Universidade do Minho
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Glial plasticity as a key mechanism underlying the pathophysiology of depression

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UMinho | 2020

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Tese de Doutoramento
Programa Interuniversitário de Doutoramento em Envelhecimento e Doenças Crónicas

Trabalho efetuado sob a orientação da
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fevereiro de 2020

ACKNOWLEDGEMENTS/AGRADECIMENTOS

Esta tese foi o resultado de um trabalho de equipa e de muita dedicação que, além deste trabalho escrito, teve também um grande impacto no meu crescimento pessoal. Ao Programa Doutoral Inter-Universitário em Envelhecimento e Doenças Crónicas (PhDOC), em nome da Professora Catarina Resende, Professora Margarida Correia Neves, Professor Duarte Barral e Professor Henrique Girão. Ao Instituto de Ciências da Vida e da Saúde (ICVS/3B's), em nome do Professor Jorge Correia Pinto, à Escola de Medicina em nome do Professor Nuno Sousa e ao Domínio das Neurociências (NeRD), em nome do Professor João Bessa. À Professora Doutora Luísa Pinto, agradeço do fundo do coração pela inspiração e por ser um modelo a seguir, pela presença diária, entusiasmo e pela amizade. Ao Professor Doutor João Peça, agradeço a amabilidade e disponibilidade de co-supervisionar o meu trabalho. À Pat (Patrícia Patrício), não tenho palavras para demonstrar o carinho e admiração que sinto por ti. Foste e és uma inspiração para mim e, por isso, só te posso deixar o meu sincero agradecimento pelo apoio, amizade e conselhos sempre tão sábios. Ao Dinis Alves, agradeço todo o empenho e rigor, a amizade e o bom-humor que foi tão bem-vindo. Ao António Pinheiro, agradeço a visão crítica dos resultados e a partilha de conhecimentos. Ao Professor Doutor João Oliveira, agradeço o entusiasmo, disponibilidade e pela visão crítica do trabalho. A todos os investigadores e funcionários do ICVS e da EM que trabalham para promover o bom funcionamento dos serviços. Aos meus queridos amigos que esta escola me deu: Cláudia, Ana Maria, Mika, Rita Silva, Bárbara, Joana Correia, Joana Silva, Eduardo e Vanessa, agradeço as constantes risadas, a troca de mensagens de força e o facto de acreditarem sempre em mim e me guiarem. Às minhas fiéis amigas Mariana, Rita, Filipa e Sofia, agradeço a forte amizade que dura há mais de 20 anos. À minha irmã gémea, Joana, agradeço por seres a minha melhor amiga, pela força incansável que sempre me transmitiste e pelo facto de seres um exemplo de esforço, dedicação e sacrifício! À minha família, agradeço o bom exemplo e as oportunidades que sempre me deram para crescer longe, mas poder sempre voltar para casa! À minha mãe, agradeço por ser um grande exemplo de força e superação, por me ajudar a cumprir os objetivos a que me propunha, e por me relembrar sempre que só eu tenho o poder de mudar a minha vida e de fazer cumprir os meus sonhos.

The work presented in this thesis was performed in the Life and Health Sciences Research Institute (ICVS), at the School of Medicine, University of Minho. Financial support was provided by a PhD Grant (PD/BD/52288/2013 to Ana Rita Santos) via Inter-University Doctoral Programme in Ageing and Chronic Diseases – PhDOC. The work was supported by FCT project Grant (IF/01079/2014), by BIAL Foundation grants (427/14 to LP), by Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER) (NORTE-01-0145-FEDER-000013), through the Competitiveness Factors Operational Programme (COMPETE), and The National Fund, through the FCT (POCI-01-0145-FEDER-007038).

STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

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RESUMO

TÍTULO: PLASTICIDADE GLIAL COMO UM MECANISMO FUNDAMENTAL SUBJACENTE À PATOFISIOLOGIA DA DEPRESSÃO

A depressão é uma doença altamente prevalente que, atualmente, representa um peso importante na sociedade, afetando mais de 264 milhões de pessoas mundialmente. A fisiopatologia desta doença é ainda pouco compreendida, mas evidências demonstraram a importância dos astrócitos nesta doença, sendo cruciais para a neurotransmissão e acoplamento neurovascular, evidenciada por uma perda de astrócitos em episódios depressivos, tanto em humanos como animais. No entanto, a importância das alterações dos astrócitos pré-existentes e da astrogliogénese na precipitação, recuperação e recorrência desta doença é, ainda, amplamente desconhecida no adulto. Por isso, propusemo-nos a estudar o papel dos astrócitos e da astrogliogénese adulta na precipitação e recuperação de défices cognitivos associados à depressão. Este estudo foi elaborado com ratos tratados e não tratados com dois antidepressivos (ADs), fluoxetina (inibidor seletivo de recaptação de serotonina) e imipramina (agente tricíclico), usando um modelo pré-validado de depressão (uCMS). Além disso, decidimos perceber as alterações dinâmicas que ocorrem nos astrócitos num episódio de recorrência, usando um modelo de dupla exposição ao stress e tratamento com os mesmos ADs. Quanto à avaliação do comportamento cognitivo, embora ambos os ADs tenham revertido completamente os défices cognitivos causados após a exposição ao protocolo de stress a longo termo, o tratamento com imipramina exerceu uma melhoria mais rápida desses défices. Além disso, a imipramina provocou um efeito pró-astrogliogénico, quer *in vivo* quer *in vitro*, enquanto a fluoxetina induziu um efeito hipertrófico nos astrócitos recém-gerados e pré-existentes. Em relação às alterações dinâmicas nos astrócitos induzidas pela exposição repetida ao stress, a exposição cumulativa ao stress induziu um aumento no número de astrócitos maduros no girus dentado do hipocampo. No entanto, o tratamento com fluoxetina ou imipramina durante a primeira exposição ao stress impediu o aumento do número de células S100B+ promovido pela exposição recorrente ao uCMS, sugerindo uma capacidade plástica dos astrócitos para alterar a sua população e um papel protetor desses ADs para reverter as alterações numéricas produzidas. Mais ainda, ambos os antidepressivos causaram uma diminuição da complexidade astrocítica imediatamente após a exposição ao stress. Curiosamente, essas alterações foram evitadas a longo prazo pelo tratamento com fluoxetina ou imipramina. Assim, na nossa opinião, este estudo adiciona informações relevantes sobre as alterações astrocíticas dinâmicas no contexto da depressão e o impacto particular do tratamento com diferentes classes de antidepressivos.

Palavras-chave: antidepressivos, astrócitos, astrogliogénese, depressão, recorrência.

ABSTRACT

Title: GLIAL PLASTICITY AS A KEY MECHANISM UNDERLYING THE PATHOPHYSIOLOGY OF DEPRESSION

Major depression (MDD) is a highly prevalent disorder that poses a significant social burden in society, affecting more than 264 million people worldwide. The pathophysiology of this disease is still poorly understood but growing evidence showed an important role for astrocytes in the pathophysiology of this disorder, which are crucial for neurotransmission and neurovascular coupling, evidenced by astrocytes loss in MDD in specific brain regions, both in humans and animals. However, the importance and the alterations of astrocytes as astrogliogenesis in the precipitation, recovery and recurrence from MDD is still largely unknown. Therefore, we proposed to study the role of pre-existent astrocytes and adult astrogliogenesis in the precipitation of and recovery from depressive-like cognitive behavior in rats both untreated and treated with two ADs, fluoxetine (selective serotonin reuptake inhibitor) and imipramine (tricyclic AD). For that purpose, we used a pre-validated model of depression, the unpredictable chronic mild stress (uCMS). Moreover, we also studied the astrocytic dynamic changes that occur in the hippocampal dentate gyrus (DG) under repeated exposure to uCMS and upon treatment with the ADs described above.

Regarding the cognitive behavior assessment, although both ADs showed to fully reverse the cognitive impairments caused by stress exposure at a long-term, imipramine treatment exerted a faster improvement of those deficits. Moreover, imipramine elicited a strong pro-astrogliogenic effect on the hippocampal DG, both *in vivo* and *in vitro*, while fluoxetine induced a hypertrophic effect on pre-existent and newborn astrocytes. Regarding the dynamic alterations on astrocytes induced by repeated exposure to stress, cumulative stress exposure was shown to impact on the number of mature astrocytes in the hippocampal DG. Contrastingly, treatment with fluoxetine or imipramine during a first stress exposure prevented the increase in the number of S100B+ cells in the DG promoted by recurrent uCMS exposure, suggesting a plastic astrocytic capacity to alter its population and a protective role of these drugs to revert numerical alterations evoked by recurrent stress exposure. Moreover, both ADs decreased astrocytic complexity immediately after uCMS exposure. Interestingly, these alterations were prevented at the long-term by either fluoxetine or imipramine treatment. In our view, this study adds relevant information about the dynamic astrocytic changes in the context of depression, and the particular impact of treatment with different classes of ADs.

Keywords: antidepressants, astrocytes, astrogliogenesis, depression, recurrence.

TABLE OF CONTENTS

ABBREVIATIONS LIST

%	Percentage
µm	Micrometre
ACTH	Adrenocorticotropic hormone
ADs	Antidepressants
ANS	Autonomic nervous system
AVP	Vasopressin
BLPB	Brain lipid binding-protein
BD	Bipolar disease
BDNF	Brain-derived neurotrophic factor
BrdU	Bromodeoxyuridine
CNS	Central nervous system
CRF	Adrenocorticotropin hormone releasing factor
CTRL	Control
DAPI	4´-6´- diamidino-2-phenylindole
DESIP	Desipramine
DG	Dentate gyrus
DSM	Diagnostic and Statistical Manual of Mental Disorders
FLX	Fluoxetine
FST	Forced swimming test
GC	Glucocorticoid
GCL	Granular cell layer
GFAP	Glial fibrillary acidic protein
GLAST	Glutamate aspartate transporter
HPA	Hypothalamic pituitary adrenal
IMIP	Imipramine
LT	Long-term
LTD	Long-term depression
LTP	Long-term potentiation
MAM	Methylazoximethanol
MAOI	Monoamine oxidase inhibitor
MDD	Major depressive disorder

MDE	Major depressive episode
MIN	Minutes
NMDA	N-methyl-D-aspartate
NOR	Novel object recognition
NORFLX	Norfluoxetine
NSC	Neural stem cell
OB	Olfactory bulb
OF	Open field
PDFG	Platelet derived growth factor
PHQ	Patient Health Questionnaire
PFA	Paraformaldehyde
PFC	Prefrontal cortex
PVN	Paraventricular nucleous
REC	Recurrence
RMS	Rostral migratory stream
RT	Room temperature
SAL	Saline
SEM	Standard error of the mean
SERT	Serotonin transporter
SEZ	Subependymal zone
SGZ	Subgranular zone
SHAM	Serotonin, histamine, alpha, muscarinic
SNRI	Selective norepinephrine reuptake inhibitor
SPT	Sucrose preference test
SSRI	Selective serotonin reuptake inhibitor
ST	Short-term
TBS	Tris-buffered saline
TCA	Tryciclic agent
TP1	Time-point 1
TP2	Time-point 2
uCMS	unpredictable chronic mild stress

THESIS LAYOUT

Chapter 1 presents a general introduction on depression epidemiology, treatment and the main neurobiological mechanisms involved in its pathophysiology, with a particular emphasis on the role of stress on astrocytic plasticity and astrogliogenesis in the hippocampus.

Chapter 2 presents the rationale and major aims of this thesis.

Chapter 3 displays the research work on astrocytic and astrogliogenesis longitudinal changes, in an animal model of depression and after fluoxetine and imipramine treatment.

Chapter 4 comprises the research work on the longitudinal plastic astrocytic changes in the context of recurrent depression in the dentate gyrus of an animal model of depression and after treatment with two different classes of antidepressants. This chapter is presented as a final original paper published in Neuroscience in 2019 (Machado-Santos *et al*. 2019).

Chapter 5 contains a general discussion, in which the major findings of the present research work are discussed in light of the current relevant literature, as well as main limitations. Future perspectives on how the field may evolve are also herein briefly discussed.

Chapter 1

INTRODUCTION

1. DEPRESSION

1.1. Epidemiology of depression

Depression is a complex and common illness, affecting more than 264 million people worldwide (WHO 2019). This disorder poses a massive burden in current society, is known to affect several behavioral domains in patients such as mood, anxiety and cognition and is characterized by emotion dysregulation – the hallmark of depression – and sustained negative effect (Bessa *et al.*, 2009; Clelland *et al.*, 2009).

Although this disorder is a major public health concern, there are still several open questions and research hypothesis regarding the cause behind its onset and progression. In this view, and in an attempt to understand if major depressive disorder (MDD) derives from a genetic cause, a meta-analysis of twin studies estimated that MDD heritability is only 37.2% (Sullivan *et al.*, 2000; Smith, 2014), in contrast with other psychiatric diseases including schizophrenia and bipolar disorder - which is around 70-80% (Kendler, 1983). Moreover, other studies with identical twins have shown a 50% discordance rate for MDD, showing the importance of non-genetic factors for this disease (Fraga *et al.*, 2005), such as environmental factors.

Indeed, some epidemiological studies suggest that stressful life events are extremely associated with a high risk for MDD (Hammen, 2015). They hypothesized that the resilience and susceptibility to MDD is determined by genetic and environmental factors, being both crucial for the onset of depression (shown on Figure 1). Moreover, those studies also suggest that chronic exposure to environmental insults can induce adaptative changes in brain neuroplasticity of the individuals.

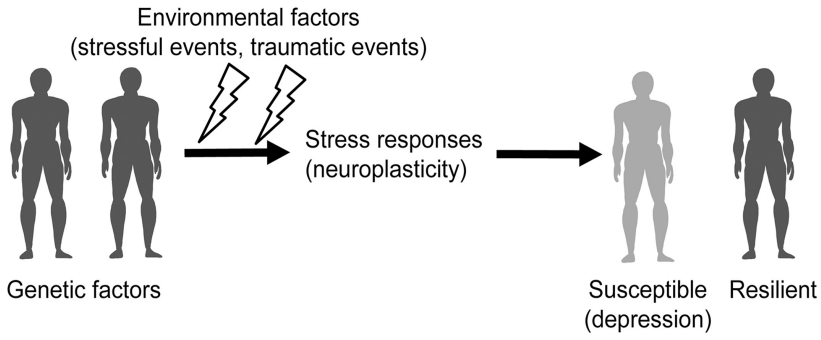

Figure 1: A proposed model of the etiology of major depression. Both genetic and environmental factors are important for the onset of depression. Exposure to environmental insults induces adaptive changes in neuroplasticity within the brain. Retrieved from S. Uchida *et al.* (2017).

Furthermore, the prevalence of depression in children is relatively low (<1% in most studies), increasing through adolescence with a one-year prevalence of 4–5% in mid to late adolescence. Indeed, depression not only stands as the major risk factor for suicide in adolescents, but it also leads to numerous social and educational impairments and associated increased rate of smoking, substance abuse and obesity (El Refaey & Amri, 2011; Thapar *et al.*, 2012). In this context, the strongest risk factors for depression in adolescents are a family history of depression and exposure to psychosocial stress (Thapar *et al.*, 2012).

Regarding the prevalence of depression among adults, during 2013–2016, 8.1% of American adults had a depressive episode in a given 2-week period (Brody *et al.*, 2018). Moreover, and as observed in other research studies (Blanco *et al.*, 2010; Pratt & Brody, 2014), depression was almost two times as common among women as among men. Interestingly, it was shown that the number of adults with depression linearly increased with decreasing family income level. More so, about 80% of adults with depression reported at least some difficulty with work, social activities or at home due to their depression symptoms (Brody *et al.*, 2018).

1.2. Symptoms of depression

As previously described, MDD is a highly complex and heterogeneous clinical disorder that is poorly characterized in terms of cellular and molecular pathophysiological factors, being diagnosed based on structured clinical tools.

Therefore, clinicians worldwide rely on diagnostic tools such as the Diagnostic and Statistical Manual of Mental Disorders (DSM) to diagnose depression. According to DSM-V, symptoms such as “depressed mood” (mood) or “loss of interest or pleasure in nearly all activities” (i.e. anhedonia) represent the necessary requirements to diagnose a Depressive Episode (Charney & Nelson, 1981). Indeed, when these two core symptoms were used to screen for MDD using a 2-item version of the Patient Health Questionnaire (PHQ-2), they displayed a sensitivity of 83%, which clearly shows their statistical power (Kennedy, 2008).

In addition to these two core experiences, there are some other symptoms of MDD (listed on Figure 2), including loss of interest in activities, sleep and appetite changes, guilt and hopelessness feelings, fatigue, restlessness, concentration problems, and suicidal ideation. Confirmatory diagnosis of a Major Depressive Episode (MDE), according to DSM-V, requires a minimum of five symptoms (with at least one being mood or anhedonia) for a minimum period of 2 weeks, in terms of duration (Kennedy, 2008). Additionally, the symptoms usually cause clinically significant distress in relevant areas such as social, professional or occupational (American Psychiatric Association, 2013). To corroborate those symptoms,

studies have shown that depressed people often fail in their quest to satisfy their need for belonging in relationships (Hagerty *et al.*, 1996). Depressed people also reported fewer intimate relationships, and elicit less positive, caring responses and more negative and rejecting responses from others (Joiner *et al.*, 2004).

Therefore, it is obvious to understand the multiple possible combinations of factors that contribute to this disorder's inherent heterogeneity.

Depression symptoms can vary from mild to severe and can include:

- Feeling sad or having a depressed mood
- Loss of interest or pleasure in activities once enjoyed
- Changes in appetite — weight loss or gain unrelated to dieting
- Trouble sleeping or sleeping too much
- Loss of energy or increased fatigue
- Increase in purposeless physical activity (e.g., handwringing or pacing) or slowed movements and speech (actions observable by others)
- Feeling worthless or guilty
- Difficulty thinking, concentrating or making decisions

Figure 2: Depression Symptoms described by the American Psychiatric Association.

1.3. Relapse and recurrence in depression

For most people with MDD, recurrence after recovery from a depressive episode is the most probable scenario. Therefore, one of the current challenges for the mental health field is to discover and understand how the state of well-being can be sustained over an individual's life.

Several studies have reported that up to a third of all patients will have episodes that last longer than two years, and that over 85% of remitted patients suffer recurrent episodes of depression within 15 years after an initial episode (Mueller *et al.*, 1999; Baldessarini *et al.*, 2015). Although a first depressive episode has been highly linked to stressful events (Simons *et al.*, 1993), recurrent depression has also been associated to the persistence of subclinical residual symptoms and the number of previous episodes (Hardeveld *et al.*, 2009).

Several predictors of recurrence were shown to occur, such as being female, having a longer depressive episode before, having more prior episodes, and never marrying (Hollon *et al.*, 2006).

Descriptively, recurrent patients reported higher levels of depressive symptoms and a greater number and higher total scores on comorbid medical conditions. These patients also reported an higher anxiety and more somatic and cognitive symptoms, than the first episode patients did (Mueller *et al.*, 1999). Indeed, although impairments in cognitive processes, such as attention and memory, can be correlated with depressive episodes, they can also increase patient´s susceptibility for a first hit and recurrence of this disorder (Gotlib & Joormann, 2010). This led us to believe that there are some specific factors and molecular changes that are empowered to increase patients´ risk for a new depressed episode. However, there are still very few studies investigating the determinants of recurrence or relapse episodes.

1.4. Aetiology of depression

1.4.1. The role of stress

The word 'stress' is used in physics to refer to the interaction between a force and the resistance to counter that force. However, in 1936 Hans Selye introduced this term in a letter to Nature to describe the "nonspecific response of the body to any demand", when he was focused on studying the universal patient reactions to illness (Selye, 1936).

The stress response is part of any adaptative biological system and is active when an individual's wellbeing, health, homeostasis or survival is threatened. In these cases, after a stimulus (which can be endogenous or exogenous) is perceived as unpleasant or threatening, several systems and processes are together activated to generate a response to it – the stress response (Sousa & Almeida, 2012; Lucassen *et al.*, 2014). These responses occur to permit the individual to cope with the situation.

However, stress is not a single unit and there are several types of stressful events that can occur: acute or chronic stressful events, occurring only once or being repetitive in time, predictable or unpredictable stressful events, and mild or severe stressful events (Lucassen *et al.*, 2014a). Moreover, the physiological stress response can be divided in: 1) the quick stress response, or called the "fight-fright-or flight" response, which involves the rapid activation of the autonomic nervous system (ANS) and leads to the norepinephrine and epinephrine release from the adrenal medulla; or 2) the later stage response which is known to activate the hypothalamic–pituitary–adrenal (HPA) axis, which has the ability to ultimately determine the magnitude and specificity of an individual's behavioral, neural and hormonal responses to stress (Lucassen *et al.*, 2014a).

1.4.2. The Hypothalamic-Pituitary-Adrenal (HPA) axis in the stress response

Upon a stress stimulus, a highly complex and dynamic response is activated, which results in changes on autonomic, physical, behavioral and neuroendocrinal systems (Fulford & Harbuz, 2005). The brain plays a central role in this process, as it perceives both sensory inputs from the external environment and

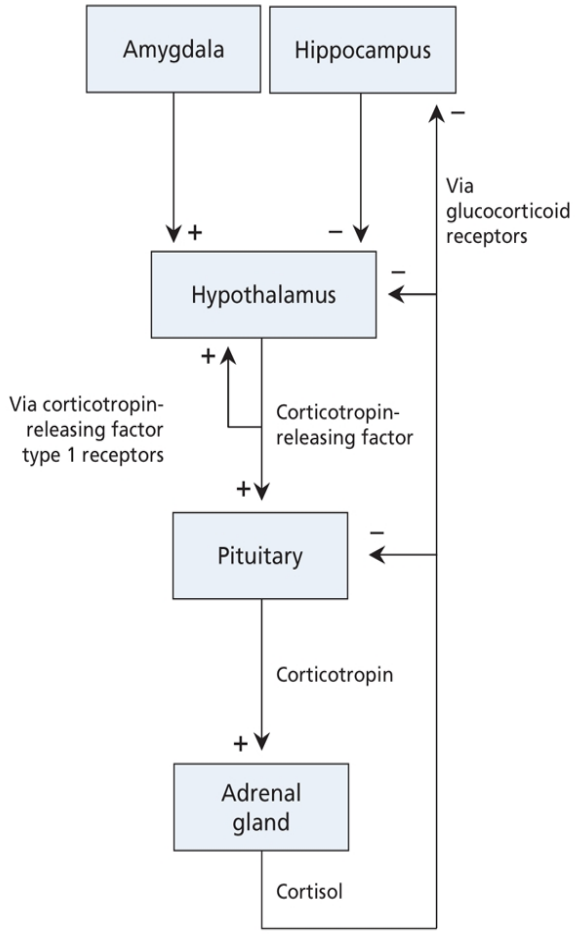

Figure 3: Overview of the HPA axis. This system is activated by stress directly at the level of the hypothalamus or indirectly at the level of the amygdala, that will stimulate the hypothalamus. The hypothalamus produces and releases CRF. Local stimulation of CRF type 1 receptors results in additional release of CRF. This creates a feed-forward loop, which facilitates a rapid response to the stressor. In the pituitary, stimulation of CRF type 1 receptors results in release of corticotropin (ACTH). The adrenal glands are stimulated by ACTH to produce cortisol – the stress hormone, which affects many organs, including the brain. The hippocampus is an important target of cortisol. Local activation of GC receptors helps the hippocampus control the HPA axis. GC receptors are also found in the hypothalamus and pituitary. Chronic stress increases the level of CRF and cortisol and decreases expression of CRF type 1 receptors and GC receptors. Similar changes have been found in some patients with MDD. Stress- and depression-associated changes at the level of the hippocampus are thought to underlie the structural changes seen in this brain region, which in turn may contribute to chronic disinhibition of the HPA axis. Image and caption retrieved and adapted from *Rot M., et al.* (2009).

internal inputs from the body, coordinating and adjusting all the responses to cope and answer to the perceived challenges (Femenía *et al.*, 2012).

