017/9781108683319.015

Anisman, H., & Merali, Z. (1999). Understanding stress: characteristics and caveats. *Alcohol Research & Health : The Journal of the National Institute on Alcohol Abuse and Alcoholism*, *23*(4), 241–249.

Arciniegas Ruiz, S. M., & Eldar-Finkelman, H. (2022). Glycogen Synthase Kinase-3 Inhibitors: Preclinical and Clinical Focus on CNS-A Decade Onward . In *Frontiers in Molecular Neuroscience* (Vol. 14).

Aresti Sanz, J., & El Aidy, S. (2019). Microbiota and gut neuropeptides: a dual action of antimicrobial activity and neuroimmune response. *Psychopharmacology*, *236*(5), 1597–1609. https://doi.org/10.1007/s00213-019-05224-0

Arumugam, V., John, V., Augustine, N., Jacob, T., Joy, S., Sen, S., & Sen, T. (2017). The impact of antidepressant treatment on brain-derived neurotrophic factor level: An evidence-based approach through systematic review and meta-analysis. *Indian Journal of Pharmacology*, *49*(3), 236. https://doi.org/10.4103/IJP.IJP_700_16

Aswendt, M., Green, C., Sadler, R., Llovera, G., Dzikowski, L., Heindl, S., Gomez de Agüero, M., Diedenhofen, M., Vogel, S., Wieters, F., Wiedermann, D., Liesz, A., & Hoehn, M. (2021). The gut microbiota modulates brain network connectivity under physiological conditions and after acute brain ischemia. *IScience*, *24*(10), 103095. https://doi.org/10.1016/j.isci.2021.103095

Aten, S., Du, Y., Taylor, O., Dye, C., Collins, K., Thomas, M., Kiyoshi, C., & Zhou, M. (2023). Chronic Stress Impairs the Structure and Function of Astrocyte Networks in an Animal Model of Depression. *Neurochemical Research*, *48*(4), 1191–1210. https://doi.org/10.1007/s11064-022-03663-4

Avery, D., & Silverman, J. (1984). Psychomotor retardation and agitation in depression: Relationship to age, sex, and response to treatment. *Journal of Affective Disorders*, *7*(1), 67–76. https://doi.org/10.1016/0165-0327(84)90066-1

Avila, C., & Angulo-Preckler, C. (2021). A Minireview on Biodiscovery in Antarctic Marine Benthic Invertebrates. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.686477

Azar, R., & Mercer, D. (2013). Mild Depressive Symptoms Are Associated with Elevated C-Reactive Protein and Proinflammatory Cytokine Levels During Early to Midgestation: A Prospective Pilot Study. *Journal of Women's Health*, *22*(4), 385. https://doi.org/10.1089/JWH.2012.3785

Ban, T. A. (2001). Pharmacotherapy of depression: a historical analysis. *Journal of Neural Transmission*, *108*(6), 707–716. https://doi.org/10.1007/s007020170047

Bartsch, T., & Wulff, P. (2015). The hippocampus in aging and disease: From plasticity to vulnerability. In *Neuroscience* (Vol. 309, pp. 1–16).

https://doi.org/10.1016/j.neuroscience.2015.07.084

Bearden, C. E., Thompson, P. M., Avedissian, C., Klunder, A. D., Nicoletti, M., Dierschke, N., Brambilla, P., & Soares, J. C. (2009). Altered hippocampal morphology in unmedicated patients with major depressive illness. *ASN Neuro*, *1*(4). https://doi.org/10.1042/AN20090026

Becker, J. B., Prendergast, B. J., & Liang, J. W. (2016). Female rats are not more variable than male rats: a meta-analysis of neuroscience studies. *Biology of Sex Differences*, *7*, 34. https://doi.org/10.1186/s13293-016-0087-5

Becker, M., Pinhasov, A., & Ornoy, A. (2021). Animal Models of Depression: What Can They Teach Us about the Human Disease? *Diagnostics 2021, Vol. 11, Page 123*, *11*(1), 123. https://doi.org/10.3390/DIAGNOSTICS11010123

Beckman, G., Beckman, L., Cedergren, B., Perris, C., & Strandman, E. (1978). Serum protein and red cell enzyme polymorphisms in affective disorders. *Human Heredity*, *28*(1), 41–47. https://doi.org/10.1159/000152929

Beckmann, A. M., Davidson, M. S., Goodenough, S., & Wilce, P. A. (1997). Differential Expression of Egr-1-Like DNA-Binding Activities in the Naive Rat Brain and After Excitatory Stimulation. *Journal of Neurochemistry*, *69*(6), 2227–2237. https://doi.org/https://doi.org/10.1046/j.1471-4159.1997.69062227.x

Beery, A. K. (2018). Inclusion of females does not increase variability in rodent research studies. *Current Opinion in Behavioral Sciences*, *23*, 143–149. https://doi.org/10.1016/j.cobeha.2018.06.016

Begum N, Mandhare A, Tryphena KP, Srivastava S, Shaikh MF, S. S. and K. D. (2022). Epigenetics in depression and gut-brain axis: A molecular crosstalk. *Frontiers in Aging Neuroscience*, *14*.

Belovicova, K., Bogi, E., Csatlosova, K., & Dubovicky, M. (2017). Animal tests for anxiety-like and depression-like behavior in rats. *Interdisciplinary Toxicology*, *10*(1), 40–43. https://doi.org/10.1515/intox-2017-0006

Belujon, P., & Grace, A. A. (2017). Dopamine System Dysregulation in Major Depressive Disorders. *The International Journal of Neuropsychopharmacology*, *20*(12), 1036–1046. https://doi.org/10.1093/ijnp/pyx056

Belzung, C., Willner, P., & Philippot, P. (2015). Depression: from psychopathology to pathophysiology. *Current Opinion in Neurobiology*, *30*, 24–30. https://doi.org/10.1016/j.conb.2014.08.013

Berlim, M. T., & Turecki, G. (2007). Definition, assessment, and staging of treatment-resistant refractory major depression: a review of current concepts and methods. *Canadian Journal of Psychiatry. Revue Canadienne de Psychiatrie*, *52*(1), 46–54. https://doi.org/10.1177/070674370705200108

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S., & Krystal, J. H. (2000). Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry*, *47*(4), 351–354. https://doi.org/10.1016/s0006-3223(99)00230-9

Berrio, J. P., & Kalliokoski, O. (2023). Rethinking data treatment: The sucrose preference threshold for anhedonia in stress-induced rat models of depression. *BioRxiv*, 2023.03.31.535101. https://doi.org/10.1101/2023.03.31.535101

Berthoud, H.-R., & Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Autonomic Neuroscience: Basic and Clinical*, *85*(1), 1–17. https://doi.org/10.1016/S1566-0702(00)00215-0

Berthoud, H. R., Blackshaw, L. A., Brookes, S. J. H., & Grundy, D. (2004). Neuroanatomy of extrinsic afferents supplying the gastrointestinal tract. *Neurogastroenterology & Motility*, *16*(s1), 28–33. https://doi.org/https://doi.org/10.1111/j.1743-3150.2004.00471.x

Besnard, A., Caboche, J., & Laroche, S. (2013). Recall and Reconsolidation of Contextual Fear Memory: Differential Control by ERK and Zif268 Expression Dosage. *PLoS ONE*, *8*(8), 72006. https://doi.org/10.1371/journal.pone.0072006

Beurel, E., Grieco, S. F., & Jope, R. S. (2015). Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacology & Therapeutics*, *148*, 114–131. https://doi.org/10.1016/J.PHARMTHERA.2014.11.016

Bharate, S. B., Yadav, R. R., Battula, S., & Vishwakarma, R. A. (2012). Meridianins: marine-derived potent kinase inhibitors. *Mini Reviews in Medicinal Chemistry*, *12*(7), 618–631. https://doi.org/10.2174/138955712800626728

Bhat, R. V., Andersson, U., Andersson, S., Knerr, L., Bauer, U., & Sundgren-Andersson, A. K. (2018). The Conundrum of GSK3 Inhibitors: Is it the Dawn of a New Beginning? *Journal of Alzheimer's Disease*, *64*(s1), S547–S554. https://doi.org/10.3233/JAD-179934

Bicknell, B., Liebert, A., Johnstone, D., & Kiat, H. (2019). Photobiomodulation of the microbiome: implications for metabolic and inflammatory diseases. *Lasers in Medical Science*, *34*(2), 317–327. https://doi.org/10.1007/s10103-018-2594-6

Bin Saif, G. A., Alotaibi, H. M., Alzolibani, A. A., Almodihesh, N. A., Albraidi, H. F., Alotaibi, N. M., & Yosipovitch, G. (2018). Association of psychological stress with skin symptoms among medical students. *Saudi Medical Journal*, *39*(1), 59–66. https://doi.org/10.15537/smj.2018.1.21231

Bird, C. M., & Burgess, N. (2008). The hippocampus and memory: insights from spatial processing. *Nature Reviews Neuroscience*, *9*(3), 182–194. https://doi.org/10.1038/nrn2335

Blake, J. A., Baldarelli, R., Kadin, J. A., Richardson, J. E., Smith, C. L., & Bult, C. J. (2021). Mouse Genome Database (MGD): Knowledgebase for mouse-human comparative biology. *Nucleic Acids Research*, *49*(D1), D981–D987. https://doi.org/10.1093/nar/gkaa1083

Blivet, G., Meunier, J., Roman, F. J., & Touchon, J. (2018). Neuroprotective effect of a new photobiomodulation technique against Aβ(25-35) peptide-induced toxicity in mice: Novel hypothesis for therapeutic approach of Alzheimer's disease suggested. *Alzheimer's & Dementia (New York, N. Y.)*, *4*, 54–63. https://doi.org/10.1016/j.trci.2017.12.003

Boku, S., Nakagawa, S., Toda, H., & Hishimoto, A. (2018). Neural basis of major depressive disorder: Beyond monoamine hypothesis. *Psychiatry and Clinical Neurosciences*, *72*(1), 3–12. https://doi.org/https://doi.org/10.1111/pcn.12604

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

Border, R., Johnson, E. C., Evans, L. M., Smolen, A., Berley, N., Sullivan, P. F., & Keller, M. C. (2019). No Support for Historical Candidate Gene or Candidate Gene-by-Interaction

Hypotheses for Major Depression Across Multiple Large Samples. *The American Journal of Psychiatry*, *176*(5), 376–387. https://doi.org/10.1176/appi.ajp.2018.18070881

Bouayed, J., Bohn, T., Tybl, E., Kiemer, A. K., & Soulimani, R. (2012). Benzo[α]pyrene-induced anti-depressive-like behaviour in adult female mice: role of monoaminergic systems. *Basic & Clinical Pharmacology & Toxicology*, *110*(6), 544–550. https://doi.org/10.1111/j.1742-7843.2011.00853.x

Boulle, F., Massart, R., Stragier, E., Païzanis, E., Zaidan, L., Marday, S., Gabriel, C., Mocaer, E., Mongeau, R., & Lanfumey, L. (2014). Hippocampal and behavioral dysfunctions in a mouse model of environmental stress: normalization by agomelatine. *Translational Psychiatry*, *4*(11), e485. https://doi.org/10.1038/tp.2014.125

Braidy, N., Guillemin, G. J., Mansour, H., Chan-Ling, T., Poljak, A., & Grant, R. (2011). Age related changes in NAD+ metabolism oxidative stress and Sirt1 activity in wistar rats. *PloS One*, *6*(4), e19194. https://doi.org/10.1371/journal.pone.0019194

Bruce, M. L. (2002). Psychosocial risk factors for depressive disorders in late life. *Biological Psychiatry*, *52*(3), 175–184. https://doi.org/10.1016/s0006-3223(02)01410-5

Buckley, M. M., O'Brien, R., Brosnan, E., Ross, R. P., Stanton, C., Buckley, J. M., & O'Malley, D. (2020). Glucagon-Like Peptide-1 Secreting L-Cells Coupled to Sensory Nerves Translate Microbial Signals to the Host Rat Nervous System. *Frontiers in Cellular Neuroscience*, *14*, 95. https://doi.org/10.3389/fncel.2020.00095

Bunney, B. G., Li, J. Z., Walsh, D. M., Stein, R., Vawter, M. P., Cartagena, P., Barchas, J. D., Schatzberg, A. F., Myers, R. M., Watson, S. J., Akil, H., & Bunney, W. E. (2015). Circadian dysregulation of clock genes: clues to rapid treatments in major depressive disorder. *Molecular Psychiatry*, *20*(1), 48–55. https://doi.org/10.1038/mp.2014.138

Cai, N., Bigdeli, T. B., Kretzschmar, W., Li, Y., Liang, J., Song, L., Hu, J., Li, Q., Jin, W., Hu, Z., Wang, G., Wang, L., Qian, P., Liu, Y., Jiang, T., Lu, Y., Zhang, X., Yin, Y., Li, Y., … consortium, C. (2015). Sparse whole-genome sequencing identifies two loci for major depressive disorder. *Nature*, *523*(7562), 588–591. https://doi.org/10.1038/nature14659

Calcia, M. A., Bonsall, D. R., Bloomfield, P. S., Selvaraj, S., Barichello, T., & Howes, O. D. (2016). Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology*, *233*(9), 1637–1650. https://doi.org/10.1007/s00213-016-4218-9

Cameron, L. P., Tombari, R. J., Lu, J., Pell, A. J., Hurley, Z. Q., Ehinger, Y., Vargas, M. V, McCarroll, M. N., Taylor, J. C., Myers-Turnbull, D., Liu, T., Yaghoobi, B., Laskowski, L. J., Anderson, E. I., Zhang, G., Viswanathan, J., Brown, B. M., Tjia, M., Dunlap, L. E., … Olson, D. E. (2021). A non-hallucinogenic psychedelic analogue with therapeutic potential. *Nature*, *589*(7842), 474–479. https://doi.org/10.1038/s41586-020-3008-z

Campbell, S., & Macqueen, G. (2004). The role of the hippocampus in the pathophysiology of major depression. *Journal of Psychiatry & Neuroscience : JPN*, *29*(6), 417–426.

Carlessi, A. S., Borba, L. A., Zugno, A. I., Quevedo, J., & Réus, G. Z. (2021). Gut microbiota-brain axis in depression: The role of neuroinflammation. *The European Journal of Neuroscience*, *53*(1), 222–235. https://doi.org/10.1111/ejn.14631

Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., & Prinsep, M. R. (2022). Marine natural products. *Natural Product Reports*, *39*(6), 1122–1171.

https://doi.org/10.1039/D1NP00076D

Caruso-Davis, M. K., Guillot, T. S., Podichetty, V. K., Mashtalir, N., Dhurandhar, N. V, Dubuisson, O., Yu, Y., & Greenway, F. L. (2011). Efficacy of low-level laser therapy for body contouring and spot fat reduction. *Obesity Surgery*, *21*(6), 722–729. https://doi.org/10.1007/s11695-010-0126-y

Caspani, G., Kennedy, S., Foster, J. A., & Swann, J. (2019). Gut microbial metabolites in depression: understanding the biochemical mechanisms. *Microbial Cell (Graz, Austria)*, *6*(10), 454–481. https://doi.org/10.15698/mic2019.10.693

Cassano, P., Cusin, C., Mischoulon, D., Hamblin, M. R., De Taboada, L., Pisoni, A., Chang, T., Yeung, A., Ionescu, D. F., Petrie, S. R., Nierenberg, A. A., Fava, M., & Iosifescu, D. V. (2015). Near-Infrared Transcranial Radiation for Major Depressive Disorder: Proof of Concept Study. *Psychiatry Journal*, *2015*, 352979. https://doi.org/10.1155/2015/352979

Cassano, P., & Fava, M. (2004). Tolerability issues during long-term treatment with antidepressants. *Annals of Clinical Psychiatry : Official Journal of the American Academy of Clinical Psychiatrists*, *16*(1), 15–25. https://doi.org/10.1080/10401230490281618

Cassano, P., Petrie, S. R., Hamblin, M. R., Henderson, T. A., & Iosifescu, D. V. (2016). Review of transcranial photobiomodulation for major depressive disorder: targeting brain metabolism, inflammation, oxidative stress, and neurogenesis. *Neurophotonics*, *3*(3), 031404. https://doi.org/10.1117/1.nph.3.3.031404

Cassano, P., Petrie, S. R., Mischoulon, D., Cusin, C., Katnani, H., Yeung, A., De Taboada, L., Archibald, A., Bui, E., Baer, L., Chang, T., Chen, J., Pedrelli, P., Fisher, L., Farabaugh, A., Hamblin, M. R., Alpert, J. E., Fava, M., & Iosifescu, D. V. (2018). Transcranial Photobiomodulation for the Treatment of Major Depressive Disorder. The ELATED-2 Pilot Trial. *Photomedicine and Laser Surgery*, *36*(12), 634–646. https://doi.org/10.1089/pho.2018.4490

Castrén, E., & Monteggia, L. M. (2021). Brain-Derived Neurotrophic Factor Signaling in Depression and Antidepressant Action. *Biological Psychiatry*, *90*(2), 128–136. https://doi.org/10.1016/j.biopsych.2021.05.008

Castro-Mondragon, J. A., Riudavets-Puig, R., Rauluseviciute, I., Berhanu Lemma, R., Turchi, L., Blanc-Mathieu, R., Lucas, J., Boddie, P., Khan, A., Manosalva Pérez, N., Fornes, O., Leung, T. Y., Aguirre, A., Hammal, F., Schmelter, D., Baranasic, D., Ballester, B., Sandelin, A., Lenhard, B., … Mathelier, A. (2022). JASPAR 2022: the 9th release of the open-access database of transcription factor binding profiles. *Nucleic Acids Research*, *50*(D1), D165–D173. https://doi.org/10.1093/nar/gkab1113

Cembrowski, M. S., Bachman, J. L., Wang, L., Sugino, K., Shields, B. C., & Spruston, N. (2016). Spatial Gene-Expression Gradients Underlie Prominent Heterogeneity of CA1 Pyramidal Neurons. *Neuron*, *89*(2), 351–368. https://doi.org/10.1016/j.neuron.2015.12.013

Cembrowski, M. S., Wang, L., Sugino, K., Shields, B. C., & Spruston, N. (2016). Hipposeq: a comprehensive RNA-seq database of gene expression in hippocampal principal neurons. *ELife*, *5*, e14997. https://doi.org/10.7554/eLife.14997

Cerniauskas, I., Winterer, J., de Jong, J. W., Lukacsovich, D., Yang, H., Khan, F., Peck, J. R., Obayashi, S. K., Lilascharoen, V., Lim, B. K., Földy, C., & Lammel, S. (2019). Chronic Stress Induces Activity, Synaptic, and Transcriptional Remodeling of the Lateral

Habenula Associated with Deficits in Motivated Behaviors. *Neuron*, *104*(5), 899-915.e8. https://doi.org/https://doi.org/10.1016/j.neuron.2019.09.005

Chan, S. W. Y., Harmer, C. J., Norbury, R., O'Sullivan, U., Goodwin, G. M., & Portella, M. J. (2016). Hippocampal volume in vulnerability and resilience to depression. *Journal of Affective Disorders*, *189*, 199–202. https://doi.org/https://doi.org/10.1016/j.jad.2015.09.021

Chaoui, N., Anarghou, H., Laaroussi, M., Essaidi, O., Najimi, M., & Chigr, F. (2022). Long lasting effect of acute restraint stress on behavior and brain anti-oxidative status. *AIMS Neuroscience*, *9*(1), 57–75. https://doi.org/10.3934/Neuroscience.2022005

Chen, L.-C., Chen, Y.-H., Bai, Y.-M., Chen, T.-J., Chen, M.-H., & Su, T.-P. (2020). Antidepressant resistance in adolescents with major depressive disorder: A nationwide longitudinal study. *Journal of Affective Disorders*, *262*, 293–297. https://doi.org/10.1016/j.jad.2019.11.038

Chen, Y., Rex, C. S., Rice, C. J., Dubé, C. M., Gall, C. M., Lynch, G., & Barama, T. Z. (2010). Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(29), 13123–13128. https://doi.org/10.1073/PNAS.1003825107

Cho, W.-H., Noh, K., Lee, B. H., Barcelon, E., Jun, S. B., Park, H. Y., & Lee, S. J. (2022). Hippocampal astrocytes modulate anxiety-like behavior. *Nature Communications*, *13*(1), 6536. https://doi.org/10.1038/s41467-022-34201-z

Chuang, H.-W., Wei, I.-H., Lin, F.-Y., Li, C.-T., Chen, K.-T., Tsai, M.-H., & Huang, C.-C. (2020). Roles of Akt and ERK in mTOR-Dependent Antidepressant Effects of Vanillic Acid. *ACS Omega*, *5*(7), 3709–3716. https://doi.org/10.1021/acsomega.9b04271

Cid, E., Marquez-Galera, A., Valero, M., Gal, B., Medeiros, D. C., Navarron, C. M., Ballesteros-Esteban, L., Reig-Viader, R., Morales, A. V, Fernandez-Lamo, I., Gomez-Dominguez, D., Sato, M., Hayashi, Y., Bayés, À., Barco, A., Lopez-Atalaya, J. P., & de la Prida, L. M. (2021). Sublayer- and cell-type-specific neurodegenerative transcriptional trajectories in hippocampal sclerosis. *Cell Reports*, *35*(10), 109229. https://doi.org/10.1016/j.celrep.2021.109229

Clements, K. M., & Wainwright, P. E. (2010). Swim stress increases hippocampal Zif268 expression in the spontaneously hypertensive rat. *Brain Research Bulletin*, *82*(5–6), 259–263. https://doi.org/10.1016/J.BRAINRESBULL.2010.05.002

Clos-Garcia, M., Andrés-Marin, N., Fernández-Eulate, G., Abecia, L., Lavín, J. L., van Liempd, S., Cabrera, D., Royo, F., Valero, A., Errazquin, N., Vega, M. C. G., Govillard, L., Tackett, M. R., Tejada, G., Gónzalez, E., Anguita, J., Bujanda, L., Orcasitas, A. M. C., Aransay, A. M., … Falcón-Pérez, J. M. (2019). Gut microbiome and serum metabolome analyses identify molecular biomarkers and altered glutamate metabolism in fibromyalgia. *EBioMedicine*, *46*, 499–511. https://doi.org/10.1016/j.ebiom.2019.07.031

Conrad, C. D., McLaughlin, K. J., Huynh, T. N., El-Ashmawy, M., & Sparks, M. (2012). Chronic stress and a cyclic regimen of estradiol administration separately facilitate spatial memory: Relationship with hippocampal CA1 spine density and dendritic complexity. *Behavioral Neuroscience*, *126*(1), 142–156. https://doi.org/10.1037/a0025770

Cornwell, B. R., Salvadore, G., Colon-Rosario, V., Latov, D. R., Holroyd, T., Carver, F. W.,

Coppola, R., Manji, H. K., Zarate, C. A. J., & Grillon, C. (2010). Abnormal hippocampal functioning and impaired spatial navigation in depressed individuals: evidence from whole-head magnetoencephalography. *The American Journal of Psychiatry*, *167*(7), 836–844. https://doi.org/10.1176/appi.ajp.2009.09050614

Covington, H. E., Lobo, M. K., Maze, I., Vialou, V., Hyman, J. M., Zaman, S., LaPlant, Q., Mouzon, E., Ghose, S., Tamminga, C. A., Neve, R. L., Deisseroth, K., & Nestler, E. J. (2010). Antidepressant Effect of Optogenetic Stimulation of the Medial Prefrontal Cortex. *Journal of Neuroscience*, *30*(48), 16082–16090. https://doi.org/10.1523/JNEUROSCI.1731-10.2010

Cowen, P. J. (2008). Serotonin and depression: pathophysiological mechanism or marketing myth? *Trends in Pharmacological Sciences*, *29*(9), 433–436. https://doi.org/10.1016/j.tips.2008.05.004

Cryan, J. F., O'riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., … Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews*, *99*(4), 1877–2013. https://doi.org/10.1152/physrev.00018.2018

Cuijpers, P., Berking, M., Andersson, G., Quigley, L., Kleiboer, A., & Dobson, K. S. (2013). A meta-analysis of cognitive-behavioural therapy for adult depression, alone and in comparison with other treatments. *Canadian Journal of Psychiatry. Revue Canadienne de Psychiatrie*, *58*(7), 376–385. https://doi.org/10.1177/070674371305800702

Cuijpers, P., Quero, S., Noma, H., Ciharova, M., Miguel, C., Karyotaki, E., Cipriani, A., Cristea, I. A., & Furukawa, T. A. (2021). Psychotherapies for depression: a network meta-analysis covering efficacy, acceptability and long-term outcomes of all main treatment types. *World Psychiatry*, *20*(2), 283–293. https://doi.org/10.1002/WPS.20860

Cuijpers, P., van Straten, A., Andersson, G., & van Oppen, P. (2008). Psychotherapy for Depression in Adults: A Meta-Analysis of Comparative Outcome Studies. *Journal of Consulting and Clinical Psychology*, *76*(6), 909–922. https://doi.org/10.1037/A0013075

Cuijpers, P., van Straten, A., & Warmerdam, L. (2007). Behavioral activation treatments of depression: A meta-analysis. *Clinical Psychology Review*, *27*(3), 318–326. https://doi.org/10.1016/J.CPR.2006.11.001

Cullinan, W. E., Herman, J. P., Battaglia, D. F., Akil, H., & Watson, S. J. (1995). Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience*, *64*(2), 477–505. https://doi.org/10.1016/0306-4522(94)00355-9

Cusin, C., & Dougherty, D. D. (2012). Somatic therapies for treatment-resistant depression: ECT, TMS, VNS, DBS. *Biology of Mood & Anxiety Disorders*, *2*, 14. https://doi.org/10.1186/2045-5380-2-14

D'Angelo, S., Mele, E., Di Filippo, F., Viggiano, A., & Meccariello, R. (2021). Sirt1 Activity in the Brain: Simultaneous Effects on Energy Homeostasis and Reproduction. *International Journal of Environmental Research and Public Health*, *18*(3). https://doi.org/10.3390/ijerph18031243

Dahmen, N., Fehr, C., Reuss, S., & Hiemke, C. (1997). Stimulation of immediate early gene expression by desipramine in rat brain. *Biological Psychiatry*, *42*(5), 317–323. https://doi.org/https://doi.org/10.1016/S0006-3223(96)00361-7

Davis, S., Bozon, B., & Laroche, S. (2003). How necessary is the activation of the immediate early gene zif268 in synaptic plasticity and learning? *Behavioural Brain Research*, *142*(1), 17–30. https://doi.org/https://doi.org/10.1016/S0166-4328(02)00421-7

Davis, S., Renaudineau, S., Poirier, R., Poucet, B., Save, E., & Laroche, S. (2010). The formation and stability of recognition memory: What happens upon recall? *Frontiers in Behavioral Neuroscience*, *4*(NOV), 177. https://doi.org/10.3389/FNBEH.2010.00177/BIBTEX

de Freitas, L. F., & Hamblin, M. R. (2016). Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE Journal of Selected Topics in Quantum Electronics : A Publication of the IEEE Lasers and Electro-Optics Society*, *22*(3). https://doi.org/10.1109/JSTQE.2016.2561201

de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience 2005 6:6*, *6*(6), 463–475. https://doi.org/10.1038/nrn1683

de Mello, N. P., Orellana, A. M., Mazucanti, C. H., de Morais Lima, G., Scavone, C., & Kawamoto, E. M. (2019). Insulin and Autophagy in Neurodegeneration. *Frontiers in Neuroscience*, *13*, 491. https://doi.org/10.3389/fnins.2019.00491

De Paermentier, F., Mauger, J. M., Lowther, S., Crompton, M. R., Katona, C. L., & Horton, R. W. (1997). Brain alpha-adrenoceptors in depressed suicides. *Brain Research*, *757*(1), 60–68. https://doi.org/10.1016/s0006-8993(97)00138-8

De Sarno, P., Li, X., & Jope, R. S. (2002). Regulation of Akt and glycogen synthase kinase-3β phosphorylation by sodium valproate and lithium. *Neuropharmacology*, *43*, 1158–1164. https://doi.org/10.1016/S0028-3908(02)00215-0

Del Ser, T., Steinwachs, K. C., Gertz, H. J., Andrés, M. V., Gómez-Carrillo, B., Medina, M., Vericat, J. A., Redondo, P., Fleet, D., & León, T. (2013). Treatment of Alzheimer's disease with the GSK-3 inhibitor tideglusib: A pilot study. *Journal of Alzheimer's Disease*, *33*(1), 205–215. https://doi.org/10.3233/JAD-2012-120805

Delgado, P. L., & Moreno, F. A. (2000). Role of norepinephrine in depression. *The Journal of Clinical Psychiatry*, *61 Suppl 1*, 5–12.

Demin, K. A., Sysoev, M., Chernysh, M. V, Savva, A. K., Koshiba, M., Wappler-Guzzetta, E. A., Song, C., De Abreu, M. S., Leonard, B., Parker, M. O., Harvey, B. H., Tian, L., Vasar, E., Strekalova, T., Amstislavskaya, T. G., Volgin, A. D., Alpyshov, E. T., Wang, D., & Kalueff, A. V. (2019). Animal models of major depressive disorder and the implications for drug discovery and development. *Expert Opinion on Drug Discovery*, *14*(4), 365–378. https://doi.org/10.1080/17460441.2019.1575360

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179–1188. https://doi.org/10.1016/j.neuroscience.2010.08.005

Deuschle A; Niemann, H; Erb-Bies, N; Colla, M; Hamann, B; Heuser, I, M. K. (2004). Impaired Declarative Memory in Depressed Patients Is Slow To Recover: Clinical Experience. *Pharmacopsychiatry*, *37*(04), 147–151. https://doi.org/10.1055/s-2004-827168

Dieterle, F., Ross, A., Schlotterbeck, G., & Senn, H. (2006). Probabilistic Quotient

Normalization as Robust Method to Account for Dilution of Complex Biological Mixtures. Application in 1H NMR Metabonomics. *Analytical Chemistry*, *78*(13), 4281–4290. https://doi.org/10.1021/ac051632c

Ding, V. W., Chen, R.-H., & McCormick, F. (2000). Differential Regulation of Glycogen Synthase Kinase 3β by Insulin and Wnt Signaling*. *Journal of Biological Chemistry*, *275*(42), 32475–32481. https://doi.org/https://doi.org/10.1074/jbc.M005342200

Diniz, B. S., Talib, L. L., Giroud Joaquim, H. P., de Paula, V. R. J., Gattaz, W. F., & Forlenza, O. V. (2011). Platelet GSK3B activity in patients with late-life depression: Marker of depressive episode severity and cognitive impairment? *The World Journal of Biological Psychiatry*, *12*(3), 216–222. https://doi.org/10.3109/15622975.2010.551408

Dompe, C., Moncrieff, L., Matys, J., Grzech-Leśniak, K., Kocherova, I., Bryja, A., Bruska, M., Dominiak, M., Mozdziak, P., Skiba, T. H. I., Shibli, J. A., Volponi, A. A., Kempisty, B., & Dyszkiewicz-Konwińska, M. (2020). Photobiomodulation—underlying mechanism and clinical applications. *Journal of Clinical Medicine*, *9*(6), 1–17. https://doi.org/10.3390/jcm9061724

Dong, Z., Shen, X., Hao, Y., Li, J., Xu, H., Yin, L., & Kuang, W. (2022). Gut microbiome: A potential indicator for predicting treatment outcomes in major depressive disorder. *Frontiers in Neuroscience*, *16*, 813075. https://doi.org/10.3389/fnins.2022.813075

Donohue, H. S., Gabbott, P. L. A., Davies, H. A., Rodríguez, J. J., Cordero, M. I., Sandi, C., Medvedev, N. I., Popov, V. I., Colyer, F. M., Peddie, C. J., & Stewart, M. G. (2006). Chronic restraint stress induces changes in synapse morphology in stratum lacunosum-moleculare CA1 rat hippocampus: A stereological and three-dimensional ultrastructural study. *Neuroscience*, *140*(2), 597–606. https://doi.org/10.1016/J.NEUROSCIENCE.2006.02.072

dos Santos Cardoso, F., dos Santos, J. C. C., Gonzalez-Lima, F., Araújo, B. H. S., Lopes-Martins, R. Á. B., & Gomes da Silva, S. (2021). Effects of Chronic Photobiomodulation with Transcranial Near-Infrared Laser on Brain Metabolomics of Young and Aged Rats. *Molecular Neurobiology*, *58*(5), 2256–2268. https://doi.org/10.1007/s12035-020-02247-z

Duclot, F., & Kabbaj, M. (2017). The Role of Early Growth Response 1 (EGR1) in Brain Plasticity and Neuropsychiatric Disorders. *Frontiers in Behavioral Neuroscience*, *0*, 35. https://doi.org/10.3389/FNBEH.2017.00035

Duda, P., Hajka, D., Wójcicka, O., Rakus, D., & Gizak, A. (2020). GSK3β: A Master Player in Depressive Disorder Pathogenesis and Treatment Responsiveness. In *Cells* (Vol. 9, Issue 3). https://doi.org/10.3390/cells9030727

Duda, P., Wiśniewski, J., Wójtowicz, T., Wójcicka, O., Jaśkiewicz, M., Drulis-Fajdasz, D., Rakus, D., McCubrey, J. A., & Gizak, A. (2018). Targeting GSK3 signaling as a potential therapy of neurodegenerative diseases and aging. *Expert Opinion on Therapeutic Targets*, *22*(10), 833–848. https://doi.org/10.1080/14728222.2018.1526925

Duerkop, B. A., Vaishnava, S., & Hooper, L. V. (2009). Immune Responses to the Microbiota at the Intestinal Mucosal Surface. *Immunity*, *31*(3), 368–376. https://doi.org/10.1016/j.immuni.2009.08.009

Duman, E. A., & Canli, T. (2015). Influence of life stress, 5-HTTLPR genotype, and SLC6A4 methylation on gene expression and stress response in healthy Caucasian males. *Biology of Mood & Anxiety Disorders*, *5*, 2. https://doi.org/10.1186/s13587-015-0017-x

Duman, R. S., Aghajanian, G. K., Sanacora, G., & Krystal, J. H. (2016). Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nature Medicine*, *22*(3), 238–249. https://doi.org/10.1038/nm.4050

Duman, R. S., & Monteggia, L. M. (2006). A Neurotrophic Model for Stress-Related Mood Disorders. *Biological Psychiatry*, *59*(12), 1116–1127. https://doi.org/10.1016/J.BIOPSYCH.2006.02.013

Duman, R. S., Sanacora, G., & Krystal, J. H. (2019). Altered Connectivity in Depression: GABA and Glutamate Neurotransmitter Deficits and Reversal by Novel Treatments. *Neuron*, *102*(1), 75–90. https://doi.org/10.1016/j.neuron.2019.03.013

Duman, R. S., & Voleti, B. (2012). Signaling pathways underlying the pathophysiology and treatment of depression: Novel mechanisms for rapid-acting agents. *Trends in Neurosciences*, *35*(1), 47–56. https://doi.org/10.1016/j.tins.2011.11.004

Dunn, N., Holmes, C., & Mullee, M. (2005). Does lithium therapy protect against the onset of dementia? *Alzheimer Disease and Associated Disorders*, *19*(1), 20–22. https://doi.org/10.1097/01.WAD.0000155068.23937.9B

Dutta, A., McKie, S., & Deakin, J. F. W. (2014). Resting state networks in major depressive disorder. *Psychiatry Research*, *224*(3), 139–151. https://doi.org/10.1016/j.pscychresns.2014.10.003

Echalier, A., Bettayeb, K., Ferandin, Y., Lozach, O., Clément, M., Valette, A., Liger, F., Marquet, B., Morris, J. C., Endicott, J. A., Joseph, B., & Meijer, L. (2008). Meriolins (3-(pyrimidin-4-yl)-7-azaindoles): Synthesis, kinase inhibitory activity, cellular effects, and structure of a CDK2/Cyclin A/meriolin complex. *Journal of Medicinal Chemistry*, *51*(4), 737–751. https://doi.org/10.1021/jm700940h

Ehrich, E., Turncliff, R., Du, Y., Leigh-Pemberton, R., Fernandez, E., Jones, R., & Fava, M. (2015). Evaluation of opioid modulation in major depressive disorder. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *40*(6), 1448–1455. https://doi.org/10.1038/npp.2014.330

Eisch, A. J., & Petrik, D. (2012). Depression and hippocampal neurogenesis: a road to remission? *Science (New York, N.Y.)*, *338*(6103), 72–75. https://doi.org/10.1126/science.1222941

Eldar-Finkelman, H., & Martinez, A. (2011). GSK-3 Inhibitors: Preclinical and Clinical Focus on CNS. *Frontiers in Molecular Neuroscience*, *4*, 32. https://doi.org/10.3389/fnmol.2011.00032

Elibol, B., & Kilic, U. (2018). High Levels of SIRT1 Expression as a Protective Mechanism Against Disease-Related Conditions. *Frontiers in Endocrinology*, *9*, 614. https://doi.org/10.3389/fendo.2018.00614

Embi, N., Rylatt, D. B., & Cohen, P. (1980). Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *European Journal of Biochemistry*, *107*(2), 519–527.

Erny, D., Hrabě de Angelis, A. L., & Prinz, M. (2017). Communicating systems in the body: how microbiota and microglia cooperate. *Immunology*, *150*(1), 7–15. https://doi.org/https://doi.org/10.1111/imm.12645

Eshaghi, E., Sadigh-Eteghad, S., Mohaddes, G., & Rasta, S. H. (2019). Transcranial photobiomodulation prevents anxiety and depression via changing serotonin and nitric

oxide levels in brain of depression model mice: A study of three different doses of 810 nm laser. *Lasers in Surgery and Medicine*, *51*(7), 634–642. https://doi.org/https://doi.org/10.1002/lsm.23082

Fang, X., Jiang, S., Wang, J., Bai, Y., Kim, C. S., Blake, D., Weintraub, N. L., Lei, Y., & Lu, X.-Y. (2021). Chronic unpredictable stress induces depression-related behaviors by suppressing AgRP neuron activity. *Molecular Psychiatry*, *26*(6), 2299–2315. https://doi.org/10.1038/s41380-020-01004-x

Fasipe, O. (2018). Neuropharmacological classification of antidepressant agents based on their mechanisms of action. *Archives of Medicine and Health Sciences*, *6*(1), 81–94. https://doi.org/10.4103/amhs.amhs_7_18

Fava, M., & Davidson, K. G. (1996). Definition and epidemiology of treatment-resistant depression. *The Psychiatric Clinics of North America*, *19*(2), 179–200. https://doi.org/10.1016/s0193-953x(05)70283-5

Fava, M., Johe, K., Ereshefsky, L., Gertsik, L. G., English, B. A., Bilello, J. A., Thurmond, L. M., Johnstone, J., Dickerson, B. C., Makris, N., Hoeppner, B. B., Flynn, M., Mischoulon, D., Kinrys, G., & Freeman, M. P. (2016). A Phase 1B, randomized, double blind, placebo controlled, multiple-dose escalation study of NSI-189 phosphate, a neurogenic compound, in depressed patients. *Molecular Psychiatry*, *21*(10), 1372–1380. https://doi.org/10.1038/mp.2015.178

Fee, C., Prevot, T., Misquitta, K., Banasr, M., & Sibille, E. (2020). Chronic Stress-induced Behaviors Correlate with Exacerbated Acute Stress-induced Cingulate Cortex and Ventral Hippocampus Activation. *Neuroscience*, *440*, 113–129. https://doi.org/10.1016/J.NEUROSCIENCE.2020.05.034

Ferguson, D., Koo, J. W., Feng, J., Heller, E., Rabkin, J., Heshmati, M., Renthal, W., Neve, R., Liu, X., Shao, N., Sartorelli, V., Shen, L., & Nestler, E. J. (2013). Essential role of SIRT1 signaling in the nucleus accumbens in cocaine and morphine action. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(41), 16088–16098. https://doi.org/10.1523/JNEUROSCI.1284-13.2013

Ferland, C. L., & Schrader, L. A. (2011). Regulation of histone acetylation in the hippocampus of chronically stressed rats: a potential role of sirtuins. *Neuroscience*, *174*, 104–114. https://doi.org/10.1016/j.neuroscience.2010.10.077

Fernández-García, S., Sancho-Balsells, A., Longueville, S., Hervé, D., Gruart, A., Delgado-García, J. M., Alberch, J., & Giralt, A. (2020). Astrocytic BDNF and TrkB regulate severity and neuronal activity in mouse models of temporal lobe epilepsy. *Cell Death & Disease 2020 11:6*, *11*(6), 1–17. https://doi.org/10.1038/s41419-020-2615-9

Filatova, E. V, Shadrina, M. I., & Slominsky, P. A. (2021). Major Depression: One Brain, One Disease, One Set of Intertwined Processes. In *Cells* (Vol. 10, Issue 6). https://doi.org/10.3390/cells10061283

Finkelmeyer, A., Nilsson, J., He, J., Stevens, L., Maller, J. J., Moss, R. A., Small, S., Gallagher, P., Coventry, K., Ferrier, I. N., & McAllister-Williams, R. H. (2016). Altered hippocampal function in major depression despite intact structure and resting perfusion. *Psychological Medicine*, *46*(10), 2157–2168. https://doi.org/DOI: 10.1017/S0033291716000702

Fiori, L. M., & Turecki, G. (2008). Implication of the polyamine system in mental disorders. *Journal of Psychiatry & Neuroscience : JPN*, *33*(2), 102–110.