The HPA axis is one of the biological systems involved in the stress response (see Figure 3). The activated HPA axis is known to regulate peripheral functions, including immune and metabolic functions, but also to impact on central nervous system (CNS) (Pariante & Lightman, 2008). During stress response, the paraventricular nucleus (PVN) in the hypothalamus is activated, leading to the secretion of adrenocorticotrophic hormone releasing factor (CRF) and vasopressin (AVP), which will further promote adrenocorticotropic hormone (ACTH) secretion from the pituitary into the blood stream. Finally, this hormone – ACTH – will stimulate the secretion of the glucocorticoids (GCs) (cortisol in humans and corticosterone in rodents) from the adrenal cortex (Pariante & Lightman, 2008). Those GCs will later interact with their receptors in multiple target tissues, including the HPA axis itself, being responsible for feedback inhibition both on CRF and AVP from the hypothalamus and directly on secretion of ACTH (Herman *et al.*, 2016). Importantly, GCs were shown to regulate neuronal survival, neurogenesis, the sizes of anatomical structures as the hippocampus, the acquisition of new memories and the emotional assessment of events (Herbert *et al.*, 2006). With such a leading role in the brain, HPA axis was found dysregulated in psychiatric disorders, particularly in major depression, where depressed patients revealed increased levels of cortisol in plasma, urine and saliva (Nemeroff & Vale, 2005).

Furthermore, several crucial biological responses are combined and orchestrated to ensure that the stress response is terminated, and the homeostasis is re-established. In this case, the hippocampus is highly involved in the termination of stress response, as the stimulation of hippocampal neurons decreases the neuronal activity in the PVN, which will inhibit GCs secretion. Also, the Prefrontal cortex (PFC) negatively regulates the HPA axis in response to stress, by decreasing both ACTH and GCs secretion. Moreover, other brain structures play a role in this feedback loop to cope with the stress response, being dynamically changed either on structure or in activity (Fulford & Harbuz, 2005).

2. Chronic stress and depression

There is a huge variability – both genetic and epigenetic – in the individual response and predisposition to the effects of stress (see Figure 1). Therefore, the stressors can be differentially perceived among individuals, which will ultimately lead to different responses.

The stress response and the related consequences depend not only on the individual susceptibility, but also on the timing of the stressor, its intensity duration, nature and predictability (Sousa, 2016). If the

duration or the intensity (combined with the fact that it can be occurring in specific neurodevelopmental stages that are more prone to lead to a future imbalance) of the stressor is exceeded, a dysregulated maladaptive response is elicited, leading to neuropathological problems such as depression (Bessa *et al.*, 2009). As an example of this graded action of the stress in the individual's system, transient mild stress is able to enhance learning and memory (Luine *et al.*, 1996), while chronic or severe stress was able to disrupt the hippocampus-dependent memory in experimental animals (which was also confirmed on humans after prolonged GCs treatment) (Sapolsky, 2003; Shors, 2006).

Indeed, chronic stress is one of the most powerful precipitating factors of depression. With the HPA axis being continuously activated due to a prolonged exposure to stress, a response that will impact on neurobiological systems involved in the pathophysiology of depression will be activated (Lucassen *et al.*, 2014b). Furthermore, those impacts are physically perceived. Specifically, sustained levels of stress or GCs were shown to damage the hippocampus at the morphological neuroplasticity level (reviewed in (Sapolsky, 2000)), with atrophy and retraction of the apical dendrites of hippocampal pyramidal cells or even death of those cells (Sapolsky, 1985).

However, there is a great body of evidence showing that, with time, the way the brain deals with stressors in terms of regions and patterns of activation suffers a considerable shift, suggesting a clear distinction between the acute and the chronic neuromatrix. Indeed, in the chronic maladaptive stress situation, in contrast to acute or subacute states, there is an involvement of additional neurons with different projections, resulting in unique modifications of stress control nodes and networks (Sousa, 2016). However, it is crucial to further explore this transition from a beneficial moderated stress response to a chronic maladaptive one, thus understanding how stress increases vulnerability to several neuropsychiatric disorders (including depression).

3. Treatment in depression

3.1. Main antidepressants used in the clinics

Antidepressants (ADs) are the first line treatment for depression. However, and despite the significant advances in the pharmacotherapy of depression during the last decades, our current knowledge on the mechanisms of action of these drugs is still very incomplete. In fact, most of the current understanding of depression has been built upon the discovery, by serendipity during clinical trials for antituberculosis agents, of the mood-elevating effects of iproniazid (in the earlies 1950s) - a monoamine oxidase inhibitor (MAOI). It happened when the patients with tuberculosis treated with this drug showed euphoric mood effects (Boku *et al.*, 2018).

However, and despite the monoamine hypothesis has dominated our understanding of the pharmacotherapy of depression for more than half a century, 35% of patients still do not benefit from the therapeutic effects of the current ADs, including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (SNRIs), MAOIs, and rapid-acting agents such as ketamine (Krishnan & Nestler, 2008; Hashimoto, 2019).

Importantly, a clinical trial completed in 2006 – STAR*D trial - which encompassed 4041 outpatients with non-psychotic depression, showed that some patients may possibly need to try more than one treatment strategy in order to achieve full remission. Curiously, the probability of treatment decreased with every additional treatment needed (Rush *et al.*, 2006). Moreover, during this trial, a great proportion of patients never achieved remission with the available treatments, which stands to show the need for better understanding the mechanisms of action of current available ADs to further develop novel and more effective ones.

In a general way, some ADs are classified according to their primary pharmacological targets, while others are named by their chemical structure, having no uniform classification. The following subsection gives a brief overview of the main classes of current available ADs and their pharmacological mechanisms.

Monoamine oxidase inhibitors (MAOIs)

MAOIs were firstly introduced in the 1950s (Culpepper, 2013). They treat different types of depression, as well as other nervous system disorders, such as panic disorder social phobia and depression with atypical features such as oversleeping and overeating (Henkel *et al.*, 2006). Although MAOIs were the first class of ADs to be introduced, they are not the first clinical choice for depression treatment due to several dietary restrictions, side effects, and safety concerns. MAOIs are only a treatment option in the clinic when all other possible options are unsuccessful.

Regarding their mechanism of action, MAOIs are responsible for blocking the monoamine oxidase enzyme. The monoamine oxidase enzyme breaks down different types of neurotransmitters from the brain: norepinephrine, serotonin, dopamine, as well as tyramine. MAOIs inhibit the breakdown of these neurotransmitters, increasing their levels in the synaptic cleft (Krishnan, 2007).

Since Iproniazid, a related compound that was the “prototype version” of this class of ADs, had to be withdrawn due to toxicity and serious side-effects, the research on MAOIs has been driven to develop newer and safer drugs, namely reversible inhibitors of MAO and MAO-A specific inhibitors (Millan et al., 2015). This class includes the antidepressants tranylcypromine, phenelzine and moclobemide.

Tricyclics (TCAs)

TCAs are among the earliest ADs developed. In 1969, the finding that TCAs could block the reuptake of serotonin in presynaptic neurons allowed Lapin and Oxenkrug to postulate the serotonergic theory of depression. This theory was based on a deficit of serotonin at an inter-synaptic level in certain brain regions (Pereira & Hiroaki-Sato, 2018). Although effective, this class of ADs have generally been replaced by others that cause fewer side effects, such as SSRIs. However, cyclic antidepressants still represent a viable option for some patients when other treatments have failed. Some possible common side effects of this class of ADs include drowsiness, blurred vision, constipation, dry mouth, weight loss, increased appetite leading to weight gain, excessive sweating, tremor, sexual problems, and others.

Regarding its mode of action, TCAs can act through NMDA antagonism, opioidergic effects, sodium, potassium and calcium channel blocking, through interfering with the reuptake of serotonin and acting as antagonists to SHAM (serotonin, histamine, alpha, muscarinic) receptors.

Real data from clinical practice suggests that initial choice of either fluoxetine or a TCA eventually results in similar relief of depressive symptoms, but an initial choice of fluoxetine led to fewer adverse effects and fewer medication changes (Simon, 1996).

Importantly, the inhibition of norepinephrine and serotonin reuptake contributes to therapeutic effects of TCAs. Besides imipramine, TCAs include amitriptyline, clomipramine and desipramine.

Selective Serotonin Reuptake Inhibitors (SSRIs)

SSRIs are among the most currently frequently prescribed therapeutic agents in the clinic. The emergence of SSRIs, which are ADs with relatively less side effects and limited risks in overdose, significantly contributed to the decreased use of the TCAs and MAOIs.

SSRIs's major pharmacological mechanism is the blockade of serotonin reuptake through inhibition of serotonin transporter (SERT) on presynaptic neurons (Rizvi & Kennedy, 2013). Consequently, serotonin accumulates in the synapse and promotes long-term disinhibition of serotonergic neurotransmission (M. Stahl *et al.*, 2013).

Their therapeutic actions range from efficacy in depression to obsessive/compulsive disorder, bulimia and other important conditions. The excess of biological substrates, receptors and pathways for serotonin are candidates to mediate not only the therapeutic actions of SSRIs, but also their side effects. There are six compounds included in this class that share the same major pharmacological mechanism - fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram and escitalopram. However, each has secondary pharmacological targets (Rizvi & Kennedy, 2013) that may allow differences in efficacy.

Serotonin and Norepinephrine reuptake Inhibitors (SNRIs)

Introduced in the early 1990's, this class of ADs is also currently used as first-line treatment for depression (Dale *et al.*, 2015). As suggested by their name, these ADs are inhibitors of serotonin and norepinephrine reuptake, thus increasing the levels of both neurotransmitters in the synaptic cleft. Moreover, NET inhibition in the PFC promotes dopamine increases in this specific brain region (M. Stahl *et al.*, 2013). ADs that fall into the SNRI class are venlafaxine, desvenlafaxine, duloxetine, milnacipran, and levomilnacipran.

The pharmacodynamic properties of these ADs differ substantially from those of the TCAs, the MOIs, the SSRIs, and, in some cases, from each other.

Ketamine: fast-acting agent

Ketamine is a non-competitive antagonist of N-methyl-D-aspartate (NMDA) receptor that now is considered as a fast activity AD in treatment-resistant depression (Pham & Gardier, 2019). In fact, studies have suggested that the N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine generates rapid and sustained AD effects in treatment-resistant patients with MDD or Bipolar Disorder (BD) (Hashimoto, 2019).

Ketamine was FDA-approved in the U.S. as an anesthetic around 50 years ago. It is primarily used by anesthesiologists in both hospital and surgical settings. As an NMDA receptor antagonist with dissociative properties, NMDA receptors have high calcium permeability, which allows ketamine to reach its target rapidly. After a large body of evidence concerning the maintenance of neurochemical and behavioral changes 24h after administration of this compound have emerged, ketamine has jumped to the spotlight, being believed to initiate a cascade of cellular mechanisms supporting its fast AD-like activity. The underlying mechanism involves glutamate release, followed by downstream activation of AMPA receptors, which trigger mammalian target of rapamycin (mTOR)-dependent structural plasticity via brain-derived neurotrophic factor (BDNF) and protein neo-synthesis in the medial PFC (Pereira & Hiroaki-Sato, 2018).

To understand the effect of Ketamine in a depressive context, some studies have shown that this fast-acting agent has the potential to improve the action of classic ADs in brain regions affected by stress exposure (Melo *et al*, 2015).

4. The Neuronal plasticity theory of depression

Neurons can mediate and respond to activity with their processes and synapses, a property that makes them highly plastic. Therefore, we can define neural plasticity as the capacity of neurons and neural elements to adapt in response to intrinsic and extrinsic signals, which will impact in our ability to process

and synthesize information, ultimately producing behavioral responses (Wilbrecht *et al.*, 2010). In this way, is more than predictable that dysregulation or disruption of neural plasticity is associated with neuropsychiatric and neurodegenerative disorders. Indeed, altered neuronal and structural plasticity has been shown to occur in both animal models (Mateus-Pinheiro, Patrício, *et al.*, 2013) and human patients with MDD (Wainwright & Galea, 2013).

The hippocampus is the brain region most studied in the neuroplastic theory of depression. Being part of the limbic system, this structure develops nerve fiber connectivity with emotion-related brain areas, such as the PFC and amygdala. In addition, the hippocampus not only contains high levels of GC receptors and glutamate, but also regulates the HPA axis, like previously explained, being highly susceptible to stress and depression. Therefore, stress and negative stimuli can change the hippocampal plasticity in several ways. In detail, chronic stress was shown to impair hippocampus-dependent explicit memory in animal models of depression, for which hippocampal synaptic plasticity was shown to have a role on its formation (Pittenger & Duman, 2008). Stress can also decrease neuronal dendrite branching and plasticity in the hippocampus (Son *et al.*, 2012). In addition, stress can trigger activation of the HPA axis, increase the level of GCs, and decrease hippocampal neurogenesis (Masi & Brovedani, 2011). Hippocampal plasticity alteration in depression is known to involve hippocampal volumetric changes, hippocampal neurogenesis, and apoptosis of hippocampal neurons.

On Table 1, we summarize the main changes that are known to occur in this specific brain region, the hippocampus, regarding neural plasticity and its related mechanisms.

Table 1: Changes of neural plasticity induced by depression in several brain regions. Table retrieved from Wei Liu *et al.* (2017).

Brain region	Changes of neural plastiticy	Mechanisms
Hippocampus	Synaptic plasticity	Impairment of LTP in CA3
		Facilitation of LTD and lLTD in CA1
		Downregulation of synaptic proteins and growth factors
	Volumetric changes	Disruption and atrophy of neurons and glia
		Neurodegenerative reaction to high levels of glucocorticoid
	Neurogenesis	Hindered by high levels of glucocorticoids and enhanced by adrenalectomy
		Additive effects in mice, while reduced in humans
		Additive function in the circuitry
	Apoptosis	Depression promotes apoptosis in the hippocampus
		The effects caused by chronic depression last longer than those of acute depression

5. CYTOGENESIS IN THE ADULT BRAIN: RELEVANCE FOR THE PATHOLOGY OF DEPRESSION

The scientific insufficiency to disclose the fundamental mechanisms underlying MDD is substantiated by the multiple neurological systems that are implicated in the etiopathogenesis of depression, as well as by the interindividual variations that are reflected in the different levels of susceptibility to the disease. Such complex etiological nature poses important challenges to the understanding of the precise pathophysiological mechanisms underlying this neuropsychiatric disorder and the effects of AD drugs, accounting for the many unmet medical needs in this field. Therefore, a better understanding of these mechanisms will help to unravel alternative options to effectively revert and cure MDD. In this context, several hypotheses have been proposed to explain the neurobiological mechanisms underlying the onset, maintenance and recovery from this disorder (Duman & Monteggia, 2006; Maletic *et al.*, 2007; Sahay & Hen, 2007; Patrício *et al.*, 2013). During the last four decades, a significant number of studies in this field revealed cell loss and neuronal atrophy, particularly in brain regions relevant for emotional behavior control (Radley *et al.*, 2004; Lucassen *et al.*, 2006; Rajkowska *et al.*, 2007; Rajkowska & Miguel-Hidalgo, 2007a; Banasr & Duman, 2008; Liu & Aghajanian, 2008; Maciag *et al.*, 2010). Therefore, it was the basis for constituting the neurogenic hypothesis of depression, where the involvement of adult neurogenesis imbalances along with dendritic arborization impairments were implicated both in the pathophysiology of MDD and in the action of ADs (Warner-Schmidt & Duman, 2006). In few words, this hypothesis states that new neurons are needed for proper mood control and AD efficacy in the adult brain (Petrik *et al.*, 2012). This hypothesis completely refuted Cajal's theory of the immutability of the CNS, showing that it has both plastic and regenerative potential. Moreover, neurogenesis, a process that includes the generation, differentiation and integration of new neurons in the existent neuronal network, was shown to occur in the adult brain and to endure throughout life in specific brain locus (Doetsch *et al.*, 1999; Gage, 2002).

Nowadays, it is currently accepted that adult neurogenesis occurs mostly in two mammalian brain regions: the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) and the subependymal zone of the lateral ventricles (SEZ). These two "canonical" sites of adult neurogenesis were found in all animal species studied so far, including humans (reviewed in (Lindsey & Tropepe, 2006; Bonfanti & Ponti, 2008; Kempermann, 2012; Grandel & Brand, 2013). However, several studies from last year have been challenging the existence of human adult neurogenesis, justified by the lack of high-quality in-vivo studies and conflicting results (Kumar *et al.*, 2019; Lucassen el al., 2019).

Besides, at these specific subregions, resident progenitor cells, also known as neural stem cells (NSCs), are known to be located. These cells have both morphological and antigenic glial properties, being overall described as stem cells with glial properties and radial-glia like form (Filippov *et al.*, 2003; Rakic, 2003; Ninkovic & Götz, 2013). They can give rise to intermediate progenitor cells, called transit amplifying neural progenitors (Type-2 cells), that are mitotically active and divide to give rise to neuroblasts (Type-3 cells). Neuroblasts can then fully mature into granule neurons, elongating their axons and making the needed axonal connections. In one hand, the neuroblasts that are generated in the SEZ and migrate along the rostral migratory stream (RMS), are turned into mature GABAergic granule and periglomerular interneurons in the olfactory bulb (OB). On the other hand, those which are born in the adult SGZ migrate into the granular cell layer (GCL) of the DG and differentiate into glutamatergic granule cells. The adult-born neurons become integrated in the pre-existing neuronal network 4 to 8 weeks after their generation (Van Praag *et al.*, 2002; Ambrogini *et al.*, 2004; Espósito *et al.*, 2005; Zhao *et al.*, 2006).

Crucially, before the integration in the neuronal circuitry and along the maturation process, adult-born cells undergo a critical period of selection, with only a fraction of cells surviving, integrating and establishing functional connections (Biebl *et al.*, 2000; Kuhn *et al.*, 2005). In light of the functional segregation along the septotemporal axis of the hippocampus, suggesting an association between the dorsal pole to cognitive regulation and ventral to emotional behavior (Fanselow & Dong, 2010; Tanti & Belzung, 2013; Wu *et al.*, 2015), it is expectable that adult-born cells generated in the hippocampal DG may present different properties, depending on the location in which they integrate. Most likely, the location in which adult-generated cells integrate and establish their contacts within the preexisting DG network may determine their functional significance.

Furthermore, and besides those two "canonical" sites, new examples of cell genesis have been shown to occur in the so-called nonneurogenic regions of the mammalian CNS. Those regions include Neocortex, Corpus callosum, Piriform cortex, Olfactory tubercle, Striatum, Septum, Amygdala, Thalamus, Hypothalamus, *Substantia nigra*, Cerebellum and Brain Stem (Feliciano *et al.*, 2015), suggesting that structural plasticity involving *de novo* neural cell genesis could be more widespread than previously thought.

Hippocampal cytogenesis in now known to encompass not only neurogenesis, but also gliogenesis, the process by which newborn glial cells are generated. Studies in this field had shown that stress can have an impact in the integration of these new generated cells (Rajkowska & Miguel-Hidalgo, 2007). In this way, some studies had suggested that slower neuroplastic changes, regarding neurogenesis and remodeling of the neuro-glial networks, are apparently necessary to determine the extent of recovery from

depressive symptoms (Mateus-Pinheiro *et al.*, 2013). In fact, while stress has been found to inhibit adult cytogenesis in the hippocampus – which is a region central to emotion, memory and learning regulation – ADs treatment has the ability to reverse the anti-cytogenic effects (Fuchs *et al.*, 2004; Banasr & Duman, 2007; Rajkowska & Miguel-Hidalgo, 2007). However, according to the current view, newborn cells in the hippocampus *per se* may not be critical for the development of depression but may be required for certain behavioral effects of ADs (Sahay & Hen, 2007).

In this way, several ADs can not only stimulate neurogenesis, but also exert similar stimulatory effects on gliogenesis (Czéh & Di Benedetto, 2013). Animal studies have shown that chronic stress can inhibit glial cell proliferation in the PFC, and that this inhibitory effect can be counteracted by AD treatment (Czéh *et al.*, 2007a). The significance of these observations is strengthened by *in vivo* neuroimaging studies in patients with mood disorders that consistently point to the involvement of prefrontal brain sites in the pathophysiology of this disorder. These imaging findings are further supported by reports on human post-mortem tissues revealing that the number of glial cells in the prefrontal cortex is adversely affected in patients with mood disorders (Rajkowska, 2000).

Concluding, several evidences show that a basal and stable physiological cytogenesis – generation of new neurons and glial cells - is crucial for a preserved emotional and cognitive domain, and that an impairment in this generation leads to cognitive and emotional deficits (Figure 4; capture on next page).

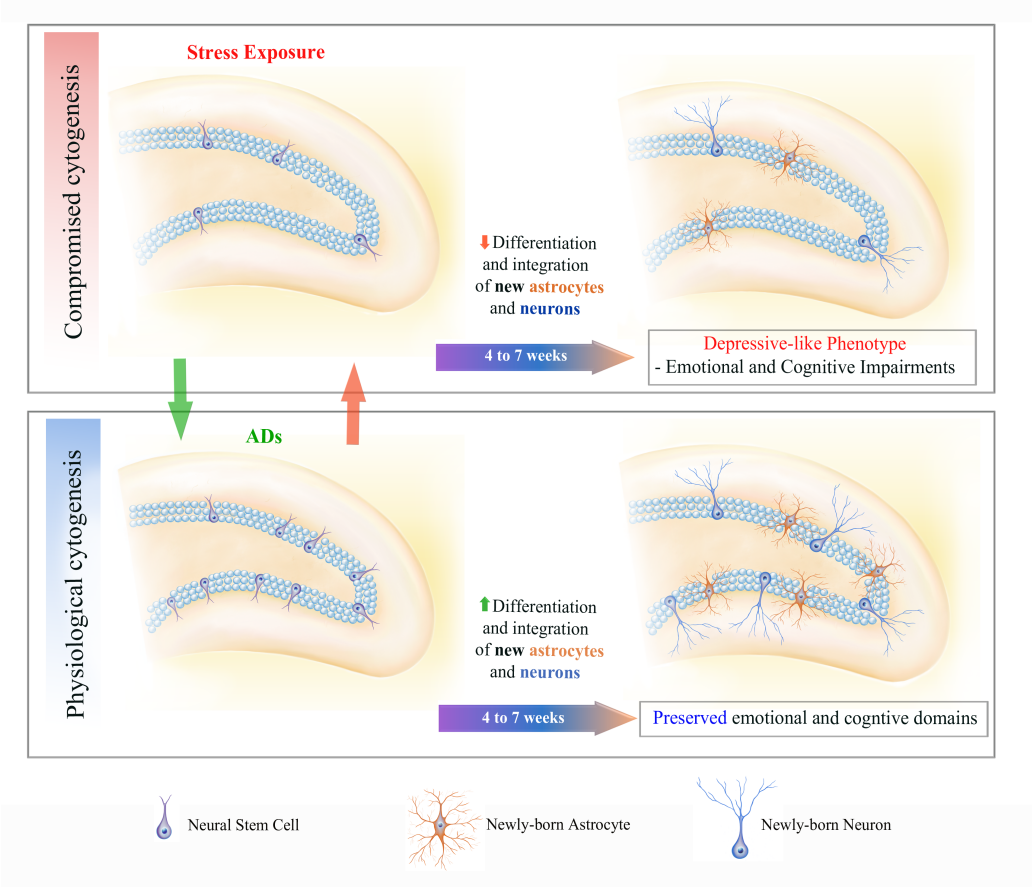

Figure 4: Preserved hippocampal neurogenesis and astrogliogenesis are required for normal emotional and cognitive functions. Chronic exposure to stress, a major precipitating factor of depression, produces a decrease in hippocampal neural stem cells (NSCs) pool, thus leading to a long-term decrease in integration of new neurons and astrocytes. Such compromised cytogenesis will contribute to the development of multi-dimensional behavioral deficits, such as behavioral despair, anhedonic behavior, anxiety-like behavior, impaired executive function and memory deficits. ADs can restore hippocampal cytogenesis, thus reverting this pathological behavioral profile. Although the importance of adult cytogenesis is well known, the individual contribution of astrogliogenesis remains to be elucidated.

6. Adult Neurogenesis and Astrocytic Biology: the missing connection

Along the entire scientific history, glial cells have always been neglected in disregard of neurons, establishing a "neurocentric theory of depression". In fact, glia´s key role in cortical and neuronal function was always underestimated and glial cells were usually seen only as neuronal partners with a supportive and secondary role (Coyle & Schwarz, 2000). However, this particular view has changed when it was found that NSCs in the developing brain and in the adult cytogenic zones exhibit astroglial properties (Morrens *et al.*, 2012). Immediately, glial cells jumped to the front-line studies, focused on uncovering the role of these cells. Glia was found to interact closely with neurons, contributing to brain metabolism, synaptic neurotransmission and in interneuron communication (Volterra & Meldolesi, 2005). Moreover, glial cells occupy about half the volume of the brain (Jessen, 2004) and are responsible for actively maintaining the tissue homeostasis (Devinsky *et al.*, 2013). These neuron-partners, are subdivided into distinct classes, possessing different structural and functional characteristics: Astrocytes, Oligodendrocytes, Microglia and NG2-positive cells.

As this work is mainly focused on astrocytes, we will only make a brief overview of the other glial cell types and give more emphasis to astrocytes.

Oligodendrocytes

These mature glial cells are mostly involved in the production of myelin in the brain and spinal cord (Bradl & Lassmann, 2010). Axons are unsheathed with this lipoprotein – Myelin – and, periodically, some gaps named as Nodes of Ranvier are formed (Morrens *et al.*, 2012), enabling axons to accelerate the conduction of the action potential (Hartline & Colman, 2007). The absence of myelin, called demyelination, severely compromises the conduction of the action potential trough the axon, resulting in several neurological deficits (Patel & Balabanov,2012). Due to the absence of myelin, axons can be even

more predisposed to severe injury because of the deprivation of the trophic effects of the oligodendrocytes (Trapp *et al.*, 1998; Waxman, 2001).

Oligodendrocyte progenitor cells are also generated in the SEZ (Morrens *et al.*, 2012) being, just like adult oligodendrocytes, very vulnerable to certain conditions such as inflammation and oxidative stress. Hence, several pathologies, such as spinal cord injury, Parkinson´s and Alzheimer´s diseases, lead to oligodendrocytes dysfunction or dead (McTigue & Tripathi, 2008).

Microglia

These parenchymal tissue macrophages constitute about 10% of all cells in the CNS (Aguzzi *et al.*, 2013). Microglia can have a ramified appearance, ordinarily found in the brain parenchyma, or be attached to the vasculature and within the perivascular extracellular matrix (ECM), named as perivascular microglia. Although microglia activation seems not to be pro- or anti-neurogenic *per se*, the molecules secreted by them are the ones responsible for the cellular net outcome (Morrens *et al.*, 2012). These cells can be activated by neurons and are responsible for eliminating and maintaining synapses, thus leading to a normal function of the neural circuit (Aguzzi *et al.*, 2013).

Microglia functions are closely related with phagocytosis, being these cells the ones responsible for eliminating the neural precursor cells and thus regulating adult neurogenesis (Sierra *et al.*, 2010). Several studies regarding the contribution of microglia to disease states have shown beneficial, adverse and dispensable functions of these cells, putting forward an angel/devil perspective of microglia (Morrens *et al.*, 2012). However, more studies are needed to really address the microglial function on both health and disease states.

NG2$^+$ cells

NG2 chondroitin Sulfate Proteoglycan expressing cells are the fifth main cell population in the CNS (Xu *et al.*, 2011), representing the most abundant population of proliferating cells in the adult brain (Dawson *et al.*, 2003). NG2 expressing cells are identified as oligodendrocyte progenitor cells due to their capability to differentiate into oligodendrocytes (Nishiyama *et al.*, 1997; Lu *et al.*, 2002; Zhou & Anderson, 2002; Kitada & Rowitch, 2006; Ligon *et al.*, 2006; Zhu *et al.*, 2008; Komitova *et al.*, 2009). However, data have shown that these cells can also give rise to subpopulations of astrocytes during normal development. Besides acting as a plastic progenitor pool for more differentiated cells, this cell population may constitute a unique glial network, interacting with neurons (Jabs *et al.*, 2005; Bergles *et al.*, 2010). NG2 expressing cells can be found in both neurogenic zones, although in a fewer percentage on the SGZ.

Some reports suggest a multipotency of these cells but are highly controversial (Nishiyama *et al.*, 2009; Richardson *et al.*, 2011). However, most of the studies have showed that NG2+ cells receive synaptic input from neurons and may be involved in glutamate signaling modulation (Bergles *et al.*, 2010; Mangin & Gallo, 2011).

Astrocytes

Astrocytes are the main glial subtype. Given that a single astrocyte can interact with as many as 100.000 synapses in mice and possibly with up to 2.000.000 synapses in humans (Bushong *et al.*, 2002; Oberheim *et al.*, 2009), astrocytes are much more than simple supportive cells. In fact, astrocytic dysfunction influences synaptic activity, shown by the modulation of neuronal circuits and behavior alterations by astrocytes (Volterra & Meldolesi, 2005).

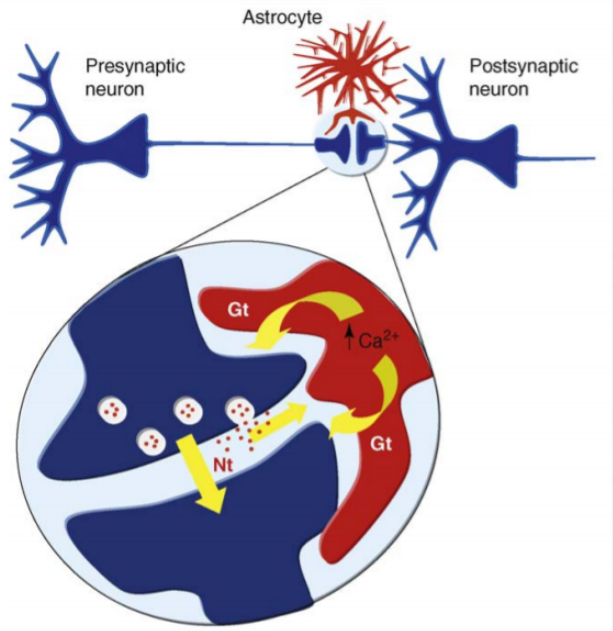

Figure 5: The tripartide synapse. Astrocytes respond with Ca^{2+} elevations to neurotransmitters released during synaptic activity and, in turn, control neuronal excitability and synaptic transmission through the Ca^{2+}- dependent release of gliotransmitters. Astrocytes express many of the same receptors as neurons. When neurotransmitters are released from the presynaptic terminal of a neuron, astrocytic receptors are thought to be activated, leading to a rise in calcium ions in the astrocyte and the release of various active substances, such as ATP, which act back on neurons to either inhibit or enhance neuronal activity. Astrocytes also release proteins, which control synapse formation, regulate presynaptic function and modulate the response of the postsynaptic neuron to neurotransmitters. Image and captions adapted from Allen & Barres, (2009).

As increased number of astrocyte-derived active substances were found, such as glutamate and D-serine, the concept of the "tripartite synapse" has been established (Araque *et al.*, 1999; Perea *et al.*, 2009; Perez-Alvarez *et al.*, 2014), which represents the capacity of astrocytes to interact closely with neurons and participating in the regulation of synaptic neurotransmission by releasing chemical transmitters (Araque *et al.*, 1999) (see Figure 5). Therefore, the term 'tripartite synapse' refers to a concept in synaptic physiology based on the demonstration of this bidirectional communication between astrocytes and neurons (Perea *et al.*, 2009).

These star-shaped cells have functional receptors for neurotransmitters and respond to their stimulation by releasing gliotransmitters, including glutamate. Astrocytes can increase their intracellular calcium levels ($[Ca2+]_i$) upon an elevation of synaptically released neurotransmitters, resulting in the release of glutamate via regulated exocytosis (Rossi & Volterra, 2009). Studies showed that this increase in $[Ca2+]_i$ is crucial, in a functional view, for astrocyte-astrocyte and also astrocyte-neuron intercellular communication (Sofroniew & Vinters, 2010; Cornell-Bell *et al.*, 1990; Charles *et al.*, 1991).

Astrocytes can also couple to neighboring astrocytes through gap junctions and play a role in both normal function and CNS disorders (Nedergaard *et al.*, 2003; Seifert *et al.*, 2006). These findings put astrocytes on the spotlight, giving rise to a new concept of neuron–astroglia intercommunication where astrocytes play an active role by integrating neuronal inputs and modulating synaptic activity (Rossi & Volterra, 2009). Moreover, unlike neurons, astrocytes can synthesize glutamate *de novo* and store glucose in the form of glycogen (Hertz & Zielke, 2004), thus contributing to brain metabolism (Hertz *et al.*, 2007). This phenomenon is only possible due to astrocytes´ high oxidative metabolism.

As previously mentioned, NSCs express several radial glia and astrocytic markers, including brain lipid binding-protein (BLPB) and the glutamate aspartate transporter - GLAST (Steiner *et al.*, 2006). The fact that NSCs in both neurogenic zones of the adult brain (SGZ and SEZ) have several astroglial properties, the link between adult neurogenesis and glial cells is obvious (Morrens *et al.*, 2012). In fact, genetic ablation of GFAP-expressing cells led to the elimination of adult neurogenesis (A. D. R. Garcia *et al.*, 2004; Imura *et al.*, 2003; Morshead *et al.*, 2003). Moreover, astrocytes from SEZ and SGZ were shown to promote the proliferation of progenitor cells and their neuronal differentiation *ex vivo* (Lim & Alvarez-Buylla, 1999; Song *et al.*, 2002). Although astroglia seems enough to support synaptic integration and functional maturation of newly born neurons (Hong-jun Song *et al.*, 2002), the same cells derived from a non-neurogenic region fosters glial over neurogenic fate (Lie *et al.*, 2002). *In vivo*, astrocytes seem to provide highly physical support to progenitor cells and newly born neurons (Shapiro *et al.*, 2005; Plümpe *et al.*, 2006), thus also playing a possible role in adult neurogenesis regulation.

7. GLIAL PLASTICITY AND ASTROGLIOGENESIS IN DEPRESSION

7.1. Glial plasticity of resident mature astrocytes

During the past 25 years, astrocytes have been pointed out as important players in brain function (Wang & Bordey, 2008; Perea *et al.*, 2009; Araque *et al.*, 2014). These star-shaped cells are known to possess unique phenotypic features that allow them to monitor their neighborhood and to dynamically respond to neurovascular changes (Wang & Bordey, 2008; Parpura & Verkhratsky, 2012). Astrocytes are in close contact with neurons, which favors the cross-talk between them, complementing and modulating the communication between pre- and post-synaptic structures (Araque *et al.*, 1999; Halassa, Fellin, *et al.*, 2009). Moreover, by expressing functional receptors for neurotransmitters, such as glutamate, ATP, acetylcholine and GABA, astrocytes have also the ability to sense the surrounding activity. Astrocytic activation may, in turn, lead to intracellular calcium signaling and to further release neuro- and vasoactive substances (e.g. D-serine, ATP, glutamate, GABA, prostaglandins or peptides) in a process named gliotransmission (Pascual *et al.*, 2005; Halassa, Florian, *et al.*, 2009; Henneberger *et al.*, 2010; Perea & Araque, 2010; Panatier *et al.*, 2011; Navarrete *et al.*, 2012; Perez-Alvarez *et al.*, 2014). This process may ultimately modulate synaptic function, metabolic support and homeostatic regulation (reviewed by Nedergaard et al., 2003; Maragakis and Rothstein, 2006; Wang and Bordey, 2008; Allen and Barres, 2009; Halassa and Haydon, 2010; Perea and Araque, 2010; Clarke and Barres, 2013; Araque et al., 2014; Volterra et al., 2014). Astrocytes from neurogenic regions of the adult brain, namely in the hippocampus, can even regulate adult neurogenesis by, either instructing neuronal fate commitment or/and promoting the adult neural stem cells proliferation (Song *et al.*, 2002a). This regulation is highly fine-tuned and helps the system to fulfill its needs during a pathological process triggered, for instance, by stress exposure.

Moreover, and regarding the functional properties of these cells, astrocytes were also found to respond to somatosensory stimuli (Navarrete *et al.*, 2012; Thrane *et al.*, 2012) and even to modulate spontaneous neuronal activity in vivo (Perea *et al.*, 2014).

Over the last years, a considerable number of studies has supported the putative involvement of astrocytes in several brain disorders (Parpura *et al.*, 2012a), namely in MDD (Rajkowska & Miguel-Hidalgo, 2007a; Hercher *et al.*, 2009; Miguel-Hidalgo *et al.*, 2010; Parpura *et al.*, 2012b; Verkhratsky *et al.*, 2013, 2014). In particular, several studies reported prominent decreases in the packing density and number of GFAP-positive astrocytes in different frontolimbic areas, including the medial prefrontal cortex (PFC), as well as in the dorsolateral and orbitofrontal cortex, the amygdala and the hippocampus, either

in animal models of depressive-like behavior (Czéh *et al.*, 2006; Banasr *et al.*, 2007; Banasr & Duman, 2007; Czéh *et al.*, 2007b; Gosselin *et al.*, 2009) or in postmortem brain tissue of subjects diagnosed with MDD (Ongür *et al.*, 1998; Rajkowska *et al.*, 1998, 1999; Rajkowska & Miguel-Hidalgo, 2007a).

Furthermore, other different markers of astrocytes were also found altered in postmortem brain tissue of depressive patients. Notably, the calcium-binding protein S100B was found altered in both serum and cerebrospinal fluid (Gos *et al.*, 2013) and the mRNA expression levels of glutamine synthetase – an enzyme that converts glutamate to glutamine - were reduced in the anterior cingulate and dorsolateral prefrontal cortex (Choudary *et al.*, 2005; Rajkowska & Miguel-Hidalgo, 2007a).

To further evidence the critical functions played by astroglial cells in behavior, the selective deletion of astrocytes in the medial PFC was enough to induce depressive-like behavior and to trigger cognitive impairments in rodents (Banasr & Duman, 2008; Lima *et al.*, 2014). Indeed, these studies suggested that the astrocytic damage will impair their homeostatic and modulatory duties and, therefore, will precede dendritic atrophy and neuronal loss, which could be largely responsible for the behavior impairments, similarly to those observed in models of depression.

Besides cell density alterations, astrocytic size and morphology also undergo alterations in MDD. Increased size of glial cell nuclei (Rajkowska *et al.*, 1999, 2001; Chana *et al.*, 2003) were also noted in depressed individuals and this alteration was proposed to be a compensatory mechanism for responding to the metabolic needs of the surrounding neurons. Since reduction in astrocytic density was followed by an increased nuclei volume of those cells, these authors explained that the remaining functional astrocytes could be "forced" to work, offsetting the overall astrocytic cell loss (Rajkowska & Miguel-Hidalgo, 2007a). Curiously, this adaptation of astrocytic nuclei appears to occur particularly in the context of depression, as it has not been found in other disorders, such as schizophrenia or Huntington's disease (Rajkowska *et al.*, 1998; Selemon *et al.*, 1998).

What could be called an "integrative neuron-glia theory" of depression has been progressively put forward, suggesting the requirement of functional astrocytes to process and integrate the neuronal information. Furthermore, this "integrative neuron-glia theory" of the neurobiology of depression is not only grounded in pathophysiological aspects of MDD but also in therapeutic actions promoted by ADs.

While AD treatments were believed to affect only neurons, a number of recent studies suggested that ADs also activate astrocytes (reviewed by Czéh and Di Benedetto, 2012). This astrocytic activation may, in turn, lead to reactivation of neuronal plasticity in cortical regions and cause the readjustment of the networks, thus helping depressed individuals to recover. Moreover, AD therapies were found to regulate not only the expression of GFAP and other astrocytic-related proteins, but also to regulate the expression

of astrocytic membrane receptors and to activate specific intracellular signaling pathways in astrocytes, such as the ERK/MAPK pathway (Mercier *et al.*, 2004; Hisaoka *et al.*, 2007; Li *et al.*, 2009; Czéh & Di Benedetto, 2013).

Along with these changes in astroglial physiology and morphology, ADs may also affect the generation of new astrocytes, a process named astrogliogenesis, which will have a final impact in cell densities by most likely counteracting the cellular loss observed in MDD (Czéh and Di Benedetto, 2012). However, it is still unclear whether different classes of ADs will have a differential pattern of astrogliogenesis modulation.

Overall, these pieces of evidence suggest that astrocytes are fundamental elements for both pathogenesis and treatment of MDD due to their close relationship and interdependence with neurons. Further studies are needed to dissect the importance of astrocytic alterations at the onset, maintenance and treatment of MDD. We have discussed the role of the resident pool of post-mitotic astrocytes in MDD. Next, we discuss the roles for newborn astrocytes in MDD and its treatment.

7.2. Astrogliogenesis

The formation of "new" astrocytes in the adult brain is becoming largely accepted due to the evidence gathered so far (Steiner *et al.*, 2004; Banasr & Duman, 2007; Rajkowska & Miguel-Hidalgo, 2007a; von Bohlen und Halbach, 2011; Ninkovic & Götz, 2013). However, the process that leads to astrogliogenesis and functional importance of these newly formed astrocytes to the emotional and cognitive domains in the context of MDD remains poorly understood.

After several years of controversy, it is now well accepted that radial glial cells in the developing central nervous system (CNS) are multipotent cells that have the capacity to give rise to separate precursors for neurons and mature glial cells (Campbell & Götz, 2002; Malatesta *et al.*, 2003). These glial precursors follow distinct differentiation pathways and may culminate in the generation of astrocytes due to specific factors in the microenvironment of the cell (Bonni *et al.*, 1997; Rajan & McKay, 1998; Freeman, 2010; Ge *et al.*, 2012). Indeed, unlike neurons, glial cells can retain their ability to proliferate in most brain areas of postnatal and adult subjects (Kraus-Ruppert *et al.*, 1975; Gensert & Goldman, 2001; Kornack & Rakic, 2001; Rajkowska & Miguel-Hidalgo, 2007a). Particularly, the generation of astrocytes is also detectable in the neocortex and hippocampus of the adult human brain (Eriksson *et al.*, 1998; Bhardwaj *et al.*, 2006). The vast majority of newborn cells in the adult rat hippocampal DG are neurons (about 75%), around 15% of new cells are positive for the astrocytic marker glial fibrillary acidic protein (GFAP) (Rajkowska & Miguel-Hidalgo, 2007a). Interestingly, this neuron-to-glia ratio does not suffer alterations

with AD treatment, suggesting that these treatments are also able to increase the number of newborn astrocytes in the adult brain (Rajkowska & Miguel-Hidalgo, 2007a). Additionally, pro-gliogenic factors are described to act in the proliferative zones of the adult brain and to participate in the pathophysiology of depression (Horner & Palmer, 2003). Those factors may be manipulated to achieve an effective AD action; for instance, bFGF, a known stimulator of astrocyte and oligodendrocyte proliferation (Hunter *et al.*, 1993; Castrén & Rantamäki, 2010; Ray *et al.*, 2014), was shown to be remarkably reduced in terms of mRNA levels on the dorsolateral PFC of depressed patients. Accordingly, ADs treatment was shown to increase bFGF expression in the hippocampus and neocortex in rats (Mallei *et al.*, 2002; Evans *et al.*, 2004; Maragnoli *et al.*, 2004). Moreover, studies showed that behavioral and cytogenesis impairments induced by unpredictable chronic mild stress (uCMS) exposure, a validated animal model of depression (Bessa *et al.*, 2009), were reversed both ADs fluoxetine and imipramine (Mateus-Pinheiro *et al.*, 2013). Interestingly, whereas fluoxetine failed to restore working memory when neurogenesis was blocked by a cytostatic agent, the cognitive-improving efficacy of imipramine did not depend on active neurogenesis. Fluoxetine treatment, as previously reported (Boldrini *et al.*, 2009), was more effective at promoting differentiation of newborn cells into neurons, contrarily to imipramine treatment that elicited a strong pro-gliogenic effect (Mateus-Pinheiro *et al.*, 2013). These results suggest that the efficacy of antidepressant drugs, namely tryciclic agents, in the recovery from depression may trigger preferentially astrogliogenesis-dependent mechanisms. However, the molecular changes that impair adult astrogliogenesis in the context of MDD, as well as its modulation by ADs are still largely unknown and need further clarification.

Furthermore, another abundant glial cell population – called NG2-glial cells - that is widely distributed throughout the mature CNS, was also found to change its proliferation profile after stress exposure (Alonso, 2000; Wennström *et al.*, 2006). These cells are known to possess many of the morphological features of astrocytes (Butt *et al.*, 2002, 2005) , being in close contact with neurons, astrocytes, oligodendrocytes and myelin-positive structures and are able to form direct synaptic junctions with both gray and white matter axons (Lin *et al.*, 2005; Ge *et al.*, 2009; Nishiyama *et al.*, 2009). Moreover, recent studies have shown that these stellate cells, after glucocorticoid treatment, decreased their proliferation in the adult rat hippocampus (Alonso, 2000; Wennström *et al.*, 2006). Besides this effect, exposure to chronic unpredictable stress produced the same decrease in proliferation in the prelimbic cortex of rats, which was counteracted by fluoxetine treatment (Banasr *et al.*, 2007; Czéh *et al.*, 2007b). These alterations in gliogenesis may be related with the changes in glial cell number reported in the fronto-limbic areas of depressed patients (Rajkowska & Miguel-Hidalgo, 2007a).

Besides all the recent findings regarding this topic, a fundamental problem lies on the adoption of experimental approaches for cytogenesis ablation that simultaneously ablate both neuronal or astroglial lineages. The use of such approaches has precluded our ability to withdraw valid conclusions concerning the individual participation of each newborn cell type for the pathogenesis and treatment of MDD. Hence, overcoming the previous mentioned technical impediment will surely pave the way to a better understanding of the importance of astrogliogenesis in MDD and eventually allow the identification of astrogliogenic factors as therapeutic targets in the treatment of the disease.

References

Aguzzi, A., Barres, B.A., & Bennett, M.L. (2013) Microglia: Scapegoat, Saboteur, or Something Else? *Science (80).*, **339**, 156–161.

Allen, N.J. & Barres, B.A. (2009) Neuroscience: glia—more than just brain glue. *Nature*, **457**, 675–677.

Alonso, G. (2000) Prolonged corticosterone treatment of adult rats inhibits the proliferation of oligodendrocyte progenitors present throughout white and gray matter regions of the brain. *Glia*, **31**, 219–231.

Ambrogini, P., Lattanzi, D., Ciuffoli, S., Agostini, D., Bertini, L., Stocchi, V., Santi, S., & Cuppini, R. (2004) Morpho-functional characterization of neuronal cells at different stages of maturation in granule cell layer of adult rat dentate gyrus. *Brain Res.*, **1017**, 21–31.

Araque, A., Carmignoto, G., Haydon, P.G., Oliet, S.H.R., Robitaille, R., & Volterra, A. (2014) Gliotransmitters travel in time and space. *Neuron*, **81**, 728–739.

Araque, A., Parpura, V., Sanzgiri, R.P., & Haydon, P.G. (1999) Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.*, **22**, 208–215.

Baldessarini, R.J., Lau, W.K., Sim, J., Sum, M.Y., & Sim, K. (2015) Duration of Initial Antidepressant Treatment and Subsequent Relapse of Major Depression. *J. Clin. Psychopharmacol.*, **35**, 75–76.

Banasr, M. & Duman, R.S. (2007) Regulation of neurogenesis and gliogenesis by stress and antidepressant treatment. *CNS Neurol. Disord. Drug Targets*, **6**, 311–320.

Banasr, M. & Duman, R.S. (2008) Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol. Psychiatry*, **64**, 863–870.

Banasr, M., Valentine, G.W., Li, X.Y., Gourley, S.L., Taylor, J.R., & Duman, R.S. (2007) Chronic Unpredictable Stress Decreases Cell Proliferation in the Cerebral Cortex of the Adult Rat. *Biol. Psychiatry*, **62**, 496–504.

Bergles, D.E., Jabs, R., & Steinhäuser, C. (2010) Neuron-glia synapses in the brain. *Brain Res. Rev.*, **63**, 130–137.

Bessa, J.M., Mesquita, A.R., Oliveira, M., Pêgo, J.M., Cerqueira, J.J., Palha, J. a, Almeida, O.F.X., & Sousa, N. (2009) A trans-dimensional approach to the behavioral aspects of depression. *Front. Behav. Neurosci.*, **3**, 1.

Bhardwaj, R.D., Curtis, M.A., Spalding, K.L., Buchholz, B.A., Fink, D., Björk-Eriksson, T., Nordborg, C., Gage, F.H., Druid, H., Eriksson, P.S., & Frisén, J. (2006) Neocortical neurogenesis in humans is restricted to development. *Proc. Natl. Acad. Sci. U. S. A.*, **103**, 12564–12568.

Biebl, M., Cooper, C.M., Winkler, J., & Kuhn, H.G. (2000) Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. *Neurosci. Lett.*, **291**, 17–20.