Fjell, A. M., McEvoy, L., Holland, D., Dale, A. M., & Walhovd, K. B. (2014). What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. *Progress in Neurobiology*, *117*, 20–40. https://doi.org/10.1016/j.pneurobio.2014.02.004

Flint, J. (2023). The genetic basis of major depressive disorder. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-023-01957-9

Flint, J., & Kendler, K. S. (2014). The Genetics of Major Depression. *Neuron*, *81*(3), 484–503. https://doi.org/10.1016/j.neuron.2014.01.027

Floriou-Servou, A., von Ziegler, L., Stalder, L., Sturman, O., Privitera, M., Rassi, A., Cremonesi, A., Thöny, B., & Bohacek, J. (2018). Distinct Proteomic, Transcriptomic, and Epigenetic Stress Responses in Dorsal and Ventral Hippocampus. *Biological Psychiatry*, *84*(7), 531–541. https://doi.org/10.1016/j.biopsych.2018.02.003

Fogaça, M. V, & Duman, R. S. (2019). Cortical GABAergic Dysfunction in Stress and Depression: New Insights for Therapeutic Interventions. *Frontiers in Cellular Neuroscience*, *13*, 87. https://doi.org/10.3389/fncel.2019.00087

Fonagy, P. (2015). The effectiveness of psychodynamic psychotherapies: An update. *World Psychiatry : Official Journal of the World Psychiatric Association (WPA)*, *14*(2), 137–150. https://doi.org/10.1002/WPS.20235

Foster, J. A., Rinaman, L., & Cryan, J. F. (2017). Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiology of Stress*, *7*, 124–136. https://doi.org/10.1016/j.ynstr.2017.03.001

Franco, L. H., De Kier Joffé, E. B., Puricelli, L., Tatian, M., Seldes, A. M., & Palermo, J. A. (1998). Indole alkaloids from the tunicate Aplidium meridianum. *Journal of Natural Products*, *61*(9), 1130–1132. https://doi.org/10.1021/NP970493U

Franco, L. H., & Palermo, J. A. (2003). Synthesis of 2-(pyrimidin-4-yl)indoles. *Chemical & Pharmaceutical Bulletin*, *51*(8), 975–977. https://doi.org/10.1248/CPB.51.975

Fritschy, J.-M., Weinmann, O., Wenzel, A., & Benke, D. (1998). Synapse-specific localization of NMDA and GABAA receptor subunits revealed by antigen-retrieval immunohistochemistry. *Journal of Comparative Neurology*, *390*(2), 194–210. https://doi.org/https://doi.org/10.1002/(SICI)1096-9861(19980112)390:2<194::AID-CNE3>3.0.CO;2-X

Frodl, T., Meisenzahl, E. M., Zetzsche, T., Born, C., Groll, C., Jäger, M., Leinsinger, G., Bottlender, R., Hahn, K., & Möller, H.-J. (2002). Hippocampal changes in patients with a first episode of major depression. *The American Journal of Psychiatry*, *159*(7), 1112–1118. https://doi.org/10.1176/appi.ajp.159.7.1112

Frühauf-Perez, P. K., Temp, F. R., Pillat, M. M., Signor, C., Wendel, A. L., Ulrich, H., Mello, C. F., & Rubin, M. A. (2018). Spermine protects from LPS-induced memory deficit via BDNF and TrkB activation. *Neurobiology of Learning and Memory*, *149*, 135–143. https://doi.org/https://doi.org/10.1016/j.nlm.2018.02.012

Fuchikami, M., Morinobu, S., Segawa, M., Okamoto, Y., Yamawaki, S., Ozaki, N., Inoue, T., Kusumi, I., Koyama, T., Tsuchiyama, K., & Terao, T. (2011). DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. *PloS One*, *6*(8), e23881. https://doi.org/10.1371/journal.pone.0023881

Furness, J. B. (2012). The enteric nervous system and neurogastroenterology. *Nature Reviews Gastroenterology & Hepatology*, *9*(5), 286–294. https://doi.org/10.1038/nrgastro.2012.32

Gallo, F. T., Katche, C., Morici, J. F., Medina, J. H., & Weisstaub, N. V. (2018). Immediate Early Genes, Memory and Psychiatric Disorders: Focus on c-Fos, Egr1 and Arc. *Frontiers in Behavioral Neuroscience*, *0*, 79. https://doi.org/10.3389/FNBEH.2018.00079

Gąska, M., Kuśmider, M., Solich, J., Faron-Górecka, A., Krawczyk, M. J., Kułakowski, K., & Dziedzicka-Wasylewska, M. (2012). Analysis of region-specific changes in gene expression upon treatment with citalopram and desipramine reveals temporal dynamics in response to antidepressant drugs at the transcriptome level. *Psychopharmacology*, *223*(3), 281–297. https://doi.org/10.1007/s00213-012-2714-0

Gautam, M., Tripathi, A., Deshmukh, D., & Gaur, M. (2020). Cognitive Behavioral Therapy for Depression. *Indian Journal of Psychiatry*, *62*(8), S223–S229. https://doi.org/10.4103/PSYCHIATRY.INDIANJPSYCHIATRY_772_19

Ge, L., Liu, L., Liu, H., Liu, S., Xue, H., Wang, X., Yuan, L., Wang, Z., & Liu, D. (2015). Resveratrol abrogates lipopolysaccharide-induced depressive-like behavior, neuroinflammatory response, and CREB/BDNF signaling in mice. *European Journal of Pharmacology*, *768*, 49–57. https://doi.org/https://doi.org/10.1016/j.ejphar.2015.10.026

Genedani, S., Saltini, S., Benelli, A., Filaferro, M., & Bertolini, A. (2001). Influence of SAMe on the modifications of brain polyamine levels in an animal model of depression. *Neuroreport*, *12*(18), 3939–3942. https://doi.org/10.1097/00001756-200112210-00017

Gerhard, D. M., Wohleb, E. S., & Duman, R. S. (2016). Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity. *Drug Discovery Today*, *21*(3), 454–464. https://doi.org/10.1016/j.drudis.2016.01.016

Gillman, P. K. (2007). Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. *British Journal of Pharmacology*, *151*(6), 737–748. https://doi.org/10.1038/sj.bjp.0707253

Giralt, A., Brito, V., Chevy, Q., Simonnet, C., Otsu, Y., Cifuentes-DÍaz, C., De Pins, B., Coura, R., Alberch, J., Ginés, S., Poncer, J. C., & Girault, J. A. (2017). Pyk2 modulates hippocampal excitatory synapses and contributes to cognitive deficits in a Huntington's disease model. *Nature Communications*, *8*(1), 1–16. https://doi.org/10.1038/ncomms15592

Giraud, F., Alves, G., Debiton, E., Nauton, L., Théry, V., Durieu, E., Ferandin, Y., Lozach, O., Meijer, L., Anizon, F., Pereira, E., & Moreau, P. (2011). Synthesis, protein kinase inhibitory potencies, and in vitro antiproliferative activities of meridianin derivatives. *Journal of Medicinal Chemistry*, *54*(13), 4474–4489. https://doi.org/10.1021/JM200464W/ASSET/IMAGES/MEDIUM/JM-2011-00464W_0009.GIF

Girotti, M., Pace, T. W. W., Gaylord, R. I., Rubin, B. A., Herman, J. P., & Spencer, R. L. (2006). Habituation to repeated restraint stress is associated with lack of stress-induced c-fos expression in primary sensory processing areas of the rat brain. *Neuroscience*, *138*(4), 1067–1081. https://doi.org/10.1016/j.neuroscience.2005.12.002

Golovatscka, V., Ennes, H., Mayer, E. A., & Bradesi, S. (2012). Chronic stress-induced changes in pro-inflammatory cytokines and spinal glia markers in the rat: a time course study. *Neuroimmunomodulation*, *19*(6), 367–376. https://doi.org/10.1159/000342092

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., Ellis, R. J., Richie, C. T., Harvey, B. K., Dannals, R. F., Pomper, M. G., Bonci, A., & Michaelides, M. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science (New York, N.Y.)*, *357*(6350), 503–507. https://doi.org/10.1126/science.aan2475

Gompel, M., Leost, M., Bal De Kier Joffe, E., Puricelli, L., Hernandez Franco, L., Palermo, J., & Meijer, L. (2004). Meridianins, a new family of protein kinase inhibitors isolated from the Ascidian Aplidium meridianum. *Bioorganic & Medicinal Chemistry Letters*, *14*(7), 1703–1707. https://doi.org/10.1016/J.BMCL.2004.01.050

Goodkind, M., Eickhoff, S. B., Oathes, D. J., Jiang, Y., Chang, A., Jones-Hagata, L. B., Ortega, B. N., Zaiko, Y. V, Roach, E. L., Korgaonkar, M. S., Grieve, S. M., Galatzer-Levy, I., Fox, P. T., & Etkin, A. (2015). Identification of a common neurobiological substrate for mental illness. *JAMA Psychiatry*, *72*(4), 305–315. https://doi.org/10.1001/jamapsychiatry.2014.2206

Greenberg, P. E., Fournier, A.-A., Sisitsky, T., Simes, M., Berman, R., Koenigsberg, S. H., & Kessler, R. C. (2021). The Economic Burden of Adults with Major Depressive Disorder in the United States (2010 and 2018). *PharmacoEconomics*, *39*(6), 653–665. https://doi.org/10.1007/s40273-021-01019-4

Grimes, C. A., & Jope, R. S. (2001). CREB DNA binding activity is inhibited by glycogen synthase kinase-3 beta and facilitated by lithium. *Journal of Neurochemistry*, *78*(6), 1219–1232. https://doi.org/10.1046/j.1471-4159.2001.00495.x

Guilloux, J.-P., Seney, M., Edgar, N., & Sibille, E. (2011). Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: Relevance to emotionality and sex. *Journal of Neuroscience Methods*, *197*(1), 21–31. https://doi.org/https://doi.org/10.1016/j.jneumeth.2011.01.019

Guo, H., Deji, C., Peng, H., Zhang, J., Chen, Y., Zhang, Y., & Wang, Y. (2021). The role of SIRT1 in the basolateral amygdala in depression-like behaviors in mice. *Genes, Brain and Behavior*, *20*(8), e12765. https://doi.org/https://doi.org/10.1111/gbb.12765

Gururajan, A., Reif, A., Cryan, J. F., & Slattery, D. A. (2019). The future of rodent models in depression research. *Nature Reviews Neuroscience 2019 20:11*, *20*(11), 686–701. https://doi.org/10.1038/s41583-019-0221-6

Hage, M. P., & Azar, S. T. (2012). The Link between Thyroid Function and Depression. *Journal of Thyroid Research*, *2012*, 590648. https://doi.org/10.1155/2012/590648

Hall, J., Thomas, K. L., & Everitt, B. J. (2001). Cellular Imaging of Zif268 Expression in the Hippocampus and Amygdala during Contextual and Cued Fear Memory Retrieval: Selective Activation of Hippocampal CA1 Neurons during the Recall of Contextual Memories. *The Journal of Neuroscience*, *21*(6), 2186 LP – 2193. https://doi.org/10.1523/JNEUROSCI.21-06-02186.2001

Hamblin, M. R. (2016). Shining light on the head: Photobiomodulation for brain disorders. *BBA Clinical*, *6*, 113–124. https://doi.org/10.1016/j.bbacli.2016.09.002

Hamblin, M. R. (2017). Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophysics*, *4*(3), 337–361. https://doi.org/10.3934/biophy.2017.3.337

Hamilton, C., Liebert, A., Pang, V., Magistretti, P., & Mitrofanis, J. (2022). Lights on for

Autism: Exploring Photobiomodulation as an Effective Therapeutic Option. In *Neurology International* (Vol. 14, Issue 4, pp. 884–893). https://doi.org/10.3390/neurolint14040071

Hamilton, J. P., Etkin, A., Furman, D. J., Lemus, M. G., Johnson, R. F., & Gotlib, I. H. (2012). Functional Neuroimaging of Major Depressive Disorder: A Meta-Analysis and New Integration of Baseline Activation and Neural Response Data. *American Journal of Psychiatry*, *169*(7), 693–703. https://doi.org/10.1176/appi.ajp.2012.11071105

Hamon, M., & Blier, P. (2013). Monoamine neurocircuitry in depression and strategies for new treatments. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *45*, 54–63. https://doi.org/10.1016/j.pnpbp.2013.04.009

Han, L. K. M., Aghajani, M., Clark, S. L., Chan, R. F., Hattab, M. W., Shabalin, A. A., Zhao, M., Kumar, G., Xie, L. Y., Jansen, R., Milaneschi, Y., Dean, B., Aberg, K. A., van den Oord, E. J. C. G., & Penninx, B. W. J. H. (2018). Epigenetic Aging in Major Depressive Disorder. *American Journal of Psychiatry*, *175*(8), 774–782. https://doi.org/10.1176/appi.ajp.2018.17060595

Hartley, T., Bird, C. M., Chan, D., Cipolotti, L., Husain, M., Vargha-Khadem, F., & Burgess, N. (2007). The hippocampus is required for short-term topographical memory in humans. *Hippocampus*, *17*(1), 34–48. https://doi.org/10.1002/hipo.20240

Hashmi, J. T., Huang, Y. Y., Sharma, S. K., Kurup, D. B., De Taboada, L., Carroll, J. D., & Hamblin, M. R. (2010). Effect of pulsing in low-level light therapy. *Lasers in Surgery and Medicine*, *42*(6), 450–466. https://doi.org/10.1002/LSM.20950

Hennessy, M., & Hamblin, M. R. (2017). Photobiomodulation and the brain: a new paradigm. *Journal of Optics*, *19*(1), 13003. https://doi.org/10.1088/2040-8986/19/1/013003

Henter, I. D., Park, L. T., & Zarate, C. A. (2021). Novel Glutamatergic Modulators for the Treatment of Mood Disorders: Current Status. *CNS Drugs*, *35*(5), 527–543. https://doi.org/10.1007/s40263-021-00816-x

Herdegen, T., & Leah, J. D. (1998). Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Research Reviews*, *28*(3), 370–490. https://doi.org/https://doi.org/10.1016/S0165-0173(98)00018-6

Hermida, M. A., Dinesh Kumar, J., & Leslie, N. R. (2017). GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. *Advances in Biological Regulation*, *65*, 5–15. https://doi.org/10.1016/j.jbior.2017.06.003

Hernández, F., Langa, E., Cuadros, R., Avila, J., & Villanueva, N. (2010). Regulation of GSK3 isoforms by phosphatases PP1 and PP2A. *Molecular and Cellular Biochemistry*, *344*(1), 211–215. https://doi.org/10.1007/s11010-010-0544-0

Hernando, A., Galvez, F., García, M. A., Soto-León, V., Alonso-Bonilla, C., Aguilar, J., & Oliviero, A. (2020). Effects of Moderate Static Magnetic Field on Neural Systems Is a Non-invasive Mechanical Stimulation of the Brain Possible Theoretically? *Frontiers in Neuroscience*, *14*, 419. https://doi.org/10.3389/fnins.2020.00419

Hill, M. N., Hellemans, K. G. C., Verma, P., Gorzalka, B. B., & Weinberg, J. (2012). Neurobiology of chronic mild stress: Parallels to major depression HHS Public Access. *Neurosci Biobehav Rev*, *36*(9), 2085–2117. https://doi.org/10.1016/j.neubiorev.2012.07.001

Hobara, T., Uchida, S., Otsuki, K., Matsubara, T., Funato, H., Matsuo, K., Suetsugi, M., & Watanabe, Y. (2010). Altered gene expression of histone deacetylases in mood disorder patients. *Journal of Psychiatric Research*, *44*(5), 263–270. https://doi.org/https://doi.org/10.1016/j.jpsychires.2009.08.015

Hodges, T. E., Green, M. R., Simone, J. J., & McCormick, C. M. (2014). Effects of social context on endocrine function and Zif268 expression in response to an acute stressor in adolescent and adult rats. *International Journal of Developmental Neuroscience*, *35*(1), 25–34. https://doi.org/10.1016/J.IJDEVNEU.2014.03.001

Holanda, V. A. D., Santos, W. B., Asth, L., Guerrini, R., Calo', G., Ruzza, C., & Gavioli, E. C. (2018). NOP agonists prevent the antidepressant-like effects of nortriptyline and fluoxetine but not R-ketamine. *Psychopharmacology*, *235*(11), 3093–3102. https://doi.org/10.1007/s00213-018-5004-7

Hong, N., Kim, H. J., Kang, K., Park, J. O., Mun, S., Kim, H.-G., Kang, B. H., Chung, P.-S., Lee, M. Y., & Ahn, J.-C. (2023). Photobiomodulation improves the synapses and cognitive function and ameliorates epileptic seizure by inhibiting downregulation of Nlgn3. *Cell & Bioscience*, *13*(1), 8. https://doi.org/10.1186/s13578-022-00949-6

Howard, D. M., Adams, M. J., Clarke, T. K., Hafferty, J. D., Gibson, J., Shirali, M., Coleman, J. R. I., Hagenaars, S. P., Ward, J., Wigmore, E. M., Alloza, C., Shen, X., Barbu, M. C., Xu, E. Y., Whalley, H. C., Marioni, R. E., Porteous, D. J., Davies, G., Deary, I. J., … McIntosh, A. M. (2019). Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. *Nature Neuroscience*, *22*(3), 343–352. https://doi.org/10.1038/s41593-018-0326-7

Hu, H., Cui, Y., & Yang, Y. (2020). Circuits and functions of the lateral habenula in health and in disease. *Nature Reviews. Neuroscience*, *21*(5), 277–295. https://doi.org/10.1038/s41583-020-0292-4

Huang, M.-C., & Lin, S.-K. (2020). *Ketamine Abuse: Past and Present BT - Ketamine: From Abused Drug to Rapid-Acting Antidepressant* (K. Hashimoto, S. Ide, & K. Ikeda (Eds.); pp. 1–14). Springer Singapore. https://doi.org/10.1007/978-981-15-2902-3_1

Hyman, S. (2014). Mental health: depression needs large human-genetics studies. *Nature*, *515*(7526), 189–191. https://doi.org/10.1038/515189a

Ieraci, A., Mallei, A., & Popoli, M. (2016). Social Isolation Stress Induces Anxious-Depressive-Like Behavior and Alterations of Neuroplasticity-Related Genes in Adult Male Mice. *Neural Plasticity*, *2016*. https://doi.org/10.1155/2016/6212983

Iliodromiti, Z., Triantafyllou, A.-R., Tsaousi, M., Pouliakis, A., Petropoulou, C., Sokou, R., Volaki, P., Boutsikou, T., & Iacovidou, N. (2023). Gut Microbiome and Neurodevelopmental Disorders: A Link Yet to Be Disclosed. In *Microorganisms* (Vol. 11, Issue 2). https://doi.org/10.3390/microorganisms11020487

Inkster, B., Nichols, T. E., Saemann, P. G., Auer, D. P., Holsboer, F., Muglia, P., & Matthews, P. M. (2009). Association of GSK3β Polymorphisms With Brain Structural Changes in Major Depressive Disorder. *Archives of General Psychiatry*, *66*(7), 721–728. https://doi.org/10.1001/archgenpsychiatry.2009.70

Iorio, C., Pacitti, F., Rossi, A., Iorio, P., & Pompili, A. (2022). Declarative Memory Impairment and Emotional Bias in Recurrent Depression with a Seasonal Pattern: The Interplay between Emotion and Cognition in Seasonal Affective Disorder. *Brain Sciences*, *12*(10). https://doi.org/10.3390/brainsci12101352

Ising, M., & Holsboer, F. (2006). Genetics of stress response and stress-related disorders. *Dialogues in Clinical Neuroscience*, *8*(4), 433–444. https://doi.org/10.31887/DCNS.2006.8.4/mising

Isingrini, E., Camus, V., Le Guisquet, A. M., Pingaud, M., Devers, S., & Belzung, C. (2010). Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: A model of fluoxetine resistance in mice. *PLoS ONE*, *5*(4). https://doi.org/10.1371/journal.pone.0010404

Izquierdo, I., Medina, J. H., Vianna, M. R. M., Izquierdo, L. A., & Barros, D. M. (1999). Separate mechanisms for short- and long-term memory. *Behavioural Brain Research*, *103*(1), 1–11. https://doi.org/https://doi.org/10.1016/S0166-4328(99)00036-4

Jagdeo, J. R., Adams, L. E., Brody, N. I., & Siegel, D. M. (2012). Transcranial Red and Near Infrared Light Transmission in a Cadaveric Model. *PLOS ONE*, *7*(10), e47460.

Jakobsson, J., Cordero, M. I., Bisaz, R., Groner, A. C., Busskamp, V., Bensadoun, J.-C., Cammas, F., Losson, R., Mansuy, I. M., Sandi, C., & Trono, D. (2008). KAP1-mediated epigenetic repression in the forebrain modulates behavioral vulnerability to stress. *Neuron*, *60*(5), 818–831. https://doi.org/10.1016/j.neuron.2008.09.036

Jiang, H., Alahmad, A., Fu, S., Fu, X., Liu, Z., Han, X., Li, L., Song, T., Xu, M., Liu, S., Wang, J., Albash, B., Alaqeel, A., Catalina, V., Prokisch, H., Taylor, R. W., McFarland, R., & Fang, F. (2022). Identification and characterization of novel MPC1 gene variants causing mitochondrial pyruvate carrier deficiency. *Journal of Inherited Metabolic Disease*, *45*(2), 264–277. https://doi.org/10.1002/jimd.12462

Jiang, R., Wang, Y., Liu, J., Wu, Z., Wang, D., Deng, Q., Yang, C., & Zhou, Q. (2022). Gut microbiota is involved in the antidepressant effects of adipose-derived mesenchymal stem cells in chronic social defeat stress mouse model. *Psychopharmacology*, *239*(2), 533–549. https://doi.org/10.1007/s00213-021-06037-w

Johnstone, D M, el Massri, N., Moro, C., Spana, S., Wang, X. S., Torres, N., Chabrol, C., De Jaeger, X., Reinhart, F., Purushothuman, S., Benabid, A.-L., Stone, J., & Mitrofanis, J. (2014). Indirect application of near infrared light induces neuroprotection in a mouse model of parkinsonism - an abscopal neuroprotective effect. *Neuroscience*, *274*, 93–101. https://doi.org/10.1016/j.neuroscience.2014.05.023

Johnstone, Daniel M, Mitrofanis, J., & Stone, J. (2015). Targeting the body to protect the brain: inducing neuroprotection with remotely-applied near infrared light. *Neural Regeneration Research*, *10*(3), 349–351. https://doi.org/10.4103/1673-5374.153673

Jones, M. W., Errington, M. L., & French, P. J. (2001). A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories, A Bliss, T V Garel, S Charnay, P Bozon, B Laroche, S Davis, S Zif268 in the expression of late LTP and long-term memoriesTitle. *Nature Neuroscience*, *4*(3), 289–296.

Jope, R., & Roh, M.-S. (2012). Glycogen Synthase Kinase-3 (GSK3) in Psychiatric Diseases and Therapeutic Interventions. *Current Drug Targets*, *7*(11), 1421–1434. https://doi.org/10.2174/1389450110607011421

Jope, R. S. (2011). Glycogen Synthase Kinase-3 in the Etiology and Treatment of Mood Disorders. *Frontiers in Molecular Neuroscience*, *4*(August), 1–11. https://doi.org/10.3389/fnmol.2011.00016

Jung, S. H., Brownlow, M. L., Pellegrini, M., & Jankord, R. (2017). Divergence in Morris

Water Maze-Based Cognitive Performance under Chronic Stress Is Associated with the Hippocampal Whole Transcriptomic Modification in Mice. *Frontiers in Molecular Neuroscience*, *10*, 275. https://doi.org/10.3389/fnmol.2017.00275

Kaidanovich-Beilin, O., Milman, A., Weizman, A., Pick, C. G., & Eldar-Finkelman, H. (2004). Rapid antidepressive-like activity of specific glycogen synthase kinase-3 inhibitor and its effect on beta-catenin in mouse hippocampus. *Biological Psychiatry*, *55*(8), 781–784. https://doi.org/10.1016/j.biopsych.2004.01.008

Kaiser, R. H., Andrews-Hanna, J. R., Wager, T. D., & Pizzagalli, D. A. (2015). Large-Scale Network Dysfunction in Major Depressive Disorder: A Meta-analysis of Resting-State Functional Connectivity. *JAMA Psychiatry*, *72*(6), 603–611. https://doi.org/10.1001/jamapsychiatry.2015.0071

Kaiser, T., Herzog, P., Voderholzer, U., & Brakemeier, E. L. (2021). Unraveling the comorbidity of depression and anxiety in a large inpatient sample: Network analysis to examine bridge symptoms. *Depression and Anxiety*, *38*(3), 307–317. https://doi.org/10.1002/da.23136

Kallarackal, A. J., Kvarta, M. D., Cammarata, E., Jaberi, L., Cai, X., Bailey, A. M., & Thompson, S. M. (2013). Chronic Stress Induces a Selective Decrease in AMPA Receptor-Mediated Synaptic Excitation at Hippocampal Temporoammonic-CA1 Synapses. *Journal of Neuroscience*, *33*(40), 15669–15674. https://doi.org/10.1523/JNEUROSCI.2588-13.2013

Kamran, M., Bibi, F., ur. Rehman, A., & Morris, D. W. (2022). Major Depressive Disorder: Existing Hypotheses about Pathophysiological Mechanisms and New Genetic Findings. In *Genes* (Vol. 13, Issue 4). https://doi.org/10.3390/genes13040646

Kanter, J. W., Manos, R. C., Bowe, W. M., Baruch, D. E., Busch, A. M., & Rusch, L. C. (2010). What is behavioral activation?: A review of the empirical literature. *Clinical Psychology Review*, *30*(6), 608–620. https://doi.org/10.1016/J.CPR.2010.04.001

Karege, F., Perret, G., Bondolfi, G., Schwald, M., Bertschy, G., & Aubry, J.-M. (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Research*, *109*(2), 143–148. https://doi.org/10.1016/s0165-1781(02)00005-7

Karege, F., Perroud, N., Burkhardt, S., Fernandez, R., Ballmann, E., La Harpe, R., & Malafosse, A. (2012). Protein levels of β-catenin and activation state of glycogen synthase kinase-3β in major depression. A study with postmortem prefrontal cortex. *Journal of Affective Disorders*, *136*(1–2), 185–188. https://doi.org/10.1016/j.jad.2011.09.024

Karege, F., Perroud, N., Burkhardt, S., Schwald, M., Ballmann, E., La Harpe, R., & Malafosse, A. (2007). Alteration in kinase activity but not in protein levels of protein kinase B and glycogen synthase kinase-3beta in ventral prefrontal cortex of depressed suicide victims. *Biological Psychiatry*, *61*(2), 240–245. https://doi.org/10.1016/J.BIOPSYCH.2006.04.036

Karpov, A. S., Merkul, E., Rominger, F., & Müller, T. J. J. (2005). Concise syntheses of meridianins by carbonylative alkynylation and a four-component pyrimidine synthesis. *Angewandte Chemie - International Edition*, *44*(42), 6951–6956. https://doi.org/10.1002/ANIE.200501703

Karrouri, R., Hammani, Z., Benjelloun, R., & Otheman, Y. (2021). Major depressive disorder: Validated treatments and future challenges. *World Journal of Clinical Cases*, *9*(31),

9350–9367. https://doi.org/10.12998/wjcc.v9.i31.9350

Kessler, R. C. (1997). The effects of stressful life events on depression. *Annual Review of Psychology*, *48*, 191–214. https://doi.org/10.1146/annurev.psych.48.1.191

Kim, A., García-García, E., Straccia, M., Comella-Bolla, A., Miguez, A., Masana, M., Alberch, J., Canals, J. M., & Rodríguez, M. J. (2020). Reduced Fractalkine Levels Lead to Striatal Synaptic Plasticity Deficits in Huntington's Disease. *Frontiers in Cellular Neuroscience*, *14*, 163. https://doi.org/10.3389/FNCEL.2020.00163/BIBTEX

Kim, H.-D., Hesterman, J., Call, T., Magazu, S., Keeley, E., Armenta, K., Kronman, H., Neve, R. L., Nestler, E. J., & Ferguson, D. (2016). SIRT1 Mediates Depression-Like Behaviors in the Nucleus Accumbens. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *36*(32), 8441–8452. https://doi.org/10.1523/JNEUROSCI.0212-16.2016

Kim, U.-J., Hong, N., & Ahn, J.-C. (2022). Photobiomodulation Attenuated Cognitive Dysfunction and Neuroinflammation in a Prenatal Valproic Acid-Induced Autism Spectrum Disorder Mouse Model. *International Journal of Molecular Sciences*, *23*(24). https://doi.org/10.3390/ijms232416099

Kishi, T., Yoshimura, R., Kitajima, T., Okochi, T., Okumura, T., Tsunoka, T., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., Fukuo, Y., Naitoh, H., Umene-Nakano, W., Inada, T., Nakamura, J., Ozaki, N., & Iwata, N. (2010). SIRT1 gene is associated with major depressive disorder in the Japanese population. *Journal of Affective Disorders*, *126*(1), 167–173. https://doi.org/https://doi.org/10.1016/j.jad.2010.04.003

Kivimäki, M., Bartolomucci, A., & Kawachi, I. (2023). The multiple roles of life stress in metabolic disorders. *Nature Reviews Endocrinology*, *19*(1), 10–27. https://doi.org/10.1038/s41574-022-00746-8

Klimek, V., Schenck, J. E., Han, H., Stockmeier, C. A., & Ordway, G. A. (2002). Dopaminergic abnormalities in amygdaloid nuclei in major depression: a postmortem study. *Biological Psychiatry*, *52*(7), 740–748. https://doi.org/10.1016/s0006-3223(02)01383-5

Knapska, E., & Kaczmarek, L. (2004). A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? *Progress in Neurobiology*, *74*(4), 183–211. https://doi.org/10.1016/j.pneurobio.2004.05.007

Kosten, T. A., Galloway, M. P., Duman, R. S., Russell, D. S., & D'Sa, C. (2008). Repeated Unpredictable Stress and Antidepressants Differentially Regulate Expression of the Bcl-2 Family of Apoptotic Genes in Rat Cortical, Hippocampal, and Limbic Brain Structures. *Neuropsychopharmacology*, *33*(7), 1545–1558. https://doi.org/10.1038/sj.npp.1301527

Krishnan, V., Han, M.-H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., LaPlant, Q., Graham, A., Lutter, M., Lagace, D. C., Ghose, S., Reister, R., Tannous, P., Green, T. A., Neve, R. L., Chakravarty, S., Kumar, A., Eisch, A. J., Self, D. W., … Nestler, E. J. (2007). Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. *Cell*, *131*(2), 391–404. https://doi.org/10.1016/j.cell.2007.09.018

Krishnan, V., & Nestler, E. J. (2011). Animal models of depression: molecular perspectives. *Current Topics in Behavioral Neurosciences*, *7*, 121–147. https://doi.org/10.1007/7854_2010_108

Krugers, H. J., Koolhaas, J. M., Medema, R. M., & Korf, J. (1996). Prolonged subordination stress increases Calbindin-D28k immunoreactivity in the rat hippocampal CA1 area. *Brain Research*, *729*(2), 289–293. https://doi.org/10.1016/0006-8993(96)00583-5

Kryst, J., Kawalec, P., & Pilc, A. (2020). Efficacy and safety of intranasal esketamine for the treatment of major depressive disorder. *Expert Opinion on Pharmacotherapy*, *21*(1), 9–20. https://doi.org/10.1080/14656566.2019.1683161

Krzystyniak, A., Baczynska, E., Magnowska, M., Antoniuk, S., Roszkowska, M., Zareba-Koziol, M., Das, N., Basu, S., Pikula, M., & Wlodarczyk, J. (2019). Prophylactic Ketamine Treatment Promotes Resilience to Chronic Stress and Accelerates Recovery: Correlation with Changes in Synaptic Plasticity in the CA3 Subregion of the Hippocampus. *International Journal of Molecular Sciences Article Int. J. Mol. Sci*, *20*, 1726. https://doi.org/10.3390/ijms20071726

Kuga, N., Nakayama, R., Morikawa, S., Yagishita, H., Konno, D., Shiozaki, H., Honjoya, N., Ikegaya, Y., & Sasaki, T. (2023). Hippocampal sharp wave ripples underlie stress susceptibility in male mice. *Nature Communications*, *14*(1), 2105. https://doi.org/10.1038/s41467-023-37736-x

Kuleshov, M. V, Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., Koplev, S., Jenkins, S. L., Jagodnik, K. M., Lachmann, A., McDermott, M. G., Monteiro, C. D., Gundersen, G. W., & Ma'ayan, A. (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research*, *44*(W1), W90-7. https://doi.org/10.1093/nar/gkw377

Kumar, R., Chaterjee, P., Sharma, P. K., Singh, A. K., Gupta, A., Gill, K., Tripathi, M., Dey, A. B., & Dey, S. (2013). Sirtuin1: a promising serum protein marker for early detection of Alzheimer's disease. *PloS One*, *8*(4), e61560. https://doi.org/10.1371/journal.pone.0061560

Kunji, E. R. S., King, M. S., Ruprecht, J. J., & Thangaratnarajah, C. (2020). The SLC25 Carrier Family: Important Transport Proteins in Mitochondrial Physiology and Pathology. *Physiology (Bethesda, Md.)*, *35*(5), 302–327. https://doi.org/10.1152/physiol.00009.2020

Kuti, D., Winkler, Z., Horváth, K., Juhász, B., Szilvásy-Szabó, A., Fekete, C., Ferenczi, S., & Kovács, K. J. (2022). The metabolic stress response: Adaptation to acute-, repeated- and chronic challenges in mice. *IScience*, *25*(8). https://doi.org/10.1016/j.isci.2022.104693

Kwon, J., Kim, Y. J., Choi, K., Seol, S., & Kang, H. J. (2019). Identification of stress resilience module by weighted gene co-expression network analysis in Fkbp5-deficient mice. *Molecular Brain*, *12*(1), 99. https://doi.org/10.1186/s13041-019-0521-9

Laine, M. A., Sokolowska, E., Dudek, M., Callan, S. A., Hyytiä, P., & Hovatta, I. (2017). Brain activation induced by chronic psychosocial stress in mice. *Scientific Reports*, *7*(1). https://doi.org/10.1038/S41598-017-15422-5

Lambert, K. G., Gerecke, K. M., Quadros, P. S., Doudera, E., Jasnow, A. M., & Kinsley, C. H. (2000). Activity-Stress Increases Density of GFAP-Immunoreactive Astrocytes in the Rat Hippocampus. *Stress*, *3*(4), 275–284. https://doi.org/10.3109/10253890009001133

Lapchak, P. A., Boitano, P. D., Butte, P. V, Fisher, D. J., Hölscher, T., Ley, E. J., Nuño, M., Voie, A. H., & Rajput, P. S. (2015). Transcranial Near-Infrared Laser Transmission (NILT) Profiles (800 nm): Systematic Comparison in Four Common Research Species.

PLOS ONE, *10*(6), e0127580.

Lautrup, S., Sinclair, D. A., Mattson, M. P., & Fang, E. F. (2019). NAD(+) in Brain Aging and Neurodegenerative Disorders. *Cell Metabolism*, *30*(4), 630–655. https://doi.org/10.1016/j.cmet.2019.09.001

Lebar, M. D., Hahn, K. N., Mutka, T., Maignan, P., McClintock, J. B., Amsler, C. D., van Olphen, A., Kyle, D. E., & Baker, B. J. (2011). CNS and antimalarial activity of synthetic meridianin and psammopemmin analogs. *Bioorganic & Medicinal Chemistry*, *19*(19), 5756–5762. https://doi.org/10.1016/j.bmc.2011.08.033

Lee, H. K., Takamiya, K., Han, J. S., Man, H., Kim, C. H., Rumbaugh, G., Yu, S., Ding, L., He, C., Petralia, R. S., Wenthold, R. J., Gallagher, M., & Huganir, R. L. (2003). Phosphorylation of the AMPA Receptor GluR1 Subunit Is Required for Synaptic Plasticity and Retention of Spatial Memory. *Cell*, *112*(5), 631–643. https://doi.org/10.1016/S0092-8674(03)00122-3

Lee, S. M., Dong, T. S., Krause-Sorio, B., Siddarth, P., Milillo, M. M., Lagishetty, V., Datta, T., Aguilar-Faustino, Y., Jacobs, J. P., & Lavretsky, H. (2022). The intestinal microbiota as a predictor for antidepressant treatment outcome in geriatric depression: a prospective pilot study. *International Psychogeriatrics*, *34*(1), 33–45. https://doi.org/10.1017/S1041610221000120

Leem, Y.-H., Park, J.-S., Chang, H., Park, J., & Kim, H.-S. (2019). *Exercise Prevents Memory Consolidation Defects Via Enhancing Prolactin Responsiveness of CA1 Neurons in Mice Under Chronic Stress*. https://doi.org/10.1007/s12035-019-1560-z

Legendy, C. R., & Salcman, M. (1985). Bursts and recurrences of bursts in the spike trains of spontaneously active striate cortex neurons. *Journal of Neurophysiology*, *53*(4), 926–939. https://doi.org/10.1152/JN.1985.53.4.926

Leng, L., Zhuang, K., Liu, Z., Huang, C., Gao, Y., Chen, G., Lin, H., Hu, Y., Wu, D., Shi, M., Xie, W., Sun, H., Shao, Z., Li, H., Zhang, K., Mo, W., Huang, T. Y., Xue, M., Yuan, Z., … Zhang, J. (2018). Menin Deficiency Leads to Depressive-like Behaviors in Mice by Modulating Astrocyte-Mediated Neuroinflammation. *Neuron*, *100*(3), 551-563.e7. https://doi.org/10.1016/j.neuron.2018.08.031

Leung, M. C. P., Lo, S. C. L., Siu, F. K. W., & So, K.-F. (2002). Treatment of experimentally induced transient cerebral ischemia with low energy laser inhibits nitric oxide synthase activity and up-regulates the expression of transforming growth factor-beta 1. *Lasers in Surgery and Medicine*, *31*(4), 283–288. https://doi.org/10.1002/lsm.10096

Levchenko, A., Losenkov, I. S., Vyalova, N. M., Simutkin, G. G., Bokhan, N. A., Wilffert, B., Loonen, A. J. M., & Ivanova, S. A. (2018). The functional variant RS334558 of GSK3B is associated with remission in patients with depressive disorders. *Pharmacogenomics and Personalized Medicine*, *11*, 121–126. https://doi.org/10.2147/PGPM.S171423

Levkovitz, Y., & Baraban, J. M. (2002). A dominant negative Egr inhibitor blocks nerve growth factor-induced neurite outgrowth by suppressing c-Jun activation: role of an Egr/c-Jun complex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(10), 3845–3854. https://doi.org/10.1523/JNEUROSCI.22-10-03845.2002

Li, J. T., Xie, X. M., Yu, J. Y., Sun, Y. X., Liao, X. M., Wang, X. X., Su, Y. A., Liu, Y. J., Schmidt, M. V., Wang, X. D., & Si, T. M. (2017). Suppressed Calbindin Levels in

Hippocampal Excitatory Neurons Mediate Stress-Induced Memory Loss. *Cell Reports*, *21*(4), 891–900. https://doi.org/10.1016/J.CELREP.2017.10.006/ATTACHMENT/7B93DAF3-E1BF-4EFE-B62E-B493121ED8C5/MMC1.PDF

Li, M, D'Arcy, C., & Meng, X. (2016). Maltreatment in childhood substantially increases the risk of adult depression and anxiety in prospective cohort studies: systematic review, meta-analysis, and proportional attributable fractions. *Psychological Medicine*, *46*(4), 717–730. https://doi.org/10.1017/S0033291715002743

Li, Ming, Yao, X., Sun, L., Zhao, L., Xu, W., Zhao, H., Zhao, F., Zou, X., Cheng, Z., Li, B., Yang, W., & Cui, R. (2020). Effects of Electroconvulsive Therapy on Depression and Its Potential Mechanism . In *Frontiers in Psychology* (Vol. 11).

Li, W., Guo, B., Tao, K., Li, F., Liu, Z., Yao, H., Feng, D., & Liu, X. (2019). Inhibition of SIRT1 in hippocampal CA1 ameliorates PTSD-like behaviors in mice by protections of neuronal plasticity and serotonin homeostasis via NHLH2/MAO-A pathway. *Biochemical and Biophysical Research Communications*, *518*(2), 344–350. https://doi.org/10.1016/j.bbrc.2019.08.060

Li, X., Friedman, A. B., Zhu, W., Wang, L., Boswell, S., May, R. S., Davis, L. L., & Jope, R. S. (2007). Lithium Regulates Glycogen Synthase Kinase-3β in Human Peripheral Blood Mononuclear Cells: Implication in the Treatment of Bipolar Disorder. *Biological Psychiatry*, *61*(2), 216–222. https://doi.org/10.1016/j.biopsych.2006.02.027

Li, X., & Jope, R. S. (2010). Is glycogen synthase kinase-3 a central modulator in mood regulation. *Neuropsychopharmacology*, *35*(11), 2143–2154. https://doi.org/10.1038/npp.2010.105

Li, X., & Polter, A. (2011). Glycogen Synthase Kinase-3 is an Intermediate Modulator of Serotonin Neurotransmission . In *Frontiers in Molecular Neuroscience* (Vol. 4).

Liang, J., Liu, L., & Xing, D. (2012). Photobiomodulation by low-power laser irradiation attenuates Aβ-induced cell apoptosis through the Akt/GSK3β/β-catenin pathway. *Free Radical Biology & Medicine*, *53*(7), 1459–1467. https://doi.org/10.1016/j.freeradbiomed.2012.08.003

Libert, S., Pointer, K., Bell, E. L., Das, A., Cohen, D. E., Asara, J. M., Kapur, K., Bergmann, S., Preisig, M., Otowa, T., Kendler, K. S., Chen, X., Hettema, J. M., van den Oord, E. J., Rubio, J. P., & Guarente, L. (2011). SIRT1 Activates MAO-A in the Brain to Mediate Anxiety and Exploratory Drive. *Cell*, *147*(7), 1459–1472. https://doi.org/https://doi.org/10.1016/j.cell.2011.10.054

Lieberman, J. A., Girgis, R. R., Brucato, G., Moore, H., Provenzano, F., Kegeles, L., Javitt, D., Kantrowitz, J., Wall, M. M., Corcoran, C. M., Schobel, S. A., & Small, S. A. (2018). Hippocampal dysfunction in the pathophysiology of schizophrenia: a selective review and hypothesis for early detection and intervention. *Molecular Psychiatry*, *23*(8), 1764–1772. https://doi.org/10.1038/mp.2017.249

Liebert, A., Bicknell, B., Johnstone, D. M., Gordon, L. C., Kiat, H., & Hamblin, M. R. (2019). "Photobiomics": Can Light, Including Photobiomodulation, Alter the Microbiome? *Photobiomodulation, Photomedicine, and Laser Surgery*, *37*(11), 681–693. https://doi.org/10.1089/photob.2019.4628

Lim, W., Kim, J., Kim, S., Karna, S., Won, J., Jeon, S. M., Kim, S. Y., Choi, Y., Choi, H., & Kim, O. (2013). Modulation of lipopolysaccharide-induced NF-κB signaling pathway

by 635 nm irradiation via heat shock protein 27 in human gingival fibroblast cells. *Photochemistry and Photobiology*, *89*(1), 199–207. https://doi.org/10.1111/j.1751-1097.2012.01225.x

Lin, E., & Tsai, S.-J. (2019). Epigenetics and Depression: An Update. *Psychiatry Investigation*, *16*(9), 654–661. https://doi.org/10.30773/pi.2019.07.17.2

Liu, L., Wang, H., Chen, X., Zhang, Y., Zhang, H., & Xie, P. (2023). Gut microbiota and its metabolites in depression: from pathogenesis to treatment. *EBioMedicine*, *90*. https://doi.org/10.1016/j.ebiom.2023.104527

Liu, S. C., Hu, W. Y., Zhang, W. Y., Yang, L., Li, Y., Xiao, Z. C., Zhang, M., & He, Z. Y. (2019). Paeoniflorin attenuates impairment of spatial learning and hippocampal long-term potentiation in mice subjected to chronic unpredictable mild stress. *Psychopharmacology*, *236*(9), 2823–2834. https://doi.org/10.1007/s00213-019-05257-5

Liu, W., Ge, T., Leng, Y., Pan, Z., Fan, J., Yang, W., & Cui, R. (2017). The Role of Neural Plasticity in Depression: From Hippocampus to Prefrontal Cortex. *Neural Plasticity*, *2017*. https://doi.org/10.1155/2017/6871089

Liu, Ying, Poon, V., Sanchez-Watts, G., Watts, A. G., Takemori, H., & Aguilera, G. (2012). Salt-inducible kinase is involved in the regulation of corticotropin-releasing hormone transcription in hypothalamic neurons in rats. *Endocrinology*, *153*(1), 223–233. https://doi.org/10.1210/en.2011-1404

Liu, Yu, Lin, D., Wu, B., & Zhou, W. (2016). Ketamine abuse potential and use disorder. *Brain Research Bulletin*, *126*, 68–73. https://doi.org/https://doi.org/10.1016/j.brainresbull.2016.05.016

Liu, Yunhui, Shao, J., Gao, D., Zhang, L., & Yang, F. (2021). Astrocytes in the Ventromedial Hypothalamus Involve Chronic Stress-Induced Anxiety and Bone Loss in Mice. *Neural Plasticity*, *2021*, 7806370. https://doi.org/10.1155/2021/7806370

Llorach-Pares, L., Nonell-Canals, A., Avila, C., & Sanchez-Martinez, M. (2022). Computer-Aided Drug Design (CADD) to De-Orphanize Marine Molecules: Finding Potential Therapeutic Agents for Neurodegenerative and Cardiovascular Diseases. *Marine Drugs*, *20*(1), 53. https://doi.org/10.3390/md20010053

Llorach-Pares, L., Nonell-Canals, A., Sanchez-Martinez, M., & Avila, C. (2017). Computer-Aided Drug Design Applied to Marine Drug Discovery: Meridianins as Alzheimer's Disease Therapeutic Agents. *Marine Drugs*, *15*(12), 366. https://doi.org/10.3390/md15120366

Llorach-Pares, L., Rodriguez-Urgelles, E., Nonell-Canals, A., Alberch, J., Avila, C., Sanchez-Martinez, M., & Giralt, A. (2020). Meridianins and Lignarenone B as Potential GSK3β Inhibitors and Inductors of Structural Neuronal Plasticity. *Biomolecules*, *10*(4). https://doi.org/10.3390/BIOM10040639

Long-Smith, C., O'Riordan, K. J., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2020). Microbiota-Gut-Brain Axis: New Therapeutic Opportunities. *Annual Review of Pharmacology and Toxicology*, *60*(1), 477–502. https://doi.org/10.1146/annurev-pharmtox-010919-023628

Longueville, S., Nakamura, Y., Brami-Cherrier, K., Coura, R., Hervé, D., & Girault, J.-A. (2021). Long-lasting tagging of neurons activated by seizures or cocaine administration in Egr1-CreERT2 transgenic mice. *European Journal of Neuroscience*, *53*(5), 1450–1472.

https://doi.org/10.1111/EJN.15060

Lu, G., Li, J., Zhang, H., Zhao, X., Yan, L.-J., & Yang, X. (2018). Role and Possible Mechanisms of Sirt1 in Depression. *Oxidative Medicine and Cellular Longevity*, *2018*, 8596903. https://doi.org/10.1155/2018/8596903

Luczynski, P., Whelan, S. O., O'Sullivan, C., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2016). Adult microbiota-deficient mice have distinct dendritic morphological changes: differential effects in the amygdala and hippocampus. *The European Journal of Neuroscience*, *44*(9), 2654–2666. https://doi.org/10.1111/ejn.13291

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Luscher, B., Shen, Q., & Sahir, N. (2011). The GABAergic deficit hypothesis of major depressive disorder. *Molecular Psychiatry*, *16*(4), 383–406. https://doi.org/10.1038/mp.2010.120

Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., Kubera, M., Bob, P., Lerer, B., & Maj, M. (2009). The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metabolic Brain Disease*, *24*(1), 27–53. https://doi.org/10.1007/s11011-008-9118-1

Mahler, S. V, & Aston-Jones, G. (2018). CNO Evil? Considerations for the Use of DREADDs in Behavioral Neuroscience. *Neuropsychopharmacology*, *43*(5), 934–936. https://doi.org/10.1038/npp.2017.299

Maiello, M., Losiewicz, O. M., Bui, E., Spera, V., Hamblin, M. R., Marques, L., & Cassano, P. (2019). Transcranial Photobiomodulation with Near-Infrared Light for Generalized Anxiety Disorder: A Pilot Study. *Photobiomodulation, Photomedicine, and Laser Surgery*, *37*(10), 644–650. https://doi.org/10.1089/photob.2019.4677

Malberg, E. J., & Schechter, E. L. (2005). Increasing Hippocampal Neurogenesis: A Novel Mechanism for Antidepressant Drugs. In *Current Pharmaceutical Design* (Vol. 11, Issue 2, pp. 145–155). https://doi.org/http://dx.doi.org/10.2174/1381612053382223

Marco, E. M., Valero, M., de la Serna, O., Aisa, B., Borcel, E., Ramirez, M. J., & Viveros, M.-P. (2013). Maternal deprivation effects on brain plasticity and recognition memory in adolescent male and female rats. *Neuropharmacology*, *68*, 223–231. https://doi.org/10.1016/j.neuropharm.2012.08.014

Markov, D. D., & Novosadova, E. V. (2022). Chronic Unpredictable Mild Stress Model of Depression: Possible Sources of Poor Reproducibility and Latent Variables. *Biology*, *11*(11). https://doi.org/10.3390/biology11111621

Masurkar, A. V, Srinivas, K. V, Brann, D. H., Warren, R., Lowes, D. C., & Siegelbaum, S. A. (2017). Medial and Lateral Entorhinal Cortex Differentially Excite Deep versus Superficial CA1 Pyramidal Neurons. *Cell Reports*, *18*(1), 148–160. https://doi.org/10.1016/j.celrep.2016.12.012

Matsumoto, K., Ono, K., Ouchi, H., Tsushima, R., & Murakami, Y. (2012). Social isolation stress down-regulates cortical early growth response 1 (Egr-1) expression in mice. *Neuroscience Research*, *73*, 257–262. https://doi.org/10.1016/j.neures.2012.04.004

Matys, V., Kel-Margoulis, O. V, Fricke, E., Liebich, I., Land, S., Barre-Dirrie, A., Reuter, I.,

Chekmenev, D., Krull, M., Hornischer, K., Voss, N., Stegmaier, P., Lewicki-Potapov, B., Saxel, H., Kel, A. E., & Wingender, E. (2006). TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. *Nucleic Acids Research*, *34*(Database issue), D108-10. https://doi.org/10.1093/nar/gkj143

Mayr, J. A., Havlíčková, V., Zimmermann, F., Magler, I., Kaplanová, V., Ješina, P., Pecinová, A., Nůsková, H., Koch, J., Sperl, W., & Houštěk, J. (2010). Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F1 ε subunit. *Human Molecular Genetics*, *19*(17), 3430–3439. https://doi.org/10.1093/hmg/ddq254

Mazucanti, C. H., Cabral-Costa, J. V., Vasconcelos, A. R., Andreotti, D. Z., Scavone, C., & Kawamoto, E. M. (2015). Longevity Pathways (mTOR, SIRT, Insulin/IGF-1) as Key Modulatory Targets on Aging and Neurodegeneration. *Current Topics in Medicinal Chemistry*, *15*(21), 2116–2138. https://doi.org/10.2174/1568026615666150610125715

McEwen, B. S. (2006). Protective and damaging effects of stress mediators: Central role of the brain. *Dialogues in Clinical Neuroscience*, *8*(4), 367–381. https://doi.org/10.31887/dcns.2006.8.4/bmcewen

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: Central role of the brain. *Physiological Reviews*, *87*(3), 873–904. https://doi.org/10.1152/physrev.00041.2006

McEwen, B. S. (2013). Allostasis and allostatic load: Implications for neuropsychopharmacology. *The Science of Mental Health: Stress and the Brain*, *9*(99), 2–18.

McEwen, B. S., Bowles, N. P., Gray, J. D., Hill, M. N., Hunter, R. G., Karatsoreos, I. N., & Nasca, C. (2015). Mechanisms of stress in the brain. *Nature Neuroscience*, *18*(10), 1353. https://doi.org/10.1038/NN.4086

McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonté, B., Szyf, M., Turecki, G., & Meaney, M. J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience*, *12*(3), 342–348. https://doi.org/10.1038/nn.2270

McLaughlin, K. J., Wilson, J. O., Harman, J., Wright, R. L., Wieczorek, L., Gomez, J., Korol, D. L., & Conrad, C. D. (2010). Chronic 17β-estradiol or cholesterol prevents stress-induced hippocampal CA3 dendritic retraction in ovariectomized female rats: Possible correspondence between CA1 spine properties and spatial acquisition. *Hippocampus*, *20*(6), 768–786. https://doi.org/10.1002/HIPO.20678

McLean, M. J., Engström, S., Qinkun, Z., Spankovich, C., & Polley, D. (2008). Effects of a static magnetic field on audiogenic seizures in black Swiss mice. *Epilepsy Research*, *80*(2), 119–131. https://doi.org/https://doi.org/10.1016/j.eplepsyres.2008.03.022

Medina-Rodriguez, E. M., Lowell, J. A., Worthen, R. J., Syed, S. A., & Beurel, E. (2018). Involvement of Innate and Adaptive Immune Systems Alterations in the Pathophysiology and Treatment of Depression. *Frontiers in Neuroscience*, *12*, 547. https://doi.org/10.3389/fnins.2018.00547

Melia, K. R., Ryabinin, A. E., Schroeder, R., Bloom, F. E., Wilson, M. C., & Anokhin, P. K. (1994). Induction and Habituation of Immediate Early Gene Expression in Rat Brain by Acute and Repeated Restraint Stress. *The Journal of Neuroscience*, *14*(10), 5929–5938.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J.-F., Rougeot, C., Pichelin, M., Cazaubiel, M., & Cazaubiel, J.-M. (2011). Assessment of

psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *The British Journal of Nutrition*, *105*(5), 755–764. https://doi.org/10.1017/S0007114510004319

Meynaghizadeh-Zargar, R., Sadigh-Eteghad, S., Mohaddes, G., Salehpour, F., & Rasta, S. H. (2020). Effects of transcranial photobiomodulation and methylene blue on biochemical and behavioral profiles in mice stress model. *Lasers in Medical Science*, *35*(3), 573–584. https://doi.org/10.1007/s10103-019-02851-z

Meza, D., Wang, D., Wang, Y., Borwege, S., Sanai, N., & Liu, J. T. C. (2015). Comparing high-resolution microscopy techniques for potential intraoperative use in guiding low-grade glioma resections. *Lasers in Surgery and Medicine*, *47*(4), 289–295. https://doi.org/https://doi.org/10.1002/lsm.22347

Mikulska, J., Juszczyk, G., Gawrońska-Grzywacz, M., & Herbet, M. (2021). HPA Axis in the Pathomechanism of Depression and Schizophrenia: New Therapeutic Strategies Based on Its Participation. In *Brain Sciences* (Vol. 11, Issue 10). https://doi.org/10.3390/brainsci11101298

Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. *Biological Psychiatry*, *65*(9), 732. https://doi.org/10.1016/J.BIOPSYCH.2008.11.029

Minatohara, K., Akiyoshi, M., & Okuno, H. (2016). Role of Immediate-Early Genes in Synaptic Plasticity and Neuronal Ensembles Underlying the Memory Trace . In *Frontiers in Molecular Neuroscience* (Vol. 8).

Miskowiak, K. W., Ott, C. V, Petersen, J. Z., & Kessing, L. V. (2016). Systematic review of randomized controlled trials of candidate treatments for cognitive impairment in depression and methodological challenges in the field. *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology*, *26*(12), 1845–1867. https://doi.org/10.1016/j.euroneuro.2016.09.641

Mizuseki, K., Diba, K., Pastalkova, E., & Buzsáki, G. (2011). Hippocampal CA1 pyramidal cells form functionally distinct sublayers. *Nature Neuroscience*, *14*(9), 1174–1181. https://doi.org/10.1038/nn.2894

Mohammed, H. S. (2016). Transcranial low-level infrared laser irradiation ameliorates depression induced by reserpine in rats. *Lasers in Medical Science*, *31*(8), 1651–1656. https://doi.org/10.1007/s10103-016-2033-5

Mohan, B., Salfeena, C. T. F., Ashitha, K. T., Krishnan, G. V., Jesmina, A. R. S., Varghese, A. M., Patil, S. A., Kumar, B. N. S. D., & Sasidhar, B. S. (2018). Functionalized Pyrimidines from Alkynes and Nitriles: Application towards the Synthesis of Marine Natural Product Meridianin Analogs. *ChemistrySelect*, *3*(23), 6394–6398. https://doi.org/10.1002/SLCT.201801126

Mohr, P., Pecenák, J., Svestka, J., Swingler, D., & Treuer, T. (2005). Treatment of acute agitation in psychotic disorders. *Neuro Endocrinology Letters*, *26*(4), 327–335.

Molinski, T. F., Dalisay, D. S., Lievens, S. L., & Saludes, J. P. (2009). Drug development from marine natural products. *Nature Reviews Drug Discovery*, *8*(1), 69–85. https://doi.org/10.1038/nrd2487

Montalban, E., Al-Massadi, O., Sancho-Balsells, A., Brito, V., de Pins, B., Alberch, J., Ginés, S., Girault, J. A., & Giralt, A. (2019). Pyk2 in the amygdala modulates chronic stress

sequelae via PSD-95-related micro-structural changes. *Translational Psychiatry*, *9*(1), 1–12. https://doi.org/10.1038/s41398-018-0352-y

Montazeri, K., Farhadi, M., Fekrazad, R., Chaibakhsh, S., & Mahmoudian, S. (2022). Photobiomodulation therapy in mood disorders: a systematic review. *Lasers in Medical Science*, *37*(9), 3343–3351. https://doi.org/10.1007/s10103-022-03641-w

Morais, L. H., Schreiber, H. L., & Mazmanian, S. K. (2021). The gut microbiota–brain axis in behaviour and brain disorders. *Nature Reviews Microbiology*, *19*(4), 241–255. https://doi.org/10.1038/s41579-020-00460-0

Morató, L., Astori, S., Zalachoras, I., Rodrigues, J., Ghosal, S., Huang, W., Guillot de Suduiraut, I., Grosse, J., Zanoletti, O., Cao, L., Auwerx, J., & Sandi, C. (2022). eNAMPT actions through nucleus accumbens NAD(+)/SIRT1 link increased adiposity with sociability deficits programmed by peripuberty stress. *Science Advances*, *8*(9), eabj9109. https://doi.org/10.1126/sciadv.abj9109

Mu, Y., & Gage, F. H. (2011). Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Molecular Neurodegeneration*, *6*, 85. https://doi.org/10.1186/1750-1326-6-85

Murrough, J. W., Abdallah, C. G., & Mathew, S. J. (2017). Targeting glutamate signalling in depression: Progress and prospects. *Nature Reviews Drug Discovery*, *16*(7), 472–486. https://doi.org/10.1038/nrd.2017.16

Musazzi, L., Tornese, P., Sala, N., & Popoli, M. (2017). Acute or Chronic? A Stressful Question. *Trends in Neurosciences*, *40*(9), 525–535. https://doi.org/10.1016/J.TINS.2017.07.002

Naskar, S., & Chattarji, S. (2019). Stress Elicits Contrasting Effects on the Structure and Number of Astrocytes in the Amygdala versus Hippocampus. *Eneuro*, *6*(1), ENEURO.0338-18.2019. https://doi.org/10.1523/ENEURO.0338-18.2019

Nemeroff, C. B., Krishnan, K. R., Reed, D., Leder, R., Beam, C., & Dunnick, N. R. (1992). Adrenal gland enlargement in major depression. A computed tomographic study. *Archives of General Psychiatry*, *49*(5), 384–387. https://doi.org/10.1001/archpsyc.1992.01820050048008

Nibuya, M., Morinobu, S., & Duman, R. S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *15*(11), 7539–7547. https://doi.org/10.1523/JNEUROSCI.15-11-07539.1995

Nifosì, F., Toffanin, T., Follador, H., Zonta, F., Padovan, G., Pigato, G., Carollo, C., Ermani, M., Amistà, P., & Perini, G. I. (2010). Reduced right posterior hippocampal volume in women with recurrent familial pure depressive disorder. *Psychiatry Research*, *184*(1), 23–28. https://doi.org/10.1016/j.pscychresns.2010.05.012

Ning, L., Paetau, S., Nyman-Huttunen, H., Tian, L., & Gahmberg, C. G. (2015). ICAM-5 affects spine maturation by regulation of NMDA receptor binding to α-actinin. *Biology Open*, *4*(2), 125–136. https://doi.org/10.1242/bio.201410439

Nollet, M. (2021). Models of Depression: Unpredictable Chronic Mild Stress in Mice. *Current Protocols*, *1*(8), e208. https://doi.org/https://doi.org/10.1002/cpz1.208

Northcutt, K. V, & Lonstein, J. S. (2009). Social contact elicits immediate-early gene expression in dopaminergic cells of the male prairie vole extended olfactory amygdala. *Neuroscience*, *163*(1), 9–22. https://doi.org/10.1016/j.neuroscience.2009.06.018

Noto, C., Rizzo, L. B., Mansur, R. B., McIntyre, R. S., Maes, M., & Brietzke, E. (2014). Targeting the inflammatory pathway as a therapeutic tool for major depression. *Neuroimmunomodulation*, *21*(2–3), 131–139. https://doi.org/10.1159/000356549

Núñez-Pons, L., & Avila, C. (2015). Natural products mediating ecological interactions in Antarctic benthic communities: a mini-review of the known molecules. *Natural Product Reports*, *32*(7), 1114–1130. https://doi.org/10.1039/C4NP00150H

Núñez-Pons, Laura, Carbone, M., Vázquez, J., Rodríguez, J., Nieto, R. M., Varela, M. M., Gavagnin, M., & Avila, C. (2012). Natural Products from Antarctic Colonial Ascidians of the Genera Aplidium and Synoicum: Variability and Defensive Role. *Marine Drugs*, *10*(8), 1741. https://doi.org/10.3390/MD10081741

Nutt, D., Wilson, S., & Paterson, L. (2008). Sleep disorders as core symptoms of depression. *Dialogues in Clinical Neuroscience*, *10*(3), 329–336. https://doi.org/10.31887/DCNS.2008.10.3/dnutt

O'Leary, L. A., Belliveau, C., Davoli, M. A., Ma, J. C., Tanti, A., Turecki, G., & Mechawar, N. (2021). Widespread Decrease of Cerebral Vimentin-Immunoreactive Astrocytes in Depressed Suicides . In *Frontiers in Psychiatry* (Vol. 12).

Oh, D. H., Park, Y. C., & Kim, S. H. (2010). Increased glycogen synthase kinase-3β mRNA level in the hippocampus of patients with major depression: A study using the Stanley neuropathology consortium integrative database. *Psychiatry Investigation*, *7*(3), 202–207. https://doi.org/10.4306/pi.2010.7.3.202

Olfson, M., Mojtabai, R., Merikangas, K. R., Compton, W. M., Wang, S., Grant, B. F., & Blanco, C. (2017). Reexamining associations between mania, depression, anxiety and substance use disorders: results from a prospective national cohort. *Molecular Psychiatry*, *22*(2), 235–241. https://doi.org/10.1038/mp.2016.64

Onaolapo, A. Y., & Onaolapo, O. J. (2021). Glutamate and depression: Reflecting a deepening knowledge of the gut and brain effects of a ubiquitous molecule. *World Journal of Psychiatry*, *11*(7), 297–315. https://doi.org/10.5498/wjp.v11.i7.297

Ormel, J., Hartman, C. A., & Snieder, H. (2019). The genetics of depression: successful genome-wide association studies introduce new challenges. *Translational Psychiatry*, *9*(1). https://doi.org/10.1038/s41398-019-0450-5

Otte, C., Gold, S. M., Penninx, B. W., Pariante, C. M., Etkin, A., Fava, M., Mohr, D. C., & Schatzberg, A. F. (2016). Major depressive disorder. *Nature Reviews Disease Primers 2016 2:1*, *2*(1), 1–20. https://doi.org/10.1038/nrdp.2016.65

Overmier, J. B., & Seligman, M. E. (1967). EFFECTS OF INESCAPABLE SHOCK UPON SUBSEQUENT ESCAPE AND AVOIDANCE RESPONDING. *Journal of Comparative and Physiological Psychology*, *63*(1), 28–33. https://doi.org/10.1037/h0024166

Pan, L.-C., Hang, N.-L.-T., Colley, M. M. S., Chang, J., Hsiao, Y.-C., Lu, L.-S., Li, B.-S., Chang, C.-J., & Yang, T.-S. (2022). Single Cell Effects of Photobiomodulation on Mitochondrial Membrane Potential and Reactive Oxygen Species Production in Human Adipose Mesenchymal Stem Cells. In *Cells* (Vol. 11, Issue 6). https://doi.org/10.3390/cells11060972

Pardon, M. C., Pérez-Diaz, F., Joubert, C., & Cohen-Salmon, C. (2000). Influence of a chronic ultramild stress procedure on decision-making in mice. *Journal of Psychiatry & Neuroscience : JPN*, *25*(2), 167–177.

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends in Neurosciences*, *31*(9), 464–468. https://doi.org/10.1016/j.tins.2008.06.006

Parikh, S. V., Quilty, L. C., Ravitz, P., Rosenbluth, M., Pavlova, B., Grigoriadis, S., Velyvis, V., Kennedy, S. H., Lam, R. W., MacQueen, G. M., Milev, R. V., Ravindran, A. V., & Uher, R. (2016). Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: Section 2. Psychological treatments. *Canadian Journal of Psychiatry*, *61*(9), 524–539. https://doi.org/10.1177/0706743716659418

Park, C., Rosenblat, J. D., Brietzke, E., Pan, Z., Lee, Y., Cao, B., Zuckerman, H., Kalantarova, A., & McIntyre, R. S. (2019). Stress, epigenetics and depression: A systematic review. *Neuroscience and Biobehavioral Reviews*, *102*(April), 139–152. https://doi.org/10.1016/j.neubiorev.2019.04.010

Park, H.-S., Kim, J., Ahn, S. H., & Ryu, H.-Y. (2021). Epigenetic Targeting of Histone Deacetylases in Diagnostics and Treatment of Depression. *International Journal of Molecular Sciences*, *22*(10). https://doi.org/10.3390/ijms22105398

Patel, D., Anilkumar, S., Chattarji, S., & Buwalda, B. (2018). Repeated social stress leads to contrasting patterns of structural plasticity in the amygdala and hippocampus. *Behavioural Brain Research*, *347*, 314–324. https://doi.org/10.1016/J.BBR.2018.03.034

Peng, H., Wang, H., Wang, L., Zhou, B., Li, X., & Tan, J. (2018). Gsk3 β aggravates the depression symptoms in chronic stress mouse model. *Journal of Integrative Neuroscience*, *17*, 217–226. https://doi.org/10.3233/JIN-170050

Penke, Z., Morice, E., Veyrac, A., Gros, A., Chagneau, C., LeBlanc, P., Samson, N., Baumgärtel, K., Mansuy, I. M., Davis, S., & Laroche, S. (2014). Zif268/Egr1 gain of function facilitates hippocampal synaptic plasticity and long-term spatial recognition memory. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1633). https://doi.org/10.1098/RSTB.2013.0159

Penninx, B. W. J. H., Milaneschi, Y., Lamers, F., & Vogelzangs, N. (2013). Understanding the somatic consequences of depression: Biological mechanisms and the role of depression symptom profile. *BMC Medicine*, *11*(1), 1–14. https://doi.org/10.1186/1741-7015-11-129

Pérez-Sisqués, L., Martín-Flores, N., Masana, M., Solana-Balaguer, J., Llobet, A., Romaní-Aumedes, J., Canal, M., Campoy-Campos, G., García-García, E., Sánchez-Fernández, N., Fernández-García, S., Gilbert, J. P., Rodríguez, M. J., Man, H. Y., Feinstein, E., Williamson, D. L., Soto, D., Gasull, X., Alberch, J., & Malagelada, C. (2021). RTP801 regulates motor cortex synaptic transmission and learning. *Experimental Neurology*, *342*, 113755. https://doi.org/10.1016/J.EXPNEUROL.2021.113755

Perini, G., Cotta Ramusino, M., Sinforiani, E., Bernini, S., Petrachi, R., & Costa, A. (2019). Cognitive impairment in depression: recent advances and novel treatments. *Neuropsychiatric Disease and Treatment*, *15*, 1249–1258. https://doi.org/10.2147/NDT.S199746

Pillai, V. B., Sundaresan, N. R., & Gupta, M. P. (2014). Regulation of Akt signaling by sirtuins: its implication in cardiac hypertrophy and aging. *Circulation Research*, *114*(2), 368–378. https://doi.org/10.1161/CIRCRESAHA.113.300536

Planchez, B., Surget, A., & Belzung, C. (2019). Animal models of major depression:

drawbacks and challenges. *Journal of Neural Transmission 2019 126:11*, *126*(11), 1383–1408. https://doi.org/10.1007/S00702-019-02084-Y

Pláteník, J., Fišar, Z., Buchal, R., Jirák, R., Kitzlerová, E., Zvěřová, M., & Raboch, J. (2014). GSK3β, CREB, and BDNF in peripheral blood of patients with Alzheimer's disease and depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *50*, 83–93. https://doi.org/10.1016/j.pnpbp.2013.12.001

Polter, A., Beurel, E., Yang, S., Garner, R., Song, L., Miller, C. A., Sweatt, J. D., McMahon, L., Bartolucci, A. A., Li, X., & Jope, R. S. (2010). Deficiency in the inhibitory serine-phosphorylation of glycogen synthase kinase-3 increases sensitivity to mood disturbances. *Neuropsychopharmacology*, *35*(8), 1761–1774. https://doi.org/10.1038/npp.2010.43

Pothion, S., Bizot, J.-C., Trovero, F., & Belzung, C. (2004). Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. *Behavioural Brain Research*, *155*(1), 135–146. https://doi.org/10.1016/j.bbr.2004.04.008

Proudman, D., Greenberg, P., & Nellesen, D. (2021). The Growing Burden of Major Depressive Disorders (MDD): Implications for Researchers and Policy Makers. *PharmacoEconomics*, *39*(6), 619–625. https://doi.org/10.1007/s40273-021-01040-7

Pryce, C. R., & Fuchs, E. (2017). Chronic psychosocial stressors in adulthood: Studies in mice, rats and tree shrews. *Neurobiology of Stress*, *6*, 94–103. https://doi.org/10.1016/j.ynstr.2016.10.001

Qiao, H., An, S. C., Ren, W., & Ma, X. M. (2014). Progressive alterations of hippocampal CA3-CA1 synapses in an animal model of depression. *Behavioural Brain Research*, *275*, 191–200. https://doi.org/10.1016/J.BBR.2014.08.040

Qiao, H., Li, M. X., Xu, C., Chen, H. Bin, An, S. C., & Ma, X. M. (2016). Dendritic Spines in Depression: What We Learned from Animal Models. *Neural Plasticity*, *2016*, 20–24. https://doi.org/10.1155/2016/8056370

Qiao, Y., Zhao, J., Li, C., Zhang, M., Wei, L., Zhang, X., Kurskaya, O., Bi, H., & Gao, T. (2020). Effect of combined chronic predictable and unpredictable stress on depression-like symptoms in mice. *Annals of Translational Medicine*, *8*(15), 942. https://doi.org/10.21037/atm-20-5168

Qin, X., Jiang, Y., Tse, Y. C., Wang, Y., Wong, T. P., & Paudel, H. K. (2015). Early Growth Response 1 (Egr-1) Regulates N-Methyl-d-aspartate Receptor (NMDAR)-dependent Transcription of PSD-95 and α-Amino-3-hydroxy-5-methyl-4-isoxazole Propionic Acid Receptor (AMPAR) Trafficking in Hippocampal Primary Neurons. *The Journal of Biological Chemistry*, *290*(49), 29603–29616. https://doi.org/10.1074/JBC.M115.668889

Qu, Y., Yang, C., Ren, Q., Ma, M., Dong, C., Hashimoto, K., & Regional, H. K. (2017). *Regional differences in dendritic spine density confer resilience to chronic social defeat stress*. https://doi.org/10.1017/neu.2017.16

Racagni, G., & Popoli, M. (2008). Cellular and molecular mechanisms in the long-term action of antidepressants. *Dialogues in Clinical Neuroscience*, *10*(4), 385–400. https://doi.org/10.31887/DCNS.2008.10.4/gracagni

Rajkowska, G., & Stockmeier, C. A. (2013). Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Current Drug Targets*, *14*(11), 1225–1236. https://doi.org/10.2174/13894501113149990156

Ramirez, S., Liu, X., MacDonald, C. J., Moffa, A., Zhou, J., Redondo, R. L., & Tonegawa, S. (2015). Activating positive memory engrams suppresses depression-like behaviour. *Nature*, *522*(7556), 335–339. https://doi.org/10.1038/nature14514

Ramsey, K. M., Mills, K. F., Satoh, A., & Imai, S. (2008). Age-associated loss of Sirt1-mediated enhancement of glucose-stimulated insulin secretion in beta cell-specific Sirt1-overexpressing (BESTO) mice. *Aging Cell*, *7*(1), 78–88. https://doi.org/https://doi.org/10.1111/j.1474-9726.2007.00355.x

Rasmussen, A. H., Rasmussen, H. B., & Silahtaroglu, A. (2017). The DLGAP family: neuronal expression, function and role in brain disorders. *Molecular Brain*, *10*(1), 43. https://doi.org/10.1186/s13041-017-0324-9

Rathour, D., Shah, S., Khan, S., Singh, P. K., Srivastava, S., Singh, S. B., & Khatri, D. K. (2023). Role of gut microbiota in depression: Understanding molecular pathways, recent research, and future direction. *Behavioural Brain Research*, *436*, 114081. https://doi.org/https://doi.org/10.1016/j.bbr.2022.114081

Ratti, E., Bettica, P., Alexander, R., Archer, G., Carpenter, D., Evoniuk, G., Gomeni, R., Lawson, E., Lopez, M., Millns, H., Rabiner, E. A., Trist, D., Trower, M., Zamuner, S., Krishnan, R., & Fava, M. (2013). Full central neurokinin-1 receptor blockade is required for efficacy in depression: evidence from orvepitant clinical studies. *Journal of Psychopharmacology (Oxford, England)*, *27*(5), 424–434. https://doi.org/10.1177/0269881113480990

Richards, D. (2011). Prevalence and clinical course of depression: A review. *Clinical Psychology Review*, *31*(7), 1117–1125. https://doi.org/10.1016/j.cpr.2011.07.004

Riederer, P., & Laux, G. (2011). MAO-inhibitors in Parkinson's Disease. *Experimental Neurobiology*, *20*(1), 1–17. https://doi.org/10.5607/en.2011.20.1.1

Rizvi, S. J., Donovan, M., Giacobbe, P., Placenza, F., Rotzinger, S., & Kennedy, S. H. (2011). Neurostimulation therapies for treatment resistant depression: A focus on vagus nerve stimulation and deep brain stimulation. *International Review of Psychiatry*, *23*(5), 424–436. https://doi.org/10.3109/09540261.2011.630993

Rodríguez-Urgellés, E., Sancho-Balsells, A., Chen, W., López-Molina, L., Ballasch, I., Castillo, I. del, Avila, C., Alberch, J., & Giralt, A. (2022). Meridianins Rescue Cognitive Deficits, Spine Density and Neuroinflammation in the 5xFAD Model of Alzheimer's Disease. *Frontiers in Pharmacology*, *13*. https://doi.org/10.3389/FPHAR.2022.791666

Rosen, J. B., Adamec, R. E., & Thompson, B. L. (2005). Expression of egr-1 (zif268) mRNA in select fear-related brain regions following exposure to a predator. *Behavioural Brain Research*, *162*, 279–288. https://doi.org/10.1016/j.bbr.2005.04.001

Rouillard, A. D., Gundersen, G. W., Fernandez, N. F., Wang, Z., Monteiro, C. D., McDermott, M. G., & Ma'ayan, A. (2016). The harmonizome: a collection of processed datasets gathered to serve and mine knowledge about genes and proteins. *Database : The Journal of Biological Databases and Curation*, *2016*. https://doi.org/10.1093/database/baw100

Roy, B., Wang, Q., & Dwivedi, Y. (2018). Long Noncoding RNA-Associated Transcriptomic Changes in Resiliency or Susceptibility to Depression and Response to Antidepressant Treatment. *The International Journal of Neuropsychopharmacology*, *21*(5), 461–472. https://doi.org/10.1093/ijnp/pyy010

Sakkas, P. N., Soldatos, C. R., Bergiannaki, J. D., Paparrigopoulos, T. J., & Stefanis, C. N. (1998). Growth hormone secretion during sleep in male depressed patients. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *22*(3), 467–483. https://doi.org/10.1016/s0278-5846(98)00018-9

Salehpour, F., Farajdokht, F., Cassano, P., Sadigh-Eteghad, S., Erfani, M., Hamblin, M. R., Salimi, M. M., Karimi, P., Rasta, S. H., & Mahmoudi, J. (2019). Near-infrared photobiomodulation combined with coenzyme Q10 for depression in a mouse model of restraint stress: reduction in oxidative stress, neuroinflammation, and apoptosis. *Brain Research Bulletin*, *144*, 213–222. https://doi.org/https://doi.org/10.1016/j.brainresbull.2018.10.010

Salehpour, F., Farajdokht, F., Mahmoudi, J., Erfani, M., Farhoudi, M., Karimi, P., Rasta, S. H., Sadigh-Eteghad, S., Hamblin, M. R., & Gjedde, A. (2019). Photobiomodulation and Coenzyme Q10 Treatments Attenuate Cognitive Impairment Associated With Model of Transient Global Brain Ischemia in Artificially Aged Mice . In *Frontiers in Cellular Neuroscience* (Vol. 13).

Sanacora, G., Treccani, G., & Popoli, M. (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*, *62*(1), 63–77. https://doi.org/10.1016/j.neuropharm.2011.07.036

Sanacora, G., Yan, Z., & Popoli, M. (2022). The stressed synapse 2.0: pathophysiological mechanisms in stress-related neuropsychiatric disorders. *Nature Reviews Neuroscience*, *23*(2), 86–103. https://doi.org/10.1038/s41583-021-00540-x

Sancho-Balsells, A., Borràs-Pernas, S., Brito, V., Alberch, J., Girault, J.-A., & Giralt, A. (2023). Cognitive and Emotional Symptoms Induced by Chronic Stress Are Regulated by EGR1 in a Subpopulation of Hippocampal Pyramidal Neurons. *International Journal of Molecular Sciences*, *24*(4). https://doi.org/10.3390/ijms24043833

Saus, E., Soria, V., Escaramís, G., Crespo, J. M., Valero, J., Gutiérrez-Zotes, A., Martorell, L., Vilella, E., Menchón, J. M., Estivill, X., Gratacòs, M., & Urretavizcaya, M. (2010). A haplotype of glycogen synthase kinase 3β is associated with early onset of unipolar major depression. *Genes, Brain and Behavior*, *9*(7), 799–807. https://doi.org/https://doi.org/10.1111/j.1601-183X.2010.00617.x

Savitz, J., Rauch, S. L., & Drevets, W. C. (2013). Clinical application of brain imaging for the diagnosis of mood disorders: The current state of play. *Molecular Psychiatry*, *18*. https://doi.org/10.1038/mp.2013.25

Schaub, A.-C., Schneider, E., Vazquez-Castellanos, J. F., Schweinfurth, N., Kettelhack, C., Doll, J. P. K., Yamanbaeva, G., Mählmann, L., Brand, S., Beglinger, C., Borgwardt, S., Raes, J., Schmidt, A., & Lang, U. E. (2022). Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial. *Translational Psychiatry*, *12*(1), 227. https://doi.org/10.1038/s41398-022-01977-z

Schiffer, F., Johnston, A. L., Ravichandran, C., Polcari, A., Teicher, M. H., Webb, R. H., & Hamblin, M. R. (2009). Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety. *Behavioral and Brain Functions*, *5*(1), 46. https://doi.org/10.1186/1744-9081-5-46

Schmaal, L., Veltman, D. J., Van Erp, T. G. M., Smann, P. G., Frodl, T., Jahanshad, N., Loehrer, E., Tiemeier, H., Hofman, A., Niessen, W. J., Vernooij, M. W., Ikram, M. A.,

Wittfeld, K., Grabe, H. J., Block, A., Hegenscheid, K., Völzke, H., Hoehn, D., Czisch, M., … Hibar, D. P. (2016). Subcortical brain alterations in major depressive disorder: Findings from the ENIGMA Major Depressive Disorder working group. *Molecular Psychiatry*, *21*(6), 806–812. https://doi.org/10.1038/mp.2015.69

Schnell, C., Janc, O. A., Kempkes, B., Callis, C. A., Flügge, G., Hülsmann, S., & Müller, M. (2012). Restraint stress intensifies interstitial K+ accumulation during severe hypoxia. *Frontiers in Pharmacology*, *3 MAR*(March), 1–16. https://doi.org/10.3389/fphar.2012.00053

Schreiber, S. S., Tocco, G., Shors, T. J., & Thompson, R. F. (1991). Activation of immediate early genes after acute stress. *Neuroreport*, *2*(1), 17–20. https://doi.org/10.1097/00001756-199101000-00004

Schroeder, F. A., Lin, C. L., Crusio, W. E., & Akbarian, S. (2007). Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biological Psychiatry*, *62*(1), 55–64. https://doi.org/10.1016/j.biopsych.2006.06.036

Seldes, A. M., Brasco, M. F. R., Franco, L. H., & Palermo, J. A. (2007). Identification of two meridianins from the crude extract of the tunicate Aplidium meridianum by tandem mass spectrometry. *Http://Dx.Doi.Org/10.1080/14786410601133517*, *21*(6), 555–563. https://doi.org/10.1080/14786410601133517

Senior, T. J., Huxter, J. R., Allen, K., O'Neill, J., & Csicsvari, J. (2008). Gamma oscillatory firing reveals distinct populations of pyramidal cells in the CA1 region of the hippocampus. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(9), 2274–2286. https://doi.org/10.1523/JNEUROSCI.4669-07.2008

Seo, M. K., Ly, N. N., Lee, C. H., Cho, H. Y., Choi, C. M., Nhu, L. H., Lee, J. G., Lee, B. J., Kim, G.-M., Yoon, B. J., Park, S. W., & Kim, Y. H. (2016). Early life stress increases stress vulnerability through BDNF gene epigenetic changes in the rat hippocampus. *Neuropharmacology*, *105*, 388–397. https://doi.org/10.1016/j.neuropharm.2016.02.009

Sequeira-Cordero, A., Salas-Bastos, A., Fornaguera, J., & Brenes, J. C. (2019). Behavioural characterisation of chronic unpredictable stress based on ethologically relevant paradigms in rats. *Scientific Reports 2019 9:1*, *9*(1), 1–21. https://doi.org/10.1038/s41598-019-53624-1

Shadrina, M., Bondarenko, E. A., & Slominsky, P. A. (2018). Genetics factors in major depression disease. *Frontiers in Psychiatry*, *9*(JUL), 1–18. https://doi.org/10.3389/fpsyt.2018.00334

Shah, A. D., Goode, R. J. A., Huang, C., Powell, D. R., & Schittenhelm, R. B. (2020). LFQ-Analyst: An Easy-To-Use Interactive Web Platform To Analyze and Visualize Label-Free Proteomics Data Preprocessed with MaxQuant. *Journal of Proteome Research*, *19*(1), 204–211. https://doi.org/10.1021/acs.jproteome.9b00496

Sharma, S. K., Kharkwal, G. B., Sajo, M., Huang, Y.-Y., De Taboada, L., McCarthy, T., & Hamblin, M. R. (2011). Dose response effects of 810 nm laser light on mouse primary cortical neurons. *Lasers in Surgery and Medicine*, *43*(8), 851–859. https://doi.org/10.1002/lsm.21100

Sharpley, A. L., & Cowen, P. J. (1995). Effect of pharmacologic treatments on the sleep of depressed patients. *Biological Psychiatry*, *37*(2), 85–98. https://doi.org/10.1016/0006-3223(94)00135-P

Sheline, Y. I., Barch, D. M., Price, J. L., Rundle, M. M., Vaishnavi, S. N., Snyder, A. Z., Mintun, M. A., Wang, S., Coalson, R. S., & Raichle, M. E. (2009). The default mode network and self-referential processes in depression. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(6), 1942–1947. https://doi.org/10.1073/pnas.0812686106

Shen, J., Qu, C., Xu, L., Sun, H., & Zhang, J. (2019). Resveratrol exerts a protective effect in chronic unpredictable mild stress–induced depressive-like behavior: involvement of the AKT/GSK3β signaling pathway in hippocampus. *Psychopharmacology*, *236*(2), 591–602. https://doi.org/10.1007/s00213-018-5087-1

Sheng, J. A., Bales, N. J., Myers, S. A., Bautista, A. I., Roueinfar, M., Hale, T. M., & Handa, R. J. (2021). The Hypothalamic-Pituitary-Adrenal Axis: Development, Programming Actions of Hormones, and Maternal-Fetal Interactions . In *Frontiers in Behavioral Neuroscience* (Vol. 14).