Blanco, C., Okuda, M., Markowitz, J.C., Liu, S.-M., Grant, B.F., & Hasin, D.S. (2010) The Epidemiology of Chronic Major Depressive Disorder and Dysthymic Disorder. *J. Clin. Psychiatry*, **71**, 1645–1656.

Boku, S., Nakagawa, S., Toda, H., & Hishimoto, A. (2018) Neural basis of major depressive disorder: Beyond monoamine hypothesis. *Psychiatry Clin. Neurosci.*, **72**, 3–12.

Boldrini, M., Underwood, M.D., Hen, R., Rosoklija, G.B., Dwork, A.J., John Mann, J., & Arango, V. (2009) Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology*, **34**, 2376–2389.

Bonfanti, L. & Ponti, G. (2008) Adult mammalian neurogenesis and the New Zealand white rabbit. *Vet. J.*, **175**, 310–331.

Bonni, A., Sun, Y., Nadal-Vicens, M., Bhatt, A., Frank, D.A., Rozovsky, I., Stahl, N., Yancopoulos, G.D., & Greenberg, M.E. (1997) Regulation of gliogenesis in the central nervous system by the JAK-STAT signaling pathway. *Science (80-.).*, **278**, 477–483.

Bradl, M. & Lassmann, H. (2010) Oligodendrocytes: biology and pathology. *Acta Neuropathol.*, **119**, 37–53.

Brody, D.J., Pratt, L.A., & Hughes, J.P. (2018) Prevalence of Depression Among Adults Aged 20 and Over: United States, 2013-2016. *NCHS Data Brief*, 1–8.

Bushong, E.A., Martone, M.E., Jones, Y.Z., & Ellisman, M.H. (2002) Protoplasmic Astrocytes in CA1 Stratum Radiatum Occupy Separate Anatomical Domains. *J. Neurosci.*, **22**, 183–192.

Butt, A.M., Hamilton, N., Hubbard, P., Pugh, M., & Ibrahim, M. (2005) Synantocytes: the fifth element. *J. Anat.*, **207**, 695–706.

Butt, A.M., Kiff, J., Hubbard, P., & Berry, M. (2002) Synantocytes: new functions for novel NG2 expressing glia. *J. Neurocytol.*, **31**, 551–565.

Campbell, K. & Götz, M. (2002) Radial glia: multi-purpose cells for vertebrate brain development. *Trends Neurosci.*, **25**, 235–238.

Castrén, E. & Rantamäki, T. (2010) The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. *Dev. Neurobiol.*, **70**, 289–297.

Chana, G., Landau, S., Beasley, C., Everall, I.P., & Cotter, D. (2003) Two-dimensional assessment of cytoarchitecture in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia: evidence for decreased neuronal somal size and increased neuronal density. *Biol. Psychiatry*, **53**, 1086–1098.

Charles, A.C., Merrill, J.E., Dirksen, E.R., & Sanderson, M.J. (1991) Intercellular signaling in glial cells: calcium waves and oscillations in response to mechanical stimulation and glutamate. *Neuron*, **6**, 983–992.

Charney, D.S. & Nelson, J.C. (1981) Delusional and nondelusional unipolar depression: further evidence for distinct subtypes. *Am. J. Psychiatry*, **138**, 328–333.

Choudary, P. V, Molnar, M., Evans, S.J., Tomita, H., Li, J.Z., Vawter, M.P., Myers, R.M., Bunney, W.E., Akil, H., Watson, S.J., & Jones, E.G. (2005) Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proc. Natl. Acad. Sci. U. S. A.*, **102**, 15653–15658.

Clarke, L.E. & Barres, B.A. (2013) Emerging roles of astrocytes in neural circuit development. *Nat. Rev. Neurosci.*, **14**, 311–321.

Clelland, C.D., Choi, M., Romberg, C., Clemenson, G.D., Fragniere, A., Tyers, P., Jessberger, S., Saksida, L.M., Barker, R.A., Gage, F.H., & Bussey, T.J. (2009) A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science (80-.).*, **325**, 210–213.

Cornell-Bell, A.H., Finkbeiner, S.M., Cooper, M.S., & Smith, S.J. (1990) Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science (80-.).*, New Series, **247**, 470–473.

Coyle, J.T. & Schwarz, R. (2000) Mind glue: Implications of glial cell biology for psychiatry. *Arch. Gen. Psychiatry*, **57:90 –93**.

Culpepper, L. (2013) Reducing the Burden of Difficult-to-Treat Major Depressive Disorder. *Prim. Care Companion CNS Disord.*,.

Czéh, B. & Di Benedetto, B. (2013) Antidepressants act directly on astrocytes: Evidences and functional consequences. *Eur. Neuropsychopharmacol.*, **23**, 171–185.

Czéh, B., Müller-Keuker, J.I.H., Rygula, R., Abumaria, N., Hiemke, C., Domenici, E., & Fuchs, E. (2007a) Chronic Social Stress Inhibits Cell Proliferation in the Adult Medial Prefrontal Cortex: Hemispheric Asymmetry and Reversal by Fluoxetine Treatment. *Neuropsychopharmacology*, **32**, 1490–1503.

Czéh, B., Simon, M., Schmelting, B., Hiemke, C., & Fuchs, E. (2006) Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology*, **31**, 1616–1626.

Dale, E., Bang-Andersen, B., & Sánchez, C. (2015) Emerging mechanisms and treatments for depression beyond SSRIs and SNRIs. *Biochem. Pharmacol.*, **95**, 81–97.

Dawson, M.R.L., Polito, A., Levine, J.M., & Reynolds, R. (2003) NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol. Cell. Neurosci.*, **24**, 476–488.

Devinsky, O., Vezzani, A., Najjar, S., De Lanerolle, N.C., & Rogawski, M. a (2013) Glia and epilepsy: excitability and inflammation. *Trends Neurosci.*, **4**, 1–11.

Doetsch, F., Caillé, I., Lim, D.A., García-Verdugo, J.M., & Alvarez-Buylla, A. (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*, **97**, 703–716.

Duman, R.S. & Monteggia, L.M. (2006) A Neurotrophic Model for Stress-Related Mood Disorders. *Biol. Psychiatry*, **59**, 1116–1127.

El Refaey, H. & Amri, H.S. (2011) Effects of antidepressants on behavioral assessment in adolescent rats. *Bahrain Med. Bull.*, **33**(2).

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., & Gage, F.H. (1998) Neurogenesis in the adult human hippocampus. *Nat. Med.*, **4**, 1313–1317.

Espósito, M.S., Piatti, V.C., Laplagne, D.A., Morgenstern, N.A., Ferrari, C.C., Pitossi, F.J., & Schinder, A.F. (2005) Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J. Neurosci.*, **25**, 10074–10086.

Evans, S.J., Choudary, P. V, Neal, C.R., Li, J.Z., Vawter, M.P., Tomita, H., Lopez, J.F., Thompson, R.C., Meng, F., Stead, J.D., Walsh, D.M., Myers, R.M., Bunney, W.E., Watson, S.J., Jones, E.G., & Akil, H. (2004) Dysregulation of the fibroblast growth factor system in major depression. *Proc. Natl. Acad. Sci. U. S. A.*, **101**, 15506–15511.

Fanselow, M.S. & Dong, H.-W. (2010) Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? *Neuron*, **65**, 7–19.

Feliciano, D.M., Bordey, A., & Bonfanti, L. (2015) Noncanonical Sites of Adult Neurogenesis in the Mammalian Brain. *Cold Spring Harb. Perspect. Biol.*, **7**, a018846.

Femenía, T., Gómez-Galán, M., Lindskog, M., & Magara, S. (2012) Dysfunctional hippocampal activity affects emotion and cognition in mood disorders. *Brain Res.*, **1476**, 58–70.

Filippov, V., Kronenberg, G., Pivneva, T., Reuter, K., Steiner, B., Wang, L.P., Yamaguchi, M., Kettenmann, H., & Kempermann, G. (2003) Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. *Mol. Cell. Neurosci.*, **23**, 373–382.

Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestar, M.L., Heine-Suner, D., Cigudosa, J.C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T.D., Wu, Y.-Z., Plass, C., & Esteller, M. (2005) From The Cover: Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl. Acad. Sci.*, **102**, 10604–10609.

Freeman, M.R. (2010) Specification and morphogenesis of astrocytes. *Science*, **330**, 774–778.

Fuchs, E., Czéh, B., Kole, M.H.P., Michaelis, T., & Lucassen, P.J. (2004) Alterations of neuroplasticity in depression: the hippocampus and beyond. *Eur. Neuropsychopharmacol.*, **14**, S481–S490.

Fulford, A.J. & Harbuz, M.S. (2005) An introduction to the HPA axis. In *Techniques in the Behavioral and Neural Sciences*. pp. 43–65.

Gage, F.H. (2002) Neurogenesis in the adult brain. *J. Neurosci.*, **22**, 612–613.

Garcia, A.D.R., Doan, N.B., Imura, T., Bush, T.G., & Sofroniew, M. V (2004) GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. *Nat. Neurosci.*, **7**, 1233–1241.

Ge, W.-P., Miyawaki, A., Gage, F.H., Jan, Y.N., & Jan, L.Y. (2012) Local generation of glia is a major astrocyte source in postnatal cortex. *Nature*, **484**, 376–380.

Ge, W.-P., Zhou, W., Luo, Q., Jan, L.Y., & Jan, Y.N. (2009) Dividing glial cells maintain differentiated properties including complex morphology and functional synapses. *Proc. Natl. Acad. Sci. U. S. A.*, **106**, 328–333.

Gensert, J.M. & Goldman, J.E. (2001) Heterogeneity of cycling glial progenitors in the adult mammalian cortex and white matter. *J. Neurobiol.*, **48**, 75–86.

Gos, T., Schroeter, M.L., Lessel, W., Bernstein, H.-G., Dobrowolny, H., Schiltz, K., Bogerts, B., & Steiner, J. (2013) S100B-immunopositive astrocytes and oligodendrocytes in the hippocampus are differentially afflicted in unipolar and bipolar depression: a postmortem study. *J. Psychiatr. Res.*, **47**, 1694–1699.

Gosselin, R.-D., Gibney, S., O'Malley, D., Dinan, T.G., & Cryan, J.F. (2009) Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression. *Neuroscience*, **159**, 915–925.

Gotlib, I.H. & Joormann, J. (2010) Cognition and depression: current status and future directions. *Annu. Rev. Clin. Psychol.*, **6**, 285–312.

Grandel, H. & Brand, M. (2013) Comparative aspects of adult neural stem cell activity in vertebrates. *Dev. Genes Evol.*, **223**, 131–147.

Hagerty, B.M., Williams, R.A., Coyne, J.C., & Early, M.R. (1996) Sense of belonging and indicators of social and psychological functioning. *Arch. Psychiatr. Nurs.*, **10**, 235–244.

Halassa, M.M., Fellin, T., & Haydon, P.G. (2009) Tripartite synapses: roles for astrocytic purines in the control of synaptic physiology and behavior. *Neuropharmacology*, **57**, 343–346.

Halassa, M.M., Florian, C., Fellin, T., Munoz, J.R., Lee, S.Y., Abel, T., Haydon, P.G., & Frank, M.G. (2009) Astrocytic Modulation of Sleep Homeostasis and Cognitive Consequences of Sleep Loss. *Neuron*, **61**, 213–219.

Halassa, M.M. & Haydon, P.G. (2010) Integrated Brain Circuits: Astrocytic Networks Modulate Neuronal Activity and Behavior. *Annu. Rev. Physiol.*, **72**, 335–355.

Hammen, C.L. (2015) Stress and depression: old questions, new approaches. *Curr. Opin. Psychol.*, **4**, 80–85.

Hardeveld, F., Spijker, J., De Graaf, R., Nolen, W.A., & Beekman, A.T.F. (2009) Prevalence and predictors of recurrence of major depressive disorder in the adult population. *Acta Psychiatr. Scand.*, **122**, 184–191.

Hartline, D.K. & Colman, D.R. (2007) Rapid conduction and the evolution of giant axons and myelinated fibers. *Curr. Biol.*, **17**, R29–R35.

Hashimoto, K. (2019) Rapid-acting antidepressant ketamine, its metabolites and other candidates: A historical overview and future perspective. *Psychiatry Clin. Neurosci.*, **73**, 613–627.

Henkel, V., Mergl, R., Allgaier, A.-K., Kohnen, R., Möller, H.-J., & Hegerl, U. (2006) Treatment of depression with atypical features: A meta-analytic approach. *Psychiatry Res.*, **141**, 89–101.

Henneberger, C., Papouin, T., Oliet, S.H.R., & Dmitri, A. (2010) Europe PMC Funders Group Long term potentiation depends on release of D-serine from astrocytes **463**, 232–236.

Herbert, J., Goodyer, I.M., Grossman, A.B., Hastings, M.H., de Kloet, E.R., Lightman, S.L., Lupien, S.J., Roozendaal, B., & Seckl, J.R. (2006) Do Corticosteroids Damage the Brain? *J. Neuroendocrinol.*, **18**, 393–411.

Hercher, C., Turecki, G., & Mechawar, N. (2009) Through the looking glass: examining neuroanatomical evidence for cellular alterations in major depression. *J. Psychiatr. Res.*, **43**, 947–961.

Herman, J.P., McKlveen, J.M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., & Myers, B. (2016) Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. In *Comprehensive Physiology*. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 603–621.

Hertz, L., Peng, L., & Dienel, G.A. (2007) Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J. Cereb. blood flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab.*, **27**, 219–249.

Hertz, L. & Zielke, H.R. (2004) Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci.*, **27**, 735–743.

Hisaoka, K., Takebayashi, M., Tsuchioka, M., Maeda, N., Nakata, Y., & Yamawaki, S. (2007) Antidepressants increase glial cell line-derived neurotrophic factor production through monoamine-independent activation of protein tyrosine kinase and extracellular signal-regulated kinase in glial cells. *J. Pharmacol. Exp. Ther.*, **321**, 148–157.

Hollon, S.D., Shelton, R.C., Wisniewski, S., Warden, D., Biggs, M.M., Friedman, E.S., Husain, M., Kupfer, D.J., Nierenberg, A.A., Petersen, T.J., Shores-Wilson, K., & Rush, A.J. (2006) Presenting characteristics of depressed outpatients as a function of recurrence: Preliminary findings from the STAR*D clinical trial. *J. Psychiatr. Res.*, **40**, 59–69.

Horner, P.J. & Palmer, T.D. (2003) New roles for astrocytes: the nightlife of an “astrocyte”. La vida loca! *Trends Neurosci.*, **26**, 597–603.

Hunter, K.E., Sporn, M.B., & Davies, A.M. (1993) Transforming growth factor-betas inhibit mitogen-stimulated proliferation of astrocytes. *Glia*, **7**, 203–211.

Imura, T., Kornblum, H.I., & Sofroniew, M. V (2003) The predominant neural stem cell isolated from postnatal and adult forebrain but not early embryonic forebrain expresses GFAP. *J. Neurosci.*, **23**, 2824–2832.

Jabs, R., Pivneva, T., Hüttmann, K., Wyczynski, A., Nolte, C., Kettenmann, H., & Steinhäuser, C. (2005) Synaptic transmission onto hippocampal glial cells with hGFAP promoter activity. *J. Cell Sci.*, **118**, 3791–3803.

Jessen, K.R. (2004) Glial cells. *Int. J. Biochem. Cell Biol.*, **36**, 1861–1867.

Joiner, T., Coyne, J.C., & Blalock, J. (2004) On the interpersonal nature of depression: Overview and synthesis. In *The Interactional Nature of Depression: Advances in Interpersonal Approaches.* American Psychological Association, Washington, pp. 3–19.

Kempermann, G. (2012) New neurons for "survival of the fittest." *Nat. Rev. Neurosci.*, **13**, 727–736.

Kendler, K.S. (1983) Overview: a current perspective on twin studies of schizophrenia. *Am. J. Psychiatry*, **140**, 1413–1425.

Kennedy, S.H. (2008) Core symptoms of major depressive disorder: relevance to diagnosis and treatment. *Dialogues Clin. Neurosci.*, **10**, 271–277.

Kitada, M. & Rowitch, D.H. (2006) Transcription factor co-expression patterns indicate heterogeneity of oligodendroglial subpopulations in adult spinal cord. *Glia*, **54**, 35–46.

Komitova, M., Zhu, X., Serwanski, D.R., & Nishiyama, A. (2009) NG2 cells are distinct from neurogenic cells in the postnatal mouse subventricular zone. *J. Comp. Neurol.*, **512**, 702–716.

Kornack, D.R. & Rakic, P. (2001) Cell proliferation without neurogenesis in adult primate neocortex. *Science (80-.).*, **294**, 2127–2130.

Kraus-Ruppert, R., Laissue, J., Bürki, H., & Odartchenko, N. (1975) Kinetic studies on glial, Schwann and capsular cells labelled with [3H] thymidine in cerebrospinal tissue of young mice. *J. Neurol. Sci.*, **26**, 555–563.

Krishnan, K.R. (2007) Revisiting monoamine oxidase inhibitors. *J. Clin. Psychiatry*, **68 Suppl 8**, 35–41.

Krishnan, V. & Nestler, E.J. (2008) The molecular neurobiology of depression. *Nature*, **455**, 894–902.

Kuhn, H.G., Biebl, M., Wilhelm, D., Li, M., Friedlander, R.M., & Winkler, J. (2005) Increased generation of granule cells in adult Bcl-2-overexpressing mice: A role for cell death during continued hippocampal neurogenesis. *Eur. J. Neurosci.*, **22**, 8.

Kumar A, Pareek V, Faiq MA, Ghosh SK, Kumari C. ADULT NEUROGENESIS IN HUMANS: A Review of Basic Concepts, History, Current Research, and Clinical Implications. (2019) *Innov Clin Neurosci.*, **16**, 30–37.

Li, B., Zhang, S., Li, M., Hertz, L., & Peng, L. (2009) Chronic treatment of astrocytes with therapeutically relevant fluoxetine concentrations enhances cPLA2 expression secondary to 5-HT 2B-induced, transactivation-mediated ERK1/2 phosphorylation. *Psychopharmacology (Berl).*, **207**, 1–12.

Lie, D.C., Dziewczapolski, G., Willhoite, A.R., Kaspar, B.K., Shults, C.W., & Gage, F.H. (2002) The adult substantia nigra contains progenitor cells with neurogenic potential. *J. Neurosci.*, **22**, 6639–6649.

Ligon, K.L., Kesari, S., Kitada, M., Sun, T., Arnett, H.A., Alberta, J.A., Anderson, D.J., Stiles, C.D., & Rowitch, D.H. (2006) Development of NG2 neural progenitor cells requires Olig gene function. *Proc. Natl. Acad. Sci. U. S. A.*, **103**, 7853–7858.

Lim, D.A. & Alvarez-Buylla, A. (1999) Interaction between astrocytes and adult subventricular zone precursors stimulates neurogenesis. *Proc. Natl. Acad. Sci. U. S. A.*, **96**, 7526–7531.

Lima, A., Sardinha, V.M., Oliveira, A. F., Reis, M., Mota, C., Silva, M. A., Marques, F., Cerqueira, J.J., Pinto, L., Sousa, N., & Oliveira, J.F. (2014) Astrocyte pathology in the prefrontal cortex impairs the cognitive function of rats. *Mol. Psychiatry*, **19**, 834–841.

Lin, S.C., Huck, J.H.J., Roberts, J.D.B., Macklin, W.B., Somogyi, P., & Bergles, D.E. (2005) Climbing fiber innervation of NG2-expressing glia in the mammalian cerebellum. *Neuron*, **46**, 773–785.

Lindsey, B.W. & Tropepe, V. (2006) A comparative framework for understanding the biological principles of adult neurogenesis. *Prog. Neurobiol.*, **80**, 281–307.

Liu, R.-J. & Aghajanian, G.K. (2008) Stress blunts serotonin- and hypocretin-evoked EPSCs in prefrontal cortex: role of corticosterone-mediated apical dendritic atrophy. *Proc. Natl. Acad. Sci. U. S. A.*, **105**, 359–364.

Lu, Q.R., Sun, T., Zhu, Z., Ma, N., Garcia, M., Stiles, C.D., & Rowitch, D.H. (2002) Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. *Cell*, **109**, 75–86.

Lucassen, P.J., Heine, V.M., Muller, M.B., van der Beek, E.M., Wiegant, V.M., De Kloet, E.R., Joels, M., Fuchs, E., Swaab, D.F., & Czeh, B. (2006) Stress, depression and hippocampal apoptosis. *CNS Neurol. Disord. Drug Targets*, **5**, 531–546.

Lucassen, P.J., Pruessner, J., Sousa, N., Almeida, O.F.X., Van Dam, A.M., Rajkowska, G., Swaab, D.F., & Czéh, B. (2014a) Neuropathology of stress. *Acta Neuropathol.*, **127**, 109–135.

Lucassen, P. J., Toni, N., Kempermann, G., Frisen, J., Gage, F. H., & Swaab, D. F. (2019). Limits to human neurogenesis—really? *Molecular Psychiatry*. Nature Publishing Group.

Luine, V., Martinez, C., Villegas, M., María Magariños, A., & McEwen, B.S. (1996) Restraint stress reversibly enhances spatial memory performance. *Physiol. Behav.*, **59**, 27–32.

M. Stahl, S., Lee-Zimmerman, C., Cartwright, S., & Ann Morrissette, D. (2013) Serotonergic Drugs for Depression and Beyond. *Curr. Drug Targets*, **14**, 578–585.

Maciag, D., Hughes, J., O'Dwyer, G., Pride, Y., Stockmeier, C.A., Sanacora, G., & Rajkowska, G. (2010) Reduced Density of Calbindin Immunoreactive GABAergic Neurons in the Occipital Cortex in Major Depression: Relevance to Neuroimaging Studies. *Biol. Psychiatry*, **67**, 465–470.

Malatesta, P., Hack, M.A., Hartfuss, E., Kettenmann, H., Klinkert, W., Kirchhoff, F., & Götz, M. (2003) Neuronal or glial progeny: regional differences in radial glia fate. *Neuron*, **37**, 751–764.

Maletic, V., Robinson, M., Oakes, T., Iyengar, S., Ball, S.G., & Russell, J. (2007) Neurobiology of depression: an integrated view of key findings. *Int. J. Clin. Pract.*, **61**, 2030–2040.

Mallei, A., Shi, B., & Mocchetti, I. (2002) Antidepressant treatments induce the expression of basic fibroblast growth factor in cortical and hippocampal neurons. *Mol. Pharmacol.*, **61**, 1017–1024.

Mangin, J.-M. & Gallo, V. (2011) The curious case of NG2 cells: transient trend or game changer? *ASN Neuro*, **3**, e00052.

Maragakis, N.J. & Rothstein, J.D. (2006) Mechanisms of Disease: astrocytes in neurodegenerative disease. *Nat. Clin. Pract. Neurol.*, **2**, 679–689.

Maragnoli, M.E., Fumagalli, F., Gennarelli, M., Racagni, G., & Riva, M.A. (2004) Fluoxetine and olanzapine have synergistic effects in the modulation of fibroblast growth factor 2 expression within the rat brain. *Biol. Psychiatry*, **55**, 1095–1102.

Masi, G. & Brovedani, P. (2011) The Hippocampus, Neurotrophic Factors and Depression. *CNS Drugs*, **25**, 913–931.

Mateus-Pinheiro, A., Patrício, P., Bessa, J.M., Sousa, N., & Pinto, L. (2013) Cell genesis and dendritic plasticity: a neuroplastic pas de deux in the onset and remission from depression. *Mol. Psychiatry*, **18**, 748–750.

Mateus-Pinheiro, A., Pinto, L., Bessa, J.M., Morais, M., Alves, N.D., Monteiro, S., Patrício, P., Almeida, O.F.X., & Sousa, N. (2013) Sustained remission from depressive-like behavior depends on hippocampal neurogenesis. *Transl. Psychiatry*, **3**, e210–e210.

McTigue, D.M. & Tripathi, R.B. (2008) The life, death, and replacement of oligodendrocytes in the adult CNS. *J. Neurochem.*, **107**, 1–19.

Melo, A., Kokras, N., Dalla, C., Ferreira, C., Ventura-Silva, A. P., Sousa, N., & Pêgo, J. M. (2015) The positive effect on ketamine as a priming adjuvant in antidepressant treatment. *Transl. Psychiatry*, **5**, 5.

Mercier, G., Lennon, A.M., Renouf, B., Dessouroux, A., Ramaugé, M., Courtin, F., & Pierre, M. (2004) MAP kinase activation by fluoxetine and its relation to gene expression in cultured rat astrocytes. *J. Mol. Neurosci.*, **24**, 207–216.