Sherrill, J. T., Anderson, B., Frank, E., Reynolds, C. F. 3rd, Tu, X. M., Patterson, D., Ritenour, A., & Kupfer, D. J. (1997). Is life stress more likely to provoke depressive episodes in women than in men? *Depression and Anxiety*, *6*(3), 95–105.

Shishkina, G. T., Kalinina, T. S., Berezova, I. V, & Dygalo, N. N. (2012). Stress-induced activation of the brainstem Bcl-xL gene expression in rats treated with fluoxetine: correlations with serotonin metabolism and depressive-like behavior. *Neuropharmacology*, *62*(1), 177–183. https://doi.org/10.1016/j.neuropharm.2011.06.016

Shishov, V. A., Kirovskaya, T. A., Kudrin, V. S., & Oleskin, A. V. (2009). Amine neuromediators, their precursors, and oxidation products in the culture of Escherichia coli K-12. *Applied Biochemistry and Microbiology*, *45*(5), 494–497. https://doi.org/10.1134/S0003683809050068

Shlomi, S., Toledano, R., Nitzan, K., Shahaf, S. D., Break, E. P., Frenkel, D., & Doron, R. (2022). Imbalance in Sirt1 Alternative Splicing in Response to Chronic Stress during the Adolescence Period in Female Mice. *International Journal of Molecular Sciences*, *23*(9). https://doi.org/10.3390/ijms23094945

Si, X., Miguel-Hidalgo, J. J., O'Dwyer, G., Stockmeier, C. A., & Rajkowska, G. (2004). Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *29*(11), 2088–2096. https://doi.org/10.1038/sj.npp.1300525

Silva, R., Mesquita, A. R., Bessa, J., Sousa, J. C., Sotiropoulos, I., Leão, P., Almeida, O. F. X., & Sousa, N. (2008). Lithium blocks stress-induced changes in depressive-like behavior and hippocampal cell fate: The role of glycogen-synthase-kinase-3β. *Neuroscience*, *152*(3), 656–669. https://doi.org/10.1016/j.neuroscience.2007.12.026

Silva, Y. P., Bernardi, A., & Frozza, R. L. (2020). The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication . In *Frontiers in Endocrinology* (Vol. 11).

Simpson, C. A., Diaz-Arteche, C., Eliby, D., Schwartz, O. S., Simmons, J. G., & Cowan, C. S. M. (2021). The gut microbiota in anxiety and depression – A systematic review. *Clinical Psychology Review*, *83*, 101943. https://doi.org/https://doi.org/10.1016/j.cpr.2020.101943

Slattery, D. A., Morrow, J. A., Hudson, A. L., Hill, D. R., Nutt, D. J., & Henry, B. (2005). Comparison of Alterations in c-fos and Egr-1 (zif268) Expression Throughout the Rat Brain Following Acute Administration of Different Classes of Antidepressant

Compounds. *Neuropsychopharmacology*, *30*(7), 1278–1287. https://doi.org/10.1038/sj.npp.1300717

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychological Bulletin*, *140*(3), 774–815. https://doi.org/10.1037/a0035302

Smarr, B., & Kriegsfeld, L. J. (2022). Female mice exhibit less overall variance, with a higher proportion of structured variance, than males at multiple timescales of continuous body temperature and locomotive activity records. *Biology of Sex Differences*, *13*(1), 41. https://doi.org/10.1186/s13293-022-00451-1

Smith, D. K., Kassam, T., Singh, B., & Elliott, J. F. (1992). Escherichia coli has two homologous glutamate decarboxylase genes that map to distinct loci. *Journal of Bacteriology*, *174*(18), 5820–5826. https://doi.org/10.1128/jb.174.18.5820-5826.1992

Smith, R. S. (1991). The macrophage theory of depression. *Medical Hypotheses*, *35*(4), 298–306. https://doi.org/10.1016/0306-9877(91)90272-Z

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*, *8*(4), 383–395. https://doi.org/10.31887/DCNS.2006.8.4/ssmith

Soldatou, S., & Baker, B. J. (2017). Cold-water marine natural products, 2006 to 2016. *Natural Product Reports*, *34*(6), 585–626. https://doi.org/10.1039/C6NP00127K

Soltesz, I., & Losonczy, A. (2018). CA1 pyramidal cell diversity enabling parallel information processing in the hippocampus. In *Nature Neuroscience* (Vol. 21, Issue 4, pp. 484–493). Nature Publishing Group. https://doi.org/10.1038/s41593-018-0118-0

Song, S. B., Park, J. S., Chung, G. J., Lee, I. H., & Hwang, E. S. (2019). Diverse therapeutic efficacies and more diverse mechanisms of nicotinamide. *Metabolomics*, *15*(10), 137. https://doi.org/10.1007/s11306-019-1604-4

Spellman, T., & Liston, C. (2020). Toward Circuit Mechanisms of Pathophysiology in Depression. *American Journal of Psychiatry*, *177*(5), 381–390. https://doi.org/10.1176/appi.ajp.2020.20030280

Stankiewicz, A. M., Goscik, J., Majewska, A., Swiergiel, A. H., & Juszczak, G. R. (2015). The Effect of Acute and Chronic Social Stress on the Hippocampal Transcriptome in Mice. *PLOS ONE*, *10*(11), e0142195.

Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., Stein, T. I., Nudel, R., Lieder, I., Mazor, Y., Kaplan, S., Dahary, D., Warshawsky, D., Guan-Golan, Y., Kohn, A., Rappaport, N., Safran, M., & Lancet, D. (2016). The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. *Current Protocols in Bioinformatics*, *54*(1), 1.30.1-1.30.33. https://doi.org/https://doi.org/10.1002/cpbi.5

Sterling, P., & Eyer, J. (1988). Allostatis: a new paradigm to explain arousal pathology. *Handbook of Life Stressm Cognition and Health*, 629–649.

Strandwitz, P., Kim, K. H., Terekhova, D., Liu, J. K., Sharma, A., Levering, J., McDonald, D., Dietrich, D., Ramadhar, T. R., Lekbua, A., Mroue, N., Liston, C., Stewart, E. J., Dubin, M. J., Zengler, K., Knight, R., Gilbert, J. A., Clardy, J., & Lewis, K. (2019). GABA-modulating bacteria of the human gut microbiota. *Nature Microbiology*, *4*(3), 396–403. https://doi.org/10.1038/s41564-018-0307-3

Strekalova, T., Couch, Y., Kholod, N., Boyks, M., Malin, D., Leprince, P., & Steinbusch, H. M. (2011). Update in the methodology of the chronic stress paradigm: internal control matters. *Behavioral and Brain Functions : BBF*, *7*, 9. https://doi.org/10.1186/1744-9081-7-9

Strekalova, T., Spanagel, R., Bartsch, D., Henn, F. A., & Gass, P. (2004). Stress-Induced Anhedonia in Mice is Associated with Deficits in Forced Swimming and Exploration. *Neuropsychopharmacology*, *29*(11), 2007–2017. https://doi.org/10.1038/sj.npp.1300532

Strekalova, T., & Steinbusch, H. W. M. (2010). Measuring behavior in mice with chronic stress depression paradigm. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(2), 348–361. https://doi.org/10.1016/j.pnpbp.2009.12.014

Suda, K., & Matsuda, K. (2022). How Microbes Affect Depression: Underlying Mechanisms via the Gut-Brain Axis and the Modulating Role of Probiotics. *International Journal of Molecular Sciences*, *23*(3). https://doi.org/10.3390/ijms23031172

Sun, D. S., Zhong, G., Cao, H. X., Hu, Y., Hong, X. Y., Li, T., Li, X., Liu, Q., Wang, Q., Ke, D., Liu, G. P., Ma, R. H., & Luo, D. J. (2020). Repeated Restraint Stress Led to Cognitive Dysfunction by NMDA Receptor-Mediated Hippocampal CA3 Dendritic Spine Impairments in Juvenile Sprague-Dawley Rats. *Frontiers in Molecular Neuroscience*, *13*, 173. https://doi.org/10.3389/FNMOL.2020.552787/BIBTEX

Tartt, A. N., Mariani, M. B., Hen, R., Mann, J. J., & Boldrini, M. (2022). Dysregulation of adult hippocampal neuroplasticity in major depression: pathogenesis and therapeutic implications. *Molecular Psychiatry*, *27*(6), 2689–2699. https://doi.org/10.1038/s41380-022-01520-y

Thomas, A. J., Davis, S., Morris, C., Jackson, E., Harrison, R., & O'Brien, J. T. (2005). Increase in interleukin-1β in late-life depression. *American Journal of Psychiatry*, *162*(1), 175–177. https://doi.org/10.1176/APPI.AJP.162.1.175/ASSET/IMAGES/LARGE/N626T1.JPEG

Tian, F., Shen, Q., Hu, Y., Ye, W., Valdimarsdóttir, U. A., Song, H., & Fang, F. (2022). Association of stress-related disorders with subsequent risk of all-cause and cause-specific mortality: A population-based and sibling-controlled cohort study. *The Lancet Regional Health - Europe*, *18*, 100402. https://doi.org/https://doi.org/10.1016/j.lanepe.2022.100402

Tianzhu, Z., Shihai, Y., & Juan, D. (2014). Antidepressant-like effects of cordycepin in a mice model of chronic unpredictable mild stress. *Evidence-Based Complementary and Alternative Medicine : ECAM*, *2014*, 438506. https://doi.org/10.1155/2014/438506

Tibiletti, F., Simonetti, M., Nicholas, K. M., Palmisano, G., Parravicini, M., Imbesi, F., Tollari, S., & Penoni, A. (2010). One-pot synthesis of meridianins and meridianin analogues via indolization of nitrosoarenes. *Tetrahedron*, *66*(6), 1280–1288. https://doi.org/10.1016/J.TET.2009.12.020

Tonon, A. C., Pilz, L. K., Markus, R. P., Hidalgo, M. P., & Elisabetsky, E. (2021). Melatonin and Depression: A Translational Perspective From Animal Models to Clinical Studies . In *Frontiers in Psychiatry* (Vol. 12).

Troubat, R., Barone, P., Leman, S., Desmidt, T., Cressant, A., Atanasova, B., Brizard, B., El Hage, W., Surget, A., Belzung, C., & Camus, V. (2021). Neuroinflammation and depression: A review. *European Journal of Neuroscience*, *53*(1), 151–171.

https://doi.org/https://doi.org/10.1111/ejn.14720

Turecki, G., & Meaney, M. J. (2016). Effects of the Social Environment and Stress on Glucocorticoid Receptor Gene Methylation: A Systematic Review. *Biological Psychiatry*, *79*(2), 87–96. https://doi.org/10.1016/j.biopsych.2014.11.022

Tynan, R. J., Beynon, S. B., Hinwood, M., Johnson, S. J., Nilsson, M., Woods, J. J., & Walker, F. R. (2013). Chronic stress-induced disruption of the astrocyte network is driven by structural atrophy and not loss of astrocytes. *Acta Neuropathologica*, *126*(1), 75–91. https://doi.org/10.1007/s00401-013-1102-0

Tynan, R. J., Weidenhofer, J., Hinwood, M., Cairns, M. J., Day, T. A., & Walker, F. R. (2012). A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. *Brain, Behavior, and Immunity*, *26*(3), 469–479. https://doi.org/10.1016/j.bbi.2011.12.011

UK ECT Review Group. (2003). Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis. *Lancet (London, England)*, *361*(9360), 799–808. https://doi.org/10.1016/S0140-6736(03)12705-5

Ulrich-Lai, Y. M., Figueiredo, H. F., Ostrander, M. M., Choi, D. C., Engeland, W. C., & Herman, J. P. (2006). Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. *American Journal of Physiology-Endocrinology and Metabolism*, *291*(5), E965–E973. https://doi.org/10.1152/ajpendo.00070.2006

Ursin, H. (2014). Brain sensitization to external and internal stimuli. *Psychoneuroendocrinology*, *42*, 134–145. https://doi.org/10.1016/j.psyneuen.2014.01.008

Vaishnava, S., Behrendt, C. L., & Hooper, L. V. (2008). Innate immune responses to commensal bacteria in the gut epithelium. *Journal of Pediatric Gastroenterology and Nutrition*, *46 Suppl 1*, E10-1. https://doi.org/10.1097/01.mpg.0000313823.93841.65

Valero, M., & de la Prida, L. M. (2018). The hippocampus in depth: a sublayer-specific perspective of entorhinal–hippocampal function. *Current Opinion in Neurobiology*, *52*, 107–114. https://doi.org/10.1016/j.conb.2018.04.013

Van Hoesen, G. W., & Pandya, D. N. (1975). Some connections of the entorhinal (area 28) and perirhinal (area 35) cortices of the rhesus monkey. III. Efferent connections. *Brain Research*, *95*(1), 39–59. https://doi.org/10.1016/0006-8993(75)90206-1

van Oort, J., Tendolkar, I., Hermans, E. J., Mulders, P. C., Beckmann, C. F., Schene, A. H., Fernández, G., & van Eijndhoven, P. F. (2017). How the brain connects in response to acute stress: A review at the human brain systems level. In *Neuroscience and Biobehavioral Reviews* (Vol. 83, pp. 281–297). https://doi.org/10.1016/j.neubiorev.2017.10.015

Veselkov, K. A., Vingara, L. K., Masson, P., Robinette, S. L., Want, E., Li, J. V, Barton, R. H., Boursier-Neyret, C., Walther, B., Ebbels, T. M., Pelczer, I., Holmes, E., Lindon, J. C., & Nicholson, J. K. (2011). Optimized preprocessing of ultra-performance liquid chromatography/mass spectrometry urinary metabolic profiles for improved information recovery. *Analytical Chemistry*, *83*(15), 5864–5872. https://doi.org/10.1021/ac201065j

Vollmayr, B., & Gass, P. (2013). Learned helplessness: unique features and translational value of a cognitive depression model. *Cell and Tissue Research*, *354*(1), 171–178. https://doi.org/10.1007/s00441-013-1654-2

von Ziegler, L. M., Floriou-Servou, A., Waag, R., Das Gupta, R. R., Sturman, O., Gapp, K.,

Maat, C. A., Kockmann, T., Lin, H. Y., Duss, S. N., Privitera, M., Hinte, L., von Meyenn, F., Zeilhofer, H. U., Germain, P. L., & Bohacek, J. (2022). Multiomic profiling of the acute stress response in the mouse hippocampus. *Nature Communications*, *13*(1). https://doi.org/10.1038/s41467-022-29367-5

Vukojevic, V., Kolassa, I.-T., Fastenrath, M., Gschwind, L., Spalek, K., Milnik, A., Heck, A., Vogler, C., Wilker, S., Demougin, P., Peter, F., Atucha, E., Stetak, A., Roozendaal, B., Elbert, T., Papassotiropoulos, A., & de Quervain, D. J.-F. (2014). Epigenetic modification of the glucocorticoid receptor gene is linked to traumatic memory and post-traumatic stress disorder risk in genocide survivors. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(31), 10274–10284. https://doi.org/10.1523/JNEUROSCI.1526-14.2014

Walker, S. R., Czyz, M. L., & Morris, J. C. (2014). *Concise Syntheses of Meridianins and Meriolins Using a Catalytic Domino Amino-Palladation Reaction*. https://doi.org/10.1021/ol403390m

Wang, M., Cao, J., Amakye, W. K., Gong, C., Li, Q., & Ren, J. (2020). Mid infrared light treatment attenuates cognitive decline and alters the gut microbiota community in APP/PS1 mouse model. *Biochemical and Biophysical Research Communications*, *523*(1), 60–65. https://doi.org/https://doi.org/10.1016/j.bbrc.2019.12.015

Wang, Q., Van Heerikhuize, J., Aronica, E., Kawata, M., Seress, L., Joels, M., Swaab, D. F., & Lucassen, P. J. (2013). Glucocorticoid receptor protein expression in human hippocampus; stability with age. *Neurobiology of Aging*, *34*(6), 1662–1673. https://doi.org/10.1016/j.neurobiolaging.2012.11.019

Wang, R., Li, J. J., Diao, S., Kwak, Y.-D., Liu, L., Zhi, L., Büeler, H., Bhat, N. R., Williams, R. W., Park, E. A., & Liao, F.-F. (2013). Metabolic stress modulates Alzheimer's β-secretase gene transcription via SIRT1-PPARγ-PGC-1 in neurons. *Cell Metabolism*, *17*(5), 685–694. https://doi.org/10.1016/j.cmet.2013.03.016

Wang, X., Li, X., Zuo, X., Liang, Z., Ding, T., Li, K., Ma, Y., Li, P., Zhu, Z., Ju, C., Zhang, Z., Song, Z., Quan, H., Zhang, J., Hu, X., & Wang, Z. (2021). Photobiomodulation inhibits the activation of neurotoxic microglia and astrocytes by inhibiting Lcn2/JAK2-STAT3 crosstalk after spinal cord injury in male rats. *Journal of Neuroinflammation*, *18*(1), 1–20. https://doi.org/10.1186/s12974-021-02312-x

Wang, Y.-L., Wu, H.-R., Zhang, S.-S., Xiao, H.-L., Yu, J., Ma, Y.-Y., Zhang, Y.-D., & Liu, Q. (2021). Catalpol ameliorates depressive-like behaviors in CUMS mice via oxidative stress-mediated NLRP3 inflammasome and neuroinflammation. *Translational Psychiatry*, *11*(1), 353. https://doi.org/10.1038/s41398-021-01468-7

Webster, M. J., O'Grady, J., Kleinman, J. E., & Weickert, C. S. (2005). Glial fibrillary acidic protein mRNA levels in the cingulate cortex of individuals with depression, bipolar disorder and schizophrenia. *Neuroscience*, *133*(2), 453–461. https://doi.org/10.1016/j.neuroscience.2005.02.037

Wegrzyn, D., Juckel, G., & Faissner, A. (2022). Structural and Functional Deviations of the Hippocampus in Schizophrenia and Schizophrenia Animal Models. In *International Journal of Molecular Sciences* (Vol. 23, Issue 10). https://doi.org/10.3390/ijms23105482

Whooley, M. A., & Wong, J. M. (2013). Depression and Cardiovascular Disorders. *Annual Review of Clinical Psychology*, *9*(1), 327–354. https://doi.org/10.1146/annurev-clinpsy-050212-185526

Wilkinson, M. B., Dias, C., Magida, J., Mazei-Robison, M., Lobo, M., Kennedy, P., Dietz, D.,

Covington, H. 3rd, Russo, S., Neve, R., Ghose, S., Tamminga, C., & Nestler, E. J. (2011). A novel role of the WNT-dishevelled-GSK3β signaling cascade in the mouse nucleus accumbens in a social defeat model of depression. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(25), 9084–9092. https://doi.org/10.1523/JNEUROSCI.0039-11.2011

Willner, P. (2005). Chronic Mild Stress (CMS) Revisited: Consistency and Behavioural-Neurobiological Concordance in the Effects of CMS. *Neuropsychobiology*, *52*(2), 90–110. https://doi.org/10.1159/000087097

Willner, P, Muscat, R., & Papp, M. (1992). Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neuroscience and Biobehavioral Reviews*, *16*(4), 525–534. https://doi.org/10.1016/s0149-7634(05)80194-0

Willner, P, Towell, A., Sampson, D., Sophokleous, S., & Muscat, R. (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology*, *93*(3), 358–364. https://doi.org/10.1007/BF00187257

Willner, Paul. (2017). The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiology of Stress*, *6*, 78–93. https://doi.org/10.1016/j.ynstr.2016.08.002

Willner, Paul, & Belzung, C. (2015). Treatment-resistant depression: are animal models of depression fit for purpose? *Psychopharmacology 2015 232:19*, *232*(19), 3473–3495. https://doi.org/10.1007/S00213-015-4034-7

Winter, G., Hart, R. A., Charlesworth, R. P. G., & Sharpley, C. F. (2018). *Gut microbiome and depression: what we know and what we need to know*. *29*(6), 629–643. https://doi.org/doi:10.1515/revneuro-2017-0072

Wisden, W., Errington, M. L., Williams, S., Dunnett, S. B., Waters, C., Hitchcock, D., Evan, G., Bliss, T. V. P., & Hunt, S. P. (1990). Differential expression of immediate early genes in the hippocampus and spinal cord. *Neuron*, *4*(4), 603–614. https://doi.org/10.1016/0896-6273(90)90118-Y

Wu, J., Li, J., Gaurav, C., Muhammad, U., Chen, Y., Li, X., Chen, J., & Wang, Z. (2021). CUMS and dexamethasone induce depression-like phenotypes in mice by differentially altering gut microbiota and triggering macroglia activation. *General Psychiatry*, *34*(6), e100529. https://doi.org/10.1136/gpsych-2021-100529

Wu, X., Alberico, S. L., Moges, H., De Taboada, L., Tedford, C. E., & Anders, J. J. (2012). Pulsed Light Irradiation Improves Behavioral Outcome in a Rat Model of Chronic Mild Stress. *Lasers in Surgery and Medicine*, *44*(3), 227–232. https://doi.org/https://doi.org/10.1002/lsm.22004

Xu, J., Jackson, C. W., Khoury, N., Escobar, I., & Perez-Pinzon, M. A. (2018). Brain SIRT1 Mediates Metabolic Homeostasis and Neuroprotection. *Frontiers in Endocrinology*, *9*, 702. https://doi.org/10.3389/fendo.2018.00702

Xu, Y., Pan, J., Sun, J., Ding, L., Ruan, L., Reed, M., Yu, X., klabnik, J., Lin, D., Li, J., Chen, L., Zhang, C., Zhang, H., & O'Donnell, J. M. (2015). Inhibition of phosphodiesterase 2 reverses impaired cognition and neuronal remodeling caused by chronic stress. *Neurobiology of Aging*, *36*(2), 955–970. https://doi.org/10.1016/j.neurobiolaging.2014.08.028

Xuan, W., Vatansever, F., Huang, L., & Hamblin, M. R. (2014). Transcranial low-level laser therapy enhances learning, memory, and neuroprogenitor cells after traumatic brain injury in mice. *Journal of Biomedical Optics*, *19*(10), 108003. https://doi.org/10.1117/1.JBO.19.10.108003

Yadav, R. R., Khan, S. I., Singh, S., Khan, I. A., Vishwakarma, R. A., & Bharate, S. B. (2015). Synthesis, antimalarial and antitubercular activities of meridianin derivatives. *European Journal of Medicinal Chemistry*, *98*, 160–169. https://doi.org/10.1016/j.ejmech.2015.05.020

Yang, Yu, Ju, W., Zhang, H., & Sun, L. (2018). Effect of ketamine on LTP and NMDAR EPSC in hippocampus of the chronic social defeat stress mice model of depression. *Frontiers in Behavioral Neuroscience*, *12*(October), 1–11. https://doi.org/10.3389/fnbeh.2018.00229

Yang, Yunshu, Liu, Y., Wang, Y., Chao, Y., Zhang, J., Jia, Y., Tie, J., & Hu, D. (2022). Regulation of SIRT1 and Its Roles in Inflammation . In *Frontiers in Immunology* (Vol. 13).

Yao, H.-B., Shaw, P.-C., Wong, C.-C., & Wan, D. C.-C. (2002). Expression of glycogen synthase kinase-3 isoforms in mouse tissues and their transcription in the brain. *Journal of Chemical Neuroanatomy*, *23*(4), 291–297. https://doi.org/https://doi.org/10.1016/S0891-0618(02)00014-5

Yao, L., Chen, J., Chen, H., Xiang, D., Yang, C., Xiao, L., Liu, W., Wang, H., Wang, G., Zhu, F., & Liu, Z. (2016). Hypothalamic gastrin-releasing peptide receptor mediates an antidepressant-like effect in a mouse model of stress. *American Journal of Translational Research*, *8*(7), 3097–3105.

Yeung, F., Hoberg, J. E., Ramsey, C. S., Keller, M. D., Jones, D. R., Frye, R. A., & Mayo, M. W. (2004). Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *The EMBO Journal*, *23*(12), 2369–2380. https://doi.org/10.1038/sj.emboj.7600244

Yien, Y. Y., Robledo, R. F., Schultz, I. J., Takahashi-Makise, N., Gwynn, B., Bauer, D. E., Dass, A., Yi, G., Li, L., Hildick-Smith, G. J., Cooney, J. D., Pierce, E. L., Mohler, K., Dailey, T. A., Miyata, N., Kingsley, P. D., Garone, C., Hattangadi, S. M., Huang, H., … Paw, B. H. (2014). TMEM14C is required for erythroid mitochondrial heme metabolism. *The Journal of Clinical Investigation*, *124*(10), 4294–4304. https://doi.org/10.1172/JCI76979

Young, J. F., Mufson, L., & Davies, M. (2006). Impact of comorbid anxiety in an effectiveness study of interpersonal psychotherapy for depressed adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry*, *45*(8), 904–912. https://doi.org/10.1097/01.chi.0000222791.23927.5f

Young, W., Sun, W.-H., Kruppa, M., Sommer, G. A., & Müller, T. J. J. (2022). *molecules Concise Syntheses of Marine (Bis)indole Alkaloids Meridianin C, D, F, and G and Scalaridine A via One-Pot Masuda Borylation-Suzuki Coupling Sequence*. https://doi.org/10.3390/molecules27072233

Yu, Z., Zhu, J., Wang, H., Li, H., & Jin, X. (2022). Function of BCLAF1 in human disease. *Oncology Letters*, *23*(2), 58. https://doi.org/10.3892/ol.2021.13176

Zaki, N. F. W., Spence, D. W., BaHammam, A. S., Pandi-Perumal, S. R., Cardinali, D. P., & Brown, G. M. (2018). Chronobiological theories of mood disorder. *European Archives of*

Psychiatry and Clinical Neuroscience, *268*(2), 107–118. https://doi.org/10.1007/s00406-017-0835-5

Zalachoras, I., Hollis, F., Ramos-Fernández, E., Trovo, L., Sonnay, S., Geiser, E., Preitner, N., Steiner, P., Sandi, C., & Morató, L. (2020). Therapeutic potential of glutathione-enhancers in stress-related psychopathologies. *Neuroscience and Biobehavioral Reviews*, *114*(March), 134–155. https://doi.org/10.1016/j.neubiorev.2020.03.015

Zemla, R., & Basu, J. (2017). Hippocampal function in rodents. *Current Opinion in Neurobiology*, *43*, 187–197. https://doi.org/10.1016/j.conb.2017.04.005

Zhan, J., Komal, R., Keenan, W. T., Hattar, S., & Fernandez, D. C. (2019). Non-invasive Strategies for Chronic Manipulation of DREADD-controlled Neuronal Activity. *J. Vis. Exp*, *150*, 59439. https://doi.org/10.3791/59439

Zhang, D., Shen, Q., Wu, X., & Xing, D. (2021). Photobiomodulation Therapy Ameliorates Glutamatergic Dysfunction in Mice with Chronic Unpredictable Mild Stress-Induced Depression. *Oxidative Medicine and Cellular Longevity*, *2021*, 6678276. https://doi.org/10.1155/2021/6678276

Zhang, F., Lin, M., Abidi, P., Thiel, G., & Liu, J. (2003). Specific interaction of Egr1 and c/EBPbeta leads to the transcriptional activation of the human low density lipoprotein receptor gene. *The Journal of Biological Chemistry*, *278*(45), 44246–44254. https://doi.org/10.1074/jbc.M305564200

Zhang, G., Xiao, L., & Qi, L. (2021). Metabolite Profiling of Meridianin C in Vivo of Rat by UHPLC/Q-TOF MS. *Journal of Analytical Methods in Chemistry*, *2021*. https://doi.org/10.1155/2021/1382421

Zhang, H., Chen, Y., Wang, Z., Xie, G., Liu, M., Yuan, B., Chai, H., Wang, W., & Cheng, P. (2022). Implications of Gut Microbiota in Neurodegenerative Diseases . In *Frontiers in Immunology* (Vol. 13).

Zhang, J.-C., Yao, W., Dong, C., Yang, C., Ren, Q., Ma, M., & Hashimoto, K. (2017). Blockade of interleukin-6 receptor in the periphery promotes rapid and sustained antidepressant actions: a possible role of gut-microbiota-brain axis. *Translational Psychiatry*, *7*(5), e1138. https://doi.org/10.1038/tp.2017.112

Zhang, K., Yang, C., Xu, Y., Sun, N., Yang, H., Liu, J., Xu, Q., & Shen, Y. (2010). Genetic association of the interaction between the BDNF and GSK3B genes and major depressive disorder in a Chinese population. *Journal of Neural Transmission*, *117*(3), 393–401. https://doi.org/10.1007/s00702-009-0360-4

Zhang, L., Zhang, Y., & Xing, D. (2010). LPLI inhibits apoptosis upstream of Bax translocation via a GSK-3beta-inactivation mechanism. *Journal of Cellular Physiology*, *224*(1), 218–228. https://doi.org/10.1002/jcp.22123

Zhang, T. R., Larosa, A., Di Raddo, M.-E., Wong, V., Wong, A. S., & Wong, T. P. (2019). Negative Memory Engrams in the Hippocampus Enhance the Susceptibility to Chronic Social Defeat Stress. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(38), 7576–7590. https://doi.org/10.1523/JNEUROSCI.1958-18.2019

Zhang, W.-J., Wang, H.-H., Lv, Y.-D., Liu, C.-C., Sun, W.-Y., & Tian, L.-J. (2018). Downregulation of Egr-1 Expression Level via GluN2B Underlies the Antidepressant Effects of Ketamine in a Chronic Unpredictable Stress Animal Model of Depression. *Neuroscience*, *372*, 38–45. https://doi.org/10.1016/j.neuroscience.2017.12.045

Zhang, X., Wu, W., Luo, Y., & Wang, Z. (2022). Transcranial Photobiomodulation Therapy Ameliorates Perioperative Neurocognitive Disorder Through Modulation of Mitochondrial Function in Aged Mice. *Neuroscience*, *490*, 236–249. https://doi.org/https://doi.org/10.1016/j.neuroscience.2021.12.033

Zhang, Z., Shen, Q., Wu, X., Zhang, D., & Xing, D. (2020). Activation of PKA/SIRT1 signaling pathway by photobiomodulation therapy reduces Aβ levels in Alzheimer's disease models. *Aging Cell*, *19*(1), e13054. https://doi.org/https://doi.org/10.1111/acel.13054

Zheng, Y., Fan, W., Zhang, X., & Dong, E. (2016). Gestational stress induces depressive-like and anxiety-like phenotypes through epigenetic regulation of BDNF expression in offspring hippocampus. *Epigenetics*, *11*(2), 150–162. https://doi.org/10.1080/15592294.2016.1146850

Zhou, L., & Foster, J. A. (2015). Psychobiotics and the gut-brain axis: in the pursuit of happiness. *Neuropsychiatric Disease and Treatment*, *11*, 715–723. https://doi.org/10.2147/NDT.S61997

Zhou, X., Xiao, Q., Xie, L., Yang, F., Wang, L., & Tu, J. (2019). Astrocyte, a Promising Target for Mood Disorder Interventions . In *Frontiers in Molecular Neuroscience* (Vol. 12).

Zhu, Z., Wang, X., Song, Z., Zuo, X., Ma, Y., Zhang, Z., Ju, C., Liang, Z., Li, K., Hu, X., & Wang, Z. (2022). Photobiomodulation promotes repair following spinal cord injury by restoring neuronal mitochondrial bioenergetics via AMPK/PGC-1α/TFAM pathway . In *Frontiers in Pharmacology* (Vol. 13).

Ziegler, L. M. von, Floriou-Servou, A., Waag, R., Gupta, R. R. Das, Sturman, O., Gapp, K., Maat, C. A., Kockmann, T., Lin, H.-Y., Duss, S. N., Privitera, M., Hinte, L., Meyenn, F. von, Zeilhofer, H. U., Germain, P.-L., & Bohacek, J. (2021). Molecular roadmap of the healthy stress response in the mouse hippocampus. *BioRxiv*, 2021.03.26.436970. https://doi.org/10.1101/2021.03.26.436970

Zomkowski, A. D. E., Hammes, L., Lin, J., Calixto, J. B., Santos, A. R. S., & Rodrigues, A. L. S. (2002). Agmatine produces antidepressant-like effects in two models of depression in mice. *Neuroreport*, *13*(4), 387–391. https://doi.org/10.1097/00001756-200203250-00005

ANNEX

International Journal of
Molecular Sciences

MDPI

Article

Cognitive and Emotional Symptoms Induced by Chronic Stress Are Regulated by EGR1 in a Subpopulation of Hippocampal Pyramidal Neurons

Anna Sancho-Balsells [1,2,3], Sara Borràs-Pernas [1,2,3], Verónica Brito [1,2,3], Jordi Alberch [1,2,3,4], Jean-Antoine Girault [5,6,7] and Albert Giralt [1,2,3,4,*]

1 Departament de Biomedicina, Facultat de Medicina, Institut de Neurociències, Universitat de Barcelona, Casanova 143, 08036 Barcelona, Spain
2 Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain
3 Centro de Investigación Biomédica en Red Sobre Enfermedades Neurodegenerativas (CIBERNED), 08036 Barcelona, Spain
4 Production and Validation Center of Advanced Therapies (Creatio), Faculty of Medicine and Health Science, University of Barcelona, 08036 Barcelona, Spain
5 Inserm UMR-S 1270, 75005 Paris, France
6 Science and Engineering Faculty, Sorbonne Université, 75005 Paris, France
7 Institut du Fer à Moulin, 75005 Paris, France
* Correspondence: albertgiralt@ub.edu; Tel.: +34-934037980

Abstract: Chronic stress is a core risk factor for developing a myriad of neurological disorders, including major depression. The chronicity of such stress can lead to adaptive responses or, on the contrary, to psychological maladaptation. The hippocampus is one of the most affected brain regions displaying functional changes in chronic stress. Egr1, a transcription factor involved in synaptic plasticity, is a key molecule regulating hippocampal function, but its role in stress-induced sequels has been poorly addressed. Emotional and cognitive symptoms were induced in mice by using the chronic unpredictable mild stress (CUMS) protocol. We used inducible double-mutant Egr1-CreERT2 x R26RCE mice to map the formation of Egr1-dependent activated cells. Results show that short- (2 days) or long-term (28 days) stress protocols in mice induce activation or deactivation, respectively, of hippocampal CA1 neural ensembles in an Egr1-activity-dependent fashion, together with an associated dendritic spine pathology. In-depth characterization of these neural ensembles revealed a deep-to-superficial switch in terms of Egr1-dependent activation of CA1 pyramidal neurons. To specifically manipulate deep and superficial pyramidal neurons of the hippocampus, we then used Chrna7-Cre (to express Cre in deep neurons) and Calb1-Cre mice (to express Cre in superficial neurons). We found that specific manipulation of superficial but not deep pyramidal neurons of the CA1 resulted in the amelioration of depressive-like behaviors and the restoration of cognitive impairments induced by chronic stress. In summary, Egr1 might be a core molecule driving the activation/deactivation of hippocampal neuronal subpopulations underlying stress-induced alterations involving emotional and cognitive sequels.

Keywords: dendritic spines; hippocampus; engram; memory; behavioral despair

Citation: Sancho-Balsells, A.; Borràs-Pernas, S.; Brito, V.; Alberch, J.; Girault, J.-A.; Giralt, A. Cognitive and Emotional Symptoms Induced by Chronic Stress Are Regulated by EGR1 in a Subpopulation of Hippocampal Pyramidal Neurons. *Int. J. Mol. Sci.* **2023**, *24*, 3833. https://doi.org/10.3390/ijms24043833

Academic Editor: Irena Nalepa

Received: 10 January 2023
Revised: 7 February 2023
Accepted: 10 February 2023
Published: 14 February 2023

1. Introduction

One of the most-studied and important environmental risk factors associated with the development of neurological disorders, such as schizophrenia, major depressive disorder (MDD) and other cognitive problems, is chronic stress [1–4]. In response to acute stressful events, an organism responds with adaptive changes on many levels (psychological, behavioral and physiological) which help to cope with the situation [5]. In healthy individuals, this response is limited in time. However, if this response is prolonged in

time and disproportionate in salience, it leads to significant disorders such as anxiety and depression [6].

In recent years, many neuroimaging-based studies have shown that the hippocampus is one of the brain regions most affected in psychiatric disorders such as MDD. Specifically, they show a significant reduction in hippocampal volume [7,8], and similar results have been observed in animal models of depression [9,10]. Furthermore, chronic stress has been related to the impairment of hippocampal-dependent memory [11], although studies about associated microstructural changes, such as spine loss, have been less consistent [12,13]. Interestingly, these alterations may depend on the hippocampal subregion [14], the stressor type and its duration [15,16] and the sex [16,17] of the animals, among other things. On the other hand, the rich cellular diversity recently described in the hippocampus [18] increases the complexity of the cellular interactions in the progression of stress-induced depression. It has been proposed that neurons of the CA1 region of the hippocampus can be classified as deep and superficial pyramidal cells along the radial axis. Deep and superficial pyramidal neurons have distinct transcriptional profiles and different electrophysiological properties [19,20]. These previous reports suggest that both subtypes could play different roles in the hippocampus in both normal and stressful conditions.

Growing evidence suggests that immediate early genes (IEGs) can be altered in different neuropsychiatric conditions that are associated with deficits in neuronal activity and plasticity, including major depression [21,22]. Early growth response 1 (*Egr1*) is a classical IEG and a zinc finger transcription factor that modulates the expression of many genes involved in crucial neuronal functions, such as neurotransmission, synaptic plasticity, learning and response to stress [22]. As with other IEGs, *Egr1* expression can be induced by a variety of signals, such as injury or stress [21]. Moreover, Egr1 is a very dynamic transcription factor, being upregulated or downregulated in the brain depending on the chronicity of the stress protocol [23,24]. From these previous studies, we hypothesize that *Egr1* expression could be tightly regulated depending on the chronicity of stress and be a potential molecular event underlying the cognitive and emotional symptoms induced by chronic stress.

In the present work, we mapped in mice the Egr1-dependent activation of neural ensembles in several brain regions at different time points in a chronic unpredictable mild stress protocol (CUMS). We identified Egr1-activity-tagged neurons in CA1 that increase or decrease depending on the chronicity of the stress protocol. Furthermore, the cellular identity of these activated cells changed along the CUMS protocol, shifting from deep to superficial pyramidal neurons. Finally, we show that manipulations of superficial or deep pyramidal cells play distinct roles in the modulation of the sequelae induced by chronic stress.