Miguel-Hidalgo, J.J., Waltzer, R., Whittom, A.A., Austin, M.C., Rajkowska, G., & Stockmeier, C.A. (2010) Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. *J. Affect. Disord.*, **127**, 230–240.

Morrens, J., Van Den Broeck, W., & Kempermann, G. (2012) Glial cells in adult neurogenesis. *Glia*, **60**, 159–174.

Morshead, C.M., Garcia, a. D., Sofroniew, M. V., & van der Kooy, D. (2003) The ablation of glial fibrillary acidic protein-positive cells from the adult central nervous system results in the loss of forebrain neural stem cells but not retinal stem cells. *Eur. J. Neurosci.*, **18**, 76–84.

Mueller, T.I., Leon, A.C., Keller, M.B., Solomon, D.A., Endicott, J., Coryell, W., Warshaw, M., & Maser, J.D. (1999) Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. *Am. J. Psychiatry*, **156**, 1000–1006.

Navarrete, M., Perea, G., de Sevilla, D.F., Gómez-Gonzalo, M., Núñez, A., Martín, E.D., & Araque, A. (2012) Astrocytes Mediate In Vivo Cholinergic-Induced Synaptic Plasticity. *PLoS Biol.*, **10**, e1001259.

Nedergaard, M., Ransom, B., & Goldman, S.A. (2003) New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci.*, **26**, 523–530.

Nemeroff, C.B. & Vale, W.W. (2005) The neurobiology of depression: inroads to treatment and new drug discovery. *J. Clin. Psychiatry*, **66 Suppl 7**, 5–13.

Ninkovic, J. & Götz, M. (2013) Fate specification in the adult brain–lessons for eliciting neurogenesis from glial cells. *Bioessays*, **35**, 242–252.

Nishiyama, A., Komitova, M., Suzuki, R., & Zhu, X. (2009) Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. *Nat. Rev. Neurosci.*, **10**, 9–22.

Nishiyama, A., Yu, M., Drazba, J.A., & Tuohy, V.K. (1997) Normal and reactive NG2+ glial cells are distinct from resting and activated microglia. *J. Neurosci. Res.*, **48**, 299–312.

Oberheim, N.A., Takano, T., Han, X., He, W., Lin, J.H.C., Wang, F., Xu, Q., Wyatt, J.D., Pilcher, W., Ojemann, J.G., Ransom, B.R., Goldman, S.A., & Nedergaard, M. (2009) Uniquely Hominid Features of Adult Human Astrocytes. *J. Neurosci.*, **29**, 3276–3287.

Ongür, D., Drevets, W.C., & Price, J.L. (1998) Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc. Natl. Acad. Sci. U. S. A.*, **95**, 13290–13295.

Panatier, A., Vallée, J., Haber, M., Murai, K.K., Lacaille, J.-C., & Robitaille, R. (2011) Astrocytes are endogenous regulators of basal transmission at central synapses. *Cell*, **146**, 785–798.

Pariante, C.M. & Lightman, S.L. (2008) The HPA axis in major depression: classical theories and new developments. *Trends Neurosci.*, **31**, 464–468.

Parpura, V., Heneka, M.T., Montana, V., Oliet, S.H.R., Schousboe, A., Haydon, P.G., Stout, R.F., Spray, D.C., Reichenbach, A., Pannicke, T., Pekny, M., Pekna, M., Zorec, R., & Verkhratsky, A. (2012) Glial cells in (patho)physiology. *J. Neurochem.*, **121**, 4–27.

Parpura, V. & Verkhratsky, A. (2012) Homeostatic function of astrocytes: Ca2+ and Na+ signalling. *Transl. Neurosci.*, **3**, 334–344.

Pascual, O., Casper, K.B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J.-Y., Takano, H., Moss, S.J., McCarthy, K., & Haydon, P.G. (2005) Astrocytic purinergic signaling coordinates synaptic networks. *Science*, **310**, 113–116.

Patel, J. & Balabanov, R. (2012) Molecular mechanisms of oligodendrocyte injury in multiple sclerosis and experimental autoimmune encephalomyelitis. *Int. J. Mol. Sci.*, **13**, 10647–10659.

Patrício, P., Mateus-Pinheiro, A., Sousa, N., & Pinto, L. (2013) Re-cycling paradigms: cell cycle regulation in adult hippocampal neurogenesis and implications for depression. *Mol. Neurobiol.*, **48**, 84–96.

Perea, G. & Araque, A. (2010) GLIA modulates synaptic transmission. *Brain Res. Rev.*, **63**, 93–102.

Perea, G., Navarrete, M., & Araque, A. (2009) Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci.*, **32**, 421–431.

Perea, G., Yang, A., Boyden, E.S., & Sur, M. (2014) Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons in vivo. *Nat. Commun.*, **5**, 3262.

Pereira, V.S. & Hiroaki-Sato, V.A. (2018) A brief history of antidepressant drug development: from tricyclics to beyond ketamine. *Acta Neuropsychiatr.*, **30**, 307–322.

Perez-Alvarez, A., Navarrete, M., Covelo, A., Martin, E.D., & Araque, A. (2014) Structural and functional plasticity of astrocyte processes and dendritic spine interactions. *J. Neurosci.*, **34**, 12738–12744.

Petrik, D., Lagace, D.C., & Eisch, A.J. (2012) The neurogenesis hypothesis of affective and anxiety disorders: Are we mistaking the scaffolding for the building? *Neuropharmacology*, **62**, 21–34.

Pham, T.H. & Gardier, A.M. (2019) Fast-acting antidepressant activity of ketamine: highlights on brain serotonin, glutamate, and GABA neurotransmission in preclinical studies. *Pharmacol. Ther.*, **199**, 58–90.

Pittenger, C. & Duman, R.S. (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology*, **33**, 88–109.

Plümpe, T., Ehninger, D., Steiner, B., Klempin, F., Jessberger, S., Brandt, M., Römer, B., Rodriguez, G.R., Kronenberg, G., & Kempermann, G. (2006) Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neurosci.*, **7**, 77.

Pratt, L.A. & Brody, D.J. (2014) Depression in the U.S. household population, 2009-2012. *NCHS Data Brief*, 1–8.

Radley, J.J., Sisti, H.M., Hao, J., Rocher, A.B., McCall, T., Hof, P.R., McEwen, B.S., & Morrison, J.H. (2004) Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience*, **125**, 1–6.

Rajan, P. & McKay, R.D. (1998) Multiple routes to astrocytic differentiation in the CNS. *J. Neurosci.*, **18**, 3620–3629.

Rajkowska, G. (2000) Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. *Biol. Psychiatry*, **48**, 766–777.

Rajkowska, G., Halaris, A., & Selemon, L.D. (2001) Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biol. Psychiatry*, **49**, 741–752.

Rajkowska, G. & Miguel-Hidalgo, J. (2007) Gliogenesis and Glial Pathology in Depression. *CNS Neurol. Disord. - Drug Targets*, **6**, 219–233.

Rajkowska, G., Miguel-Hidalgo, J.J., Wei, J., Dilley, G., Pittman, S.D., Meltzer, H.Y., Overholser, J.C., Roth, B.L., & Stockmeier, C.A. (1999) Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol. Psychiatry*, **45**, 1085–1098.

Rajkowska, G., O'Dwyer, G., Teleki, Z., Stockmeier, C.A., & Miguel-Hidalgo, J.J. (2007) GABAergic neurons immunoreactive for calcium binding proteins are reduced in the prefrontal cortex in major depression. *Neuropsychopharmacology*, **32**, 471–482.

Rajkowska, G., Selemon, L.D., & Goldman-Rakic, P.S. (1998) Neuronal and glial somal size in the prefrontal cortex: a postmortem morphometric study of schizophrenia and Huntington disease. *Arch Gen Psychiatry*, **55**, 215–224.

Rakic, P. (2003) Elusive radial glial cells: historical and evolutionary perspective. *Glia*, **43**, 19–32.

Ray, M.T., Shannon Weickert, C., & Webster, M.J. (2014) Decreased BDNF and TrkB mRNA expression in multiple cortical areas of patients with schizophrenia and mood disorders. *Transl. Psychiatry*, **4**, e389.

Richardson, W.D., Young, K.M., Tripathi, R.B., & McKenzie, I. (2011) NG2-glia as multipotent neural stem cells: fact or fantasy? *Neuron*, **70**, 661–673.

Rizvi, S. & Kennedy, S. (2013) Management strategies for SSRIinduced sexual dysfunction. *J. Psychiatry Neurosci.*, **38**, E27–E28.

Rossi, D. & Volterra, A. (2009) Astrocytic dysfunction: insights on the role in neurodegeneration. *Brain Res. Bull.*, **80**, 224–232.

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., McGrath, P.J., Rosenbaum, J.F., Sackeim, H.A., Kupfer, D.J., Luther, J., & Fava, M. (2006) Acute and Longer-Term Outcomes in Depressed Outpatients Requiring One or Several Treatment Steps: A STAR*D Report. *Am. J. Psychiatry*, **163**, 1905–1917.

Sahay, A. & Hen, R. (2007) Adult hippocampal neurogenesis in depression. *Nat. Neurosci.*, **10**, 1110–1115.

Sapolsky, R.M. (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch. Gen. Psychiatry*, **57**, 925–935.

Sapolsky, R.M. (2003) Stress and plasticity in the limbic system. *Neurochem. Res.*, **28**, 1735–1742.

Sapolsky, R.M. (1985) Stress-Induced Suppression of Testicular Function in the Wild Baboon: Role of Glucocorticoids. *Endocrinology*, **116**, 2273–2278.

Seifert, G., Schilling, K., & Steinhäuser, C. (2006) Astrocyte dysfunction in neurological disorders: a molecular perspective. *Nat. Rev. Neurosci.*, **7**, 194–206.

Selemon, L.D., Rajkowska, G., & Goldman-Rakic, P.S. (1998) Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional, stereologic counting method. *J. Comp. Neurol.*, **392**, 402–412.

Selye, H. (1936) A Syndrome produced by Diverse Nocuous Agents. *Nature*, **138**, 32–32.

Shapiro, L.A., Korn, M.J., Shan, Z., & Ribak, C.E. (2005) GFAP-expressing radial glia-like cell bodies are involved in a one-to-one relationship with doublecortin-immunolabeled newborn neurons in the adult dentate gyrus. *Brain Res.*, **1040**, 81–91.

Shors, T.J. (2006) Stressful Experience and Learning Across the Lifespan. *Annu. Rev. Psychol.*, **57**, 55–85.

Sierra, A., Encinas, J.M., Deudero, J.J.P., Chancey, J.H., Enikolopov, G., Overstreet-Wadiche, L.S., Tsirka, S.E., & Maletic-Savatic, M. (2010) Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell*, **7**, 483–495.

Simon, G.E. (1996) Initial antidepressant choice in primary care. Effectiveness and cost of fluoxetine vs tricyclic antidepressants. *JAMA J. Am. Med. Assoc.*, **275**, 1897–1902.

Simons, A.D., Angell, K.L., Monroe, S.M., & Thase, M.E. (1993) Cognition and life stress in depression: Cognitive factors and the definition, rating, and generation of negative life events. *J. Abnorm. Psychol.*, **102**, 584–591.

Smith, K. (2014) Mental health: A world of depression. *Nature*, **515**, 180–181.

Sofroniew, M. V & Vinters, H. V (2010) Astrocytes: biology and pathology. *Acta Neuropathol.*, **119**, 7–35.

Son, H., Banasr, M., Choi, M., Chae, S.Y., Licznerski, P., Lee, B., Voleti, B., Li, N., Lepack, A., Fournier, N.M., Lee, K.R., Lee, I.Y., Kim, J., Kim, J.-H., Kim, Y.H., Jung, S.J., & Duman, R.S. (2012) Neuritin produces antidepressant actions and blocks the neuronal and behavioral deficits caused by chronic stress. *Proc. Natl. Acad. Sci.*, **109**, 11378–11383.

Song, H., Stevens, C.F., & Gage, F.H. (2002) Astroglia induce neurogenesis from adult neural stem cells. *Nature*, **417**, 39–44.

Song, H., Stevens, C.F., & Gage, F.H. (2002) Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat. Neurosci.*, **5**, 438–445.

Sousa, N. (2016) The dynamics of the stress neuromatrix. *Mol. Psychiatry*, **21**, 302–312.

Sousa, N., & Almeida, O. F. X. (2012) Disconnection and reconnection: The morphological basis of (mal)adaptation to stress. *Trends in Neurosciences*, **35**, 12

Steiner, B., Klempin, F., Wang, L., Kott, M., Kettenmann, H., & Kempermann, G. (2006) Type-2 Cells as Link Between Glial and Neuronal Lineage in Adult Hippocampal Neurogenesis **814**, 805–814.

Steiner, B., Kronenberg, G., Jessberger, S., Brandt, M.D., Reuter, K., & Kempermann, G. (2004) Differential Regulation of Gliogenesis in the Context of Adult Hippocampal Neurogenesis in Mice. *Glia*, **46**, 41–52.

Sullivan, P.F., Neale, M.C., & Kendler, K.S. (2000) Genetic Epidemiology of Major Depression: Review and Meta-Analysis. *Am. J. Psychiatry*, **157**, 1552–1562.

Tanti, A. & Belzung, C. (2013) Neurogenesis along the septo-temporal axis of the hippocampus: Are depression and the action of antidepressants region-specific? *Neuroscience*, **252**, 234–252.

Thapar, A., Collishaw, S., Pine, D.S., & Thapar, A.K. (2012) Depression in adolescence. *Lancet*, **379**, 1056–1067.

Thrane, A.S., Rangroo Thrane, V., Zeppenfeld, D., Lou, N., Xu, Q., Nagelhus, E.A., & Nedergaard, M. (2012) General anesthesia selectively disrupts astrocyte calcium signaling in the awake mouse cortex. *Proc. Natl. Acad. Sci.*, **109**, 18974–18979.

Trapp, B.D., Peterson, J., Ransohoff, R.M., Rudick, R., Mörk, S., & Bö, L. (1998) Axonal transection in the lesions of multiple sclerosis. *N. Engl. J. Med.*, **338**, 278–285.

Van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., & Gage, F.H. (2002) Functional neurogenesis in the adult hippocampus. *Nature*, **415**, 1030–1034.

Verkhratsky, A., Rodríguez, J.J., & Parpura, V. (2013) Astroglia in neurological diseases. *Future Neurol.*, **8**, 149–158.

Verkhratsky, A., Rodríguez, J.J., & Steardo, L. (2014) Astrogliopathology: a central element of neuropsychiatric diseases? *Neuroscientist*, **20**, 576–588.

Volterra, A., Liaudet, N., & Savtchouk, I. (2014) Astrocyte Ca(2+) signalling: an unexpected complexity. *Nat. Rev. Neurosci.*, **15**, 327–335.

Volterra, A. & Meldolesi, J. (2005) Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci.*, **6**, 626–640.

von Bohlen und Halbach, O. (2011) Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus. *Cell Tissue Res.*, **345**, 1–19.

Wainwright, S.R. & Galea, L.A.M. (2013) The Neural Plasticity Theory of Depression: Assessing the Roles of Adult Neurogenesis and PSA-NCAM within the Hippocampus. *Neural Plast.*, **2013**, 1–14.

Wang, D.D. & Bordey, A. (2008) The astrocyte odyssey. *Prog. Neurobiol.*, **86**, 342–367.

Warner-Schmidt, J.L. & Duman, R.S. (2006) Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus*, **16**, 239–249.

Waxman, S.G. (2001) Acquired channelopathies in nerve injury and MS. *Neurology*, **56**, 1621–1627.

Wennström, M., Hellsten, J., Ekstrand, J., Lindgren, H., & Tingström, A. (2006) Corticosterone-induced inhibition of gliogenesis in rat hippocampus is counteracted by electroconvulsive seizures. *Biol. Psychiatry*, **59**, 178–186.

Wilbrecht, L., Holtmaat, A., Wright, N., Fox, K., & Svoboda, K. (2010) Structural Plasticity Underlies Experience-Dependent Functional Plasticity of Cortical Circuits. *J. Neurosci.*, **30**, 4927–4932.

Wu, M. V., Sahay, A., Duman, R.S., & Hen, R. (2015) Functional Differentiation of Adult-Born Neurons along the Septotemporal Axis of the Dentate Gyrus. *Cold Spring Harb. Perspect. Biol.*, **7**, a018978.

Xu, J.-P., Zhao, J., & Li, S. (2011) Roles of NG2 glial cells in diseases of the central nervous system. *Neurosci. Bull.*, **27**, 413–421.

Zhao, C., Teng, E.M., Summers, R.G., Ming, G.-L., & Gage, F.H. (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J. Neurosci.*, **26**, 3–11.

Zhou, Q. & Anderson, D.J. (2002) The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. *Cell*, **109**, 61–73.

Zhu, X., Hill, R.A., & Nishiyama, A. (2008) NG2 cells generate oligodendrocytes and gray matter astrocytes in the spinal cord. *Neuron Glia Biol.*, **4**, 19–26.

Chapter 2

BACKGROUND AND AIMS

Despite the great advances in the past few decades, our understanding of the pathophysiology of depression and of the action of antidepressants on neuronal plasticity is still limited. Moreover, most of the studies performed so far in this field have been focusing on neurons in disregard of glial cells. Therefore, the focus of researchers must be driven forward in this field.

In this thesis, we focused our analysis in a specific brain region reported to be involved in the pathophysiology of depression – the hippocampus. Specifically in the dentate gyrus (DG), a subregion of the hippocampus, alterations in neuronal plasticity and in citogenesis have been identified and associated with depression. Specifically, and although the vast majority of newborn cells in the adult rat hippocampal DG are neurons, around 15% of new cells are positive for the astrocytic marker glial fibrillary acidic protein (GFAP). Therefore, in order to better understand the molecular mechanisms regulating the astroplasticity in this brain region, in depression and after antidepressants treatment, we used a robust and validated rat model of depression, the unpredictable chronic mild stress model (uCMS) and *in vitro* approaches.

In this context, two major aims were established:

1. Perform a comprehensive characterization of the hippocampal DG astrocytic plasticity and astrogliogenesis in depressive-like animals and after treatment with two antidepressants from different pharmacological classes (fluoxetine and imipramine); correlate this astrocytic changes with the cognitive behavior of the animals (Chapter 3).

2. Understand the dynamic changes of astrocytes in the hippocampal DG immediately after stress exposure and in a relapse episode (when animals were exposed to a second hit of stress) (Chapter 4).

Chapter 3

IMIPRAMINE TREATMENT ACTS AS A PRO-ASTROGLIOGENIC FACTOR IN THE HIPPOCAMPAL DENTATE GYRUS AND RESCUES COGNITIVE IMPAIRMENTS INDUCED BY STRESS EXPOSURE

Manuscript to be submitted

Imipramine treatment acts as a pro-astrogliogenic factor in the hippocampal dentate gyrus and rescues cognitive impairments induced by stress exposure

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Abstract

Major depression is a highly prevalent disorder that poses a significant social burden in society. The pathophysiology of this disease is still poorly understood but growing evidence suggests that impaired neuroplasticity may be a key underlying mechanism for the precipitation of the disorder. This theory is substantiated by the fact that depression leads to a decrease in neurogenesis and several antidepressants (ADs) stimulate hippocampal neurogenesis, although it is still unclear if these pro-neurogenic effects are responsible for their mood-, emotional- and cognitive-improving actions. Moreover, recent studies also showed an important role for astrocytes in the pathophysiology of this disorder, which are crucial for neurotransmission and neurovascular coupling, evidenced by astrocytes loss in major depressive disorder (MDD). However, the importance of astrocytes in the precipitation of and recovery from MDD is still largely unknown.

Therefore, we proposed to study the modulation of astrocytes and adult astrogliogenesis in the hippocampal dentate gyrus (DG) of rats exposed to unpredictable chronic mild stress (uCMS), untreated and treated with ADs in a longitudinal manner – immediately after stress exposure (short-term; tp1) and four weeks after stress exposure (long-term; tp2).

Stress exposure led to immediate cognitive impairments (tp1) both on short and long-term memory, although animals could fully recover from those deficits 4 weeks after stress exposure (tp2). Moreover, our results showed that stress did not induce any immediate response on hippocampal DG astrocytic density. However, 4 weeks after stress exposure, astrocytes were shown to be decreased in this specific brain region. Moreover, we show here that imipramine promotes a pro-astrogliogenic response in the hippocampal DG of rats exposed to stress, 4 weeks after treatment, and rescues cognitive impairments induced by stress exposure. On the other hand, fluoxetine modulates astrocytic morphology, promoting an astrocytic hypertrophy of both pre-existent and newborn astrocytes in the hippocampal DG 4 weeks after stress exposure.

Our results show that adult hippocampal DG astrogliogenesis is modulated by stress and AD treatment, possibly being correlated with the induced behavioral alterations. Importantly, distinct classes of ADs impact differently in the astrogliogenic process, showing different cellular mechanisms relevant for the recovery from behavioral deficits induced by stress. As such, hippocampal DG resident and newborn astrocytes might be additional promising therapeutic targets for future therapies in the neuropsychiatric field.

Keywords: Astrocytes; Dentate gyrus; Astrogliogenesis; Chronic stress; Antidepressants.

INTRODUCTION

Major depressive disorder (MDD) is a prevalent and common neuropsychiatric disorder affecting around 16% of the population worldwide, which experiences one or several episodes of depression during their lifetime. According to the WHO - data from 2018 -, more than 300 million people are affected worldwide, with around 800.000 people dying every year due to suicide. However, mechanisms involved in the emergence of depressive episodes are multifactorial and not yet fully understood (van Calker *et al.*, 2009). Several hypotheses have been proposed to explain the neurobiological mechanisms underlying the onset, maintenance and recovery from this disorder (Duman & Monteggia, 2006; Maletic *et al.*, 2007; Sahay & Hen, 2007; Patrício *et al.*, 2013). During the last three decades, a significant number of studies in this field revealed cell loss and neuronal atrophy, particularly in brain loci relevant for emotional behavior control (Radley *et al.*, 2004; Lucassen *et al.*, 2006; Rajkowska & Miguel-Hidalgo, 2007a; Rajkowska *et al.*, 2007; Banasr & Duman, 2008; Liu & Aghajanian, 2008; Maciag *et al.*, 2010). Multiple mechanisms were proposed to be responsible for this neuronal atrophy, namely glucocorticoid and glutamate toxicity for both astrocytes and neurons (Abrahám *et al.*, 1998), decreased expression of neurotrophic factors (Lee *et al.*, 2002; Sairanen *et al.*, 2005), and, more interestingly, decreased neuroplasticity (Bessa et al. 2009a; Mateus-Pinheiro *et al.* 2013) in animal models of depression. However, most of the evidence gathered so far focus on neuronal cells in disregard of glial cells.

In fact, it is now well recognized that glial cells, namely astrocytes, undergo several plastic alterations both in the healthy and depressed brain, puting behind the idea of being only neuronal supportive cells (Manji *et al.*, 2000; Rajkowska & Miguel-Hidalgo, 2007a; Bélair *et al.*, 2010; Ben Achour & Pascual, 2010; Miguel-Hidalgo *et al.*, 2010; Verkhratsky *et al.*, 2013, 2014).

In particular, several studies reported prominent decreases in the packing density and number of GFAP-positive astrocytes in different frontolimbic areas, including the medial prefrontal cortex (PFC), as well as in the dorsolateral and orbitofrontal cortex, the amygdala and the hippocampus, either in animal models of depressive-like behavior (Czéh *et al.*, 2006; Banasr *et al.*, 2007; Banasr & Duman, 2007; Czéh *et al.*, 2007; Gosselin *et al.*, 2009a) or in postmortem brain tissue of subjects diagnosed with MDD (Ongür *et al.*, 1998; Rajkowska *et al.*, 1998a, 1999a; Rajkowska & Miguel-Hidalgo, 2007a). Furthermore, S100B, a selective marker of mature astrocytes, was also found altered in postmortem brain tissue of depressive patients (Gos *et al.*, 2013).

Besides cell density alterations, astrocytic size and morphology also undergo alterations in MDD. Increased size of glial cell nuclei (Rajkowska *et al.*, 1999a, 2001; Chana *et al.*, 2003) were also noted specifically in depressed individuals and this alteration was proposed to be a compensatory mechanism

for responding to the metabolic needs of the surrounding neurons (Rajkowska *et al.*, 1998b; Selemon *et al.*, 1998; Rajkowska & Miguel-Hidalgo, 2007b; Machado-Santos *et al.*, 2019).