2. Results

2.1. Egr1-Dependent Neural Activation in Hippocampal CA1 Depends on the Chronicity of the Stress

It is widely accepted that several brain regions, such as the amygdala, cingulate cortex, septum and hippocampus, among others, play a role in chronic stress [25–28], and that persistence of stressful periods can determine the progression and severity of associated depressive symptoms [29]. Here, we mapped the forebrain by counting the density of GFP-positive cells in brain regions highly affected by chronic stress. Since previous studies suggest that Egr1 is upregulated in short periods of stress [30] whereas the same protein is downregulated in long periods of stress [11], we hypothesized that this protein could be an excellent candidate to map and manipulate the activation/deactivation of neural ensembles dependently on the chronicity of the stressful events. Thus, we took advantage of Egr1-CreERT2 BAC transgenic mice [31]. These mice express Cre recombinase fused to modified estrogen receptor (ERT2) only activated by 4-hydroxytamoxifen (4-HT), under the control of the *Egr1* promoter. When these mice are injected with saline, no recombination is observed [31,32]. To identify

which neural cells are activated/deactivated in short-term vs. long-term stress conditions, we crossed Egr1-CreERT2 mice with R26RCE mice, a reporter line in which EGFP expression requires recombination by Cre (Figure 1A). We then subjected Egr1-CreERT2xR26RCE double transgenic mice to a CUMS protocol. Mice were separated into three groups: control non-stressed (CNT group), short-term-stressed (2 days, STS group), and long-term-stressed (28 days, LTS group). Each group received one injection of 4-HT 30 min before the presentation of a stressful stimulus during the last two consecutive days of the CUMS protocol (Figure 1B). First, we observed that the density of GFP-positive cells in the septum (Figure 1C), the amygdala (Figure 1D) and the cingulate cortex (Figure 1E) was similar between groups. The LTS group showed an increase in GFP-positive cells in the paraventricular nucleus compared to the CNT and STS groups (Figure 1F). In the striatum we could not detect significant differences between any condition (Figure 1G). In the hippocampal CA3 region (Figure 1H), GFP-positive cells were reduced in the LTS group compared with CNT group. Finally, in the CA1 we observed remarkable bidirectional changes in the number of GFP-positive cells depending on the chronicity of the stress protocol (Figure 1I). Concretely, GFP-positive cells were increased in the STS group but decreased in the LTS group when compared with the CNT group. From the latter result, we hypothesize that the CA1 could be highly sensitive to the chronicity of the stress. Finally, we performed and immunofluorescence to validate that all the GFP-positive cells also expressed Egr1 (Figure 1J).

2.2. Specific Spine Density Alterations in Egr1-Positive Activated Cells

Since we observed that stress induced opposite changes in the activation of Egr1-dependent neural ensembles in the CA1 depending on its duration, we hypothesized that such changes could be accompanied by alterations in synapse numbers in these particular ensembles. To explore this, we used Egr1-CreERT2 mice (Figure 2A) injected in the dorsal CA1 with AAV-CaMKII-FLEX-EGFP to allow the quantification of dendritic spines specifically in the Egr1-positive ensembles (Figure 2B). Again, we used a control group (CNT), a group of mice subjected to stress for 2 days (STS), and a group of mice subjected to stress for 28 days (LTS) (Figure 2C). Apical dendrites from CA1 pyramidal neurons were analyzed in the three groups (Figure 2D). We observed that spine density in apical dendrites was significantly decreased in the LTS group whereas it was not changed in the STS mice (Figure 2E). Taken together, the results highlighted that, although short-term stress induced a similar trend, only long-term stress resulted in significant changes in spine density from activated pyramidal neurons of the CA1. To evaluate whether such changes in spine density were global or specific to the Egr1-dependent ensembles, we then evaluated spine density in pyramidal neurons of the dorsal CA1 by using the DioListic method in different groups of wild type mice subjected to 28 days of CUMS and compared them with unstressed control mice (Figure 2G). The results showed that the density of dendritic spines was similar between control and LTS mice when it was counted in pyramidal neurons in an unspecific manner. Although the methods employed in each experiment are different, these data suggest that not all pyramidal cells present alterations in spine density upon CUMS but mainly those that are activated in a Egr1-dependent manner.

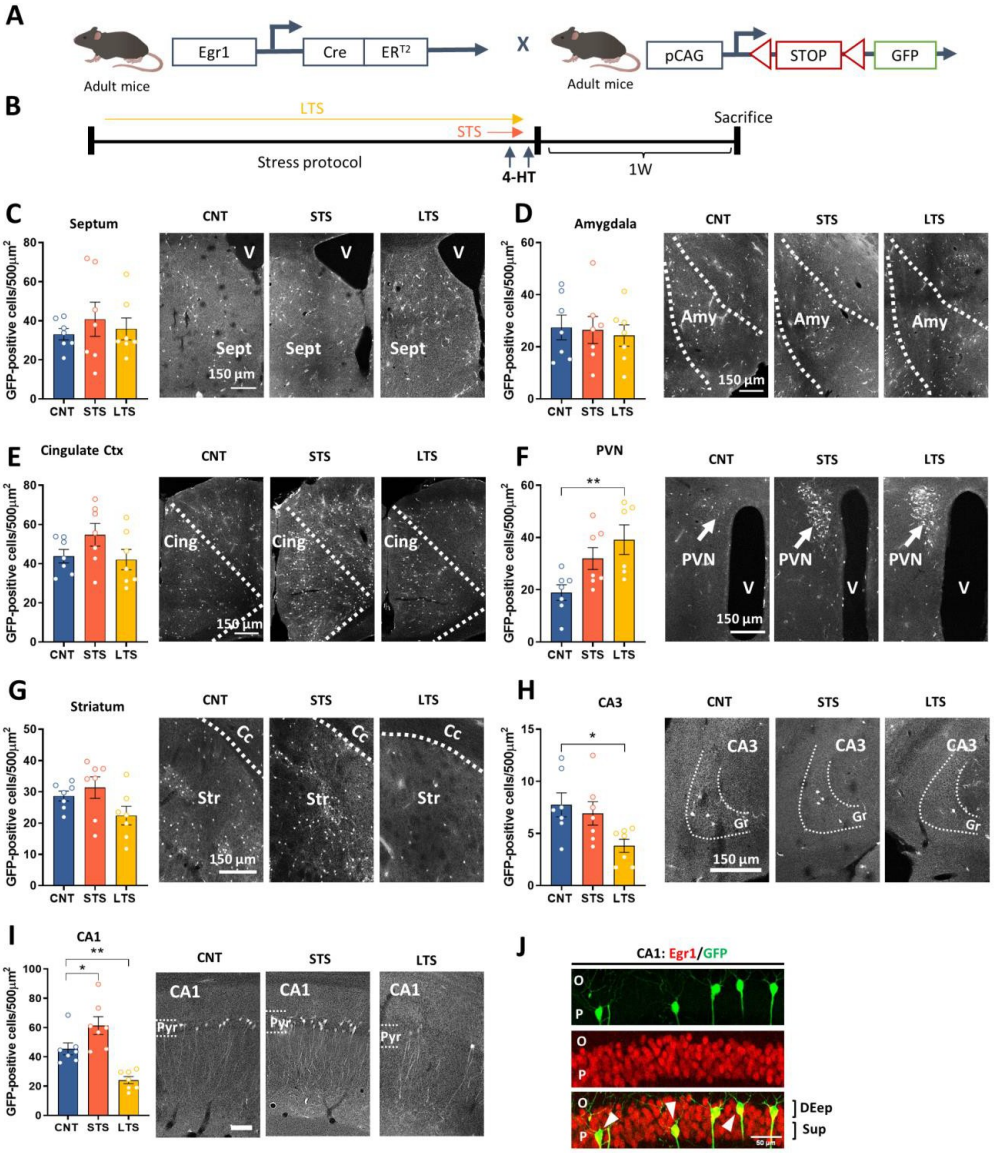


Figure 1. Egr1-dependent activated neuronal ensembles in different brain regions by chronic stress. (**A**), Schematic representation of double-heterozygous-mutant Egr1-CreERT2 × R26RCE GFP mice. (**B**), Egr1-CreERT2 × R26RCE mice were subjected to 0 (CNT), 2 (STS) or 28 (LTS) days of CUMS. For the last two days of the protocol, all mice received 50 mg/kg of 4-hydroxytamoxifen (4-HT, i.p), and one week later they were sacrificed. Representative images and quantification of Egr1-dependent activated cells (estimated number of GFP-positive cells/area of 500 µm^2) per region in: the septum (**C**), one-way ANOVA group effect: $F_{(2,18)} = 0.3858$, $p = 0.6854$; the amygdala (**D**), group effect: $F_{(2,18)} = 0.1160$, $p = 0.8911$; the cingulate cortex (**E**), group effect: $F_{(2,18)} = 1.946$, $p = 0.1718$; the paraventricular nucleus (PVN) (**F**), group effect: $F_{(2,17)} = 5.686$, $p = 0.0129$; the striatum (**G**), group effect: $F_{(2,18)} = 2.716$, $p = 0.0931$; the CA3 (**H**), group effect: $F_{(2,18)} = 4.322$, $p = 0.0293$, and the CA1 (**I**), group effect: $F_{(2,18)} = 17.63$, $p < 0.0001$; Scale bar, 50 µm. The values are expressed as the mean ± SEM, N = 7 adult mice per condition. Dunnet's post hoc test, * $p < 0.05$, ** $p < 0.005$ compared with CNT. (**J**) Representative images of the CA1 of the hippocampus showing GFP-activated cells (green) and Egr1-positive cells (red). White arrows indicate double-Egr1-positive and GFP-positive cells. V, ventricle; CC, corpus callosum; O, Stratum oriens; P, Stratum pyramidale.

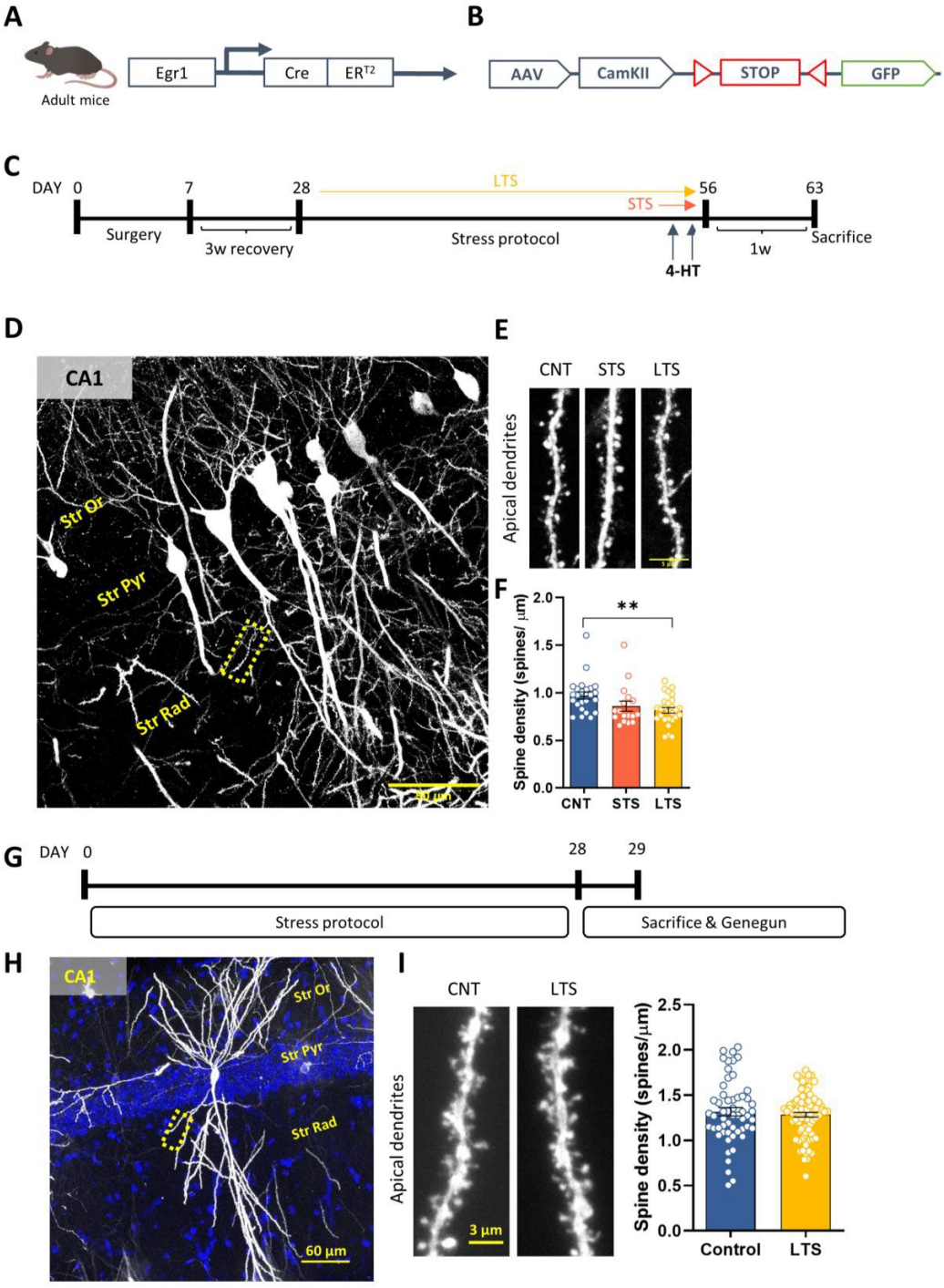


Figure 2. CUMS specifically alters dendritic spine density in Egr1-activated pyramidal neurons. (**A**), Schematic representation of the Egr1-CreERT2 mouse model and (**B**) AAV expressing GFP under the control of a STOP floxed codon. (**C**), Timeline of the experiment: Adult mice were injected with AAV-CaMKII-Flex-GFP. After recovery, they were exposed to 0 (CNT) or 2 (STS, orange arrow) or 28 days of CUMS (LTS, yellow arrow). On the last two days of CUMS, all animals received 50 mg/kg of 4-HT (i.p.) 30 min before the stressor. One week after the last stressor, animals were sacrificed for

tissue collection. (**D**), Representative image showing labelled neurons in the CA1 region of STS mice. Effect of CUMS on apical spine density (**E**,**F**). In (**F**), N = 17–27 dendrites from seven different mice per group were quantified. The values are means ± SEM. Statistical analysis with one-way ANOVA followed by Tukey's post hoc test, (group effect in (**F**), $F_{(2, 47)} = 5.86$, $p = 0.005$). ** $p < 0.005$ compared with CNT group. (**G**), WT mice were subjected to 0 (CNT) or 28 days (LTS) of CUMS. Forty-eight hours after the last stressor, mice were perfused and hippocampal slices subjected to the gene gun method. (**H**), Representative image showing a labelled neuron of the CA1. (**I**), Representative image (left panel) and quantification (right panel) showing no effect of CUMS in apical dendrites of CA1 pyramidal cells in LTS mice compared with CNT mice (unpaired *t*-test, $t_{142} = 0.6730$, $p = 0.5020$). In (**H**), 58–88 dendrites from seven different mice per group were quantified.

2.3. Different Hippocampal Subpopulations Are Activated Depending on Stress Duration

We observed that the CA1 displayed the highest contrast in neural activation depending on the duration of the stress protocol, as indicated by Egr1-activity induction. Such activation was accompanied by time-dependent changes in dendritic spine densities. This led us to hypothesize that a possible progressive cellular reorganization (in terms of Egr1-dependent activation) could take place in the CA1 during the progression of chronic stress. To characterize such potential cellular reorganization, we immunostained the CA1 of Egr1-CreERT2xR26RCE mice in the three conditions (CNT, STS and LTS) for different markers, including GFAP, a marker of astrocytes, PV, a marker of parvalbumin interneurons, and MAP2, a general marker for CA1 pyramidal cells. We first observed that neither GFAP (Figure 3A,B) nor PV (Figure 3A,D) colocalized with GFP (Egr1-dependent activated neural cells) in any group. In contrast, almost all the GFP-positive cells colocalized with MAP2-positive cells in the three groups (Figure 3A,C). We also immunostained hippocampal sections of the three groups of mice for Calb1, which identifies the superficial pyramidal neurons of CA1, whereas pyramidal neurons negative for Calb1 are considered to be deep pyramidal cells [19]. Remarkably, our quantifications revealed that the percentage of GFP-positive CA1 pyramidal cells co-stained with Calb1 was different in the three groups (Figure 3E). The percentage of double-stained Calb1-positive/GFP-positive cells was low in the CNT group, significantly increased in the STS group, and reached its highest level in the LTS group (Figure 3E). However, these percentages did not reflect changes in the total number of GFP-positive cells depending on the duration of the CUMS protocol (see Figure 1I). Therefore, we also analyzed the total number of Calb1-positive/GFP-positive neurons, corresponding to activated superficial pyramidal neurons. This number was increased in the STS group, whereas in the LTS group the number of Calb1-positive/GFP-double-positive neurons was similar to in the CNT group (Figure 3F). In contrast, the density of Calb1-negative/GFP-positive neurons (activated deep pyramidal neurons) was unchanged in the STS group compared to the CNT group but significantly decreased in the LTS group (Figure 3G). To confirm that these alterations were not due to global changes in total Calb1 levels, we measured Calb1 levels using western blotting (Figure 3H,I) and immunofluorescence (Figure 3J,K). Our quantifications (Figure 3H,J) confirmed that total Calb1 levels are unaltered by stress. All these results taken together indicate that, although there is a progressive reduction of CA1 GFP-positive pyramidal neurons at the end of the CUMS protocol (Figure 1I), there is a transient and concomitant increase in the percentage of Egr1-dependent activated neurons, which are in turn Calb1-positive neurons (superficial pyramidal neurons). In contrast, Calb1-negative neurons (deep pyramidal neurons) are severely and progressively deactivated (in terms of Egr1-dependent activation) at the end of the CUMS protocol.

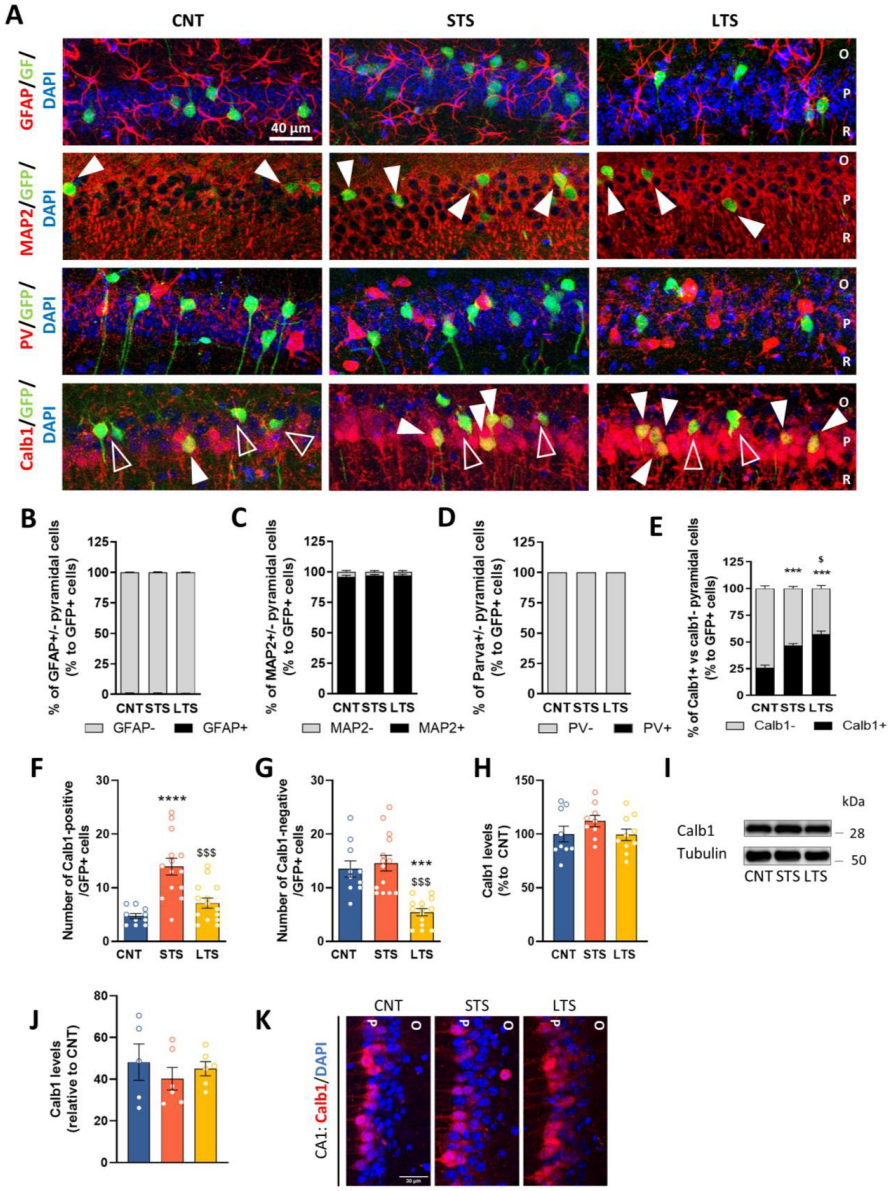


Figure 3. Neural identity characterization of Egr1-dependent activated cells by short- or long-term stress in the CA1. (**A**), Representative images showing the identity of the activated cells (green) by co-labeling with specific cells markers (red). In all rows, GFP-positive cells are in green. First row: representative images from all conditions (CNT, STS and LTS) showing labeling of astrocytes (red). Second row: labeling of MAP2 (red). White arrows point to GFP- and MAP2-double-positive neurons. Third row: labeling of parvalbumin (PV, red). Fourth row: labeling of calbindin1 (Calb1, red). White arrows point to double-positive GFP- and calbindin-double-positive neurons. Open arrows point to GFP-positive calbindin-negative cells. (**B**–**E**), Quantification of the percentages of double-positive cells in CA1 for GFP and GFAP (**B**), GFP and MAP2 (**C**), GFP and PV (**D**), and GFP and Calb1 (**E**). Values are means ± SEM, N = 7 mice per group. In (**E**), statistical analysis with one-way ANOVA, group effect: $F_{(2,18)} = 42.31$, $p < 0.0001$. Total number of GFP-positive cells per surface unit that are also calbindin1-positive (**F**) or calbindin-negative (**G**). Statistical analysis with one way ANOVA, in (**F**), $F_{(2,35)} = 15.91$, $p < 0.0001$ and in (**G**), $F_{(2,35)} = 17.40$, $p < 0.0001$. Densitometry quantification (**H**) and representative immunoblots (**I**) of calbindin levels in the hippocampal lysates of CNT, STS and LTS mice. N = 9–10 mice per group. Quantification of calbindin intensity in the CA1 (**J**) and representative images (**K**) of CNT, STS and LTS mice, N= 5–6 mice per group. All values are expressed as means ± SEM. Tukey's post hoc test, **** $p < 0.0001$ or *** $p < 0.001$ compared to CNT and \$ < 0.05, \$\$\$ $p < 0.001$ compared to STS mice. O, Stratum oriens; R, Stratum radiatum; P, Stratum pyramidale.

2.4. Chemogenetic Activation of Dorsal CA1 Deep Pyramidal Cells Does Not Prevent Sequelae Induced by CUMS

We observed that chronic stress induced a reduction in the number of Egr-1-dependent activated deep pyramidal cells in the CA1 (Figure 3G). To test whether such Egr1-dependent deactivation was relevant for the sequelae induced by chronic stress we aimed to activate them by using designer-receptors-exclusively-activated-by-designer-drugs technology (DREADDs). To selectively express hM3Dq DREADDs in deep pyramidal neurons we used Chrna7-Cre mice (Figure 4A). Chrna7-mice were injected in the dorsal hippocampus with an AAV vector construct containing a double-floxed inverted open-reading frame (DIO) sequence encoding hM3Dq-mCherry (Figure 4B). With such a design, only those Chrna7-positive neurons (deep pyramidal neurons) expressing Cre-recombinase would be able to recombine and express the DREADD in the correct orientation and only when CNO is given to the animal (in drinking water) would those cells become activated. We then subjected the mice to CUMS and divided them into four different groups: non-stressed treated with vehicle (CNT VEH group), non-stressed treated with CNO (CNT CNO), long-term (28 days)-stressed treated with VEH (LTS VEH) and long-term-stressed (28 days) treated with CNO (LTS CNO) (Figure 4C). In parallel, we used a small subgroup of mice treated with CNO for 2 days but without behavioral manipulation to avoid unspecific changes due to extensive experimentation. In these mice, immunofluorescence staining confirmed that only deep pyramidal cells were transduced and that CNO in drinking water induced the activation of Chrna7-positive neurons (cFos+ cells) (Figure 4D–F). We then analyzed the % of cFos+ cells out of those transduced and clearly observed that treatment with CNO induced activation of those transduced neurons (Figure 4E). Regarding the mice subjected to the CUMS procedure, we only used mice with correct allocation of the viral transduction in the CA1 (Figure 4F). Interestingly, we first observed that both groups of LTS mice presented increased locomotor activity in the open field test (Figure 4G). Next, to assess cognitive sequelae after CUMS, we performed the novel object location test, which is a task sensitive to detecting cognitive impairment upon CUMS [11,33,34]. In this test, only the CNT VEH and CNT CNO groups were capable of discriminating between the new and the old locations of the object (Figure 4H). Finally, we analyzed the time spent struggling in the forced-swim test. Both groups of LTS mice spent less time struggling specifically during the first minutes of the test compared to both groups of CNT mice (Figure 4I,J). To exclude the possibility of unspecific or off-target effects of CNO per se, we repeated the same experimental design as in Figure 4C without surgery. Hence, control mice received normal housing or the CUMS protocol while they were treated with VEH or CNO in drinking water. CNO did not produce any effect on locomotion in the open field when comparing the vehicle and CNO conditions (Figure 4K). Similarly, we did not find any effect mediated by CNO either in the novel object location task (Figure 4L) or in the forced-swim test (Figure 4M,N). These results ruled out unspecific off-target effects induced by CNO in the behavioral tasks performed. Altogether, our data suggest that the activation of Chrna7-positive (deep) neurons during the CUMS protocol has no effect on the CUMS-induced sequelae.

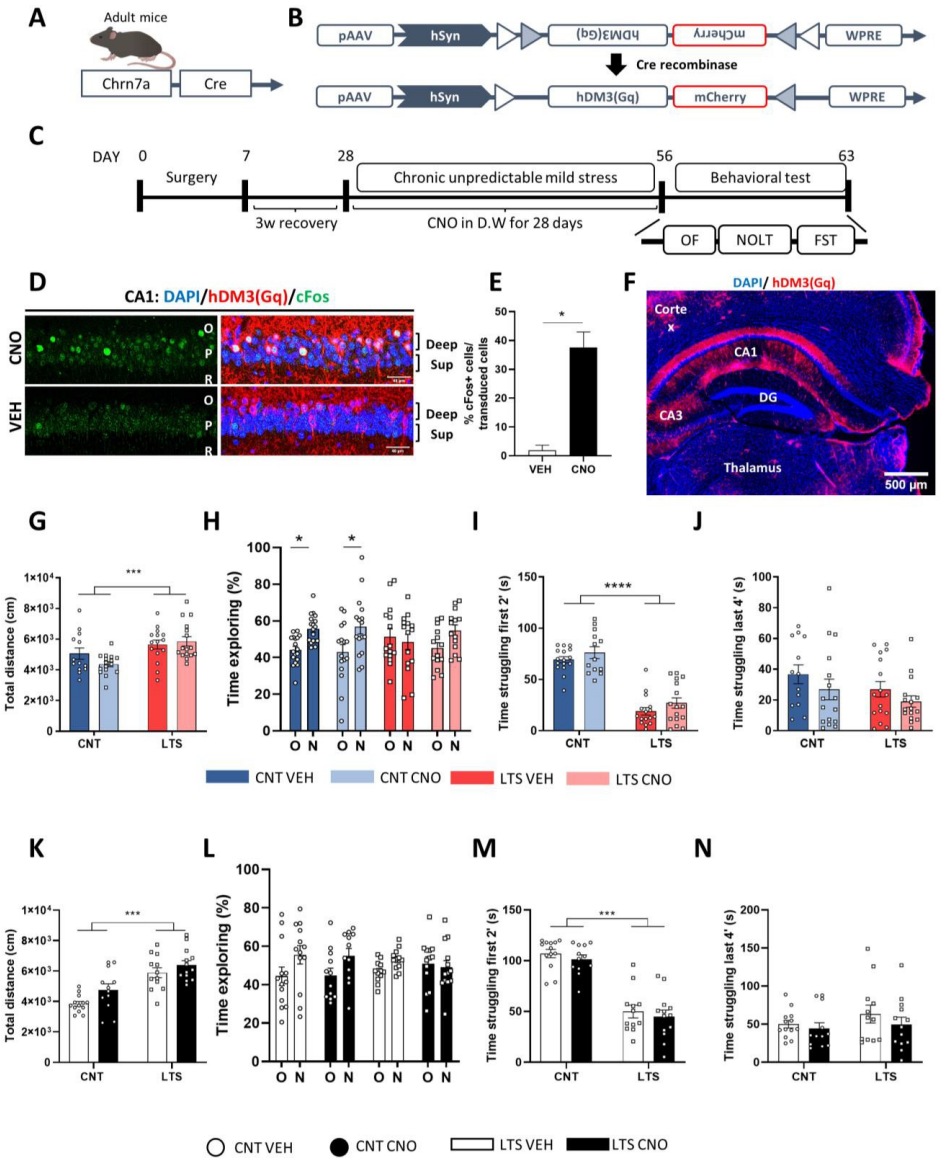


Figure 4. Effects of the chemogenetic activation of CA1 deep pyramidal neurons during CUMS. (**A**), Schematic illustration of the Chrn7a-Cre mouse model and of the AAV (**B**) used to express the activator DREADD (hDM3(Gq)) after Cre recombination. (**C**), Timeline of the experiment. Mice were injected with the vector shown in (**B**) and, after recovery, they were subjected to the CUMS protocol. During the stress protocol, mice received either CNO (1 mg/kg) or vehicle (VEH) solution in drinking water. Twenty-four h after the last stressor, mice were subjected to a battery of behavioral tests. (**D**), Representative image of CA1 showing cFos-positive cells (green) and viral transduction (mCherry, red) and cell nuclei (blue, DAPI). (**E**), Percentage of cFos+ cells (green) co-labelled with mCherry (red) in mice treated with VEH or CNO (unpaired *t*-test, $t_{17} = 2.83$, $p = 0.01$). A representative global image (**F**) from (**D**) of viral transduction specifically in the hippocampal CA1. In the open field, locomotor activity (**G**) was monitored for 15 min. Two-way ANOVA, stress effect: $F_{(1,56)} = 13.23$, $p = 0.0006$. (**H**), In the novel object location test, spatial memory was evaluated 24 h after a training trial as the percentage of total time spent exploring either the object placed at a new location (**N**) or the object placed at the old location (**O**). Two-way ANOVA, new location effect: $F_{(1,122)} = 8.64$, $p = 0.0039$, N = 13–18 mice per condition. In the forced-swim test, the immobility time was evaluated during the first 2 min (**I**) and the last 4 min (**J**) of the 6 min trial in all groups. In the

first 2 min, two-way ANOVA, group effect: $F_{(1,57)} = 128.6$, $p < 0.0001$. An independent new cohort of WT mice underwent CUMS or not and were treated with vehicle or CNO to determine undesired effects of CNO. We used the same experimental design as in **c** without surgery. (**K**), In the open field, locomotor activity was monitored for 15 min. Two-way ANOVA, stress effect: $F_{(1,46)} = 37.43$, $p < 0.0001$. (**L**), Spatial memory was evaluated in the novel object location test. Two-way ANOVA, new location effect: $F_{(1,92)} = 6.1$, $p = 0.0152$. In the forced-swim test, the immobility time was evaluated during the first 2 min (**M**) and the last 4 min (**N**) of the 6 min trial in all groups. In the first 2 min, two-way ANOVA, group effect, $F_{(1,46)} = 113$, $p < 0.0001$. N= 12–13 mice per condition. All values are means ± SEM. Tukey's post hoc in (**G**,**I**–**K**,**M**); Bonferroni post hoc in (**H**,**L**). * $p < 0.05$, *** $p < 0.001$ and **** $p < 0.0001$. O, Stratum oriens; R, Stratum radiatum; P, Stratum pyramidale.

2.5. Specific Downregulation of Egr1 in CA1 Superficial Pyramidal Cells during CUMS Improves Depressive Sequelae

Trying to upregulate the activity of the deep pyramidal cells by using DREADDs was not efficient in the prevention of the sequelae induced by stress. Next, we decided to prevent effects mediated by the early increase in Egr1-dependent activated cells in the superficial pyramidal cells (Figure 3E–G) by reducing their endogenous Egr1 levels. We hypothesized that suppressing the increase in Egr1 in superficial neurons found at the beginning of the stress response could prevent some alterations induced by CUMS. To specifically modulate superficial neurons, we used Calb1-cre mice (Figure 5A). We thus transduced only superficial CA1 pyramidal (Calb1-positive) cells with shRNA against *Egr1* (Figure 5B) or with a GFP CNT virus. Mice were then divided into four different conditions: CNT mice injected with control AAV (AAV-CNT), CNT mice injected with an AAV-shRNA against *Egr1* (AAV-shEGR1), chronically stressed mice injected with control AAV (AAV-CNT+CUMS) and chronically stressed mice injected with an AAV-shRNA against *Egr1* (AAV-shEGR1+CUMS). After the CUMS protocol, mice were tested for different behavioral tasks as illustrated in our experimental design (Figure 5*C*), and, finally, they were sacrificed for viral transduction verification. We first showed widespread viral transduction in the CA1 superficial pyramidal neurons of the dorsal hippocampus (Figure 5D–F). Egr1 levels were specifically downregulated in those superficial pyramidal cells of the CA1 that were transduced with the SH-viral vector (Figure 5E). Then, in the open field test, both groups of mice subjected to the CUMS presented hyperlocomotion compared to the CNT groups (Figure 5G). We then used the NOLT to test spatial memory. AAV-CNT mice were able to distinguish between the old and new object positions. Conversely, AAV-shEGR1 mice could not differentiate the new location of the object. Interestingly, the deficits in spatial memory caused by CUMS (as observed in the AAV-CNT+CUMS mice) were alleviated in the AAV-shEGR1+CUMS mice (Figure 5H). We then subjected the mice to the forced-swim test to evaluate behavioral despair. In the first two minutes of the test (Figure 5I), both groups of mice subjected to CUMS significantly spent less time swimming compared to non-stressed mice. This was maintained for the last four minutes of the task (Figure 5J). Interestingly, in the last four minutes of the test, we observed that stressed mice injected with SH- against *Egr1* swam significantly more than stressed mice injected with the control AAV (Figure 5J). Finally, we also verified that Calb1 levels in superficial CA1 neurons were not affected by our experimental conditions (Supplementary Figure S1). In summary, inhibition of *Egr1* expression in Calb1-positive (superficial) neurons corrected different sequelae caused by CUMS. It is noteworthy that since in this experiment we used AAV5-CAG-FLEX-GFP as a control instead of a scramble sequence the results should be taken with caution.

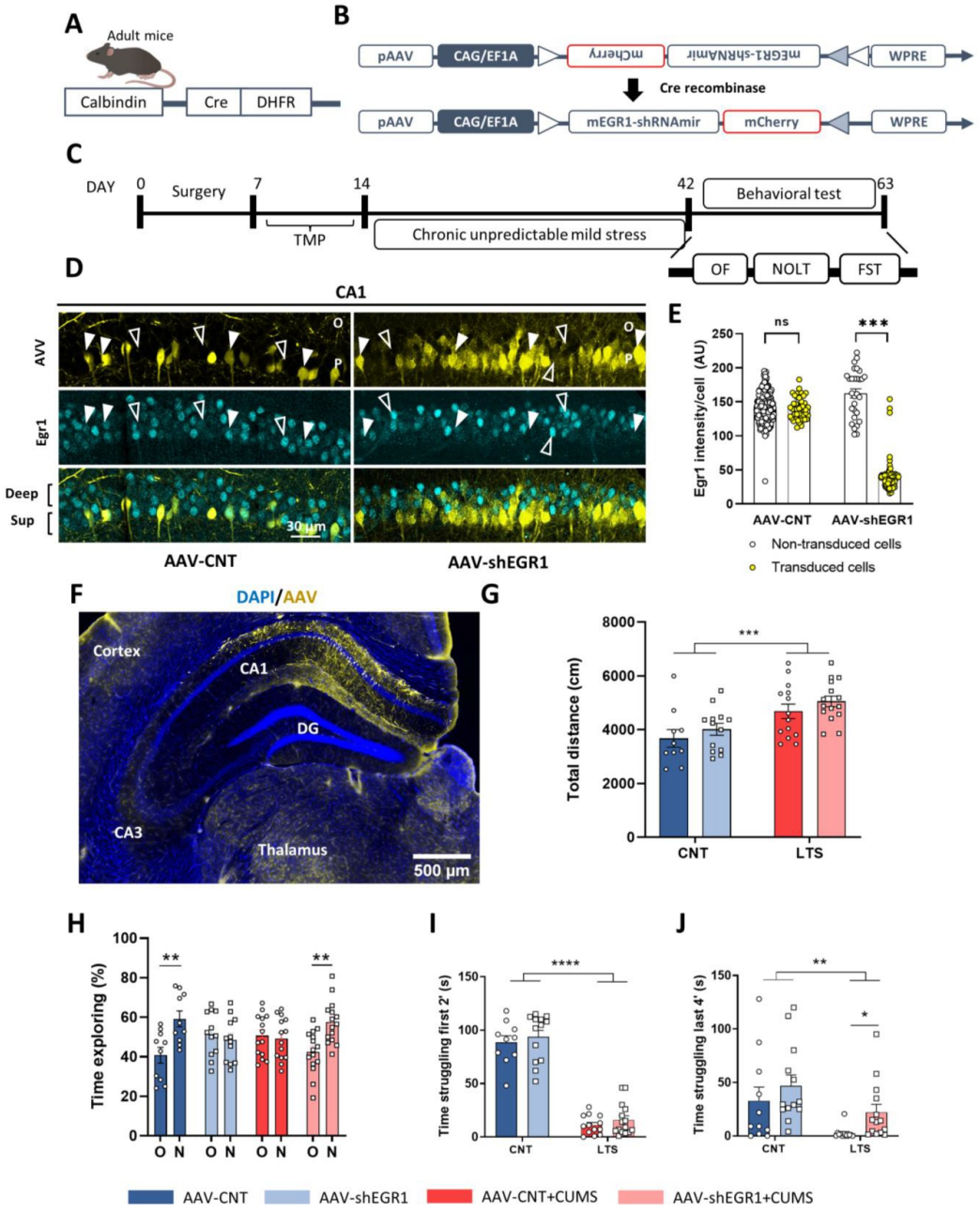


Figure 5. Effects of Egr1 shRNA delivery in CA1 superficial pyramidal neurons during CUMS. (**A**), Schematic representation of the calbindin-Cre mice. (**B**), Schematic diagram showing the AAV-mCherry-floxed-shEGR1 viral construct. (**C**), Timeline of the experiment. Mice were injected with the construct shown in (**B**) or with a control floxed construct and, after surgery, they received TMP to induce Cre expression. Three weeks after surgery, they were subjected to the CUMS protocol or stayed in their home cage. (**D**), Representative images in CA1 showing Egr1 levels (cyan) in mice injected with control floxed virus (AAV-CNT, left panel) or with the AAV containing the floxed shRNA against Egr1 (AAV-shEGR1). Viral expression is depicted in yellow. White arrows designate transduced neurons expressing the virus. Open arrows designate non-transduced neurons. (**E**), Quantification of Egr1 optical density (IOD) in non-transduced cells (white dots) and in transduced cells with the AAV

(yellow dots) in each condition (AAV-CNT or AAV-shEGR1). T-test: $t_{167} = 56.77$, *** $p < 0.001$ (ns, not significant). A representative global image (**F**), from (**D**) of viral transduction specifically in the hippocampal CA1. In the open field, locomotor activity (**G**) was monitored for 15 min. Locomotor activity, two-way ANOVA, group effect: $F_{(1,48)} = 16.36$, $p < 0.0001$. In the novel object location test (**H**), spatial memory was evaluated 24 h after a training trial as percentage of time exploring the object placed in a new location (N) versus the time exploring the object placed in an old location (O). Two-way ANOVA, object in a new location effect: $F_{(1,94)} = 10.33$, $p = 0.0018$. Interaction effect: $F_{(3,95)} = 5.878$, $p = 0.001$. In the forced-swim test, the immobility time was evaluated during the first 2 min (**I**) and the last 4 min (**J**) of the 6 min trial in all groups. In (**I**), two-way ANOVA, group effect: $F_{(1, 47)} = 257.7$, $p < 0.0001$. In **J**, two-way ANOVA, group effect: $F_{(1\ 47)} = 10.28$, $p = 0.0024$. T-test between LTS mice ($t_{25} = 2.568$, $p = 0.0166$). N= 12–13 mice per condition. All data is represented as mean ± SEM. Tukey's was used as a post hoc * $p < 0.05$, ** $p < 0.005$, **** $p < 0.001$. O, Stratum oriens; P, Stratum pyramidale.