The crucial functions played by astroglial cells in behavior were also explored in several contexts (reviewed by Oliveira, Sardinha, Guerra-Gomes, Araque, & Sousa, 2015). Specifically, selective deletion of astrocytes in the medial PFC was enough to induce depressive-like behavior and to trigger cognitive impairments in rodents (Banasr & Duman, 2008; Lima *et al.*, 2014; Oliveira *et al.*, 2015). Therefore, these studies have gathered evidences regarding the key roles of astrocytes in the depressive contexts.

Furthermore, the formation of "new" astrocytes in the adult brain is becoming widely accepted due to the evidence gathered hitherto (Steiner *et al.*, 2004; Banasr & Duman, 2007; Rajkowska & Miguel-Hidalgo, 2007a; von Bohlen und Halbach, 2011; Ninkovic & Götz, 2013). Indeed, unlike neurons, glial cells can retain their ability to proliferate in most brain areas of postnatal and adult subjects (Kraus-Ruppert *et al.*, 1975; Gensert & Goldman, 2001; Kornack & Rakic, 2001; Rajkowska & Miguel-Hidalgo, 2007a). Particularly, the generation of astrocytes is also detectable in the neocortex and hippocampus of the adult human brain (Eriksson *et al.*, 1998; Bhardwaj *et al.*, 2006). It is now known that around 15% of newborn cells in the adult rat hippocampal DG are positive for the astrocytic marker glial fibrillary acidic protein (GFAP) (Rajkowska & Miguel-Hidalgo, 2007a). Interestingly, the neuron-to-glia ratio does not suffer alterations with AD treatment, suggesting that these treatments are also able to increase the number of newborn astrocytes in the adult brain (Rajkowska & Miguel-Hidalgo, 2007a).

Moreover, glucocorticoid treatment was able to decrease astrocytic proliferation in the adult rat hippocampus (Alonso, 2000; Wennström *et al.*, 2006). Besides this effect, exposure to chronic unpredictable stress produced the same decrease in proliferation in the prelimbic cortex of rats, which was counteracted by fluoxetine treatment (Banasr *et al.*, 2007; Czéh *et al.*, 2007). Therefore, it becomes necessary to clarify how the newborn astrocytes are modulated by stress and ADs treatment, and which molecular changes are impairing adult astrogliogenesis in the context of MDD.

Hence, to better understand the modulation of astrogliogenic plasticity in the context of depression, we longitudinally assessed dynamic alterations of resident and newborn astrocytes, namely expression of genes, density and morphology, in the hippocampal DG under exposure to unpredictable chronic mild stress (uCMS) and AD treatment. To unravel the short- and long-term astrocytic alterations promoted by AD treatment, subsets of animals were treated with two typical ADs, fluoxetine – a selective inhibitor of serotonin reuptake - and imipramine – a tricyclic agent and a potent inhibitor of serotonin and norepinephrine reuptake. We showed that imipramine acts as a pro-astrogliogenic factor, increasing the number of newborn astrocytes in the hippocampal DG 4 weeks after stress exposure, while fluoxetine

treatment induces hypertrophy of both resident and newborn astrocytes in the same region. Interestingly, only imipramine could significantly rescue the cognitive impairments transiently induced by stress exposure. Therefore, this study can pave the way to a better understanding of the importance of astrogliogenesis in MDD and eventually allow the identification of astrogliogenic factors as therapeutic targets in the treatment of the disease.

MATERIALS AND METHODS

Animals

Experiments were conducted in adult male (2 months old) Wistar Han rats (Charles River Laboratories, L'Arbresle, France) group housed and kept under standard laboratory conditions at 22±1 °C, 55% relative humidity, 12 h light/dark cycle, food and water *ad libitum*. Rats (n=14-16 per group for behavioral analysis, of which 6-8 were considered for gene expression quantification, immunofluorescence and morphologic studies) from three independent sets were randomly divided into four groups, next described in detail. In detail, we used samples from three independent sets for the short-term analysis (tp1) and samples from two independent sets for the long-term analysis (tp2), for gene expression quantification, immunofluorescence and morphologic purposes. All the procedures were conducted in accordance with EU Directive 2010/63/EU and the Portuguese National Authority for animal experimentation, *Direção-Geral de Alimentação e Veterinária (DGAV)*.

Unpredictable chronic mild stress (uCMS) and drug treatment

Rats were exposed to a pre-validated uCMS protocol for 6 weeks, as previously described (Bessa *et al.*, 2009a; Mateus-Pinheiro *et al.*, 2013a). This stress paradigm was shown to induce depressive-like behavior, anxiety-like phenotype and cognitive deficits in rats through a random and unpredictable exposure to several different mild stressors, which include: confinement to a restricted space for 1h; housing on damp bedding for 8 h; overnight illumination; placement in a tilted cage (30°) for 3h; 15h of food deprivation followed by exposure to inaccessible food for 1 h; water deprivation for 15 h followed by exposure to an empty bottle for 1h; exposure to stroboscopic lights during 4h; replacement of bedding material by cold water (4°C) for 2h and reversed light/dark cycle for 48 h, every 7 days. In accordance with previous studies (Bessa *et al.*, 2009a; Mateus-Pinheiro *et al.*, 2013a), in the last 2 weeks of the protocol, animals were daily injected intraperitoneally with saline (SAL) or with ADs - either fluoxetine (FLX;

10 mg.kg^{-1}, Kemprotec, Middlesbrough, UK) or imipramine (IMIP; 10 mg.kg^{-1}; Kemprotec). At the same time, a group of animals not exposed to uCMS, was also injected with saline (CTRL).

All groups received for 5 days (2 days before and 3 days after the cessation of the uCMS protocol) intraperitoneal injections of bromodeoxyuridine (BrdU, 50 mg kg–1; Sigma-Aldrich, St. Louis, MO, USA) to label newly adult-born cells generated immediately after ADs treatment. A subset of animals (n= 8-10) were not subjected to any stressor in the following 4 weeks after uCMS exposure (long-term time point of analysis).

Behavioral analysis

Along the experimental protocol, behavior was continuously monitored for depressive- and anhedonic-like behavior, anxiety and cognition (Figure 1 and supplementary Figure 1). At week 6, in order to validate the depressive-like phenotype and ADs action, animals were submitted to the sucrose consumption test to assess anhedonic-like behavior, to the forced swimming test to assess the depressive-like behavior and to the open field test to assess anxious-like behavior (description of the behavioral tests in the Supplementary information; Supplementary Figure 1). As we wanted to understand the cognitive alterations in a longitudinal manner, animals were exposed to the novel object recognition test for cognitive assessment immediately after stress exposure and 4 weeks after stress exposure (Figure 1).

Serum corticosterone levels measurement

Corticosterone levels were measured in the rat's blood serum using a [125I] radioimmunoassay (RIA) kit (MP Biomedicals), according to manufacturer's instructions. Blood sampling (tail venipuncture) was performed during the diurnal and nocturnal nadir (N, 08:00-09:00) at the sixth week of the uCMS protocol (Figure 1b).

Hippocampal DG primary cultures

Postnatal day 3-5 Wistar Han rats were rapidly decapitated using a scissors and their brains collected. The meninges were removed, the hemispheres separated and the hippocampus macrodissected in ice-cold DMEM+10% FBS. After mechanical trituration and washes in DMEM+10% FBS, hippocampal cells were seeded in 12-well plates (NUNC) in 3 mL of neurospheres medium (DMEM-F12-GlutaMAX™, B27 supplement 2%, Pen-Strep 1%, HEPES buffer 8 mM, bFGF and EGF (10 ng/μL)). Besides the control plates, Dexamethasone (DEX, Fortecortin, Merck; 1 μM) was added to the plates. Cells were maintained

at 37° C, in 5% CO2 and humid atmosphere. On the following day, the plates were carefully washed 3 times with differentiation medium (Neurobasal A, B27 supplement 2%, Pen-Strep 1%, HEPES buffer 8mM), and 500µL of medium was added in every plate. Every 2 days, DEX was added to the medium and in the last 4 days of culturing, Desipramine (10µM, Sigma-Aldrich, St. Louis, MO, USA), Norfluoxetine hydrochloride (10µM, Sigma-Aldrich, St. Louis, MO, USA) and BrdU (10µM, Sigma-Aldrich, St. Louis, MO, USA) was added to the culture. Cells were fixed at day 8 for further immunostaining analysis.

Immunostaining procedures

In vivo

Animals (n=6-8 *per* group) were deeply anaesthetized with sodium pentobarbital (20%; Eutasil®, Sanofi, Gentilly, France) and transcardially perfused with 0.9% saline followed by cold 4% paraformaldehyde (PFA). Brains were removed, post-fixed in 4% PFA, cryoprotected in 30% sucrose overnight, and then embedded in Optimal Cutting Temperature compound (OCT, ThermoScientific, Waltham, MA, USA), snap-frozen and stored at -20°C. Coronal sections (20 µm) containing the dorsal pole of the hippocampal dentate gyrus (DG) were further stained to assess alterations on astroglial populations. Sections were stained with GFAP (1:200; Dako, Glostrup, Denmark), BrdU (1:100; Abcam, Cambridge, UK) and S100B (1:100; Dako, Glostrup, Denmark). Cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, 1:200; Sigma Aldrich). The total number of cells *per* DG was analyzed individually for distinct DG and the density of each cell population in the DG was determined by the ratio of the total number of cells and the respective area. Double stained cells ($GFAP^+BrdU^+$ cells) were analyzed in the same way. Analysis and cell counting were performed using a confocal microscope (Olympus FluoViewTM FV1000, Hamburg, Germany) and each area was determined using an optical microscope (Olympus BX51). Importantly, $GFAP^+$ cells in the subgranular layer of the DG exhibiting a radial morphology were not included in the analysis as these are cells typically classified as (type-1) neural stem cells. Observers were blind to the experimental condition of each subject. Cell densities are reported as number of cells *per* $100\mu m^2$.

In vitro

Viable progenitor hippocampal DG cells from p3-p5 animals were counted by trypan blue exclusion assay in a hemocytometer and platted in PDL-coated 24-well plates at a density of 80.000 cells-100.000/well. Cells were maintained at 37° C in 5% CO_2 and humid atmosphere for 7 days following the procedure explained before. All these cultures were fixed in 4% PFA for 10 min at RT and then washed with PBS.

PBS-T 0.5% was used to permeabilize cellular membranes for 10 min. Incubation with primary antibodies for GFAP (Dako; 1:200) or BrdU (Dako, 1:50) was performed overnight at 4°C. Primary antibodies were detected using subclass specific secondary antibodies (1:1000; anti-mouse Alexa-fluor® 488; anti-rabbit Alexa-fluor® 568; Life Technologies, Thermo Fisher Scientific), incubated for 2h at RT.

Finally, a 10 min incubation with DAPI (Invitrogen) was performed. Slides were then washed in PBS 1x and mounted with PermaFluor mountant medium (Thermo Scientific). Sections were analyzed using an Olympus BX-61 Fluorescence Microscope (Olympus).

For specific astrocytic genesis analysis BrdU/GFAP double positive cells were counted. For this purpose, three coverslips and ten randomly selected microscope fields per condition were analyzed. Results are shown as the average number of $GFAP^+$ or $GFAP^+BrdU^+$ cells per DAPI.

Morphological Analyses

To analyze astrocytic morphology, we applied the previously described open-source tool Simple Neurite Tracer, that was previously reported to enable the tridimensional reconstruction of astrocytic main processes in GFAP-stained sections as this marker specifically stains astrocyte main processes and its expression is tightly related to morphological alterations (Tavares *et al.*, 2017). After immunostaining, z-stacks of confocal images (magnification: 40x; n=10-15 astrocytes per subregion/animal) were used to determine total processes length (in µm), number of processes, maximum intersection radius (in µm) and number of intersections from the soma.

This analysis was performed for each DG subsection: granule cell layer (GCL), subgranular zone (SGZ), defined as the three deepest rows of granule cells, Inner molecular layer (IML) and hilus.

RT-PCR measurements

For dorsal DG macrodissection, animals were firstly anesthetized with pentobarbital (20%; Eutasil®, Sanofi) and transcardially perfused with 0.9% saline. Immediately after dissection, tissues were frozen and stored at -80 °C until further analysis.

Total RNA was isolated from the macrodissected DGs using the Direct-Zol™ RNA Mini Prep (Zymo Research, CA, USA), according to the manufacturer's instructions.

Total RNA (500 ng) was reverse-transcribed using qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA).

For real-time RT-PCR, oligonucleotide primers for S100 calcium-binding protein b (S100b, sense CACCGACTGGGCAAAATACT, antisense TCCGAACTTCCATGTCC), Glial Fibrillary Acidic Protein (GFAP, sense GGACCAGCTTACTACCAACAGTGCC, antisense TGGTTTCATCTTGGAGCTTCTGCCT), Signal transducer and activator of transcription 3 (STAT3, sense TGGACCGTCTGGAAAACTGGATAAC, antisense CTCCACCACGAAGGCACTCTTCATTA), Bone morphogenetic protein 4 (BMP4, sense TCCATCACGAAGAACATCTGGAGAA, antisense GTCCACCTGCTCCCGAAATAGC) and jmjd3 (sense CGGTTCTGCCCAGTCTGTGAAACCG, antisense ATGCTGGGTGTAGGAGGGTTG) and B2M (sense GTGCTTGCCATTCAGAAAACTCC, antisense AGGTGGGTGGAACTGAGACA) were designed using Primer-BLAST software (NCBI). Reactions were performed in an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, LLC, CA, USA) using 5X HOT FIREPol EvaGreen qPCR Mix Plus, ROX (Solis Biodyne, Tartu, Estonia), Target gene expression levels were normalized against the housekeeping gene Beta-2-Microglobulin (B2M). The relative expression was calculated using the DDCt method. Results are presented as fold-change of mRNA levels between the respective experimental groups after normalization to B2M levels.

Statistical analysis

Statistical analysis was performed using Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA). Animals were randomly assigned to the experimental groups. All presented data satisfied normal distribution in Kolmogorov–Smirnov testing. After confirmation of homogeneity of group variances, data was subjected to the appropriate statistical tests. Student's t-test was used for statistical comparisons between experimental groups when appropriate. The comparison between stressed groups was evaluated using One-way analysis of variance. Analysis of variance repeated measures was used to analyze the number of intersections from the soma. Descriptive statistical results are presented as mean ± standard error of the mean (SEM). Differences between groups were determined by Bonferroni's *post-hoc* multiple comparison test and statistical significance was set at $P < 0.05$.

RESULTS

Imipramine, but not fluoxetine, rescues cognitive impairments induced by stress exposure

In order to validate the phenotype typically observed in rodents exposed to the uCMS protocol, we analyzed the short-term behavioral and molecular effects induced by stress exposure (Bessa *et al.*, 2009a). In the current study, we could confirm that rats exposed to the uCMS protocol presented a depressive-like phenotype (Supplementary Fig. 1). In detail, stressed animals were significantly less active in the Forced Swimming Test (as it is a swimming-based test) and present lower sucrose preference in the Sucrose Consumption Test (FST: P=0.0235, Supplementary Fig. 1a; SCT: P= 0.0006, Supplementary Fig.1b), being normalized to control group values by the administration of both ADs, namely fluoxetine and imipramine (FST: $F_{2,13}$= 5.803, P=0.0158; SCT: $F_{2,14}$ = 6.924, P=0.0081; Supplementary Fig. 1 a and b). Moreover, stress-exposed animals also showed a tendency for an anxiety-like behavior in the Open Field test, although results did not reach statistical significance (P>0.1; Supplementary Fig. 1c).

As a molecular validation of the model and knowing that a sustained stress exposure leads to an hyperactivation of the Hypothalamic-Pituitary-Adrenal (HPA) axis, with consequent alteration of corticosterone blood levels and the circadian rhythm (Herman *et al.*, 2016), we analyzed corticosterone levels in animals' blood on night and day activity peaks. Assessment of corticosterone levels of stressed animals revealed a disruption in the HPA axis, with similar values of corticosterone at nadir and zenith (P>0.1; Fig. 1b). However, animals treated with fluoxetine and imipramine showed a similar response to control animals, with higher levels of corticosterone at zenith, suggesting that both ADs were able to re-establish the HPA axis function (CTRL: P<0.0001; FLX: P= 0.0006; IMIP: P= 0.0049; Fig. 1b).

We further characterized cognitive alterations upon stress exposure and ADs administration in a longitudinal manner. As such, we assessed changes at two different time-points: 1) at week 6 (called tp1 from now on), immediately after exposure to a 6-week uCMS protocol that included 2-weeks of AD treatment and, 2) at week 10 (called tp2 from now on), after four weeks of exposure to the uCMS protocol (see Fig.1a for a schematic representation of the experimental timeline).

We observed that on tp1, stressed animals presented short-term memory deficits (P=0.0277; Fig. 1c) and imipramine, but not fluoxetine, treatment promoted rescue of this cognitive impairment ($F_{2,10}$=6.318, P=0.0168; Fig. 1c). Regarding long-term memory assessment, which is reported to be more dependent on hippocampal function (Sawangjit *et al.*, 2018), stressed animals showed long-term memory deficits at tp1 (P=0.0004; Fig. 1d), with only imipramine treatment being able to restore the long-term memory performance to similar levels of the control group ($F_{2,11}$=6.982; P=0.0110; Fig. 1d). Despite these cognitive impairments at short-term (tp1), at tp2 (4 weeks after the end of the uCMS

protocol) stress-exposed animals could recover from short-term and long-term memory deficits when compared with control animals (short-term memory: P=0.1609, Fig. 1c; long-term memory: P=0.7172; Fig. 1d). Furthermore, animals treated with both ADs did not exhibit any alterations at tp2, either on long-term or short-term memory.

Imipramine promotes the generation of new astrocytes in the hippocampal dentate gyrus

To understand the longitudinal impact of stress exposure and ADs treatment on the density of both pre-existent astrocytes and newborn astrocytes, we assessed the density of astrocytes (GFAP+ cells: includes immature and mature astrocytes and S100B+ cells: includes mature astrocytes) and of newborn astrocytes (GFAP+BrdU+ cells). To quantify newborn astrocytes animals were injected with BrdU 3 days before and 2 days after cessation of the stress protocol and ADs administration. BrdU is a synthetic nucleoside, analog of thymidine, which is incorporated in the DNA of proliferating cells during the S-phase, thus allowing us to follow these cells by immunofluorescence (Cavanagh *et al.*, 2011).

Assessment of astrocytic changes in the dorsal dentate gyrus (dDG) immediately after stress exposure (at tp1) revealed no major differences between control and stress groups, on S100B+ cells (P=0.0714; Fig.2b) and on GFAP+BrdU+ cells (P=0.2119; Fig.2c and P=0.1518; Fig.2d). However, stress exposure could decrease the density of GFAP+ cells population when compared to control (P= 0.0280; Fig.2a),

However, assessment of quantitative changes in the density of astrocytes 4 weeks after the end of the uCMS protocol – tp2 - revealed that exposure to the uCMS protocol significantly decreased the number of GFAP+ cells (P=0.0397: Fig.2e), as previously supported by several studies (Gosselin *et al.*, 2009b; Tynan *et al.*, 2013a). Treatment with ADs did not induce alterations on those cellular levels (F2,31=0.9464, P=0.3991; Fig.2e). When exploring mature astrocytes - cells labeled with S100β (Raponi *et al.*, 2007) – stress exposure induces a reduction on the number of S100B+ cells in the hippocampal DG at tp2 (P=0.0397; Fig.2f). Imipramine, but not fluoxetine, treatment significantly increased the number of S100B+ cells, to levels even higher than those presented by the control group (F2,31=0.9464, P=0.3991; Fig.2f). Furthermore, when exploring the effect of stress and ADs treatment on newborn astrocytes at tp2, a time-point in which newborn cells that incorporated BrdU already differentiated and started integrating into the circuitry (a process that takes 4–8 weeks in rodents; (Mateus-Pinheiro *et al.*, 2013), stressed animals presented a significant reduction on newborn astrocytes number (P=0.0252; Fig.2g). Interestingly, imipramine treatment elicited a strong pro-astrogliogenic response by increasing the number of both GFAP+BrdU+ cells (F2,9=6.167, P=0.0206; Fig.2g) and the proportion of GFAP+BrdU+ cells among all BrdU+ cells (GFAP+BrdU+/BrdU+; P=0.8815, F2,33=13.99, P<0,0001;

Fig.2h). Fluoxetine did not exert any alteration on the density of these cells, which is aligned with having a more pro-neuronal response (Mateus-Pinheiro *et al.*, 2013). To verify if this effect was specific to newborn mature astrocytes and not to glial-like precursor cells, we analyzed the effect of stress and ADs administration on the number of GFAP+S100B+ cells among all BrdU+ cells (Supplementary Fig. 2). No alterations were found on the number of GFAP+S100B+/BrdU+ cells at tp1 ($P=0.2197$; Supplementary Fig. 2a). However, at tp2, stress exposure decreased the number of these newborn mature astrocytes ($P=0.0298$; Supplementary Fig. 2b) and although not significantly different, imipramine treatment showed a tendency to increase the density of these cells ($F_{2,6}=2.613$, $P=0.1527$; Supplementary Fig. 2b).

Furthermore, we analyzed astrocytes differentiation *in vitro*, using primary hippocampal cell cultures from p5 rats, after conditioning the cells with dexamethasone (DEX) and with the active metabolites of the ADs used in the experimental approach *in vivo*, namely norfluoxetine and desipramine. By labeling the cells with GFAP and β3-tubulin antibodies (Sup.Fig.3), after 8 days *in vitro*, we show that conditioning the cells with DEX induces a significant decrease in the number of astrocytes (GFAP+ cells; $P= 0.0220$; Fig.2i), being restored to levels similar to the control group after conditioning the cells with desipramine, but not norfluoxetine ($F_{2,33}=14.30$, $P<0,0001$; Fig.2i). Moreover, analyses of the number of double GFAP+/BrdU+ cells, revealed that DEX-treated cells present a reduced number of newborn astrocytes ($P= 0.0332$; Fig.2j). Although not statistically significant, hippocampal cells treated with desipramine presented a tendency for an increased number of newborn astrocytes, similar to the control untreated cells ($F_{2,11}=1.183$, $P=0.3424$; Fig.2j).

As dynamic changes of astrocytes are not limited to changes in their number, we further analyzed gene expression levels. Therefore, we studied the levels of several genes expressed by resident astrocytes, such as GFAP and S100B, and other genes that are more related with the promotion of astrocytes differentiation, such as BMP4, STAT3 and jmjd3. Results showed that, at tp1, both GFAP and S100B expression levels are not significantly changed by stress exposure or ADs treatment (GFAP: $P=0.2028$; $F_{2,7}=6.220$, $P=0,0280$, Fig. 3a; S100B: $P=0.3673$; $F_{2,11}=1.764$, $P=0.2166$, Fig. 3b), which is in accordance with the density analysis, although stress exposure could decrease the number of GFAP+ cells (Fig. 2a). Analyses of the genes known to induce astrocytic differentiation, revealed that imipramine treatment shows a tendency to increase both BMP4 and STAT3 expression levels (BMP4: $F_{2,5}= 5.780$, $P=0.0501$, Fig. 3c; STAT3: $F_{2,5}=2.890$, $P=0.1465$, Fig. 3d) and significantly increases jmjd3 expression levels when compared to the stress-exposed group ($F_{2,5}= 13.60$, $P=0.0095$; Fig. 3e). However, no alterations in the expression levels of these genes were induced by stress exposure (BMP4: $P=0.8693$, Fig. 3c; STAT3: $P=0.5799$, Fig. 3d; jmjd3: $P= 0.8955$, Fig. 3e).

At tp2, no differences were found on GFAP expression levels between control and stress group (P= 0.2224; Fig. 3f) and upon ADs treatment (F2,6= 0.2874, P=0.7600; Fig. 3f). Interestingly, stress exposure did not induce statistically significant changes in S100B expression, but imipramine treatment was able to increase S100B expression levels by comparison to the stress-exposed group (P= 0,2535; F2,5= 8.858, P=0.0227; Fig. 3g). Curiously, when analyzing BMP4 and STAT3 expression levels, both genes presented increased expression in the stress-exposed group, when compared to the control group (BMP4: P= 0.0512, Fig. 3h; STAT3: P= 0.0399, Fig. 3i), and decreased expression in the rats treated with imipramine, when comparing to the stress-exposed group (BMP4: F2,9= 8.353, P=0.0089, Fig. 3h; STAT3: F2,6= 8.697, P=0.0169, Fig. 3i). The expression levels of jmjd3 were not changed among groups at tp2, neither after stress exposure (P=0.3344; Fig. 3j) nor after ADs treatment (F2,6= 0.02682, P=0.9737; Fig. 3j).