3. Discussion

In the present study, we identified changes in Egr1-dependent activated cells by CUMS in several brain regions. In the CA1 of the hippocampus, Egr1-dependent activated cells were of neuronal identity and differently affected by short- and long-term stress. Secondly, we found that those Egr1-dependent neurons activated during stress display dendritic spine alterations, and that these changes also depend on the duration of the CUMS protocol. We then determined the identity of the activated neurons and demonstrated that there is a change in the activated CA1 subpopulations, from being mostly Calb1-negative (deep) in non-stressed mice to being Calb1-positive (superficial) in long-term-stressed mice. We then used chemogenetic and genetic approaches to directly modulate these two hippocampal subpopulations. We found that inhibiting Egr1 expression in superficial pyramidal (Calb1-positive) neurons but not activating deep pyramidal neurons (Calb1-negative) alleviated some of the CUMS-induced sequelae.

Previous literature has reported effects on several brain regions in terms of neural activity in stress-related disorders [26,27]. Here, using mice expressing inducible Cre recombinase under the promoter of the immediate early gene *Egr1*, we describe changes in different brain areas depending on the chronicity of the CUMS protocol. First, long-term-stressed (LTS) mice presented an increased number of Egr1-dependent activated cells in the paraventricular nucleus compared to CNT mice, confirming previous literature [28]. In contrast, in the CA3, we found fewer Egr1-dependent activated cells in the LTS group. Interestingly, in the CA1 of the hippocampus we found opposite differences in the numbers of active neurons depending on stress duration. Egr1-dependent activated cells were increased after a short period of stress, while they were decreased after long periods of stress. In line with this, other studies have also shown dynamic changes in Egr1 using different stress paradigms, including social defeat, exposure to predators and chronic mild stress [21,22]. Moreover, some groups have found increased levels of Egr1 in the hippocampus upon acute stress [24,35–41] whereas other groups found decreased Egr1 levels when the stress was continuous in time ([42–44], but see [45–47]). Thus, our work reinforces the idea that chronicity is a core characteristic of stress, which determines if Egr1 is going to be up- or downregulated. Altogether, this indicates that Egr1 is very dynamic and sensitive to stress duration [46,48,49] and places the CA1 as a core brain region that differentially reacts depending on stress chronicity.

Concomitant with a progressive decrease in Egr1-dependent activated CA1 pyramidal cells we found specific alterations in dendritic spine density in the same cells, whereas the same parameter was not observed when using general methods to analyze spine density in neurons. It is widely accepted that stress induces changes in spine density, and that these changes depend on the brain region studied and the type of stress presented [13]. Here, we observed that when spine density is only measured in those significantly Egr1-dependent activated neurons, relevant alterations are found. Supporting this idea, although stress-induced dendritic spine pathology is evident in CA3 pyramidal cells [50–55], it is much less clear in CA1 pyramidal cells. Some authors have shown reduced spine density [46,55–57]

or unchanged spine density [51–53,58] or even increased spine density [54,59,60] in CA1 pyramidal neurons in different stress paradigms. Based on our results and previous literature, we conclude that changes in dendritic spines in CA1 pyramidal neurons upon chronic stress could depend, at least, on two dimensions: first, they could depend on the duration of the stress, and, second, these changes in spine density could be limited to different neuronal subpopulations. Thus, our results may shed light on the discrepancies in the literature regarding changes in spine density in the CA1 upon stress. Finally, a potential mechanism by which increased Egr1 activity may lead to decreased spine density in these particular neuronal ensembles could be by reducing PSD-95 expression and inducing AMPAR endocytosis, as previously described [61].

We also observed that the type of hippocampal neuron activated is different depending on stress duration. Concretely, Egr1-dependent activated cells were preferentially deep CA1 pyramidal cells in control conditions, whereas Egr1-dependent activated cells were preferentially CA1 superficial pyramidal cells in chronic stress conditions. Our results also indicate that the modulation of Calb1-positive (superficial) cells per se has an impact on the pathophysiology of stress, since decreasing Egr1 levels in only this subpopulation produced an improvement in behavioral despair and spatial memory loss. Supporting this idea, it has been shown that Calb1 levels increase in CA1 pyramidal neurons upon stress [62]. Furthermore, reducing Calb1 levels in the CA1 is enough to alleviate memory loss in stress models [63]. Our results probably also indicate that, with only few days of stress, molecular Egr1-dependent events in superficial pyramidals are initiated and all the changes that we observed in 28 days of stress could be just a consequence triggered by those initial changes. This reinforces the idea that preventing this initial Egr1 increase in superficial cells would prevent both spatial learning deficits and behavioral despair induced by CUMS. In line with this, since Egr1 is a critical factor in encoding the enduring behavioral effects of stress in the hippocampus [64–66] we speculate that, possibly, the increase in Egr1 at the beginning of the stress induces the depressive-like symptoms whereas the Egr1 downregulation at the end of stress would induce the deficiencies in spatial learning. If our hypothesis is true, the fact that inhibiting Egr1 at the beginning of CUMS improves both FST and NOLT would indicate that the late effects (NOLT deficiencies and decreased Egr1-positive engrams) are dependent on the first effects (increase in Egr1-positive engrams and emotional sequelae). Finally, we also conclude that appropriate levels of this transcription factor in the superficial CA1 pyramidal neurons are mandatory for proper hippocampal function, as described elsewhere [67–69], since aberrant increases (induced by stress) or decreases in Egr1 (induced by hippocampal delivery of shRNA against *Egr1* in naïve mice) can both provoke deleterious effects on hippocampal-dependent memory.

4. Materials and Methods

4.1. Animals

For this study, we used Egr1-CreER^{T2} mice [31]. These mice carry a bacterial artificial chromosome (BAC) including the Egr1 gene in which the coding sequence was replaced by that of the CreERT2 fusion protein. Egr1-CreER^{T2} mice were crossed with R26RCE mice (Gt(ROSA)26Sortm1.1(CAG-EGFP)Fsh/Mmjax, Strain 004077, The Jackson Laboratory. RRID:IMSR_JAX:004077), which harbor the R26R CAG-boosted EGFP (RCE) reporter allele with a loxP-flanked STOP cassette upstream of the enhanced green fluorescent protein (EGFP) gene, to create double-heterozygous-mutant Egr1-CreER^{T2} × R26RCE mice. We also used Chrna7-cre mice (Tg(Chrna7-cre)NP348Gsat/Mmucd, GENSAT) and Calb1-cre mice (Calb1-T2A-dgCre-D, Jackson Labs, #023531. RRID:IMSR_JAX:023531). All the animals employed were males. Genotypes were determined from an ear biopsy as described elsewhere [70] and following manufacturer's instructions. All mice were maintained in a C57BL/6 background strain and housed together in numerical birth order in groups of mixed genotypes (3–5 mice per cage). The animals were housed with access to food and water ad libitum in a colony room kept at 19–22 ˚C and 40–60% humidity, under an inverted 12:12 h light/dark cycle (from 08:00 to 20:00). All animal procedures were

approved by local committees [Universitat de Barcelona, CEEA (10141) and Generalitat de Catalunya (DAAM 315/18)], in accordance with the European Communities Council Directive (86/609/EU).

For the experiments shown in Figures 1 and 3, 7 adult (4-month-old) male mice were used per group (total n = 21 mice). For the experiments shown in Figure 2A–F, 7 adult (6–7-month-old) male mice were used per group (total n= 21 mice). For the experiments shown in Figure 2G–I, 3–4-month old mice were employed. For the experiments shown in Figure 4A–G,I,J, 60 adult (5–6-month-old) mice were used (CNT VEH n= 13; CNT CNO n = 16; LTS VEH n = 15 and LTS CNO n = 16). In Figure 4H, adult (5–6 month-old) mice were used (CNT VEH = 18; CNT CNO n = 16; LTS VEH n = 15 and LTS CNO n = 16). For the experiments shown in Figure 4K–N, 50 male adult mice (5–6-month-old) were used (CNT VEH n = 12; CNT CNO n = 12; LTS VEH n = 13 and LTS CNO n= 13). For the experiments shown in Figure 5, 52 male adult mice (7–8-month-old) were used (CNT GFP n = 10; CNT SH n = 13; LTS GFP n = 14 and LTS SH n = 15).

4.2. Drugs

4-hydroxytamoxifen (H6278, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 1 mL ethanol (100%). Corn oil (C8267, Sigma-Aldrich) was then added, and ethanol was evaporated by incubating the open tubes at 50 ˚C overnight in the dark. The volume of corn oil was adjusted for injecting the dose of active drug at 50 mg/kg.

Clozapine N-oxide (BML-NS105-0025, Enzo) was administered via drinking water as previously described [71]. The required CNO stock solution was then added to 1% sucrose water to reach a final concentration of 1 mg/kg/day. Water was changed 3 times per week.

Trimethoprim (Santa Cruz, sc-237332, CA, USA) was reconstituted in water at a concentration of 30 mg/100 mL. After surgery, all mice received TMP in drinking water for one week to induce Cre recombinase activity specifically in Calb1-cre mice.

4.3. Stereotaxic Surgery and Viral Transduction In Vivo

Animals were stereotaxically injected with one of the following adeno-associated viruses (AAVs): pAAV5-hSyn-DIO-hM3D(Gq)-mCherry (Addgene, #44361. RRID:Addgene_44361), AAV5-CAG⁄EF1a-DIO-mCherry-mEGR1-shRNAmir (#shAAV-258146, Vector Biolabs) and AAV5-CAG-FLEX-GFP (UNC, Vector Core). Following anesthesia with isoflurane (2% induction, 1.5% maintenance) and 2% oxygen, mice were bilaterally injected with AAVs (3×10^{12} GS per injection) in the CA1 of the hippocampus. We used the following coordinates (millimeters) from bregma: antero-posterior, −2.0; lateral, ±1.5; and dorso-ventral, −1.3. AAV injection was carried out in 4 min. The needle was left in place for 2 min for complete virus diffusion. After recovery, mice were returned to their home cage for 3 weeks.

4.4. Chronic Unpredictable Mild Stress (CUMS)

One day before the beginning of the CUMS procedure described elsewhere [72], mice were individually housed and maintained in isolation for the entire experiment. The CUMS procedure (Table 1) followed a random weekly schedule of commonly used mild stressors (one per day) including restraint, food or water deprivation, home cage inclination and others. The CUMS protocol lasted 28 days for the long-term-stressed (LTS) group. Short-term-stressed (STS) mice were housed individually for one day prior to the stress procedure and received two days of stress (forced swim and restraint). Both the STS and LTS groups received the same stressors during the last two days (when they received 4-HT) in order to make them comparable. CNT mice remained undisturbed in their home cages for all the procedure.

Table 1. Schematic organization of the chronic unpredictable mild stress protocol.

		GROUP		
TIME	**STRESSOR**	**CNT**	**STS**	**LTS**
Day 0	Isolation			Isolation
Day 1	Home cage inclination (1 h)			
Day 2	Food deprivation (24 h)			
Day 3	Restraint (1 h)			
Day 4	Water deprivation (24 h)			
Day 5	Light–dark cycle alterations (24 h)			
Day 6	Forced Swim (5 min)			
Day 7	Exposition to rat sawdust (4 h)			
Day 8	Home cage inclination (1 h)			
Day 9	Water deprivation (24 h)			
Day 10	Restraint (1 h)			
Day 11	Light–dark cycle alterations (24 h)			
Day 12	Food deprivation (24 h)			
Day 13	Restraint (1 h)			
Day 14	Exposition to rat sawdust (4 h)			
Day 15	Home cage inclination (1 h)			
Day 16	Light–dark cycle alterations (24 h)			
Day 17	Forced Swim (5 min)			
Day 18	Exposition to rat sawdust (4 h)			
Day 19	Water deprivation (24 h)			
Day 20	Forced Swim (5 min)			
Day 21	Exposition to rat sawdust (4 h)			
Day 22	Home cage inclination (1 h)			
Day 23	Food deprivation (24 h)			
Day 24	Water deprivation (24 h)			
Day 25	Light–dark cycle alterations (24 h)			
Day 26	Food deprivation (24 h)		Isolation	
Day 27	Restraint (1 h)	+4-HT	+4-HT	+4-HT
Day 28	Forced Swim (5 min)	+4-HT	+4-HT	+4-HT

Left column: day of stress; second column: stressor type; third column: control non-stressed group of mice; fourth column: short-term-stressed group of mice (2 days of stress); fifth column: long-term-stressed group of mice (28 days of stress). Note that gray cells illustrate real days in which each group of mice received a stressful stimulus. 4-HT: 4-Hydroxytamoxifen.

4.5. Behavioral Tests

Open field (OF) and novel object location test (NOL): For the novel object location test (NOL), an open-top arena (40 × 40 × 40 cm) with visual cues placed in the walls of the apparatus was used. Mice were first habituated to the arena (1 day, 15 min). We considered this first exposition to the open arena as an open field paradigm. We monitored total travelled distance (in cm) as a measure of locomotor activity. On day 2, two identical objects (A1 and a2) were placed on one side of the arena and mice were allowed to explore them for 10 min. Exploration was considered when the mouse was in contact with object and sniffed it. 24 h later (D3), one object was moved from its original location to the diagonally opposite corner and mice were allowed to explore the arena and the objects for 5 min. At the end of each trial, defecations were removed, and the apparatus was cleaned with 30% ethanol. Animal tracking and recording were performed using the automated SMART junior 3.0 software (Panlab, Spain).

Forced swim test: The forced-swim test was used to evaluate behavioral despair. Animals were subjected to a 6 min trial during which they are forced to swim in an acrylic glass (35 cm height × 20 cm diameters) filled with water, and from which they cannot escape. The time that the test animal spent in the cylinder without making any movements except those required to keep its head above water was measured.

All the tests were conducted during the light cycle and all mice were randomized throughout the day. Only one test was conducted per day.

4.6. Tissue Fixation, Immunofluorescence and Confocal Imaging

Animals were deeply anaesthetized and subsequently intracardially perfused with 4% (weight/vol) paraformaldehyde in 0.1 M phosphate buffer. Brains were dissected out and kept for 48 h in 4% paraformaldehyde 0.1 M phosphate buffer in agitation. After fixation, free-floating coronal sections (40 µm) were obtained using a vibratome (VT1000. Leica Microsystems CMS GmbH, Mannheim, Germany).

For immunofluorescence, sections were first washed twice in PBS and incubated in 50 mM NH4Cl for 30 min. Blocking and permeabilization were performed for 1 h in PBS-T with 0.02% azide, 3% NGS and 0.2% BSA. Primary antibodies were diluted in blocking solution, and sections were incubated overnight at 4 ˚C in agitation. The following primary antibodies were used: MAP2 (1:500; #M1406. RRID:AB_477171. Sigma-Aldrich, St. Louis, MO, USA), GFP FITC-conjugated (1:500, #Ab6662. RRID:AB_305635. Abcam, Cambridge, UK), Egr1 (1:1000, #4154S, Cell Signalling, Danvers, Massachusetts, USA), cFos (1:150, #sc-52. RRID:AB_2106783. Santa Cruz Biotechnology, Dallas, TX, USA), GFAP (1:500, Dako #z0334. RRID:AB_10013382) and Parvalbumin (1:1250; Sigma #P3088. RRID:AB_477329). Secondary antibodies (Cy3- or Cy2-coupled fluorescent secondary antibodies, 1:200, Jackson ImmunoResearch Laboratories catalogue #715-165-150, RRID:AB_2340813 and #715-545-150, RRID:AB_2340846, respectively) were diluted in blocking solution, and tissues were incubated for 2 h at room temperature. Nuclei were stained for 10 min with 4^{t},6-diamidino-2-phenylindole (DAPI; catalogue #D9542, Sigma-Aldrich). The sections were mounted onto slides and cover-slipped with Mowiol. Images (at 1024 × 1024-pixel resolution) in a mosaic format were acquired with a Leica Confocal SP5 with 63× or 40× oil-immersion objectives and standard (1 Airy disc) pinhole (1 AU), and frame averaging (3 frames per z step) were held constant throughout the study.

4.7. Immunobloting

Animals (n = 9–10 per group) were sacrificed by cervical dislocation. The hippocampus was dissected out, frozen using CO2 pellets, and stored at −80 ˚C until use. Briefly, the tissue was lysed by sonication in 250 µL of lysis buffer (50 mM Tris base (pH 7.5), 10 mM EDTA and 1% Triton X−100, supplemented with 1 mM sodium orthovanadate, 1 mM phenylmethylsulphonyl fluoride, 1 mg/mL leupeptin, and 1 mg/mL aprotinin). After lysis, samples were centrifuged at 15,000× *g* for 20 min. Supernatant proteins (15 mg) from total brain region extracts were loaded in SDS–PAGE and transferred to nitrocellulose membranes (GE Healthcare, LC, UK; Calle Gobelas 35- 37, 28023, Madrid, Spain). Membranes were blocked in TBS-T (150 mM NaCl, 20 mM Tris- HCl, pH 7.5, 0.5 mL Tween 20) with 5% BSA and 5% non-fat dry milk. Immunoblots were incubated overnight at 4˚ with anti-calbindin (Swant CB38) at 1:2000 in PBS with 0.2% Sodium Azide. After three washes in TBS-T, blots were incubated for 1 h at room temperature with anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:2000; Promega, Madison, WI, USA) and washed again with TBS-T. Immunoreactive bands were visualized using the Western Blotting Luminol Reagent (Santa Cruz Biotechnology) and quantified by a computer-assisted densitometer (Gel-Pro Analyzer, version 4, Media Cybernetics, Rockville, MD, USA). For loading control, a mouse monoclonal antibody for tubulin was used (1:50,000, Sigma 083M4847V).

4.8. Gene Gun

Hippocampal neurons were labeled using the Helios Gene Gun System (Bio-Rad) as previously described [73]. Briefly, dye-coated particles were delivered by shooting into 200-µm coronal brain sections (obtained in a vibratome, Leica VT100), pointing at the hippocampus, at 80 psi through a membrane filter of 3 µm pore size and 8 × 10 pores/cm^2 (Millipore, Burlington, MA, USA). Sections were stored at room temperature in PBS for 3 h protected from light and then subjected DAPI (#D1306, Invitrogen, Waltham, MA, USA) staining and mounted in Mowiol to be analyzed.

4.9. Imaging Analysis

Cell counting was performed in area estimates for striatum, dorsal hippocampal CA1, dorsal hippocampal CA3, septum, para-ventricular nucleus, cingulate cortex, and amygdala and was performed in double-blind counting fashion relative to the different conditions using the ImageJ software (ImageJ 2.3.0/ 1.53q; Java 1.8). Images (at 512 × 512-pixel resolution) in a mosaic format were acquired with a Leica Confocal SP5 with a 20× normal objective and standard (1 Airy disc) pinhole (1 AU) and frame averaging (3 frames) were held constant throughout the study. No z-stacks were taken. To determine the neuronal subpopulation densities, we used the online Gaidi atlas as a reference, and we counted GFP-positive cells in coronal sections spaced 240 µm apart. The numbers of GFP-positive cells were relativized per area (500 μm^2). For identification of the activated neurons shown in Figure 3, three 40-micron-thick slices per mouse separated 240 microns from each other were used to quantify these percentages. Percentages (%) of GFP-positive cells colocalizing with different markers (MAP2, GFAP, parvalbumin and calbindin1) were estimated per area as described above. For calbindin1 intensity quantification, 20 µm coronal sections were counted with a step size of 5 µm. Briefly, the ROI of the CA1 was delimited using the DAPI channel, and this ROI was applied in the calbindin1 channel, in which mean intensity was determined. Spine density counting was performed as previously described [73] using the ImageJ software. GFP- or DiI-labeled pyramidal neurons from CA1 of the dorsal hippocampus were imaged using a Leica Confocal SP5 with a ×63 oil-immersion objective (digital zoom 5×). Conditions such as pinhole size (1 AU) and frame averaging (4 frames per z-step) were held constant throughout the study. Confocal z-stacks were taken with a digital zoom of 5 and a z-step of 0.2 µm at 1024 × 1024 pixel resolution, yielding an image with pixel dimensions of 49.25 × 49.25 µm. For spine density analysis, we examined second-order dendrites. Thus, ramifications from the apical dendrite were analyzed. On average, 30 µm ± 2 of length was evaluated per dendrite, and the ratio was calculated as the number of dendrites/total length analyzed. A total of 17–27 dendrites from 7 different mice per group were quantified. For double-positive cell quantification, the criterion was to count as double-positive those cells presenting yellow pixels indicating colocalization of the marker with GFP.

4.10. Statistics

All data are expressed as mean ± SEM, and statistics were calculated using the GraphPad Prism 8.0 software. In experiments with normal distribution, statistical analyses were performed using the unpaired two-sided Student's *t* test, one-way ANOVA and two-way ANOVA with Bonferroni's or Tukey's post hoc tests. T test with Welch's correction was applied when variances were unequal. Values of $p < 0.05$ were considered statistically significant. Grubbs and ROUT tests were performed to determine the significant outlier values.

5. Conclusions

In conclusion, our study points out the hippocampal CA1 as a brain region sensitive to the duration of chronic stress and that Egr1 in superficial CA1 pyramidal neurons may play a crucial role in stress-induced sequelae such as behavioral despair and impaired spatial learning. Finally, future studies should focus on the identification of Egr1 upstream and downstream molecular mechanisms underlying stress-induced sequelae.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms24043833/s1.

Author Contributions: Conceptualization, A.S.-B. and A.G.; methodology, A.S.-B., V.B. and A.G.; validation, A.S.-B. and V.B.; formal analysis, A.S.-B. and A.G.; investigation, A.S.-B., S.B.-P., V.B. and A.G.; resources, J.A., J.-A.G. and A.G.; data curation, A.S.-B.; writing—original draft preparation, A.S.-B. and A.G.; writing—review and editing, A.S.-B., J.A., J.-A.G. and A.G.; visualization, A.S.-B. and A.G.; supervision, A.G.; project administration, A.S.-B. and A.G.; funding acquisition, J.-A.G., J.A. and A.G. All authors have read and agreed to the published version of the manuscript.

Funding: A.G. is a Ramón y Cajal fellow (RYC-2016-19466). This work was supported by grants from Ministerio de Ciencia e Innovación/ AEI/10.13039/501100011033/ "FEDER": A.G.: PID2021-122258OB-I00. This work was also supported by the NARSAD foundation (Young Investigator Grant 2016 (Ref. 24803) to A.G. J.-A.G. was supported by grants from Fondation pour la Recherche Médicale (FRM # DPA20140629798) and ANR (Epitraces, ANR-16-CE16-0018). We thank Ana López (María de Maeztu Unit of Excellence, Institute of Neurosciences, University of Barcelona, MDM-2017-0729, Ministry of Science, Innovation and Universities) for technical support.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and all animal procedures were approved by local committees [Universitat de Barcelona, CEEA (10141) and Generalitat de Catalunya (DAAM 315/18)], in accordance with the European Communities Council Directive (86/609/EU).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We thank Ana López (María de Maeztu Unit of Excellence, Institute of Neurosciences, University of Barcelona, MDM-2017–0729, Ministry of Science, Innovation and Universities) for technical support. We thank María Calvo from the Advanced Microscopy Service (Centres Científics i Tecnològics Universitat de Barcelona) for her help in the acquisition, analysis, and interpretation of the confocal images. The mouse strain lines were shared by our collaborator Liset M de la Prida for internal research purposes only according to donor agreements. The line Chrna7-cre RRID:MMRRC_034694-UCD was obtained from the Mutant Mouse Resource and Research Center (MMRRC) at University of California Davis, an NIH-funded strain repository, and was donated to the MMRRCC by Nathaniel Heintz, PhD, Rockefeller University GENSAT, and Charles Gerfen, PhD, National Institutes of Health, National Institute of Mental Health. The line Calb1-cre was obtained from the Jackson Laboratory JAX stock #028532.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Scott, S.B.; Graham-Engeland, J.E.; Engeland, C.G.; Smyth, J.M.; Almeida, D.M.; Katz, M.J.; Lipton, R.B.; Mogle, J.A.; Munoz, E.; Ram, N.; et al. The Effects of Stress on Cognitive Aging, Physiology and Emotion (ESCAPE) Project. *BMC Psychiatry* **2015**, *15*, 146. [CrossRef]
2. Vargas, T.; Conley, R.E.; Mittal, V.A. Chronic stress, structural exposures and neurobiological mechanisms: A stimulation, discrepancy and deprivation model of psychosis. *Int. Rev. Neurobiol.* **2020**, *152*, 41–69. [CrossRef]
3. Nabeshima, T.; Kim, H.-C. Involvement of Genetic and Environmental Factors in the Onset of Depression. *Exp. Neurobiol.* **2013**, *22*, 235. [CrossRef]
4. Richter-Levin, G.; Xu, L. How could stress lead to major depressive disorder? *IBRO Rep.* **2018**, *4*, 38–43. [CrossRef] [PubMed]
5. Van Oort, J.; Tendolkar, I.; Hermans, E.J.; Mulders, P.C.; Beckmann, C.F.; Schene, A.H.; Fernández, G.; van Eijndhoven, P.F. How the brain connects in response to acute stress: A review at the human brain systems level. *Neurosci. Biobehav. Rev.* **2017**, *83*, 281–297. [CrossRef]
6. McEwen, B.S.; Bowles, N.P.; Gray, J.D.; Hill, M.N.; Hunter, R.G.; Karatsoreos, I.N.; Nasca, C. Mechanisms of stress in the brain. *Nat. Neurosci.* **2015**, *18*, 1353. [CrossRef]
7. Schmaal, L.; Veltman, D.J.; Van Erp, T.G.M.; Smann, P.G.; Frodl, T.; Jahanshad, N.; Loehrer, E.; Tiemeier, H.; Hofman, A.; Niessen, W.J.; et al. Subcortical brain alterations in major depressive disorder: Findings from the ENIGMA Major Depressive Disorder working group. *Mol. Psychiatry* **2016**, *21*, 806–812. [CrossRef] [PubMed]
8. Liu, W.; Ge, T.; Leng, Y.; Pan, Z.; Fan, J.; Yang, W.; Cui, R. The Role of Neural Plasticity in Depression: From Hippocampus to Prefrontal Cortex. *Neural Plast.* **2017**, *2017*, 1–11. [CrossRef] [PubMed]
9. Lee, T.; Jarome, T.; Li, S.-J.; Kim, J.J.; Helmstetter, F.J. Chronic stress selectively reduces hippocampal volume in rats: A longitudinal MRI study. *Neuroreport* **2009**, *20*, 1554. [CrossRef] [PubMed]
10. Lyons, D.M.; Yang, C.; Sawyer-Glover, A.M.; Moseley, M.E.; Schatzberg, A.F. Early life stress and inherited variation in monkey hippocampal Volumes. *Arch. Gen. Psychiatry* **2001**, *58*, 1145–1151. [CrossRef]
11. Xu, Y.; Pan, J.; Sun, J.; Ding, L.; Ruan, L.; Reed, M.; Yu, X.; Klabnik, J.; Lin, D.; Li, J.; et al. Inhibition of phosphodiesterase 2 reverses impaired cognition and neuronal remodeling caused by chronic stress. *Neurobiol. Aging* **2015**, *36*, 955–970. [CrossRef] [PubMed]
12. Alfarez, D.N.; Joëls, M.; Krugers, H.J. Chronic unpredictable stress impairs long-term potentiation in rat hippocampal CA1 area and dentate gyrus in vitro. *Eur. J. Neurosci.* **2003**, *17*, 1928–1934. [CrossRef] [PubMed]

13. Qiao, H.; Li, M.-X.; Xu, C.; Chen, H.-B.; An, S.-C.; Ma, X.-M. Dendritic Spines in Depression: What We Learned from Animal Models. *Neural Plast.* **2016**, *2016*, 20–24. [CrossRef] [PubMed]
14. Magariños, A.M.; Li, C.J.; Gal Toth, J.; Bath, K.G.; Jing, D.; Lee, F.S.; McEwen, B.S. Effect of brain-derived neurotrophic factor haploinsufficiency on stress-induced remodeling of hippocampal neurons. *Hippocampus* **2011**, *21*, 253–264. [CrossRef]
15. Pawlak, R.; Rao, B.S.S.; Melchor, J.P.; Chattarji, S.; McEwen, B.; Strickland, S. Tissue plasminogen activator and plasminogen mediate stress-induced decline of neuronal and cognitive functions in the mouse hippocampus. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 18201. [CrossRef] [PubMed]
16. Shors, T.J.; Falduto, J.; Leuner, B. The opposite effects of stress on dendritic spines in male vs. female rats are NMDA receptor-dependent. *Eur. J. Neurosci.* **2004**, *19*, 145–150. [CrossRef] [PubMed]
17. Shors, T.J.; Chua, C.; Falduto, J. Sex Differences and Opposite Effects of Stress on Dendritic Spine Density in the Male Versus Female Hippocampus. *J. Neurosci.* **2001**, *21*, 6292–6297. [CrossRef]
18. Cembrowski, M.S.; Bachman, J.L.; Wang, L.; Sugino, K.; Shields, B.C.; Spruston, N. Spatial Gene-Expression Gradients Underlie Prominent Heterogeneity of CA1 Pyramidal Neurons. *Neuron* **2016**, *89*, 351–368. [CrossRef]
19. Valero, M.; de la Prida, L.M. The hippocampus in depth: A sublayer-specific perspective of entorhinal–hippocampal function. *Curr. Opin. Neurobiol.* **2018**, *52*, 107–114. [CrossRef] [PubMed]
20. Valero, M.; Cid, E.; Averkin, R.G.; Aguilar, J.; Sanchez-Aguilera, A.; Viney, T.J.; Gomez-Dominguez, D.; Bellistri, E.; De La Prida, L.M. Determinants of different deep and superficial CA1 pyramidal cell dynamics during sharp-wave ripples. *Nat. Neurosci.* **2015**, *18*, 1281–1290. [CrossRef]
21. Gallo, F.T.; Katche, C.; Morici, J.F.; Medina, J.H.; Weisstaub, N.V. Immediate Early Genes, Memory and Psychiatric Disorders: Focus on c-Fos, Egr1 and Arc. *Front. Behav. Neurosci.* **2018**, *12*, 79. [CrossRef]
22. Duclot, F.; Kabbaj, M. The Role of Early Growth Response 1 (EGR1) in Brain Plasticity and Neuropsychiatric Disorders. *Front. Behav. Neurosci.* **2017**, *11*, 35. [CrossRef]
23. Girotti, M.; Pace, T.W.W.; Gaylord, R.I.; Rubin, B.A.; Herman, J.P.; Spencer, R.L. Habituation to repeated restraint stress is associated with lack of stress-induced c-fos expression in primary sensory processing areas of the rat brain. *Neuroscience* **2006**, *138*, 1067–1081. [CrossRef] [PubMed]
24. Kozlovsky, N.; Matar, M.A.; Kaplan, Z.; Zohar, J.; Cohen, H. A distinct pattern of intracellular glucocorticoid-related responses is associated with extreme behavioral response to stress in an animal model of post-traumatic stress disorder. *Eur. Neuropsychopharmacol.* **2009**, *19*, 759–771. [CrossRef] [PubMed]
25. Musazzi, L.; Tornese, P.; Sala, N.; Popoli, M. Acute or Chronic? A Stressful Question. *Trends Neurosci.* **2017**, *40*, 525–535. [CrossRef]
26. Laine, M.A.; Sokolowska, E.; Dudek, M.; Callan, S.A.; Hyytiä, P.; Hovatta, I. Brain activation induced by chronic psychosocial stress in mice. *Sci. Rep.* **2017**, *7*, 1–11. [CrossRef]
27. Fee, C.; Prevot, T.; Misquitta, K.; Banasr, M.; Sibille, E. Chronic Stress-induced Behaviors Correlate with Exacerbated Acute Stress-induced Cingulate Cortex and Ventral Hippocampus Activation. *Neuroscience* **2020**, *440*, 113–129. [CrossRef]
28. Flak, J.N.; Solomon, M.B.; Jankord, R.; Krause, E.G.; Herman, J.P. Identification of chronic stress-activated regions reveals a potential recruited circuit in rat brain. *Eur. J. Neurosci.* **2012**, *36*, 2547–2555. [CrossRef] [PubMed]
29. De Kloet, E.R.; Joëls, M.; Holsboer, F. Stress and the brain: From adaptation to disease. *Nat. Rev. Neurosci.* **2005**, *6*, 463–475. [CrossRef]
30. Floriou-Servou, A.; von Ziegler, L.; Waag, R.; Schläppi, C.; Germain, P.L.; Bohacek, J. The Acute Stress Response in the Multiomic Era. *Biol. Psychiatry* **2021**, *89*, 1116–1126. [CrossRef]
31. Longueville, S.; Nakamura, Y.; Brami-Cherrier, K.; Coura, R.; Hervé, D.; Girault, J.-A. Long-lasting tagging of neurons activated by seizures or cocaine administration in Egr1-CreERT2 transgenic mice. *Eur. J. Neurosci.* **2021**, *53*, 1450–1472. [CrossRef] [PubMed]
32. Brito, V.; Montalban, E.; Sancho-Balsells, A.; Pupak, A.; Flotta, F.; Masana, M.; Ginés, S.; Alberch, J.; Martin, C.; Girault, J.-A.; et al. Hippocampal Egr1-dependent neuronal ensembles negatively regulate motor learning. *J. Neurosci.* **2022**, *42*, 5346–5360. [CrossRef] [PubMed]
33. Román-Albasini, L.; Díaz-Véliz, G.; Olave, F.A.; Aguayo, F.I.; García-Rojo, G.; Corrales, W.A.; Silva, J.P.; Ávalos, A.M.; Rojas, P.S.; Aliaga, E.; et al. Antidepressant-relevant behavioral and synaptic molecular effects of long-term fasudil treatment in chronically stressed male rats. *Neurobiol. Stress* **2020**, *13*, 100234. [CrossRef]
34. Roustazade, R.; Radahmadi, M.; Yazdani, Y. Therapeutic effects of saffron extract on different memory types, anxiety, and hippocampal BDNF and TNF-α gene expressions in sub-chronically stressed rats. *Nutr. Neurosci.* **2021**, *25*, 192–206. [CrossRef] [PubMed]
35. Rosen, J.B.; Adamec, R.E.; Thompson, B.L. Expression of egr-1 (zif268) mRNA in select fear-related brain regions following exposure to a predator. *Behav. Brain Res.* **2005**, *162*, 279–288. [CrossRef]
36. Hall, J.; Thomas, K.L.; Everitt, B.J. Cellular Imaging of Zif268 Expression in the Hippocampus and Amygdala during Contextual and Cued Fear Memory Retrieval: Selective Activation of Hippocampal CA1 Neurons during the Recall of Contextual Memories. *J. Neurosci.* **2001**, *21*, 2186–2193. [CrossRef] [PubMed]
37. Schreiber, S.S.; Tocco, G.; Shors, T.J.; Thompson, R.F. Activation of immediate early genes after acute stress. *Neuroreport* **1991**, *2*, 17–20. [CrossRef] [PubMed]
38. Melia, K.R.; Ryabinin, A.E.; Schroeder, R.; Bloom, F.E.; Wilson, M.C.; Anokhin, P.K. Induction and Habituation of Immediate Early Gene Expression in Rat Brain by Acute and Repeated Restraint Stress. *J. Neurosci.* **1994**, *14*, 5929–5938. [CrossRef] [PubMed]

39. Cullinan, W.E.; Herman, J.P.; Battaglia, D.F.; Akil, H.; Watson, S.J. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* **1995**, *64*, 477–505. [CrossRef]
40. Olsson, T.; Seckl, J.R. Ketanserin selectively blocks acute stress-induced changes in ngf1-a and mineralocorticoid receptor gene expression in hippocampal neurons. *Pergamon Neurosci.* **1997**, *76*, 441–448. [CrossRef]
41. Knapska, E.; Kaczmarek, L. A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? *Prog. Neurobiol.* **2004**, *74*, 183–211. [CrossRef]
42. Covington, H.E.; Lobo, M.K.; Maze, I.; Vialou, V.; Hyman, J.M.; Zaman, S.; LaPlant, Q.; Mouzon, E.; Ghose, S.; Tamminga, C.A.; et al. Antidepressant Effect of Optogenetic Stimulation of the Medial Prefrontal Cortex. *J. Neurosci.* **2010**, *30*, 16082–16090. [CrossRef]
43. Matsumoto, K.; Ono, K.; Ouchi, H.; Tsushima, R.; Murakami, Y. Social isolation stress down-regulates cortical early growth response 1 (Egr-1) expression in mice. *Neurosci. Res.* **2012**, *73*, 257–262. [CrossRef]
44. Hodges, T.E.; Green, M.R.; Simone, J.J.; McCormick, C.M. Effects of social context on endocrine function and Zif268 expression in response to an acute stressor in adolescent and adult rats. *Int. J. Dev. Neurosci.* **2014**, *35*, 25–34. [CrossRef]
45. Northcutt, K.V.; Lonstein, J.S. Social contact elicits immediate-early gene expression in dopaminergic cells of the male prairie vole extended olfactory amygdala. *Neuroscience* **2009**, *163*, 9–22. [CrossRef] [PubMed]
46. Zhang, W.; Wang, H.; Lv, Y.; Liu, C.; Sun, W.; Tian, L. Downregulation of Egr-1 Expression Level via GluN2B Underlies the Antidepressant Effects of Ketamine in a Chronic Unpredictable Stress Animal Model of Depression. *Neuroscience* **2018**, *372*, 38–45. [CrossRef]
47. Ieraci, A.; Mallei, A.; Popoli, M. Social Isolation Stress Induces Anxious-Depressive-Like Behavior and Alterations of Neuroplasticity-Related Genes in Adult Male Mice. *Neural Plast.* **2016**, *2016*, 1–13. [CrossRef]
48. Clements, K.M.; Wainwright, P.E. Swim stress increases hippocampal Zif268 expression in the spontaneously hypertensive rat. *Brain Res. Bull.* **2010**, *82*, 259–263. [CrossRef] [PubMed]
49. Isingrini, E.; Camus, V.; Le Guisquet, A.M.; Pingaud, M.; Devers, S.; Belzung, C. Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: A model of fluoxetine resistance in mice. *PLoS ONE* **2010**, *5*, e10404. [CrossRef] [PubMed]
50. Chen, Y.; Rex, C.S.; Rice, C.J.; Dubé, C.M.; Gall, C.M.; Lynch, G.; Barama, T.Z. Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13123–13128. [CrossRef] [PubMed]
51. Sun, D.S.; Zhong, G.; Cao, H.X.; Hu, Y.; Hong, X.Y.; Li, T.; Li, X.; Liu, Q.; Wang, Q.; Ke, D.; et al. Repeated Restraint Stress Led to Cognitive Dysfunction by NMDA Receptor-Mediated Hippocampal CA3 Dendritic Spine Impairments in Juvenile Sprague-Dawley Rats. *Front. Mol. Neurosci.* **2020**, *13*, 552787. [CrossRef]
52. Krzystyniak, A.; Baczynska, E.; Magnowska, M.; Antoniuk, S.; Roszkowska, M.; Zareba-Koziol, M.; Das, N.; Basu, S.; Pikula, M.; Wlodarczyk, J. Prophylactic Ketamine Treatment Promotes Resilience to Chronic Stress and Accelerates Recovery: Correlation with Changes in Synaptic Plasticity in the CA3 Subregion of the Hippocampus. *Int. J. Mol. Sci.* **2019**, *20*, 1726. [CrossRef]
53. Qu, Y.; Yang, C.; Ren, Q.; Ma, M.; Dong, C.; Hashimoto, K.; Regional, H.K. Regional differences in dendritic spine density confer resilience to chronic social defeat stress. *Acta Neuropsychiatr.* **2017**, *30*, 117–122. [CrossRef] [PubMed]
54. Mclaughlin, K.J.; Baran, S.E.; Wright, R.L.; Conrad, C.D. Chronic stress enhances spatial memory in ovariectomized female rats despite CA3 dendritic retraction: Possible involvement of CA1 neurons. *Neuroscience* **2005**, *135*, 1045–1054. [CrossRef]
55. Qiao, H.; An, S.C.; Ren, W.; Ma, X.M. Progressive alterations of hippocampal CA3-CA1 synapses in an animal model of depression. *Behav. Brain Res.* **2014**, *275*, 191–200. [CrossRef]
56. Patel, D.; Anilkumar, S.; Chattarji, S.; Buwalda, B. Repeated social stress leads to contrasting patterns of structural plasticity in the amygdala and hippocampus. *Behav. Brain Res.* **2018**, *347*, 314–324. [CrossRef] [PubMed]
57. Leem, Y.-H.; Park, J.-S.; Chang, H.; Park, J.; Kim, H.-S. Exercise Prevents Memory Consolidation Defects Via Enhancing Prolactin Responsiveness of CA1 Neurons in Mice Under Chronic Stress. *Mol. Neurobiol.* **2019**, *56*, 6609–6625. [CrossRef] [PubMed]
58. Donohue, H.S.; Gabbott, P.L.A.; Davies, H.A.; Rodríguez, J.J.; Cordero, M.I.; Sandi, C.; Medvedev, N.I.; Popov, V.I.; Colyer, F.M.; Peddie, C.J.; et al. Chronic restraint stress induces changes in synapse morphology in stratum lacunosum-moleculare CA1 rat hippocampus: A stereological and three-dimensional ultrastructural study. *Neuroscience* **2006**, *140*, 597–606. [CrossRef]
59. Conrad, C.D.; Mclaughlin, K.J.; Huynh, T.N.; El-Ashmawy, M.; Sparks, M. Chronic Stress and a Cyclic Regimen of Estradiol Administration Separately Facilitate Spatial Memory: Relationship with CA1 Spine Density and Dendritic Complexity. *Behav. Neurosci.* **2012**, *126*, 142. [CrossRef] [PubMed]
60. McLaughlin, K.J.; Wilson, J.O.; Harman, J.; Wright, R.L.; Wieczorek, L.; Gomez, J.; Korol, D.L.; Conrad, C.D. Chronic 17β-estradiol or cholesterol prevents stress-induced hippocampal CA3 dendritic retraction in ovariectomized female rats: Possible correspondence between CA1 spine properties and spatial acquisition. *Hippocampus* **2010**, *20*, 768–786. [CrossRef]
61. Qin, X.; Jiang, Y.; Tse, Y.C.; Wang, Y.; Wong, T.P.; Paudel, H.K. Early Growth Response 1 (Egr-1) Regulates N-Methyl-d-aspartate Receptor (NMDAR)-dependent Transcription of PSD-95 and α-Amino-3-hydroxy-5-methyl-4-isoxazole Propionic Acid Receptor (AMPAR) Trafficking in Hippocampal Primary Neurons. *J. Biol. Chem.* **2015**, *290*, 29603–29616. [CrossRef] [PubMed]
62. Krugers, H.J.; Koolhaas, J.M.; Medema, R.M.; Korf, J. Prolonged subordination stress increases Calbindin-D28k immunoreactivity in the rat hippocampal CA1 area. *Brain Res.* **1996**, *729*, 289–293. [CrossRef] [PubMed]

63. Li, J.T.; Xie, X.M.; Yu, J.Y.; Sun, Y.X.; Liao, X.M.; Wang, X.X.; Su, Y.A.; Liu, Y.J.; Schmidt, M.V.; Wang, X.D.; et al. Suppressed Calbindin Levels in Hippocampal Excitatory Neurons Mediate Stress-Induced Memory Loss. *Cell Rep.* **2017**, *21*, 891–900. [CrossRef] [PubMed]
64. Revest, J.M.; Di Blasi, F.; Kitchener, P.; Rougé-Pont, F.; Desmedt, A.; Turiault, M.; Tronche, F.; Piazza, P.V. The MAPK pathway and Egr-1 mediate stress-related behavioral effects of glucocorticoids. *Nat. Neurosci. 2005 85* **2005**, *8*, 664–672. [CrossRef]
65. Revest, J.M.; Kaouane, N.; Mondin, M.; Le Roux, A.; Rougé-Pont, F.; Vallée, M.; Barik, J.; Tronche, F.; Desmedt, A.; Piazza, P.V. The enhancement of stress-related memory by glucocorticoids depends on synapsin-Ia/Ib. *Mol. Psychiatry* **2010**, *15*, 1140–1151. [CrossRef]
66. Saunderson, E.A.; Spiers, H.; Mifsud, K.R.; Gutierrez-Mecinas, M.; Trollope, A.F.; Shaikh, A.; Mill, J.; Reul, J.M.H.M. Stress-induced gene expression and behavior are controlled by DNA methylation and methyl donor availability in the dentate gyrus. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 4830–4835. [CrossRef]
67. Davis, S.; Renaudineau, S.; Poirier, R.; Poucet, B.; Save, E.; Laroche, S. The formation and stability of recognition memory: What happens upon recall? *Front. Behav. Neurosci.* **2010**, *4*, 177. [CrossRef]
68. Besnard, A.; Caboche, J.; Laroche, S. Recall and Reconsolidation of Contextual Fear Memory: Differential Control by ERK and Zif268 Expression Dosage. *PLoS ONE* **2013**, *8*, 72006. [CrossRef]
69. Penke, Z.; Morice, E.; Veyrac, A.; Gros, A.; Chagneau, C.; LeBlanc, P.; Samson, N.; Baumgärtel, K.; Mansuy, I.M.; Davis, S.; et al. Zif268/Egr1 gain of function facilitates hippocampal synaptic plasticity and long-term spatial recognition memory. *Philos. Trans. R. Soc. B Biol. Sci.* **2014**, *369*, 20130159. [CrossRef] [PubMed]
70. Fernández-García, S.; Sancho-Balsells, A.; Longueville, S.; Hervé, D.; Gruart, A.; Delgado-García, J.M.; Alberch, J.; Giralt, A. Astrocytic BDNF and TrkB regulate severity and neuronal activity in mouse models of temporal lobe epilepsy. *Cell Death Dis.* **2020**, *11*, 411. [CrossRef]
71. Zhan, J.; Komal, R.; Keenan, W.T.; Hattar, S.; Fernandez, D.C. Non-invasive Strategies for Chronic Manipulation of DREADD-controlled Neuronal Activity. *JoVE* **2019**, *150*, e59439.
72. Montalban, E.; Al-Massadi, O.; Sancho-Balsells, A.; Brito, V.; de Pins, B.; Alberch, J.; Ginés, S.; Girault, J.-A.; Giralt, A. Pyk2 in the amygdala modulates chronic stress sequelae via PSD-95-related micro-structural changes. *Transl. Psychiatry* **2019**, *9*, 3. [CrossRef] [PubMed]
73. Giralt, A.; Brito, V.; Pardo, M.; Rubio, S.E.; Marion-Poll, L.; Martín-Ibáñez, R.; Zamora-Moratalla, A.; Bosch, C.; Ballesteros, J.J.; Blasco, E.; et al. Helios modulates the maturation of a CA1 neuronal subpopulation required for spatial memory formation. *Exp. Neurol.* **2020**, *323*, 113095. [CrossRef] [PubMed]

marine drugs

MDPI

Article

Meridianins Inhibit GSK3β In Vivo and Improve Behavioral Alterations Induced by Chronic Stress

Anna Sancho-Balsells [1,2,3], Esther García-García [1,2,3], Francesca Flotta [1,2,3], Wanqi Chen [1,2,3], Jordi Alberch [1,2,3,4], Manuel J. Rodríguez [1,2,3], Conxita Avila [5] and Albert Giralt [1,2,3,4,*]

1 Departament de Biomedicina, Facultat de Medicina, Institut de Neurociències (UBneuro), University of Barcelona, 08036 Barcelona, Spain
2 Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain
3 Centro de Investigación Biomédica en Red Sobre Enfermedades Neurodegenerativas (CIBERNED), 08036 Barcelona, Spain
4 Production and Validation Center of Advanced Therapies (Creatio), Faculty of Medicine and Health Science, University of Barcelona, 08036 Barcelona, Spain
5 Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology and Biodiversity Research Institute (IRBio), University of Barcelona, 08028 Catalonia, Spain
* Correspondence: albertgiralt@ub.edu; Tel.: +34-934037980

Abstract: Major depression disorder (MDD) is a severe mental alteration with a multifactorial origin, and chronic stress is one of the most relevant environmental risk factors associated with MDD. Although there exist some therapeutical options, 30% of patients are still resistant to any type of treatment. GSK3β inhibitors are considered very promising therapeutic tools to counteract stress-related affectations. However, they are often associated with excessive off-target effects and undesired secondary alterations. Meridianins are alkaloids with an indole framework linked to an aminopyrimidine ring from Antarctic marine ascidians. Meridianins could overcome several of the aforementioned limitations since we previously demonstrated that they can inhibit GSK3β activity without the associated neurotoxic or off-target effects in rodents. Here, we show that meridianins delivered into the lateral ventricle inhibited GSK3β in several brain regions involved with stress-related symptoms. We also observed changes in major signaling pathways in the prefrontal cortex (Akt and PKA) and hippocampus (PKC and GluR1). Moreover, meridianins increased synaptic activity, specifically in the CA1 but not in the CA3 or other hippocampal subfields. Finally, we chronically treated the mice subjected to an unpredictable mild chronic stress (CUMS) paradigm with meridianins. Our results showed improvements produced by meridianins in behavioral alterations provoked by CUMS. In conclusion, meridianins could be of therapeutic interest to patients with stress-related disorders such as MDD.

Keywords: GSK3β; PKA; PKC; Akt; GluR1; memory; synaptic activity

Citation: Sancho-Balsells, A.; García-García, E.; Flotta, F.; Chen, W.; Alberch, J.; Rodríguez, M.J.; Avila, C.; Giralt, A. Meridianins Inhibit GSK3β In Vivo and Improve Behavioral Alterations Induced by Chronic Stress. *Mar. Drugs* **2022**, *20*, 648. https://doi.org/10.3390/md20100648

Academic Editors: Helena Gaspar and Joana Silva

Received: 7 July 2022
Accepted: 17 October 2022
Published: 19 October 2022

1. Introduction

Major depression disorder (MDD) is a debilitating mental illness that affects millions of people worldwide [1]. MDD is characterized by low mood, a feeling of worthlessness, agitation, and diminished interest in activities, among others [1]. Additionally, MDD is very comorbid with other disorders such as anxiety [2]. One of the most important environmental risk factors associated with MDD is chronic stress [3,4]. Some brain regions such as the hippocampus, the nucleus accumbens (Nacc), the medial prefrontal cortex, and the amygdala interpret what is stressful and regulate an appropriate response that, when failing, may lead to major depression [5,6]. Despite years of research, current medications are still ineffective in 30% of patients and are often associated with significant side effects [7]. Therefore, it is an emergency to design more effective therapeutic approaches.

One of the molecules that have been recently associated with stress-related pathologies is the glycogen synthase kinase 3 B (GSK3β) [8,9]. GSK3β is a serine (Ser)/threonine (Thr) kinase highly enriched in the brain. GSK3β is constitutively active, and its regulation is mainly mediated by phosphorylation and dephosphorylation processes [10]. Thus, the phosphorylation of the N-terminal Ser9 inhibits GSK3β activity [10]. Akt is the major regulator of GSK3β, as it exerts inhibitory phosphorylation on Ser9 in the amino-terminal part of the protein. Additionally, other kinases such as PKA and PKC also regulate GSK3β activity [11].

Several studies showed increased activity of GSK3β in mice subjected to different stress protocols [12–14]. In line with this, some groups have found that blocking GSK3β along the stress protocol induces recovery in some of the deficits observed by the stress itself [12,14]. Furthermore, increased activity of this kinase has been found in depressed human post-mortem samples and peripheral tissues such as peripheral blood mononuclear cells (PBMCs) [15–17]. These human studies suggest that GSK3β activity correlates with the severity of maniac and depressive symptoms [15–17].

With all this evidence, GSK3β has emerged as a potential therapeutic target for mood disorders. For years, lithium has been the most common inhibitor used to modulate GSK3β [18,19]. However, the use of lithium raises some concerns. First, the therapeutic dose of lithium is lower than the IC50 of lithium for inhibition of GSK3 [20]. Secondly, lithium has other intracellular actions [21] and can induce some side effects such as a higher risk of developing dementia [22]. Third, the use of other GSK3β inhibitors has been associated with excessive undesired and secondary effects [23,24], causing the research for kinase modulators to reach an impasse. Therefore, the search for specific GSK3β inhibitors with no off-target effects is mandatory.

Marine natural products (MNPs) are a still understudied source of potentially bioactive compounds [25,26]. Among them, Antarctic MNPs are even more unknown regarding their biological function and their putative pharmacological role as drugs, mainly due to the difficulties in their collection, identification, and further laboratory synthesis [27–31]. Meridianins are a family of indole alkaloids consisting of an indole framework linked to an aminopyrimidine ring, described from marine benthic organisms from Antarctica, particularly from *Aplidium* ascidians [32–37]. In general, the species of the tunicate *Aplidium* Savigny, 1816 are the source of abundant nitrogen-containing metabolites belonging to unprecedented structural families of MNPs [30,38]. Meridianins have been isolated from different specimens of the tunicate genera *Aplidium* and *Synoicum* [36,37]; however, it is still unclear whether tunicates are the true producers of the molecules or whether the associated microbes may play a role in their synthesis and chemical ecology [28,39]. The potential of meridianins as bioactive drugs has been reported previously [33,40–53]. Recently, we showed that meridianins can act as the ATP-competitive or non-ATP-competitive inhibitors of GSK3β in vitro without altering neuronal survival [54]. Moreover, we also demonstrated that in vivo, meridianin administration can improve cognitive deficits and rescue the loss of spine density in the 5xFAD mouse model of Alzheimer's disease [31].

In the present study, we show how the intraventricular administration of meridianins is capable of modulating GSK3β activity in several brain regions involved with stress and major depression. We also describe how meridianins regulate synaptic activity in hippocampal slices, and we identified some of the potential up- and downstream molecular targets affected. Finally, we show that in vivo GSK3β inhibition could have beneficial effects on some of the cognitive and depressive-like deficits provoked by chronic unpredictable mild stress (CUMS), which is a widely used model of induced depression [55,56].

2. Results

2.1. In Vivo Inhibition of GSK3β by Meridianins

GSK3β hyperactivity has been described in different brain regions in stress-related pathologies [13,14]. Here, we first wanted to analyze GSK3β activity in chronically stressed mice. To this end, we subjected the mice to the chronic unpredictable mild stress protocol

(CUMS) for 28 days (Figure 1A). Then, 24 h after the last stressor, the mice were sacrificed to evaluate GSK3β activity. We analyzed the Ser9 phosphorylation of GSK3β as a measure of inhibition. We found that the stressed mice (CUMS) presented decreased Ser9 phosphorylation levels when compared with CNT mice, both in the prefrontal cortex (Figure 1B) and in the hippocampus (Figure 1C). These results confirm that chronic stress induces the hyperactivity of GSK3β. Then, we wanted to test if meridianins could inhibit GS3Kβ in vivo in the different brain regions related to depression such as the prefrontal cortex (PFC), the hippocampus (Hipp), the nucleus accumbens (NAcc), and the amygdala. To this end, we injected meridianins (500 nM) in the third ventricle of the brain of adult (3-month-old) mice. This dose was selected based on our previous works in which we observed it was the most efficient one [31,54]. We then sacrificed the mice at different time points to evaluate GSK3β inhibition (Figure 1D). We found GSK3β inhibition at 20 min and 1 h after injection in the prefrontal cortex (PFC) (Figure 1E) and in the hippocampus (Figure 1F). Conversely, we only found GSK3β inhibition at 20 min in the amygdala (Figure 1G), and in the NAcc, we found just a trend (Figure 1H). These results confirm that the intraventricular injection of meridianins (500 nM) inhibits GSK3β in different brain areas, with the strongest effects in the hippocampus and the PFC 20 min after injection.

2.2. Meridianins Modulate Molecular Pathways Involved with GSK3β Signaling

Once we observed that meridianins could modulate GSK3β activity in vivo, we then aimed to know if this inhibition was accompanied by changes in other proteins that are known to be involved with GSK3β signaling. To this end, we performed Western blot experiments in lysates from both the hippocampus and the PFC lysates of those mice that were treated with meridianins for 20 min and their respective controls. First, we evaluated the levels of kinases that are described as GSK3β modulators such as Akt, PKC, and PKA [10,11]. To check for Akt activity, we looked at phospho-Akt^{Ser473} levels and found that their levels were significantly increased in the PFC of those mice treated with meridianins for 20 min (Figure 2A) but not in the hippocampus when compared with controls (Figure 2B). Then, we looked for PKC activity by using an antibody that detects the phosphorylation of proteins at phospho-Ser PKC substrate motifs. We found no changes between the groups in the PFC (Figure 2C). Contrarily, we found increased activity of PKC in the hippocampus in the mice treated with meridianins with respect to the mice treated with vehicle (Figure 2D). Next, we analyzed the levels of PKA activation. To this end, we used an anti-phospho-PKA substrate antibody that detects the proteins containing phosphorylated Ser/Thr residue within the consensus sequence for PKA, giving us a readout of PKA activity. Interestingly, we found an increase in PKA activity in PFC (Figure 2E) but not in the hippocampus in the mice treated with meridianins, compared with the mice treated with vehicle (Figure 2F). These results suggest different mechanisms of action of meridianins depending on the brain region. One of the substrates under PKA signaling associated with depression and antidepressant responses is CREB [9,11]. We looked at the levels of phospho-CREB^{Ser133} in both brain regions and found no changes in the hippocampus or the PFC when comparing both groups. Lastly, we assessed the levels of a major phosphorylated substrate in terms of PKC activity, the so-called glutamate receptor subunit GluR1. We evaluated the GluR1 activity using an antibody that detects the phosphorylation of the receptor at Ser 831. We found increased phospho-GluR1^{Ser831} levels in the mice treated with meridianins, compared with the mice treated with vehicle, specifically in the hippocampus. These results suggest that meridianins can also modulate synaptic transmission, especially in this brain region.

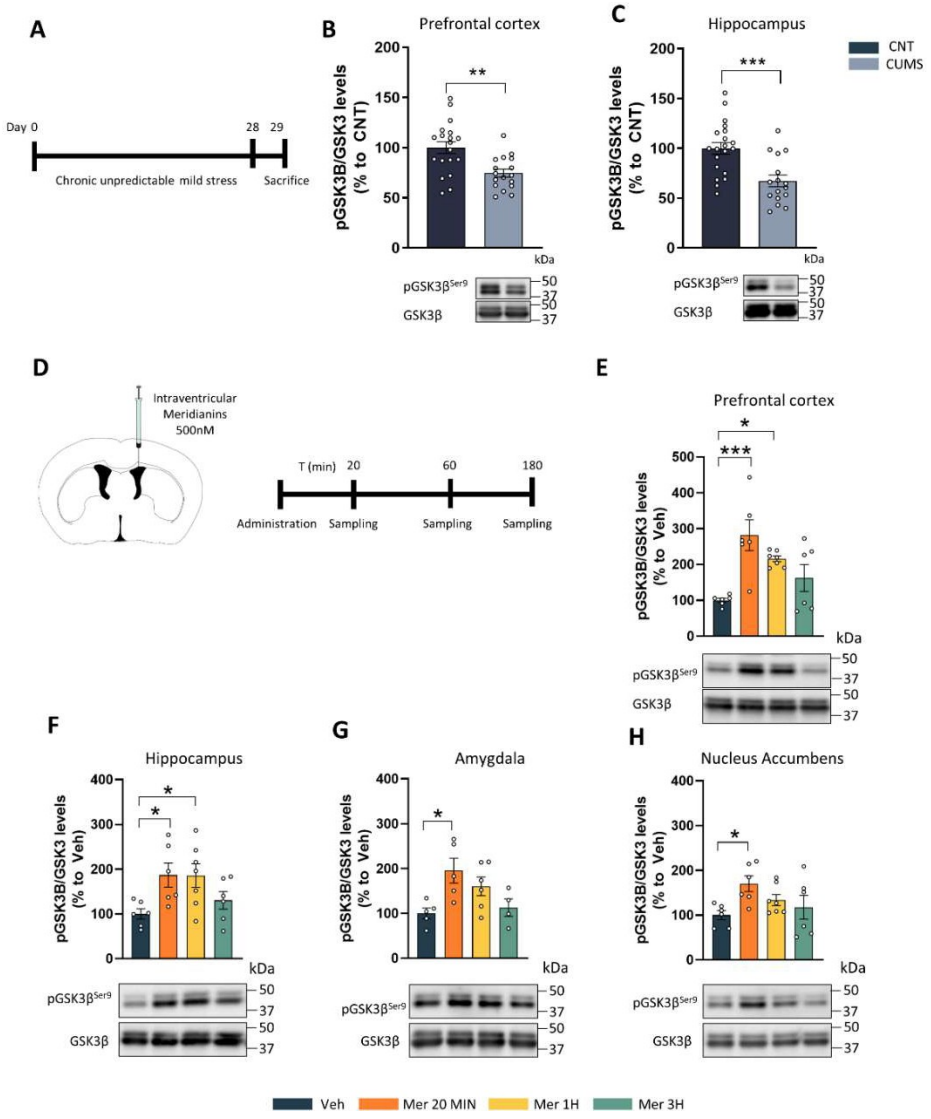


Figure 1. GSK3β phosphorylation levels upon chronic stress and its inhibition in different brain regions: (**A**) schematic representation of the experimental design. Ten-week-old C57BL6/J mice were subjected to the chronic unpredictable mild stress (CUMS) protocol for 28 days. One day after the last stressor, mice were sacrificed; (**B**) densitometry quantification and representative immunoblots of phospho-GSK3βSer9 levels in the prefrontal cortex. Unpaired *t*-test: t = 3.473, df = 34; (**C**) densitometry quantification and representative immunoblots of phospho-GSK3βSer9 levels in the hippocampus. Unpaired *t*-test: t = 3.835, df = 43. ** $p < 0.005$, *** $p < 0.0005$ compared with CNT; (**D**) three-month-old C57BL6/J male mice were stereotaxically injected into the lateral (left) ventricle with 3 µL of 500 nM meridianins and were sacrificed 20 min, 1 h or 3 h after administration; (**E**) densitometry quantification and representative immunoblots of phospho-GSK3βSer9 levels in the prefrontal cortex. One-way ANOVA: F(3,21) = 7.481, $p = 0.0014$; (**F**) densitometry quantification and representative immunoblots of phospho-GSK3βSer9 levels in the hippocampus. One-way ANOVA: F(3,21) = 3.568, $p = 0.0314$; (**G**) densitometry quantification and representative immunoblots of phospho-GSK3βSer9 levels in the amygdala. One-way ANOVA: F(3,16) = 4,191, $p = 0.0228$; (**H**) densitometry quantification and representative immunoblots of phospho-GSK3βSer9 levels in the NAcc. One-way ANOVA: F(3,21) = 2.843, $p = 0.0623$. Data are mean ± SEM. Dunnett's test as a post hoc analysis was used. * $p < 0.05$; *** $p < 0.005$; compared with Veh mice. Molecular weight markers' positions are indicated in kDa (n = 5–7 mice/group). Veh = vehicle and Mer = meridianins.

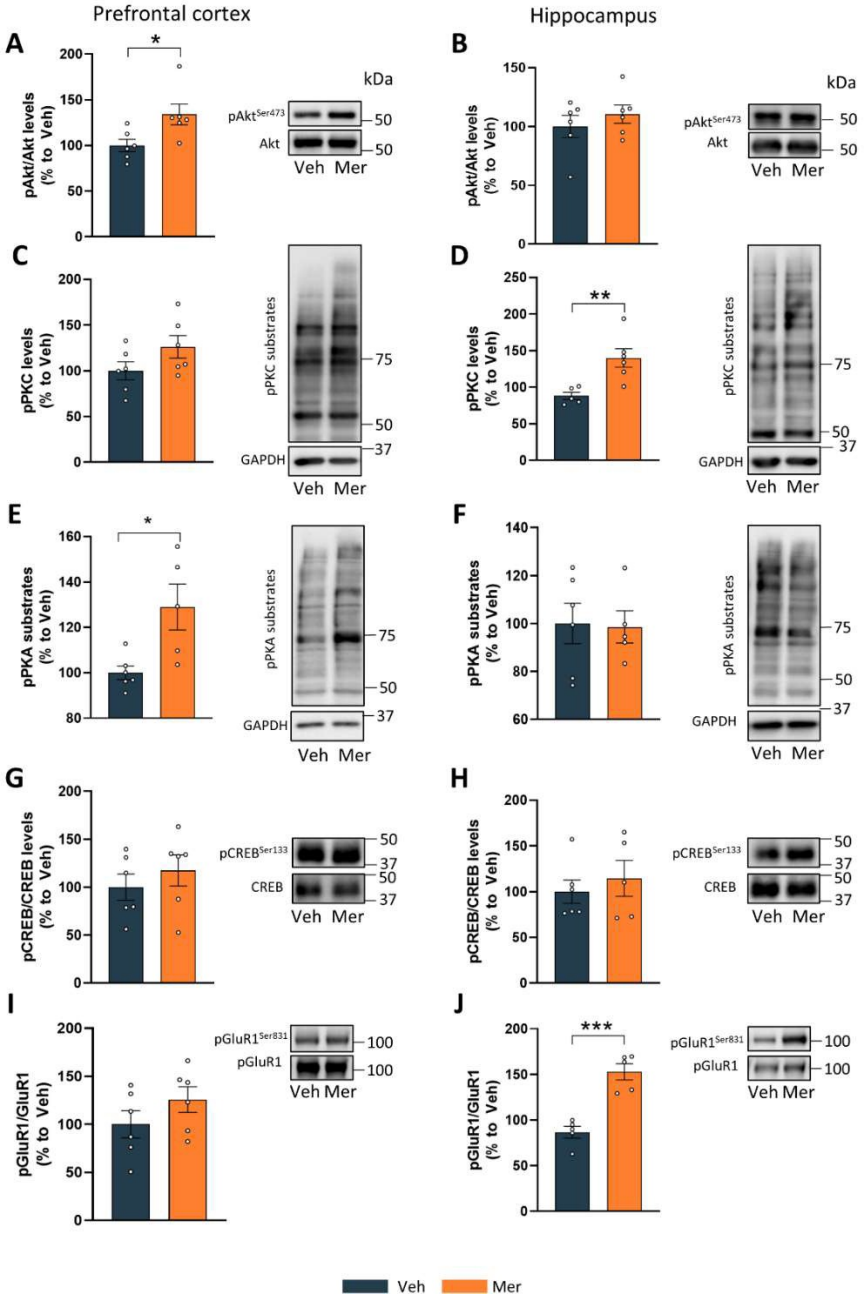


Figure 2. Biochemical assessment of mice treated with meridianins. Densitometry quantification and representative immunoblots of phosphorylated Akt at Ser 473 in the prefrontal cortex (**A**) ($t = 2.569$, $df = 10$, $p < 0.05$) and in the hippocampus (**B**) ($t = 0.8531$, $df = 10$, $p = 0.04$). Densitometry quantification and representative immunoblots of phosphorylated PKC substrates in the prefrontal cortex (**C**) ($t = 1.661$, $df = 10$, $p = 0.13$) and in the hippocampus (**D**) ($t = 3.506$, $df = 9$, $p < 0.05$). Densitometry quantification and representative immunoblots of phosphorylated PKA substrates in the prefrontal cortex (**E**) ($t = 2.984$, $df = 9$, $p < 0.05$) and in the hippocampus (**F**) ($t = 0.1293$, $df = 9$, $p = 0.9$). Densitometry quantification and representative immunoblots of phosphorylated CREB at Ser 133 in the prefrontal cortex (**G**) ($t = 0.8204$, $df = 10$, $p = 0.43$) and in the hippocampus (**H**) ($t = 0.6394$, $df = 9$, $p = 0.5385$). Densitometry quantification and representative immunoblots of phosphorylated GluR1 at Ser 831 in the prefrontal cortex (**I**) ($t = 1.326$, $df = 10$, $p = 0.21$) and in the hippocampus (**J**) ($t = 6.028$, $df = 8$, $p < 0.005$). Data are mean ± SEM. Two-tailed Student's *t*-test was used. * $p < 0.05$; ** $p < 0.05$; *** $p < 0.005$ compared with Veh mice. Molecular weight markers' positions are indicated in kDa in (**B**) (n = 5–6 mice/group). Veh = vehicle and Mer = meridianins.

2.3. Meridianins Increase Spontaneous Synaptic Activity in the Hippocampal CA1

We observed that meridianins exert GSK3β inhibition in brain regions such as the PFC and the hippocampus. Moreover, we also found that meridianins increased the activity of the excitatory glutamate receptor subunit GluR1 in the hippocampus. To further examine if this modulation was accompanied by electrophysiological changes, we assessed whether meridianins modulate hippocampal synaptic transmission in brain slices. We thus measured the neuronal spike rate and bursting in the pyramidal CA1 and CA3 and in the granular DG subfields using a multielectrode array (MEA) (Figure 3B). We found a meridianin-dependent increase in the spike rates of the pyramidal CA1 cells when compared with baseline (Figure 3C,D), but no meridianin-dependent effects were observed in the spike rates of pyramidal CA3 or granular DG layers (Figure 3C,D). We found no differences in the burst parameters (data not shown) in any of the three hippocampal areas analyzed. These results support the idea that meridianins have a moderate but specific effect on synaptic transmission or neuronal excitability in the hippocampal CA1.

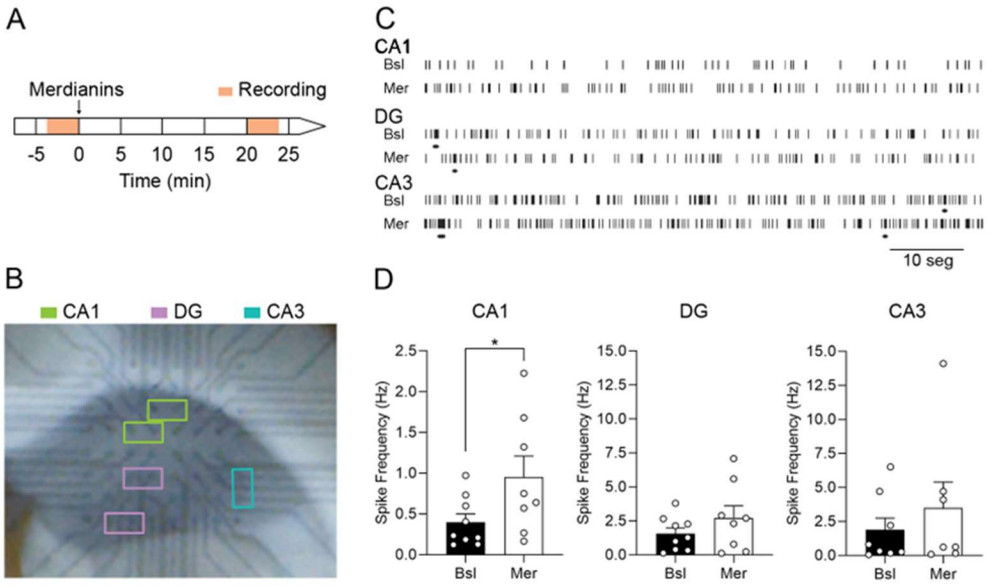


Figure 3. Meridianins specifically increase synaptic activity in pyramidal CA1 neurons: (**A**) schematic diagram of the experimental multielectrode array (MEA) recording timeline; (**B**) illustrative image of a brain coronal slice on the MEA (magnification). Recordings from the selected electrodes located on CA1, DG, and CA3 (in green, purple, and blue, respectively) were analyzed; (**C**) illustrative timescale spike raster of CA1, DG, and CA3 spontaneous activity in basal (Bsl) conditions and after incubation with 500 nM meridianins (Mer). The horizontal lines under each raster define bursts; (**D**) graphs show quantification of spike frequency in basal conditions and after treatment with meridianins in CA1 (t = 2.568, $p = 0.0371$), DG (t = 1.944, $p = 0.0930$), and CA3 (t = 1.449, $p = 0.1975$). Data are presented as mean ± SEM. * $p < 0.05$, two-tailed Student's *t*-test. (n = 7–8 mice/group).

2.4. Meridianins Improve Behavioral Deficits Induced by CUMS

To study the possible role of GSK3β inhibition in an MDD-like mouse model induced by chronic stress, we used the CUMS protocol. Several studies have already shown that CUMS induces important alterations in mice such as loss of body weight, increased anxiety, or cognitive decline [55,56]. To this end, we implanted adult (10-week-old) mice with osmotic minipumps to continuously deliver meridianins or saline (VEH) in the lateral ventricle for 28 days (Figure 4A). After surgery, the mice were randomly distributed to CNT or CUMS groups. The CUMS protocol lasted 28 days (Figure 4B). During the CUMS, we analyzed different behavioral tasks. First, in the middle of the protocol (day 13), we measured body weight and observed that only the stressed mice without treatment presented decreased body weight, compared with CNT VEH (Figure 4C). Then, we performed an open-field test to measure locomotor activity and anxiety. The results showed that all the

groups presented similar levels of covered distance (Figure 4D). We then evaluated anxiety-like behavior by measuring the distance traveled in the center of the arena (Figure 4E). We found that CUMS VEH mice spent less time in the center of the arena in comparison with CNT VEH mice, and this was totally recovered in those stressed mice treated with meridianins (CUMS MER) (Figure 4E). Moreover, we evaluated the parallel index (1.0 means walking straight) as a measure of navigation strategies in the mice (Figure 4F). We found that CUMS VEH mice presented alterations in spatial navigation in comparison with control mice, and this was recovered in those stressed mice that received meridianins (CUMS MER) (Figure 4F). We then measured behavioral despair using the forced swim test. The results indicated that both groups of stressed mice spent more time floating when compared with CNT mice. Finally, we performed the novel object location task (NOLT) to evaluate spatial memory. The results indicated that CNT mice were able to distinguish between the old and the new object position. Conversely, CUMS VEH mice could not differentiate the new location of the object. However, these deficits were totally rescued in those mice treated with meridianins (CUMS MER) (Figure 4H). These results suggest that meridianins can improve some of the deficits induced by chronic stress such as body weight loss, anxiety, navigation, and spatial memory.

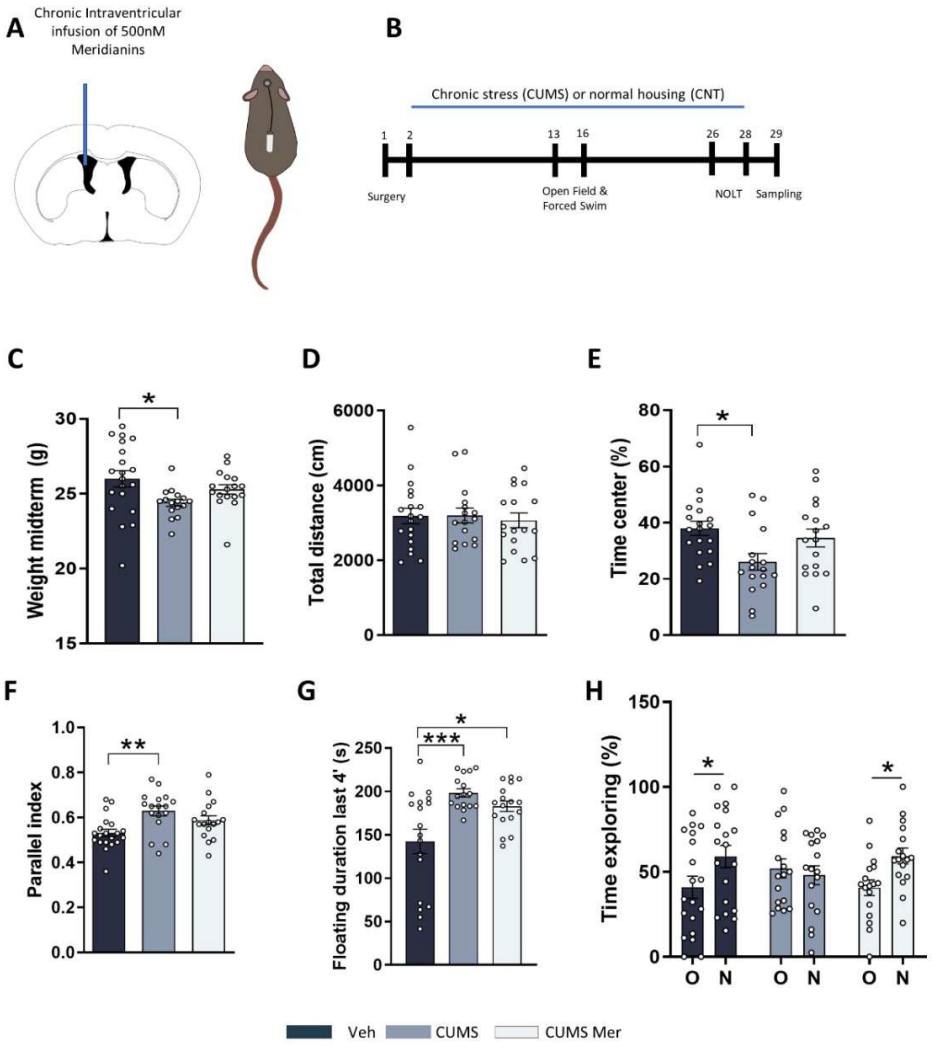


Figure 4. Effects of intraventricular meridianins' chronic delivery in stressed mice: (**A**) schematic representation of the experimental design. Ten-week-old male WT mice were chronically treated for 28 days with 500 nM meridianins or vehicle delivered into the lateral (left) ventricle; (**B**) during the stress protocol, several behavioral tasks were performed; (**C**) body weight changes induced by chronic stress. One-way ANOVA: $F(2,50) = 3.716$, $p = 0.013$. In the open field, locomotor activity (**D**),

time spent in the center (**E**), and parallel index (**F**) were monitored for 15 min. Locomotor activity, one-way ANOVA, F(2,49) = 0.1206, $p = 0.8866$. Time in center, one-way ANOVA, F(2,50) = 4.519, $p = 0.0157$. Parallel index, F(2,51) = 6.441, $p = 0.0032$; (**G**) in the forced swim test, floating duration was analyzed for the last 4 min of the test. One-way ANOVA, F(2,48) = 9.128, $p = 0.0004$; (**H**) in the novel object location task, spatial memory was evaluated 24 h after a training trial as the percentage of total time spent exploring either the object placed at a new location (N) or the object placed at the old location (O). Two-way ANOVA, new location effect, F(1,100) = 5.460, $p = 0.0214$. * $p < 0.05$ CNT; ** $p < 0.005$; *** $p < 0.0005$. N = 17–20 mice per group. Veh = vehicle and Mer = meridianins.

3. Discussion

Here, we showed that chronic treatment with meridianins in a mouse model of depression induced by chronic stress has beneficial effects. We first confirmed that chronic stress induces the hyperactivation of GSK3β. Then, we verified that meridianins can regulate in vivo GSK3β activity in different brain areas related to stress-related pathologies such as major depression disorder (MDD). We also showed that this modulation was accompanied by changes in the activity of proteins involved with GSK3β signaling or with changes in synaptic transmission. We then used a stress protocol to induce a depressive-like phenotype in mice and combined the stress protocol with meridianin administration. Interestingly, we found that some of the sequels induced by chronic stress were prevented with the meridianin treatment.

First, we confirmed that intraventricular meridianin administration can inhibit GSK3β in different brain areas. In this study, we focused on those brain regions that are known to play important roles in chronic stress and have been associated with GSK3β dysregulation. The hippocampus is one of the regions most affected by stress. Several studies reported alterations in the volume [57,58] and hippocampal-related cognitive tasks [59,60] in MDD. Moreover, changes in the activity of GSK3β in this region have been found in stress-related pathologies [16,61]. The prefrontal cortex (PFC) has emerged as another highly impaired brain region in MDD [62]. In addition, several groups have described alterations in the activity of GSK3β in this brain region [15,63]. These alterations have also been reported in the NAcc, where some groups have demonstrated that genetic GSK3β inhibition during a stress protocol is beneficial for mice [14]. Finally, recent data postulate the amygdala as a core structure in MDD [64,65], but little is known about GSK3β role in the amygdala in mood disorders. All these data suggest that GSK3β plays a crucial role in the pathophysiology of stress-related disorders including MDD. For this reason, its inhibition in different brain areas using meridianins could be a promising therapeutical approach.

An important question that is not completely understood is which GSK3β substrates could mediate its effect on mood regulation. One of the major regulators of GSK3β is Akt. Some studies reported decreased activity of Akt in depressed patients [15] and in rodent models of depression [66,67]. In our study, we observed that meridianin administration increased the Akt activity in the PFC, showing a similar effect as other antidepressants [68,69]. Moreover, we also found increased levels of phosphorylated PKA substrates in the PFC. Contrarily, this increase was not accompanied by changes in the levels of phosphorylated CREB. This could suggest that other PKA targets could be mediating GSK3β effects in the PFC. In contrast to the PFC, we saw increased levels of PKC phosphorylated substrates in the hippocampus that were accompanied by increased phosphorylation of GluR1 at Ser831. This is in line with previous research that points out PKC as one of the main kinases phosphorylating GluR1 specifically at this site [70,71]. This phosphorylation has been linked to enhanced synaptic plasticity and long-term potentiation (LTP) [72]. Moreover, studies using mice lacking this phosphorylation showed deficits in hippocampal tasks such as spatial learning [73]. This supports the idea that the GluR1 phosphorylation at specific Ser residues is crucial for memory formation and that meridianins can modulate the GluR1 activity. In this line, alterations in the levels and function of GluR1 have been already described in patients with mood disorders [74] and in mouse models of depression [75,76].

To confirm that meridianins can modulate synaptic activity, we performed electrophysiological recordings in acute hippocampal slices. Several studies reported synaptic activity alterations after CUMS [77] and in other mouse models of stress [78,79]. These alterations are often associated with cognitive deficits and with changes in the density of dendritic spines [77]. Here, we found an increase in synaptic performance specifically in the CA1 after meridianin administration that could be beneficial in a disease context such as MDD. Indeed, the GuR1 function is crucial for CA1 synaptic activity [72].

Then, we used the chronic unpredictable mild stress protocol (CUMS) to induce a depressive-like phenotype. We observed that meridianin administration was able to ameliorate some of the important sequelae induced by chronic stress. Those mice treated with meridianins presented a rescue in body weight loss and decreased anxiety levels, suggesting a role of meridianins in the emotional-like symptoms induced by chronic stress. We saw a clear effect of meridianins in cognition that could be associated with an enhancement of synaptic transmission. Although this could be due to an increase in the phosphorylation of the GluR1 receptor [72], we cannot rule out other possibilities [59,76].

Finally, this study presents some limitations. First, future work should clarify whether all the meridianins in the mixture, or only some of them, or perhaps some of them acting synergistically, are the directly responsible molecules for the observed effects. These could be achieved by using synthesized molecules, although not all the meridianins have been synthesized so far. Synthesis has been achieved for some meridianins and analogs through different pathways [80–87]. Moreover, although we demonstrated GSK3β inhibition by meridianins, we cannot exclude the possibility of alternative mechanisms of action that could be mediating the improvements observed in the stressed mice when treated with meridianins. Future studies should be focused also on elucidating these limitations. All in all, our results suggest that meridianins improve some of the deficits induced by chronic stress possibly via the inhibition of GSK3β.