Fluoxetine induces hypertrophy of resident and newborn astrocytes in the hippocampal dentate gyrus

A large body of evidence has consistently reported that depression and stress significantly impact on morphometric properties of astrocytes, including in the size and branching (reviewed in (Kim *et al.*, 2018)) either in animal (Czéh *et al.*, 2006; Tynan *et al.*, 2013b; Saur *et al.*, 2016) and human studies (Rajkowska *et al.*, 1999b; Torres-Platas *et al.*, 2011). Therefore, we decided to understand how stress and ADs treatment could affect the astrocytic morphology in the hippocampal DG.

We assessed the morphology of astrocytes from different sub-sections of the DG, such as granular cell layer (GCL) and inner molecular layer (IML), and present the results as a pool, as they did not show any differences among different areas. However, we did not included astrocytes from the subgranular zone (SGZ), as some could be stem cells (which are also GFAP+). We found that, immediately after stress exposure, at tp1, astrocytic morphology is not altered either by stress exposure (P=0.7983; Fig.4a) or ADs treatment (F2,53= 3.469, P=0.0384; Fig. 4a). However, when we analyzed the astrocytic morphology at tp2, we could see that stress exposure decreased the processes length of astrocytes (P=0.0302; Fig. 4a) and fluoxetine-treated animals presented their processes length around 2 times higher than control animals (F2,36= 49.31, P<0,0001; Fig. 4a), suggesting that this AD promotes hypertrophy of astrocytes. Moreover, in order to understand if stress exposure and ADs treatment could impact the morphology of newborn astrocytes, we analyzed the morphology of GFAP+BrdU+ cells in the GCL of the hippocampal dentate gyrus at tp2. Our results showed that newborn astrocytes from stress-exposed animals had increased processes length when compared to the control astrocytes (P=0.0108;

Fig. 4b) similar to fluoxetine-treated animals, while imipramine-treated animals revealed astrocytes with processes length equivalent to control astrocytes ($F_{2,11}$= 8.802, P=0.0052; Fig. 4b).

Discussion

Overall, the present study shows that different classes of ADs have a differential impact on resident and newborn astrocytes in the adult hippocampal DG in the longitudinal course of a depressive episode. Moreover, imipramine can rescue depression-associated cognitive impairments and acts through astrogliogenesis potentiation, while fluoxetine induces a state of astrocytic hypertrophy on both resident and newborn astrocytes.

Interestingly, this differential effect of ADs, namely of fluoxetine and imipramine, was also shown to occur in a context of stress re-exposure, where fluoxetine, contrarily to imipramine, induced an over-production of new neurons in the hippocampal DG (Alves *et al.*, 2017).

In this study, only imipramine, and not fluoxetine, could efficiently rescue cognitive impairments immediately after stress exposure. Importantly, some studies showed that noradrenaline reuptake inhibitors – imipramine - can ameliorate anxiety and cognitive deficits induced by stress independently of ongoing neurogenesis. In contrast, the anxiolytic and pro-cognitive efficacy of serotonin reuptake inhibitors – fluoxetine - required uninterrupted neurogenic processes, showing the distinct neurogenic profile of those ADs (Mateus-Pinheiro *et al.*, 2013).

Moreover, the cognitive impairments were fully re-established on the long-term perspective of the disorder (tp2 analysis). It makes sense as, in fact, previous studies have shown already that continuous proliferation and complete circuitry integration of new neurons and glial cells, a process that takes 4–8 weeks in rodents, is necessary for the maintenance of emotional and cognitive homeostasis (Mateus-Pinheiro *et al.* 2013). Therefore, it seems that imipramine is acting in a faster way to re-establish the immediate impairments while fluoxetine needs more time to act.

Moreover, with our study we could see that imipramine is eliciting a pro-astrogliogenic effect in the hippocampal DG, confirmed both *in vivo* and *in vitro*.

Importantly, several studies have already reported the direct effect of imipramine on astrocytic differentiation, either *in vivo* or *in vitro* (Kim *et al.*, 2011; Takano *et al.*, 2012; Mateus-Pinheiro *et al.*, 2013b; Kedracka-Krok *et al.*, 2018). In accordance, we found that several genes related with astrocytic differentiation – STAT3, BMP4 and jmjd3 – were upregulated in the hippocampal DG of stress-exposed animals treated with imipramine immediately after stress exposure and AD treatment, being those levels re-established 4 weeks after. However, the expression levels of these specific genes in fluoxetine-treated

animals were always similar to the stress-exposed animals, either immediately or 4 weeks after the end of the stress protocol.

Interestingly, the early effect induced by imipramine at tp1 corresponds only to the onset of a slow neuroadaptation whose neurobiological importance can only be analyzed later on, once new cells attain complete maturation and functionality, and are integrated in the local neurocircuitry – at tp2. Therefore, and as mammalian cytogenesis is known to take 4–8 weeks, the overall outcome of AD's therapeutic action regarding the impact of cell cytogenesis is only manifested after this period. Therefore, those specific genes that are known to promote astrogliogenesis have their expression levels increased immediately after stress exposure and ADs treatment. However, that potentiation only leads to a significant density increase of the newborn astrocytes, 4 weeks after (tp2 analysis).

Our data is also corroborated by the fact that, norepinephrine, in contrast to serotonin, can directly activate the resident pool of progenitor cells and stimulate neurogenesis, but also gliogenesis (Jhaveri *et al.*, 2010). *In vitro* and *ex vivo* studies have shown that norepinephrine can activate adult hippocampal precursor cells and promote neurogenesis (Masuda *et al.*, 2012). Furthermore, serotonin (5-HT) was shown to stimulate cell proliferation in both SGL and SVZ throughout different serotonergic receptor subtypes (Banasr *at al.*, 2004). In fact, fluoxetine treatment not only induced morphological alterations in resident astrocytes but also on newborn astrocytes. This AD did not rescue the reduced density of newborn astrocytes caused by stress exposure, only acting on their cellular morphology - increasing astrocytic length and inducing a state of hypertrophy.

Astrocytes respond to several forms of CNS injury and disease by a process called reactive astrogliosis, a pathological hallmark of CNS structural lesions. Based on numerous studies, reactive astrogliosis has recently been defined, but most importantly, it is always accompanied by varying degrees of cellular hypertrophy (Liu *et al.*, 2017). Functionally speaking, reactive astrocytes can absorb glutamate from the synaptic cleft, not only reducing excitotoxicity but also providing cells with the substances required for neuronal metabolism (Cheng *et al.*, 2019). Therefore, we may assume that astrocytes can be activated by fluoxetine treatment, to cope with the increased neuronal production that occurs with this AD, thus responding to the network changes and assuming a protective role.

However, importantly, treatment with fluoxetine was not effective in rescuing the immediate cognitive impairments that emerged after stress exposure, regarding both short and long-term memory. Therefore, it seems that this AD, by acting through astrocytic morphology alterations and by increasing the hippocampal neurogenesis, is not able to exert its effect in this behavioral domain in such a short timeframe.

Overall, our findings suggest that complex dynamic remodeling of astroglial networks might have an important role in the recovery from depressive symptoms.

In summary, this work shows that imipramine treatment promotes a pro-astrogliogenic response in the hippocampal DG of depressive-like animals, that might be correlated with the immediate behavioral cognitive improvements induced by this AD. Moreover, fluoxetine treatment is not able to immediately rescue the cognitive impairments induced by stress exposure and induces a hypertrophic effect on both resident and newborn astrocytes.

Funding

ARMS, NDA, PP, AMP, ELC, MM and LP received fellowships from the Portuguese Foundation for Science and Technology (FCT). This work was funded by FCT (IF/01079/2014).

This article has been developed under the scope of the project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER).

This work has been funded by FEDER funds, through the Competitiveness Factors Operational Programme (COMPETE), and by National funds, through the Foundation for Science and Technology (FCT), under the scope of the project POCI-01-0145-FEDER-007038.

Conflict of Interest

The authors declare no conflicts of interest.

REFERENCES

Abrahám, I., Juhász, G., Kékesi, K.A., & Kovács, K.J. (1998) Corticosterone peak is responsible for stress-induced elevation of glutamate in the hippocampus. *Stress*, **2**, 171–181.

Alonso, G. (2000) Prolonged corticosterone treatment of adult rats inhibits the proliferation of oligodendrocyte progenitors present throughout white and gray matter regions of the brain. *Glia*, **31**, 219–231.

Alves, N.D., Correia, J.S., Patrício, P., Mateus-Pinheiro, A., Machado-Santos, A.R., Loureiro-Campos, E., Morais, M., Bessa, J.M., Sousa, N., & Pinto, L. (2017) Adult hippocampal neuroplasticity triggers susceptibility to recurrent depression. *Transl. Psychiatry*, **7**, e1058–e1058.

Banasr, M. & Duman, R.S. (2007) Regulation of neurogenesis and gliogenesis by stress and antidepressant treatment. *CNS Neurol. Disord. Drug Targets*, **6**, 311–320.

Banasr, M. & Duman, R.S. (2008) Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol. Psychiatry*, **64**, 863–870.

Banasr, M., Hery, M., Printemps, R., & Daszuta, A. (2004) Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. *Neuropsychopharmacology*, **29**, 450–460.

Banasr, M., Valentine, G.W., Li, X.Y., Gourley, S.L., Taylor, J.R., & Duman, R.S. (2007) Chronic Unpredictable Stress Decreases Cell Proliferation in the Cerebral Cortex of the Adult Rat. *Biol. Psychiatry*, **62**, 496–504.

Bélair, E.-L., Vallée, J., & Robitaille, R. (2010) In vivo long-term synaptic plasticity of glial cells. *J. Physiol.,* **588**, 1039–1056.

Ben Achour, S. & Pascual, O. (2010) Glia: the many ways to modulate synaptic plasticity. *Neurochem. Int.*, **57**, 440–445.

Bessa, J.M., Mesquita, A.R., Oliveira, M., Pêgo, J.M., Cerqueira, J.J., Palha, J. a, Almeida, O.F.X., & Sousa, N. (2009) A trans-dimensional approach to the behavioral aspects of depression. *Front. Behav. Neurosci.*, **3**, 1.

Bhardwaj, R.D., Curtis, M.A., Spalding, K.L., Buchholz, B.A., Fink, D., Björk-Eriksson, T., Nordborg, C., Gage, F.H., Druid, H., Eriksson, P.S., & Frisén, J. (2006) Neocortical neurogenesis in humans is restricted to development. *Proc. Natl. Acad. Sci. U. S. A.*, **103**, 12564–12568.

Cavanagh, B.L., Walker, T., Norazit, A., & Meedeniya, A.C.B. (2011) Thymidine Analogues for Tracking DNA Synthesis. *Molecules*, **16**, 7980–7993.

Chana, G., Landau, S., Beasley, C., Everall, I.P., & Cotter, D. (2003) Two-dimensional assessment of cytoarchitecture in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia: evidence for decreased neuronal somal size and increased neuronal density. *Biol. Psychiatry*, **53**, 1086–1098.

Cheng, X., Wang, J., Sun, X., Shao, L., Guo, Z., & Li, Y. (2019) Morphological and functional alterations of astrocytes responding to traumatic brain injury. *J. Integr. Neurosci.,* **18**, 203.

Czéh, B., Müller-Keuker, J.I.H., Rygula, R., Abumaria, N., Hiemke, C., Domenici, E., & Fuchs, E. (2007) Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. *Neuropsychopharmacology*, **32**, 1490–1503.

Czéh, B., Simon, M., Schmelting, B., Hiemke, C., & Fuchs, E. (2006) Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology*, **31**, 1616–1626.

Duman, R.S. & Monteggia, L.M. (2006) A Neurotrophic Model for Stress-Related Mood Disorders. *Biol. Psychiatry*, **59**, 1116–1127.

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., & Gage, F.H. (1998) Neurogenesis in the adult human hippocampus. *Nat. Med.*, **4**, 1313–1317.

Gensert, J.M. & Goldman, J.E. (2001) Heterogeneity of cycling glial progenitors in the adult mammalian cortex and white matter. *J. Neurobiol.*, **48**, 75–86.

Gos, T., Schroeter, M.L., Lessel, W., Bernstein, H.-G., Dobrowolny, H., Schiltz, K., Bogerts, B., & Steiner, J. (2013) S100B-immunopositive astrocytes and oligodendrocytes in the hippocampus are differentially afflicted in unipolar and bipolar depression: a postmortem study. J*. Psychiatr. Res.*, **47**, 1694–1699.

Gosselin, R.-D., Gibney, S., O'Malley, D., Dinan, T.G., & Cryan, J.F. (2009) Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression. *Neuroscience*, **159**, 915–925.

Herman, J.P., McKlveen, J.M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., & Myers, B. (2016) Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. In Comprehensive Physiology. *John Wiley & Sons, Inc., Hoboken, NJ, USA*, 603–621.

Jhaveri, D.J., Mackay, E.W., Hamlin, A.S., Marathe, S. V., Nandam, L.S., Vaidya, V.A., & Bartlett, P.F. (2010) Norepinephrine Directly Activates Adult Hippocampal Precursors via 3-Adrenergic Receptors. *J. Neurosci.*, **30**, 2795–2806.

Kedracka-Krok, S., Swiderska, B., Bielecka-Wajdman, A.M., Prus, G., Skupien-Rabian, B., Jankowska, U., & Obuchowicz, E. (2018) Impact of imipramine on proteome of rat primary glial cells. *J. Neuroimmunol.*, **320**, 25–37.

Kim, R., Healey, K.L., Sepulveda-Orengo, M.T., & Reissner, K.J. (2018) Astroglial correlates of neuropsychiatric disease: From astrocytopathy to astrogliosis. *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, **87**, 126–146.

Kim, Y., Kim, S.H., Kim, Y.S., Lee, Y.H., Ha, K., & Shin, S.Y. (2011) Imipramine activates glial cell line-derived neurotrophic factor via early growth response gene 1 in astrocytes. *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, **35**, 1026–1032.

Kornack, D.R. & Rakic, P. (2001) Cell proliferation without neurogenesis in adult primate neocortex. *Science*, **294**, 2127–2130.

Kraus-Ruppert, R., Laissue, J., Bürki, H., & Odartchenko, N. (1975) Kinetic studies on glial, Schwann and capsular cells labelled with [3H] thymidine in cerebrospinal tissue of young mice. *J. Neurol. Sci.*, **26**, 555–563.

Lee, J., Duan, W., & Mattson, M.P. (2002) Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J. Neurochem.*, **82**, 1367–1375.

Lima, A., Sardinha, V.M., Oliveira, A. F., Reis, M., Mota, C., Silva, M. A., Marques, F., Cerqueira, J.J., Pinto, L., Sousa, N., & Oliveira, J.F. (2014) Astrocyte pathology in the prefrontal cortex impairs the cognitive function of rats. *Mol. Psychiatry*, **19**, 834–841.

Liu, B., Teschemacher, A.G., & Kasparov, S. (2017) Neuroprotective potential of astroglia. *J. Neurosci. Res.,* **95**, 2126–2139.

Liu, R.-J. & Aghajanian, G.K. (2008) Stress blunts serotonin- and hypocretin-evoked EPSCs in prefrontal cortex: role of corticosterone-mediated apical dendritic atrophy. *Proc. Natl. Acad. Sci. U. S. A.*, **105**, 359–364.

Lucassen, P.J., Heine, V.M., Muller, M.B., van der Beek, E.M., Wiegant, V.M., De Kloet, E.R., Joels, M., Fuchs, E., Swaab, D.F., & Czeh, B. (2006) Stress, depression and hippocampal apoptosis. *CNS Neurol. Disord. Drug Targets*, **5**, 531–546.

Machado-Santos, A.R., Alves, N.D., Araújo, B., Correia, J.S., Patrício, P., Mateus-Pinheiro, A., Loureiro-Campos, E., Bessa, J.M., Sousa, N., & Pinto, L. (2019) Astrocytic plasticity at the dorsal dentate gyrus on an animal model of recurrent depression. *Neuroscience.*

Maciag, D., Hughes, J., O'Dwyer, G., Pride, Y., Stockmeier, C.A., Sanacora, G., & Rajkowska, G. (2010) Reduced Density of Calbindin Immunoreactive GABAergic Neurons in the Occipital Cortex in Major Depression: Relevance to Neuroimaging Studies. *Biol. Psychiatry*, **67**, 465–470.

Maletic, V., Robinson, M., Oakes, T., Iyengar, S., Ball, S.G., & Russell, J. (2007) Neurobiology of depression: an integrated view of key findings. *Int. J. Clin. Pract.*, **61**, 2030–2040.

Manji, H.K., Moore, G.J., Rajkowska, G., & Chen, G. (2000) Neuroplasticity and cellular resilience in mood disorders. *Mol. Psychiatry*, **5**, 578–593.

Masuda, T., Nakagawa, S., Boku, S., Nishikawa, H., Takamura, N., Kato, Koyama, T. (2012) Noradrenaline increases neural precursor cells derived from adult rat dentate gyrus through beta2 receptor. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, **36**, 44–51.

Mateus-Pinheiro, A., Pinto, L., Bessa, J.M., Morais, M., Alves, N.D., Monteiro, S., Patrício, P., Almeida, O.F.X., & Sousa, N. (2013a) Sustained remission from depressive-like behavior depends on hippocampal neurogenesis. *Transl. Psychiatry*, **3**, e210–e210.

Miguel-Hidalgo, J.J., Waltzer, R., Whittom, A.A., Austin, M.C., Rajkowska, G., & Stockmeier, C.A. (2010) Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. *J. Affect. Disord.*, **127**, 230–240.

Ninkovic, J. & Götz, M. (2013) Fate specification in the adult brain–lessons for eliciting neurogenesis from glial cells. *Bioessays*, **35**, 242–252.

Oliveira, J.F., Sardinha, V.M., Guerra-Gomes, S., Araque, A., & Sousa, N. (2015) Do stars govern our actions? Astrocyte involvement in rodent behavior. *Trends Neurosci.*, **38**, 535–549.

Ongür, D., Drevets, W.C., & Price, J.L. (1998) Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc. Natl. Acad. Sci. U. S. A.*, **95**, 13290–13295.

Patrício, P., Mateus-Pinheiro, A., Sousa, N., & Pinto, L. (2013) Re-cycling paradigms: cell cycle regulation in adult hippocampal neurogenesis and implications for depression. *Mol. Neurobiol.*, **48**, 84–96.

Radley, J.J., Sisti, H.M., Hao, J., Rocher, A.B., McCall, T., Hof, P.R., McEwen, B.S., & Morrison, J.H. (2004) Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience*, **125**, 1–6.

Rajkowska, G., Halaris, A., & Selemon, L.D. (2001) Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biol. Psychiatry*, **49**, 741–752.

Rajkowska, G. & Miguel-Hidalgo, J. (2007) Gliogenesis and Glial Pathology in Depression. *CNS Neurol. Disord. - Drug Targets*, **6**, 219–233.

Rajkowska, G., Miguel-Hidalgo, J.J., Wei, J., Dilley, G., Pittman, S.D., Meltzer, H.Y., Overholser, J.C., Roth, B.L., & Stockmeier, C.A. (1999) Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol. Psychiatry*, **45**, 1085–1098.

Rajkowska, G., O'Dwyer, G., Teleki, Z., Stockmeier, C.A., & Miguel-Hidalgo, J.J. (2007) GABAergic neurons immunoreactive for calcium binding proteins are reduced in the prefrontal cortex in major depression. *Neuropsychopharmacology*, **32**, 471–482.

Rajkowska, G., Selemon, L.D., & Goldman-Rakic, P.S. (1998) Neuronal and Glial Somal Size in the Prefrontal Cortex. *Arch. Gen. Psychiatry*, **55**, 215.

Raponi, E., Agenes, F., Delphin, C., Assard, N., Baudier, J., Legraverend, C., & Deloulme, J.-C. (2007) S100B expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage. Glia, **55**, 165–177.

Sahay, A. & Hen, R. (2007) Adult hippocampal neurogenesis in depression. *Nat. Neurosci.*, **10**, 1110–1115.

Sairanen, M., Lucas, G., Ernfors, P., Castrén, M., & Castrén, E. (2005) Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J. Neurosci.*, **25**, 1089–1094.

Saur, L., Baptista, P.P.A., Bagatini, P.B., Neves, L.T., de Oliveira, R.M., Vaz, S.P., Ferreira, K., Machado, S.A., Mestriner, R.G., & Xavier, L.L. (2016) Experimental Post-traumatic Stress Disorder Decreases Astrocyte Density and Changes Astrocytic Polarity in the CA1 Hippocampus of Male Rats. *Neurochem. Res.*, **41**, 892–904.

Sawangjit, A., Oyanedel, C.N., Niethard, N., Salazar, C., & Inostroza, M. (2018) The hippocampus is crucial for forming non-hippocampal long-term memory during sleep. *Nature*, **564**, 109–113.

Selemon, L.D., Rajkowska, G., & Goldman-Rakic, P.S. (1998) Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional, stereologic counting method. *J. Comp. Neurol.*, **392**, 402–412.

Steiner, B., Kronenberg, G., Jessberger, S., Brandt, M.D., Reuter, K., & Kempermann, G. (2004) Differential Regulation of Gliogenesis in the Context of Adult Hippocampal Neurogenesis in Mice. *Glia*, **46**, 41–52.

Takano, K., Yamasaki, H., Kawabe, K., Moriyama, M., & Nakamura, Y. (2012) Imipramine Induces Brain-Derived Neurotrophic Factor mRNA Expression in Cultured Astrocytes. *J. Pharmacol. Sci.*, **120**, 176–186.

Tavares, G., Martins, M., Correia, J.S., Sardinha, V.M., Guerra-Gomes, S., das Neves, S.P., Marques, F., Sousa, N., & Oliveira, J.F. (2017) Employing an open-source tool to assess astrocyte tridimensional structure. *Brain Struct. Funct.*, **222**, 1989–1999.

Torres-Platas, S.G., Hercher, C., Davoli, M.A., Maussion, G., Labonté, B., Turecki, G., & Mechawar, N. (2011) Astrocytic Hypertrophy in Anterior Cingulate White Matter of Depressed Suicides. *Neuropsychopharmacology*, **36**, 2650–2658.

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 Neuropathol.*, **126**, 75–91.

van Calker, D., Zobel, I., Dykierek, P., Deimel, C.M., Kech, S., Lieb, K., Berger, M., & Schramm, E. (2009) Time course of response to antidepressants: Predictive value of early improvement and effect of additional psychotherapy. *J. Affect. Disord.*, **114**, 243–253.

Verkhratsky, A., Rodríguez, J.J., & Parpura, V. (2013) Astroglia in neurological diseases. *Future Neurol.*, **8**, 149–158.

Verkhratsky, A., Rodríguez, J.J., & Steardo, L. (2014) Astrogliopathology: a central element of neuropsychiatric diseases? *Neuroscientist*, **20**, 576–588.

von Bohlen und Halbach, O. (2011) Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus. *Cell Tissue Res.*, **345**, 1–19.

Wennström, M., Hellsten, J., Ekstrand, J., Lindgren, H., & Tingström, A. (2006) Corticosterone-induced inhibition of gliogenesis in rat hippocampus is counteracted by electroconvulsive seizures. *Biol. Psychiatry*, **59**, 178–186.