4. Materials and Methods

4.1. Animals

For this study, we used male adult C57BL/6JOlaHsd mice (Envigo and Charles River). All mice were housed together until starting the chronic stress procedure in groups of four or five mice per cage. All the animals were housed with access to food and water ad libitum in a colony room kept at 19–22 °C and 40–60% humidity, under an inverted 12:12 h light/dark cycle (from 08:00 to 20:00). All animal procedures were approved by local committees (Universitat de Barcelona, CEEA (10141) and Generalitat de Catalunya (DAAM 315/18)), in accordance with the European Communities Council Directive (86/609/EU).

4.2. Marine Molecules

Marine compounds were obtained from the available sample collections at the University of Barcelona (BEECA Department, Faculty of Biology) from previous Antarctic projects (BLUEBIO, CHALLENGE). Briefly, the collected Antarctic marine organisms of the species *Aplidium falklandicum* were extracted with organic solvents, and the extracts were further purified through chromatographic methods (HPLC), as previously reported [36,37]. In the current work, we used the meridianins isolated from these previous reports, which were kept frozen at −20 °C until used. In our assays, we used the mixture of several meridianins (A–G) since the total sample amount was low, and it was not possible to identify which meridianins were present in the mixture, nor in which proportions, since this is a quite complex group of compounds [35].

4.3. Experimental Design

For the experiment shown in Figures 1D–H and 2, we performed the following experimental design: Briefly, 25 male adult mice were stereotaxically injected (see Section 4.4) with meridianins and sacrificed after 20 (6 mice), 60 (7 mice), or 180 (6 mice) min. For the experiment shown in Figure 3, 9 adult mice were used for electrophysiological field

recordings. For the experiments shown in Figures 1A,B and 4, between 16 and 20 male mice were used per condition.

4.4. Immunoblot Analysis

Hippocampal samples were collected in cold lysis buffer containing 50 mM Tris base (pH 7.5), 10 mM EDTA, 1% Triton X-100, and supplemented with 1 mM sodium orthovanadate, 1 mM phenylmethylsulphonyl fluoride, 1 mg/mL leupeptin, and 1 mg/mL aprotinin. The samples were centrifuged at 16,000× *g* for 15 min, and the supernatants were collected. After incubation (1 h) in blocking buffer containing 2.5% BSA and non-fat powdered milk in Tris-buffered saline Tween (TBS-T) (50 mM Tris–HCl, 150 mM NaCl, pH 7.4, 0.05% Tween 20), the membranes were blotted overnight at 4 ˚C with primary antibodies. The antibodies used for immunoblot analysis were phospho-GSK3β at Ser9 (1:1000; Cell Signaling, #9336xz), GSK3β (1:1000; Cell Signaling, #9315), phospho-Akt Ser 473 (1:1000; Cell Signaling, #4060S), Akt (pan) (1:1000; Cell Signaling, #4691), phospho-Ser PKC substrates (1:1000; Cell Signaling, #2261), phospho-PKA substrates (RRXS*/T*) (1:1000; Cell Signaling, #9624), phospho-CREB (Ser 133) (1:1000; Millipore, #06-519), CREB (1:1000, Cell Signaling, #9197S), phospho-GluR1 at Ser 831 (1:1000; Millipore, #04-823), GluR1 (1:1000, Millipore, #ABN241), and GAPDH (1:1000; Millipore, #MAB374).

The membranes were then rinsed three times with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. After washing with TBS-T, the membranes were developed using an enhanced chemiluminescence (ECL) kit (Santa Cruz Biotechnology, Dallas, TX, USA). The ImageLab densitometry program (ImageLab from ChemiDoc system from Bio-Rad) was used to quantify the different immunoreactive bands relative to the intensity of the α-GAPDH or, in the case of phospho-GSK3β, it was relativized with respect to total GSK3β.

4.5. Stereotaxic Surgery

For Figures 1 and 2, C57BL/6JOlaHsd mice were deeply anesthetized with isoflurane (2% induction, 1.5% maintenance) and 2% oxygen and placed in a stereotaxic apparatus for injection into the third ventricle with 3 µL of 500 nM meridianins and were sacrificed 20 min, 1 h or 3 h after administration. For Figure 4, C57BL/6JOlaHsd mice were deeply anesthetized with isoflurane (2% induction, 1.5% maintenance) and 2% oxygen and placed in a stereotaxic apparatus for osmotic minipump (model 1002; Alzet, Palo Alto, CA, USA) implantation. A brain infusion kit (#0008663) was also used to deliver into the lateral (left) ventricle 0.11 µL per hour of the vehicle or 500 nM meridianins (0.1 mm posterior to bregma, ±0.8 mm lateral to the midline, and −2.5 mm ventral to the parenchyma surface). Cannulas were fixed on the skull with Loctite 454 (from Alzet). Minipumps, previously equilibrated overnight at 37 ˚C in PBS, were implanted subcutaneously in the back of the animal. After recovery, the mice were distributed between CNT and CUMS groups.

4.6. Electrophysiological Field Recordings

The brain coronal sections of 20-week-old male mice were obtained at 350 µm thickness on a vibratome (Microm HM 650 V, Thermo Scientific, Waltham, MA, USA) in an oxygenated (95% O_2, 5% CO_2) ice-cold artificial cerebrospinal fluid (aCSF). The sections were transferred to an oxygenated 32 ˚C recovery solution for 15 min, as previously described [88], and then to oxygenated aCSF, where they were incubated at room temperature for at least 1 h before electrophysiological field recording.

After recovery, the slices were transferred into 60MEA200/30iR-ITO MEA recording dishes and fully submerged in oxygenated aCSF at 37 ˚C. For the recording of spontaneous activity, the hippocampal formation slice surface was placed on MEA 60 planar electrodes arranged in an 8 × 8 array with the assistance of a digital camera. Raw traces were sampled at 5 kHz and recorded for 5 min from 58 electrodes simultaneously. To determine the effects of meridianins in the hippocampal spontaneous activity, after a 3 min baseline

recording, the slices were incubated with 500 nM meridianins in aCSF [54] for 20 min. Then, spontaneous activity was recorded for an additional 3 min.

Spikes were identified and quantified as previously described [89]. In brief, we applied a high-pass filter with a 200 Hz Butterworth 2nd-order filter. We then assessed the noise level by using the signal standard deviation on each electrode and identified the spikes as currents with slope values between 0.2 and 1 and a negative amplitude larger than −20 mV. We applied the max interval method [90] to quantify the burst activity with the following parameter values: maximum ISI beginning and end, 200 ms and 200 ms, respectively; minimum burst duration 20 ms; minimum number of spikes in a burst, 5; and minimum interburst interval, 20 ms. We used MC Rack from Multi Channel Systems software for recording and signal processing. We selected the electrodes specifically positioned on different fields of the hippocampal formation by the image taken with a digital camera (Figure 3B).

4.7. Chronic Stress

One day before the beginning of the CUMS procedure described previously [91], the mice were individually housed and maintained isolated for the entire experiment. The CUMS procedure followed a random weekly schedule of commonly used mild stressors (one per day): restrain (1 h), food or water deprivation (24 h), home cage inclination (1 h), forced swimming (5 min), exposition to rat sawdust (4 h), and alterations in the light–dark cycle (24 h). The detailed stressor used each day is described in Supplementary Table S1. The CUMS protocol lasted 28 days and

4.8. Behavioral Test

Open-field (OF) and Novel object location test (NOL): For the novel object location test (NOL), an open-top arena (40 × 40 × 40 cm) with visual cues placed in the inner part of the walls was used. The mice were first habituated to the arena (1 day, 15 min). We considered this first exposition to the open arena as an open-field paradigm. We monitored the total traveled distance and time spent in the center of the arena as measures of locomotor activity and anxiogenic behavior, respectively. On day 2, two identical objects (A1 and a2) were placed in the arena. The mice were allowed to explore the objects for 10 min. Exploration was considered when the mouse sniffed the object. Then, 24 h later (D3), one object was moved from its original location to the diagonally opposite corner, and the mice were allowed to explore the arena and the object for 5 min. At the end of each trial, defecations were removed, and the apparatus was cleaned with 30% ethanol. Animal tracking and recording were performed using the automated SMART junior software (Panlab, Barcelona, Spain).

Forced swim test: A forced swim test was used to evaluate behavioral despair. The animals were subjected to a 6 min trial during which they are forced to swim in an acrylic glass container (35 cm height × 20 cm diameter) filled with water and from which they could not escape. The time that the test animal spent in the cylinder without making any movements except those required to keep its head above water was measured.

All the tests were conducted during the light cycle, and all the mice were randomized throughout the day. Only one test was conducted per day.

4.9. Statistics

Sample sizes were determined by using the power analysis method: 0.05 alpha value, 1 estimated sigma value, and 75% of power detection. All data are expressed as mean ± SEM. Normal distribution was tested with the d'Agostino and Pearson omnibus normality test. If the test was passed, statistical analysis was performed using parametric statistical analysis. In the experiments with normal distribution, statistical analyses were performed using an unpaired two-sided Student's *t*-test (95% confidence), one-way ANOVA, and two-way ANOVA followed by Dunnett's or Tukey's post hoc tests, as appropriate and indicated in the figure legends. The values of $p < 0.05$ were considered statistically significant. Grubbs

and ROUT tests were performed to determine the significant outlier values. All statistical analyses were carried out using GraphPad Prism software version 8.0.2 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com, accessed on 6 July 2022).

5. Conclusions

In conclusion, in the present work, we showed that meridianins inhibit GSK3β in vivo in several brain regions involved with the pathogenesis of MDD. Furthermore, we deepened the understanding of the molecular pathways underlying GSK3β inhibition by meridianins such as PKA, PKC, and GluR1 and also the synaptic consequences of GSK3β inhibition by meridianins in the hippocampus. We finally showed that intraventricularly delivered meridianins ameliorate several sequelae induced by chronic stress.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/md20100648/s1, Table S1: Schematic organization of the chronic unpredictable mild stress protocol.

Author Contributions: Conceptualization, A.S.-B. and A.G.; methodology, A.S.-B., E.G.-G., M.J.R. and A.G.; validation, W.C. and F.F.; formal analysis, A.S.-B. and E.G.-G.; investigation, A.S.-B., E.G.-G., F.F. and W.C.; resources, C.A., J.A. and A.G.; data curation, A.S.-B., E.G.-G. and M.J.R.; writing—original draft preparation, A.S.-B. and A.G.; writing—review and editing, A.S.-B., M.J.R., J.A., C.A. and A.G.; visualization, A.S.-B. and A.G.; supervision, A.G.; project administration, A.S.-B. and A.G.; funding acquisition, C.A., J.A., M.J.R. and A.G. All authors have read and agreed to the published version of the manuscript.

Funding: A.G. is a Ramón y Cajal fellow (RYC-2016–19466). This work was supported by grants from Ministerio de Ciencia e Innovación/AEI/10.13039/501100011033/ and "FEDER": AG: PID2021-122258OB-I00; JA and M-JR: PD2020-119386RB-100; as well as to CA: BLUEBIO (CTM2016-78901/ANT) and CHALLENGE (CTM2019-107979/ANT).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and all animal procedures were approved by local committees (Universitat de Barcelona, CEEA (10141) and Generalitat de Catalunya (DAAM 315/18)), in accordance with the European Communities Council Directive (86/609/EU).

Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: We thank Ana López (María de Maeztu Unit of Excellence, Institute of Neurosciences, University of Barcelona, MDM-2017–0729, Ministry of Science, Innovation, and Universities) for technical support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Otte, C.; Gold, S.M.; Penninx, B.W.; Pariante, C.M.; Etkin, A.; Fava, M.; Mohr, D.C.; Schatzberg, A.F. Major Depressive Disorder. *Nat. Rev. Dis. Prim.* **2016**, *2*, 1–20. [CrossRef] [PubMed]
2. Richards, D. Prevalence and Clinical Course of Depression: A Review. *Clin. Psychol. Rev.* **2011**, *31*, 1117–1125. [CrossRef] [PubMed]
3. Nabeshima, T.; Kim, H.-C. Involvement of Genetic and Environmental Factors in the Onset of Depression. *Exp. Neurobiol.* **2013**, *22*, 235. [CrossRef] [PubMed]
4. Richter-Levin, G.; Xu, L. How Could Stress Lead to Major Depressive Disorder? *IBRO Rep.* **2018**, *4*, 38–43. [CrossRef] [PubMed]
5. McEwen, B.S. Mood Disorders and Allostatic Load. *Biol. Psychiatry* **2003**, *54*, 200–207. [CrossRef]
6. McEwen, B.S. Protective and Damaging Effects of Stress Mediators: Central Role of the Brain. *Dialogues Clin. Neurosci.* **2006**, *8*, 367–381. [CrossRef]
7. Rush, A.J.; Trivedi, M.H.; Wisniewski, S.R.; Nierenberg, A.A.; Stewart, J.W.; Warden, D.; Niederehe, G.; Thase, M.E.; Lavori, P.W.; Lebowitz, B.D.; et al. Acute and Longer-Term Outcomes in Depressed Outpatients Requiring One or Several Treatment Steps: A STAR*D Report. *Am. J. Psychiatry* **2006**, *163*, 1905–1917. [CrossRef]
8. Jope, R.S. Glycogen Synthase Kinase-3 in the Etiology and Treatment of Mood Disorders. *Front. Mol. Neurosci.* **2011**, *4*, 1–11. [CrossRef]

9. Li, X.; Jope, R.S. Is Glycogen Synthase Kinase-3 a Central Modulator in Mood Regulation. *Neuropsychopharmacology* **2010**, *35*, 2143–2154. [CrossRef]
10. Beurel, E.; Grieco, S.F.; Jope, R.S. Glycogen Synthase Kinase-3 (GSK3): Regulation, Actions, and Diseases. *Pharmacol. Ther.* **2015**, *148*, 114–131. [CrossRef]
11. Duman, R.S.; Voleti, B. Signaling Pathways Underlying the Pathophysiology and Treatment of Depression: Novel Mechanisms for Rapid-Acting Agents. *Trends Neurosci.* **2012**, *35*, 47–56. [CrossRef] [PubMed]
12. Silva, R.; Mesquita, A.R.; Bessa, J.; Sousa, J.C.; Sotiropoulos, I.; Leão, P.; Almeida, O.F.X.; Sousa, N. Lithium Blocks Stress-Induced Changes in Depressive-like Behavior and Hippocampal Cell Fate: The Role of Glycogen-Synthase-Kinase-3β. *Neuroscience* **2008**, *152*, 656–669. [CrossRef] [PubMed]
13. Wilkinson, M.B.; Dias, C.; Magida, J.; Mazei-Robison, M.; Lobo, M.; Kennedy, P.; Dietz, D.; Covington, H.; Russo, S.; Neve, R.; et al. A Novel Role of the WNT-Dishevelled-GSK3β Signaling Cascade in the Mouse Nucleus Accumbens in a Social Defeat Model of Depression. *J. Neurosci.* **2011**, *31*, 9084–9092. [CrossRef] [PubMed]
14. Aceto, G.; Colussi, C.; Leone, L.; Fusco, S.; Rinaudo, M.; Scala, F.; Green, T.A.; Laezza, F.; D'Ascenzo, M.; Grassi, C. Chronic Mild Stress Alters Synaptic Plasticity in the Nucleus Accumbens through GSK3β-Dependent Modulation of Kv4.2 Channels. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 8143–8153. [CrossRef]
15. Karege, F.; Perroud, N.; Burkhardt, S.; Schwald, M.; Ballmann, E.; La Harpe, R.; Malafosse, A. Alteration in Kinase Activity But Not in Protein Levels of Protein Kinase B and Glycogen Synthase Kinase-3β in Ventral Prefrontal Cortex of Depressed Suicide Victims. *Biol. Psychiatry* **2007**, *61*, 240–245. [CrossRef]
16. Oh, D.H.; Park, Y.C.; Kim, S.H. Increased Glycogen Synthase Kinase-3β MRNA Level in the Hippocampus of Patients with Major Depression: A Study Using the Stanley Neuropathology Consortium Integrative Database. *Psychiatry Investig.* **2010**, *7*, 202–207. [CrossRef]
17. Polter, A.; Beurel, E.; Yang, S.; Garner, R.; Song, L.; Miller, C.A.; Sweatt, J.D.; McMahon, L.; Bartolucci, A.A.; Li, X.; et al. Deficiency in the Inhibitory Serine-Phosphorylation of Glycogen Synthase Kinase-3 Increases Sensitivity to Mood Disturbances. *Neuropsychopharmacology* **2010**, *35*, 1761–1774. [CrossRef]
18. De Sarno, P.; Li, X.; Jope, R.S. Regulation of Akt and Glycogen Synthase Kinase-3β Phosphorylation by Sodium Valproate and Lithium. *Neuropharmacology* **2002**, *43*, 1158–1164. [CrossRef]
19. Li, X.; Friedman, A.B.; Zhu, W.; Wang, L.; Boswell, S.; May, R.S.; Davis, L.L.; Jope, R.S. Lithium Regulates Glycogen Synthase Kinase-3β in Human Peripheral Blood Mononuclear Cells: Implication in the Treatment of Bipolar Disorder. *Biol. Psychiatry* **2007**, *61*, 216–222. [CrossRef]
20. Jope, R.; Roh, M.-S. Glycogen Synthase Kinase-3 (GSK3) in Psychiatric Diseases and Therapeutic Interventions. *Curr. Drug Targets* **2012**, *7*, 1421–1434. [CrossRef]
21. Li, X.; Frye, M.A.; Shelton, R.C. Review of Pharmacological Treatment in Mood Disorders and Future Directions for Drug Development. *Neuropsychopharmacology* **2012**, *37*, 77–101. [CrossRef] [PubMed]
22. Dunn, N.; Holmes, C.; Mullee, M. Does Lithium Therapy Protect against the Onset of Dementia? *Alzheimer Dis. Assoc. Disord.* **2005**, *19*, 20–22. [CrossRef] [PubMed]
23. Del Ser, T.; Steinwachs, K.C.; Gertz, H.J.; Andrés, M.V.; Gómez-Carrillo, B.; Medina, M.; Vericat, J.A.; Redondo, P.; Fleet, D.; León, T. Treatment of Alzheimer's Disease with the GSK-3 Inhibitor Tideglusib: A Pilot Study. *J. Alzheimer's Dis.* **2013**, *33*, 205–215. [CrossRef]
24. Bhat, R.V.; Andersson, U.; Andersson, S.; Knerr, L.; Bauer, U.; Sundgren-Andersson, A.K. The Conundrum of GSK3 Inhibitors: Is It the Dawn of a New Beginning? *J. Alzheimer's Dis.* **2018**, *64*, S547–S554. [CrossRef] [PubMed]
25. Molinski, T.F.; Dalisay, D.S.; Lievens, S.L.; Saludes, J.P. Drug Development from Marine Natural Products. *Nat. Rev. Drug Discov.* **2009**, *8*, 69–85. [CrossRef]
26. Carroll, A.R.; Copp, B.R.; Davis, R.A.; Keyzers, R.A.; Prinsep, M.R. Marine Natural Products. *Nat. Prod. Rep.* **2022**, *39*, 1122–1171. [CrossRef] [PubMed]
27. Soldatou, S.; Baker, B.J. Cold-Water Marine Natural Products, 2006 to 2016. *Nat. Prod. Rep.* **2017**, *34*, 585–626. [CrossRef]
28. Núñez-Pons, L.; Avila, C. Natural Products Mediating Ecological Interactions in Antarctic Benthic Communities: A Mini-Review of the Known Molecules. *Nat. Prod. Rep.* **2015**, *32*, 1114–1130. [CrossRef]
29. Angulo-Preckler, C.; De Castro-Fernandez, P.; Martín-Martín, R.; Figuerola, B.; Avila, C. Chemical Ecology in the Southern Ocean. In *Life in Extreme Environments*; Cambridge University Press: Cambridge, UK, 2020; pp. 251–278.
30. Avila, C.; Angulo-Preckler, C. A Minireview on Biodiscovery in Antarctic Marine Benthic Invertebrates. *Front. Mar. Sci.* **2021**, *8*, 686477. [CrossRef]
31. Rodríguez-Urgellés, E.; Sancho-Balsells, A.; Chen, W.; López-Molina, L.; Ballasch, I.; del Castillo, I.; Avila, C.; Alberch, J.; Giralt, A. Meridianins Rescue Cognitive Deficits, Spine Density and Neuroinflammation in the 5xFAD Model of Alzheimer's Disease. *Front. Pharmacol.* **2022**, *13*, 791666. [CrossRef]
32. Seldes, A.M.; Brasco, M.F.R.; Franco, L.H.; Palermo, J.A. Identification of Two Meridianins from the Crude Extract of the Tunicate *Aplidium meridianum* by Tandem Mass Spectrometry. *Nat. Prod. Res.* **2007**, *21*, 555–563. [CrossRef] [PubMed]
33. Gompel, M.; Leost, M.; Bal De Kier Joffe, E.; Puricelli, L.; Hernandez Franco, L.; Palermo, J.; Meijer, L. Meridianins, a New Family of Protein Kinase Inhibitors Isolated from the Ascidian *Aplidium meridianum*. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1703–1707. [CrossRef] [PubMed]

34. Franco, L.H.; De Kier Joffé, E.B.; Puricelli, L.; Tatian, M.; Seldes, A.M.; Palermo, J.A. Indole Alkaloids from the Tunicate *Aplidium meridianum*. *J. Nat. Prod.* **1998**, *61*, 1130–1132. [CrossRef] [PubMed]
35. Núñez-Pons, L.; Nieto, R.M.; Avila, C.; Jiménez, C.; Rodríguez, J. Mass Spectrometry Detection of Minor New Meridianins from the Antarctic Colonial Ascidians *Aplidium falklandicum* and *Aplidium meridianum*. *J. Mass Spectrom.* **2015**, *50*, 103–111. [CrossRef]
36. Núñez-Pons, L.; Carbone, M.; Vázquez, J.; Rodríguez, J.; Nieto, R.M.; Varela, M.M.; Gavagnin, M.; Avila, C. Natural Products from Antarctic Colonial Ascidians of the Genera *Aplidium* and *Synoicum*: Variability and Defensive Role. *Mar. Drugs* **2012**, *10*, 1741. [CrossRef] [PubMed]
37. Núñez-Pons, L.; Forestieri, R.; Nieto, R.M.; Varela, M.; Nappo, M.; Rodríguez, J.; Jiménez, C.; Castelluccio, F.; Carbone, M.; Ramos-Espla, A.; et al. Chemical Defenses of Tunicates of the Genus *Aplidium* from the Weddell Sea (Antarctica). *Polar Biol.* **2010**, *33*, 1319–1329. [CrossRef]
38. Zubia, E.; Ortega, M.; Salva, J. Natural Products Chemistry in Marine Ascidians of the Genus *Aplidium*. *Mini. Rev. Org. Chem.* **2005**, *2*, 389–399. [CrossRef]
39. Dou, X.; Dong, B. Marine Drugs Origins and Bioactivities of Natural Compounds Derived from Marine Ascidians and Their Symbionts. *Mar. Drugs* **2019**, *17*, 670. [CrossRef]
40. Llorach-Pares, L.; Nonell-Canals, A.; Avila, C.; Sanchez-Martinez, M. Computer-Aided Drug Design (CADD) to De-Orphanize Marine Molecules: Finding Potential Therapeutic Agents for Neurodegenerative and Cardiovascular Diseases. *Mar. Drugs* **2022**, *20*, 53. [CrossRef]
41. Dong, J.; Huang, S.S.; Hao, Y.N.; Wang, Z.W.; Liu, Y.X.; Li, Y.Q.; Wang, Q.M. Marine-Natural-Products for Biocides Development: First Discovery of Meridianin Alkaloids as Antiviral and Anti-Phytopathogenic-Fungus Agents. *Pest Manag. Sci.* **2020**, *76*, 3369–3376. [CrossRef]
42. Llorach-Pares, L.; Nonell-Canals, A.; Avila, C.; Sanchez-Martinez, M. Kororamides, Convolutamines, and Indole Derivatives as Possible Tau and Dual-Specificity Kinase Inhibitors for Alzheimer's Disease: A Computational Study. *Mar. Drugs* **2018**, *16*, 386. [CrossRef] [PubMed]
43. Han, S.; Zhuang, C.; Zhou, W.; Chen, F. Structural-Based Optimizations of the Marine-Originated Meridianin C as Glucose Uptake Agents by Inhibiting GSK-3β. *Mar. Drugs* **2021**, *19*, 149. [CrossRef] [PubMed]
44. Park, N.S.; Park, Y.K.; Ramalingam, M.; Yadav, A.K.; Cho, H.R.; Hong, V.S.; More, K.N.; Bae, J.H.; Bishop-Bailey, D.; Kano, J.; et al. Meridianin C Inhibits the Growth of YD-10B Human Tongue Cancer Cells through Macropinocytosis and the Down-regulation of Dickkopf-related Protein-3. *J. Cell. Mol. Med.* **2018**, *22*, 5833. [CrossRef] [PubMed]
45. Bharate, B.S.; Yadav, R.R.; Battula, S.; Vishwakarma, R.A. Meridianins: Marine-Derived Potent Kinase Inhibitors. *Mini-Rev. Med. Chem.* **2012**, *12*, 618–631. [CrossRef]
46. Llorach-Pares, L.; Nonell-Canals, A.; Sanchez-Martinez, M.; Avila, C. Computer-Aided Drug Design Applied to Marine Drug Discovery: Meridianins as Alzheimer's Disease Therapeutic Agents. *Mar. Drugs* **2017**, *15*, 366. [CrossRef]
47. More, K.N.; Jang, H.W.; Hong, V.S.; Lee, J. Pim Kinase Inhibitory and Antiproliferative Activity of a Novel Series of Meridianin C Derivatives. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2424–2428. [CrossRef]
48. Shaw, S.J.; Goff, D.A.; Lin, N.; Singh, R.; Li, W.; McLaughlin, J.; Baltgalvis, K.A.; Payan, D.G.; Kinsella, T.M. Developing DYRK Inhibitors Derived from the Meridianins as a Means of Increasing Levels of NFAT in the Nucleus. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2617–2621. [CrossRef]
49. Han, S.; Zhou, W.; Zhuang, C.; Chen, F. Structure-Based Design of Marine-Derived Meridianin C Derivatives as Glycogen Synthase Kinase 3β Inhibitors with Improved Oral Bioavailability: From Aminopyrimidyl-Indoles to the Sulfonyl Analogues. *Bioorg. Chem.* **2022**, *119*, 105537. [CrossRef]
50. Zhang, G.; Xiao, L.; Qi, L. Metabolite Profiling of Meridianin C in Vivo of Rat by UHPLC/Q-TOF MS. *J. Anal. Methods Chem.* **2021**, *2021*, 1–10. [CrossRef]
51. Huggins, W.M.; Barker, W.T.; Baker, J.T.; Hahn, N.A.; Melander, R.J.; Melander, C. Meridianin D Analogues Display Antibiofilm Activity against MRSA and Increase Colistin Efficacy in Gram-Negative Bacteria. *ACS Med. Chem. Lett.* **2018**, *9*, 702–707. [CrossRef]
52. Bharate, S.B.; Yadav, R.R.; Khan, S.I.; Tekwani, B.L.; Jacob, M.R.; Khan, I.A.; Vishwakarma, R.A. Meridianin G and Its Analogs as Antimalarial Agents. *Med. Chem. Comm.* **2013**, *4*, 1042–1048. [CrossRef]
53. Cho, H.; Yadav, A.K.; Do, Y.; Heo, M.; Bishop-Bailey, D.; Lee, J.; Jang, B.C. Anti-Survival and pro-Apoptotic Effects of Meridianin C Derivatives on MV4-11 Human Acute Myeloid Leukemia Cells. *Int. J. Oncol.* **2020**, *56*, 368–378. [CrossRef] [PubMed]
54. Llorach-Pares, L.; Rodriguez-Urgelles, E.; Nonell-Canals, A.; Alberch, J.; Avila, C.; Sanchez-Martinez, M.; Giralt, A. Meridianins and Lignarenone B as Potential GSK3β Inhibitors and Inductors of Structural Neuronal Plasticity. *Biomolecules* **2020**, *10*, 639. [CrossRef]
55. Willner, P. The Chronic Mild Stress (CMS) Model of Depression: History, Evaluation and Usage. *Neurobiol. Stress* **2017**, *6*, 78–93. [CrossRef] [PubMed]
56. Mineur, Y.S.; Belzung, C.; Crusio, W.E. Effects of Unpredictable Chronic Mild Stress on Anxiety and Depression-like Behavior in Mice. *Behav. Brain Res.* **2006**, *175*, 43–50. [CrossRef]
57. Schmaal, L.; Veltman, D.J.; van Erp, T.G.M.; Smann, P.G.; Frodl, T.; Jahanshad, N.; Loehrer, E.; Tiemeier, H.; Hofman, A.; Niessen, W.J.; et al. Subcortical Brain Alterations in Major Depressive Disorder: Findings from the ENIGMA Major Depressive Disorder Working Group. *Mol. Psychiatry* **2016**, *21*, 806–812. [CrossRef]

58. Liu, W.; Ge, T.; Leng, Y.; Pan, Z.; Fan, J.; Yang, W.; Cui, R. The Role of Neural Plasticity in Depression: From Hippocampus to Prefrontal Cortex. *Neural Plast.* **2017**, *2017*, 1–11. [CrossRef]
59. Xu, Y.; Pan, J.; Sun, J.; Ding, L.; Ruan, L.; Reed, M.; Yu, X.; Klabnik, J.; Lin, D.; Li, J.; et al. Inhibition of Phosphodiesterase 2 Reverses Impaired Cognition and Neuronal Remodeling Caused by Chronic Stress. *Neurobiol. Aging* **2015**, *36*, 955–970. [CrossRef]
60. Xi, G.; Hui, J.; Zhang, Z.; Liu, S.; Zhang, X.; Teng, G.; Chan, K.C.; Wu, E.X.; Nie, B.; Shan, B.; et al. Learning and Memory Alterations Are Associated with Hippocampal N-Acetylaspartate in a Rat Model of Depression as Measured by 1h-Mrs. *PLoS ONE* **2011**, *6*, e28686. [CrossRef]
61. Omata, N.; Chiu, C.T.; Moya, P.R.; Leng, Y.; Wang, Z.; Hunsberger, J.G.; Leeds, P.; Chuang, D.M. Lentivirally Mediated GSK-3β Silencing in the Hippocampal Dentate Gyrus Induces Antidepressant-like Effects in Stressed Mice. *Int. J. Neuropsychopharmacol.* **2011**, *14*, 711–717. [CrossRef]
62. Pizzagalli, D.A.; Roberts, A.C. Prefrontal Cortex and Depression. *Neuropsychopharmacology* **2022**, *47*, 225–246. [CrossRef] [PubMed]
63. Goswami, D.B.; Jernigan, C.S.; Chandran, A.; Iyo, A.H.; May, W.L.; Austin, M.C.; Stockmeier, C.A.; Karolewicz, B. Gene Expression Analysis of Novel Genes in the Prefrontal Cortex of Major Depressive Disorder Subjects. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2013**, *43*, 126. [CrossRef] [PubMed]
64. Roddy, D.; Kelly, J.R.; Farrell, C.; Doolin, K.; Roman, E.; Nasa, A.; Frodl, T.; Harkin, A.; O'Mara, S.; O'Hanlon, E.; et al. Amygdala Substructure Volumes in Major Depressive Disorder. *Neuroimage* **2021**, *31*, 102781. [CrossRef] [PubMed]
65. Jacob, Y.; Morris, L.S.; Verma, G.; Rutter, S.B.; Balchandani, P.; Murrough, J.W. Altered Hippocampus and Amygdala Subregion Connectome Hierarchy in Major Depressive Disorder. *Transl. Psychiatry* **2022**, *12*, 1–9. [CrossRef]
66. Krishnan, V.; Han, M.-H.; Mazei-Robison, M.; Iñiguez, S.D.; Ables, J.L.; Vialou, V.; Berton, O.; Ghose, S.; Covington, H.E.; Wiley, M.D.; et al. AKT Signaling within the Ventral Tegmental Area Regulates Cellular and Behavioral Responses to Stressful Stimuli. *Biol. Psychiatry* **2008**, *64*, 691–700. [CrossRef]
67. Li, X.X.; Yu, Y.; Lang, X.Y.; Jiang, C.Y.; Lan, R.; Qin, X.Y. 2,3,5,4′-Tetrahydroxystilbene-2-O-β-d-Glucoside Restores BDNF-TrkB and FGF2-Akt Signaling Axis to Attenuate Stress-Induced Depression. *Neuroscience* **2020**, *430*, 25–33. [CrossRef]
68. Li, N.; Lee, B.; Liu, R.J.; Banasr, M.; Dwyer, J.M.; Iwata, M.; Li, X.Y.; Aghajanian, G.; Duman, R.S. MTOR-Dependent Synapse Formation Underlies the Rapid Antidepressant Effects of NMDA Antagonists. *Science* **2010**, *329*, 959. [CrossRef]
69. Vieira de Assis Lima, I.; Flávia Almeida-Santos, A.; Hélen Ferreira-Vieira, T.; Cristina Aguiar, D.; Mara Ribeiro, F.; Cristina Campos, A.; Carlos Pinheiro de Oliveira, A. Antidepressant-like Effect of Valproic Acid-Possible Involvement of PI3K/Akt/MTOR Pathway. *Behav. Brain Res.* **2017**, *329*, 166–171. [CrossRef]
70. Roche, K.W.; O'Brien, R.J.; Mammen, A.L.; Bernhardt, J.; Huganir, R.L. Characterization of Multiple Phosphorylation Sites on the AMPA Receptor GluR1 Subunit. *Neuron* **1996**, *16*, 1179–1188. [CrossRef]
71. Diering, G.H.; Heo, S.; Hussain, N.K.; Liu, B.; Huganir, R.L.; Barria, A.; Roche, K.W. Extensive Phosphorylation of AMPA Receptors in Neurons. *Proc. Natl. Acad. Sci. USA* **2016**, *33*, 8. [CrossRef]
72. Crombag, H.S.; Sutton, J.M.; Takamiya, K.; Lee, H.K.; Holland, P.C.; Gallagher, M.; Huganir, R.L. A Necessary Role for GluR1 Serine 831 Phosphorylation in Appetitive Incentive Learning. *Behav. Brain Res.* **2008**, *191*, 178. [CrossRef] [PubMed]
73. Lee, H.K.; Takamiya, K.; Han, J.S.; Man, H.; Kim, C.H.; Rumbaugh, G.; Yu, S.; Ding, L.; He, C.; Petralia, R.S.; et al. Phosphorylation of the AMPA Receptor GluR1 Subunit Is Required for Synaptic Plasticity and Retention of Spatial Memory. *Cell* **2003**, *112*, 631–643. [CrossRef]
74. Beneyto, M.; Kristiansen, L.V.; Oni-Orisan, A.; Mccullumsmith, R.E.; Meador-Woodruff, J.H. Abnormal Glutamate Receptor Expression in the Medial Temporal Lobe in Schizophrenia and Mood Disorders. *Neuropsychopharmacology* **2007**, *32*, 1888–1902. [CrossRef] [PubMed]
75. Rosa, M.L.N.M.; Guimarães, F.S.; Pearson, R.C.A.; Del Bel, E.A. Effects of Single or Repeated Restraint Stress on GluR1 and GluR2 Flip and Flop MRNA Expression in the Hippocampal Formation. *Brain Res. Bull.* **2002**, *59*, 117–124. [CrossRef]
76. Kallarackal, A.J.; Kvarta, M.D.; Cammarata, E.; Jaberi, L.; Cai, X.; Bailey, A.M.; Thompson, S.M. Chronic Stress Induces a Selective Decrease in AMPA Receptor-Mediated Synaptic Excitation at Hippocampal Temporoammonic-CA1 Synapses. *J. Neurosci.* **2013**, *33*, 15669–15674. [CrossRef]
77. Liu, S.C.; Hu, W.Y.; Zhang, W.Y.; Yang, L.; Li, Y.; Xiao, Z.C.; Zhang, M.; He, Z.Y. Paeoniflorin Attenuates Impairment of Spatial Learning and Hippocampal Long-Term Potentiation in Mice Subjected to Chronic Unpredictable Mild Stress. *Psychopharmacology* **2019**, *236*, 2823–2834. [CrossRef]
78. Schnell, C.; Janc, O.A.; Kempkes, B.; Callis, C.A.; Flügge, G.; Hülsmann, S.; Müller, M. Restraint Stress Intensifies Interstitial K+ Accumulation during Severe Hypoxia. *Front. Pharmacol.* **2012**, *3*, 1–16. [CrossRef]
79. Yang, Y.; Ju, W.; Zhang, H.; Sun, L. Effect of Ketamine on LTP and NMDAR EPSC in Hippocampus of the Chronic Social Defeat Stress Mice Model of Depression. *Front. Behav. Neurosci.* **2018**, *12*, 1–11. [CrossRef]
80. Walker, S.R.; Czyz, M.L.; Morris, J.C. Concise Syntheses of Meridianins and Meriolins Using a Catalytic Domino Amino-Palladation Reaction. *Org. Lett.* **2014**, *16*, 708–711. [CrossRef]
81. Franco, L.H.; Palermo, J.A. Synthesis of 2-(Pyrimidin-4-Yl)Indoles. *Chem. Pharm. Bull.* **2003**, *51*, 975–977. [CrossRef]
82. Tibiletti, F.; Simonetti, M.; Nicholas, K.M.; Palmisano, G.; Parravicini, M.; Imbesi, F.; Tollari, S.; Penoni, A. One-Pot Synthesis of Meridianins and Meridianin Analogues via Indolization of Nitrosoarenes. *Tetrahedron* **2010**, *66*, 1280–1288. [CrossRef]
83. Karpov, A.S.; Merkul, E.; Rominger, F.; Müller, T.J.J. Concise Syntheses of Meridianins by Carbonylative Alkynylation and a Four-Component Pyrimidine Synthesis. *Angew. Chem.-Int. Ed.* **2005**, *44*, 6951–6956. [CrossRef] [PubMed]

84. Echalier, A.; Bettayeb, K.; Ferandin, Y.; Lozach, O.; Clément, M.; Valette, A.; Liger, F.; Marquet, B.; Morris, J.C.; Endicott, J.A.; et al. Meriolins (3-(Pyrimidin-4-Yl)-7-Azaindoles): Synthesis, Kinase Inhibitory Activity, Cellular Effects, and Structure of a CDK2/Cyclin A/Meriolin Complex. *J. Med. Chem.* **2008**, *51*, 737–751. [CrossRef] [PubMed]
85. Giraud, F.; Alves, G.; Debiton, E.; Nauton, L.; Théry, V.; Durieu, E.; Ferandin, Y.; Lozach, O.; Meijer, L.; Anizon, F.; et al. Synthesis, Protein Kinase Inhibitory Potencies, and in Vitro Antiproliferative Activities of Meridianin Derivatives. *J. Med. Chem.* **2011**, *54*, 4474–4489. [CrossRef]
86. Mohan, B.; Salfeena, C.T.F.; Ashitha, K.T.; Krishnan, G.V.; Jesmina, A.R.S.; Varghese, A.M.; Patil, S.A.; Kumar, B.N.S.D.; Sasidhar, B.S. Functionalized Pyrimidines from Alkynes and Nitriles: Application towards the Synthesis of Marine Natural Product Meridianin Analogs. *ChemistrySelect* **2018**, *3*, 6394–6398. [CrossRef]
87. Young, W.; Sun, W.-H.; Kruppa, M.; Sommer, G.A.; Müller, T.J.J. Molecules Concise Syntheses of Marine (Bis)Indole Alkaloids Meridianin C, D, F, and G and Scalaridine A via One-Pot Masuda Borylation-Suzuki Coupling Sequence. *Molecules* **2022**, *27*, 2233. [CrossRef]
88. Kim, A.; García-García, E.; Straccia, M.; Comella-Bolla, A.; Miguez, A.; Masana, M.; Alberch, J.; Canals, J.M.; Rodríguez, M.J. Reduced Fractalkine Levels Lead to Striatal Synaptic Plasticity Deficits in Huntington's Disease. *Front. Cell. Neurosci.* **2020**, *14*, 163. [CrossRef]
89. Pérez-Sisqués, L.; Martín-Flores, N.; Masana, M.; Solana-Balaguer, J.; Llobet, A.; Romaní-Aumedes, J.; Canal, M.; Campoy-Campos, G.; García-García, E.; Sánchez-Fernández, N.; et al. RTP801 Regulates Motor Cortex Synaptic Transmission and Learning. *Exp. Neurol.* **2021**, *342*, 113755. [CrossRef]
90. Legendy, C.R.; Salcman, M. Bursts and Recurrences of Bursts in the Spike Trains of Spontaneously Active Striate Cortex Neurons. *J. Neurophysiol.* **1985**, *53*, 926–939. [CrossRef]
91. Montalban, E.; Al-Massadi, O.; Sancho-Balsells, A.; Brito, V.; de Pins, B.; Alberch, J.; Ginés, S.; Girault, J.A.; Giralt, A. Pyk2 in the Amygdala Modulates Chronic Stress Sequelae via PSD-95-Related Micro-Structural Changes. *Transl. Psychiatry* **2019**, *9*, 1–12. [CrossRef]