FIGURES

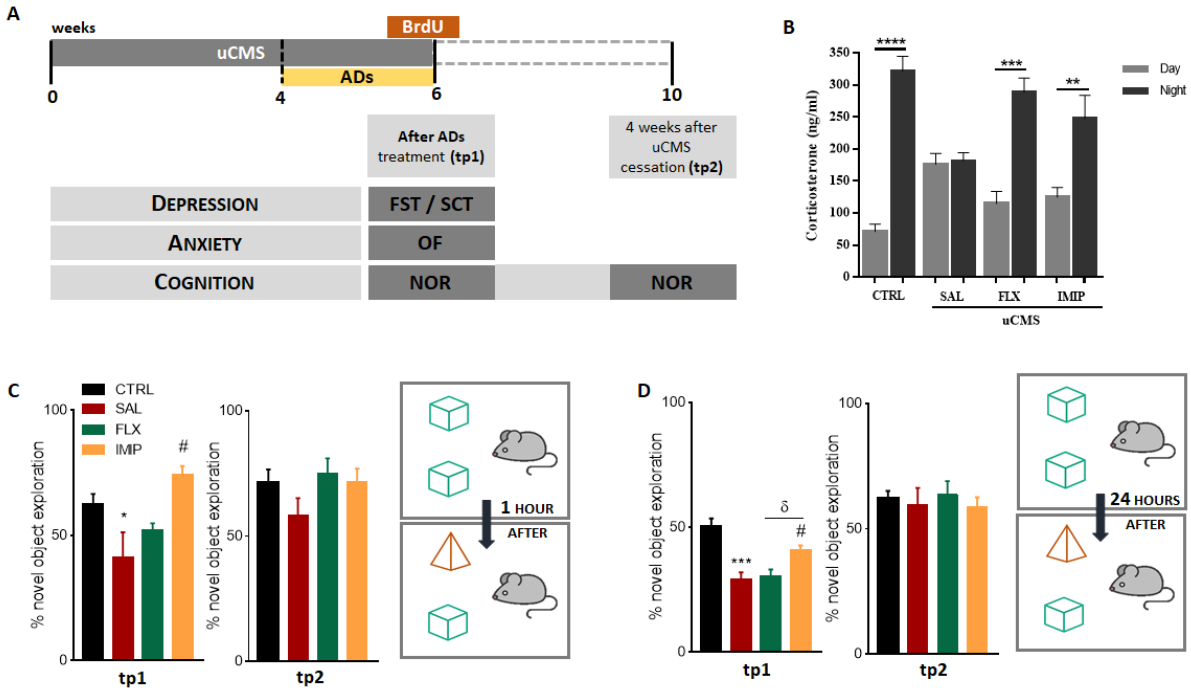


Figure 1. Imipramine is able to rescue short-term and long-term memory impairments induced by stress exposure. (A) Schematic representation of the experimental timeline used in this study, including behavioral assessments throughout the protocol and the respective treatments. (B) Costicosterone analysis on tp1 (immediately after the end of the stress protocol) on all test groups, measured in the serum of rats collected at nadir and zenith. (C) Analysis of short-term memory in the novel object recognition test (NOR) test, both at tp1 and tp2. (D) Analysis of the long-term memory in the NOR test, both at tp1 and tp2.

*Denotes the effect of uCMS analyzed by Student's t-test; #Denotes the effect of ADs, by comparison of treatment and SAL animals; and δ denotes differences between ADs, analyzed by one-way analysis of variance (ANOVA). Data are represented as mean±s.e.m. #P⩽0.05, **, ##P⩽0.01, ***, ###, δδδ P⩽0.001; n=8–10 animals per group. uCMS, unpredictable chronic mild stress protocol; AD, antidepressant; CTRL, non-stressed animals; FLX, animals exposed to uCMS and treated with fluoxetine; FST, forced-swimming test; IMIP, animals exposed to uCMS and treated with imipramine; NOR, novel object recognition; SCT, sucrose consumption test; SAL, animals exposed to uCMS and injected with saline; OF, Open field test; tp1,time point 1 (6 weeks; immediately after the stress protocol cessation); tp2, time point 2 (10 weeks; 4 weeks after the stress protocol cessation).

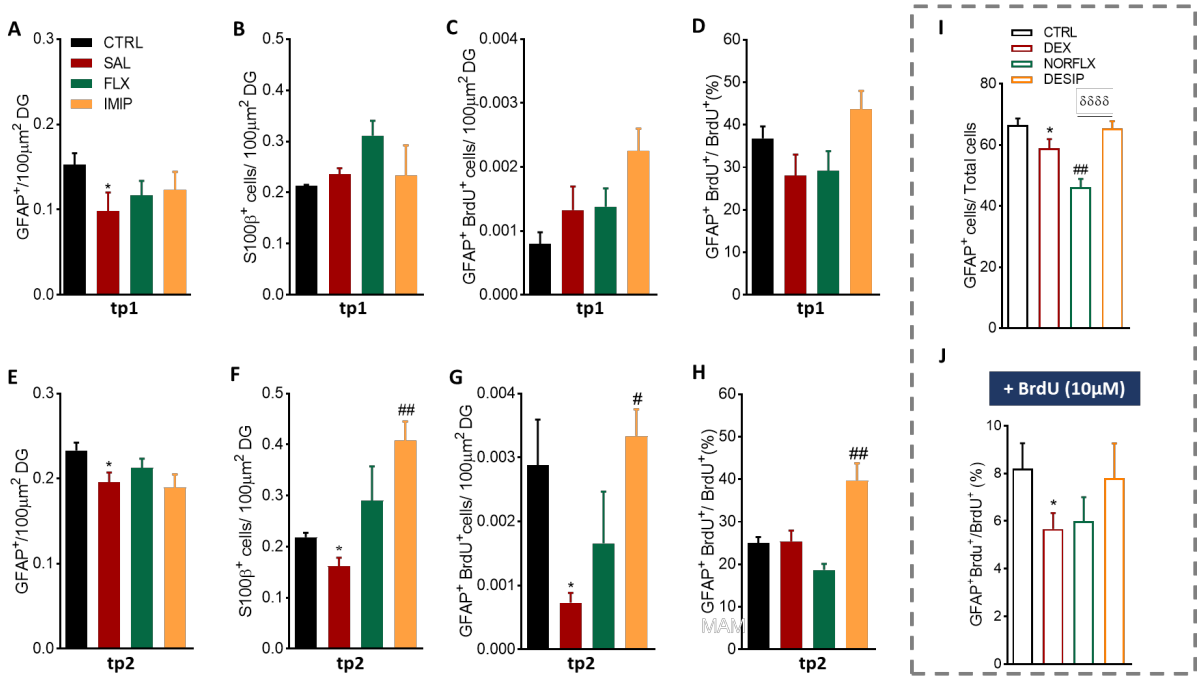


Figure 2. *In vivo* and *in vitro* longitudinal analysis of astrocytic markers in the hippocampal dentate gyrus (dDG) of an animal model of depression and in primary cultures. (A and B) Quantitative analysis of the number of glial fibrillary acidic protein (GFAP)+ cells in the dorsal dentate gyrus (dDG) on tp1 (A) and on tp2 (C), after a six-week uCMS protocol that included a treatment with different antidepressants (ADs), fluoxetine and imipramine. (B and F) Quantitative analysis of the number of S100B+ cells in the dDG, either on tp1 (B) and tp2 (F). (C and G) Quantitative analysis of the number of GFAP+BrdU+ cells, both immediately after the stress protocol, tp1, (C) and 4 weeks after the end of the stress protocol, tp2 (G). (D and H) Analysis of the number of GFAP+BrdU+ cells per total number of BrdU+ cells, both on tp1 (D) and on tp2 (H). (I and J) *In vitro* analysis of hippocampal DG primary cultures of p5 animals, regarding the number of GFAP+ astrocytic cells (I) and astrocytes differentiation – GFAP+BrdU+ (J) after incubation of the primary cell cultures with dexametasone (DEX), norfluoxetine (NORFLX) or desipramine (DESIP) and BrdU. *Denotes the effect of uCMS analyzed by Student's t-test. #Denotes the effect of ADs, by comparison of treatment and SAL animals; and δ denotes differences between ADs, analyzed by one-way analysis of variance (ANOVA). Data are represented as mean±s.e.m. #P⩽0.05, **, ##P⩽0.01, ***, ###, δδδ P⩽0.001; n = 6-8 per group. Abbreviations: GFAP, Glial Fibrillary Acidic Protein; CTRL, non-stressed animals; SAL, animals exposed to uCMS and injected with saline; FLX, animals exposed to uCMS and treated with fluoxetine; IMIP, animals exposed to uCMS and treated with imipramine; dDG, dorsal dentate gyrus; DEX, dexamethasone; NORFLX, Norfluoxetine; DESIP, Desipramine; tp1,time point 1 (6 weeks); tp2, time point 2 (10 weeks).

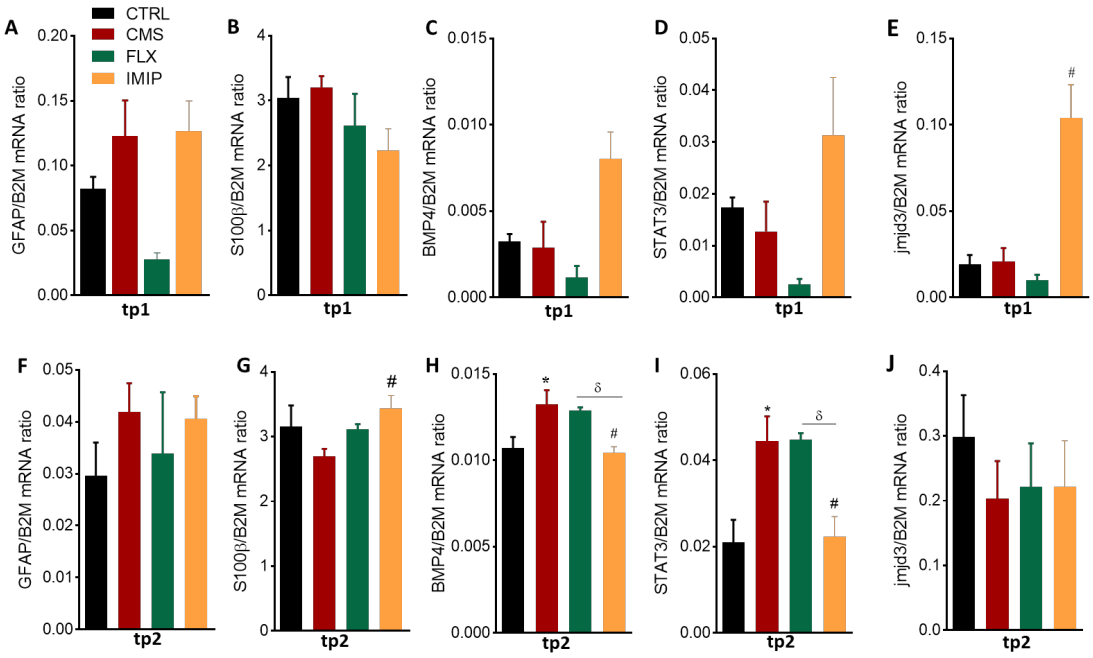


Figure 3. Relative mRNA expression levels of astrocytic and astrogliogenic related genes on tp1 and tp2 in the macrodissected DG. In detail, we analyzed the mRNA expression levels of GFAP (A and F), S100β (B and G), BMP4 (C and H), STAT3 (D and I) and jmjd3 (E and J) in macrodissected dentate gyrus tissue from control animals and uCMS-exposed animals treated with saline or with the antidepressants fluoxetine or imipramine. *Denotes the effect of unpredictable chronic mild stress (uCMS) analyzed by Student's t-test; #denotes the effect of ADs, by comparison of treatment and SAL animals, analyzed by one-way analysis of variance (ANOVA); and δ denotes differences between ADs, analyzed by one-way analysis of variance (ANOVA). Data are represented as mean±s.e.m. #P⩽0.05, **, ##P⩽0.01, ***, ###, δδδ P⩽0.001; n =± 6-8 animals per group. CTRL, non-stressed animals; FLX, animals exposed to uCMS and treated with fluoxetine; IMIP, animals exposed to uCMS and treated with imipramine; SAL, animals exposed to uCMS and injected with saline; GFAP, Glial Fibrillary Acidic Protein; S100β, S100 calcium-binding protein B; BMP4, Bone morphogenetic protein 4; STAT3, Signal transducer and activator of transcription 3; jmjd3, histone H3 Lys 27 (H3K27) demethylase; tp1,time point 1 (6 weeks); tp2, time point 2 (10 weeks).

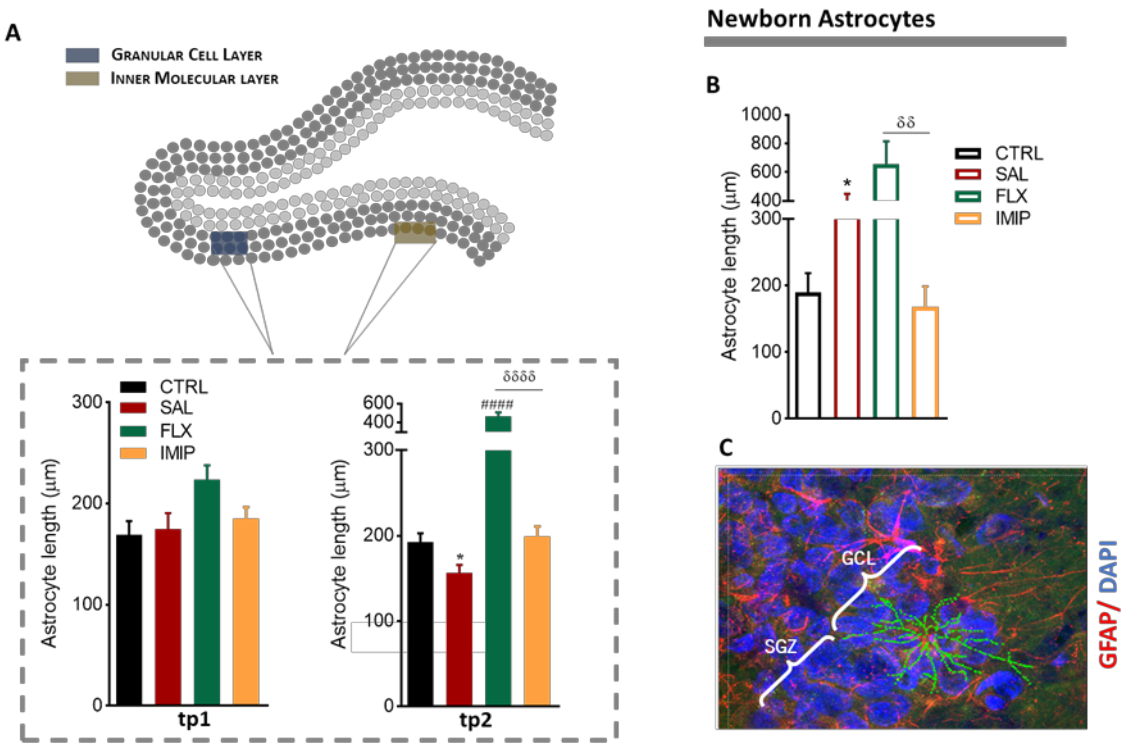


Figure 4. Morphological analysis of resident and newborn astrocytes in the hippocampal DG in a rat model of depression and after antidepressants treatment. (A) Longitudinal analysis of the astrocytic length in the dorsal hippocampal dentate gyrus (dDG) in an experimental animal model of depression, on tp1 and on tp2, specifically from GCL and IML. (B) Analysis of astrocytic length in the granular cell layer (GCL) of the hippocampal DG newborn astrocytes, 4 weeks after the cessation of the uCMS protocol and after treatment with fluoxetine and imipramine. These cells were identified by co-labeling GFAP+ and BrdU+ and were selected in the GCL to avoid stem cells analysis. (C) Representative immunostaining and morphological analysis of GFAP+ cells in the hippocampal DG.

*Denotes the effect of uCMS analyzed by Student's t-test. #Denotes the effect of ADs, by comparison of treatment and SAL animals; and δ denotes differences between ADs, analyzed by one-way analysis of variance (ANOVA). Data are represented as mean±s.e.m. #P⩽0.05, **, ##P⩽0.01, ***, ###, δδδ P⩽0.001; n = 6-8 per group. Abbreviations: GFAP, Glial Fibrillary Acidic Protein; CTRL, non-stressed animals; SAL, animals exposed to uCMS and injected with saline; FLX, animals exposed to uCMS and treated with fluoxetine; IMIP, animals exposed to uCMS and treated with imipramine; dDG, dorsal dentate gyrus; GCL, granule cell layer; SGZ, subgranular zone; tp1,time point 1 (6 weeks); tp2, time point 2 (10 weeks).

SUPPLEMENTARY INFORMATION

Supplementary Methods

Behavior Analysis

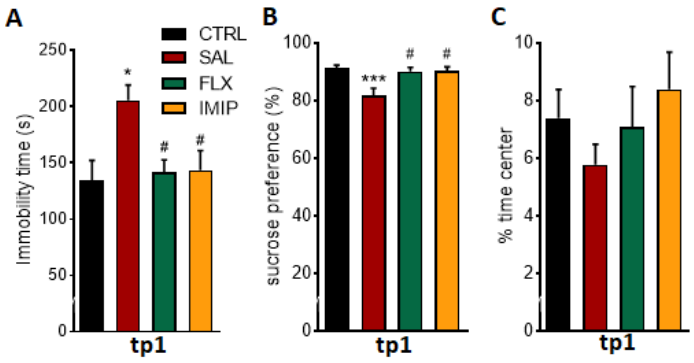
Forced swim test (FST): Depressive-like behavior was assessed at the end of the uCMS protocol using the FST. Test trials were conducted 24 h after a 5-min pre-test session. For that, rats were placed in glass cylinders filled with water (23° C; 50 cm deep) for 5 min. An increase in immobility time was taken as a measure of depressive-like behavior.

Sucrose consumption test (SCT): Anhedonia was assessed during the last week of uCMS using the SCT. Briefly, animals were allowed to habituate to the sucrose solution in the week before the beginning of the uCMS protocol to establish baseline preference levels. To test sucrose preference, animals were food and water deprived for 12 h and then presented with two pre-weighed bottles containing 2% sucrose solution or tap water for a period of 1 h (starting at the beginning of the dark period). Sucrose preference was calculated as previously described (Bessa et al., 2009; Patrício et al., 2015).

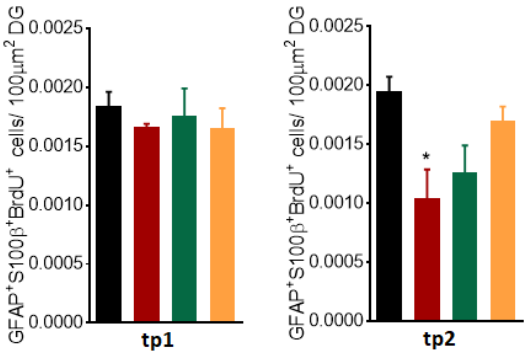
Open field test: Anxiety was investigated using the open-field test in a room brightly illuminated by white light. Briefly, rats were placed in the center of an arena (43.2 × 43.2 cm2, transparent acrylic walls and white floor, MedAssociates, St Albans, VT, USA) and instant position was monitored online over a period of 5 min with the aid of two 16-beam infrared arrays. The percentage of time spent in the center of the arena was used as a direct measure of anxiety-like behavior.

Novel object recognition (NOR): Short and long-term memory was assessed using the NOR test (Vogel-Ciernia & Wood, 2014). Rats were first familiarized to the testing arena consisting of a black acrylic box (50 x 50 x 150 cm) with an open field space, for 8 min and with no objects inside. On the following day, animals were allowed to freely explore two identical objects for 10 min. Twenty-four hours later, animals returned to the arena for 3 min, with one of the objects replaced by a novel one. The familiar and novel objects differed on size, shape, texture and color. The NOR arena was cleaned with 10% ethanol between trials to avoid odor cues. All sessions were videotaped, and the time spent exploring both objects was determined manually. Analysis were conducted blind. In case of repeated testing, distinct objects were presented in each timepoint of analysis. Analysis was conducted blindly. The percentage of time spent exploring the novel object was used as a measure of long-term and short-term memory performance.

Supplementary Figures

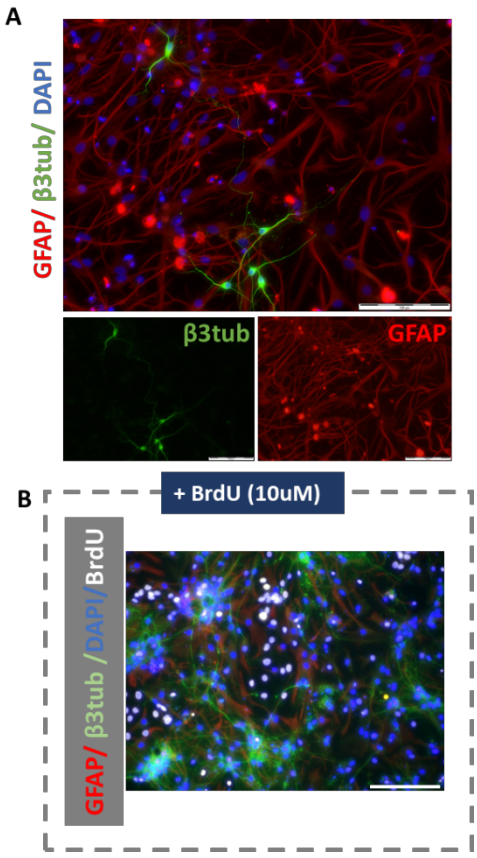


Supplementary Figure 1. Assessment of animals' behavior immediately after the stress protocol to validate the depressive-like phenotype. (A) Depressive-like behavior induced by stress exposure was assessed by the forced swimming test (FST). (B) Anhedonic-like behavior induced by stress exposure was assessed by the sucrose preference test (SPT). (C) Anxiety-like behavior was assessed by the Open field test (OF). *Denotes the effect of uCMS analyzed by Student's *t*-test; #Denotes the effect of ADs, by comparison of treatment and SAL animals, analyzed by one-way ANOVA. Data represented as mean ± SEM. $^{*,\#}$ $P \leq 0.05$, $^{**,\#\#}$ $P \leq 0.01$; $n = 8$-10 animals *per* group. Abbreviations: TP, time-point; CTRL, non-stressed animals; SAL, animals exposed to uCMS and injected with saline; FLX, animals exposed to uCMS and treated with fluoxetine; IMIP, animals exposed to uCMS and treated with imipramine.



Supplementary Figure 2. Quantitative analysis of the number of GFAP+S100B+BrdU+ cells in the dDG on tp1 (A) and on tp2 (B), after a six-week uCMS protocol that included a treatment with different antidepressants (ADs), fluoxetine and imipramine.

*Denotes the effect of uCMS analyzed by Student's t-test. Data are represented as mean±s.e.m. *P⩽0.05; n = 6-8 per group. Abbreviations: GFAP, Glial Fibrillary Acidic Protein; S100B, S100 calcium-binding protein B; CTRL, non-stressed animals; SAL, animals exposed to uCMS and injected with saline; FLX, animals exposed to uCMS and treated with fluoxetine; IMIP, animals exposed to uCMS and treated with imipramine; BrdU, Bromodeoxyuridine; tp1,time point 1 (6 weeks); tp2, time point 2 (10 weeks).



Supplementary Figure 3. A) Representative images of an immunocytochemistry of hippocampal primary cells in a control plate, with neurons labelled with β3-tubulin, astrocytes with GFAP and cell nucleus with DAPI. (B) Representative images of an immunocytochemistry of hippocampal primary cells in a control plate after incubating with BrdU, with neurons labelled with β3 tubulin, astrocytes with GFAP, proliferating cells with BrdU and cell nucleus with DAPI. Abbreviations: GFAP, Glial Fibrillary Acidic Protein; β3tub, β3-tubulin; DAPI, 4',6'-diamino-2-fenil-indol; BrdU, Bromodeoxyuridine.

Chapter 4

ASTROCYTIC PLASTICITY AT THE DORSAL DENTATE GYRUS ON AN ANIMAL MODEL OF RECURRENT DEPRESSION

Published in *Neuroscience* (2019)

doi: 10.1016/j.neuroscience.2019.10.032. [Epub ahead of print]

NEUROSCIENCE
RESEARCH ARTICLE

A. R. Machado-Santos et al. / Neuroscience xxx (2019) xxx–xxx

Astrocytic plasticity at the dorsal dentate gyrus on an animal model of recurrent depression

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Abstract—Astrocytes are now known to play crucial roles in the central nervous system, supporting and closely interacting with neurons and therefore able to modulate brain function. Both human *postmortem* studies in brain samples from patients diagnosed with Major Depressive Disorder and from animal models of depression reported numerical and morphological astrocytic changes specifically in the hippocampus. In particular, these studies revealed significant reductions in glial cell density denoted by a decreased number of S100B-positive cells and a decrease in GFAP expression in several brain regions including the hippocampus. To reveal plastic astrocytic changes in the context of recurrent depression, we longitudinally assessed dynamic astrocytic alterations (gene expression, cell densities and morphologic variations) in the hippocampal dentate gyrus under repeated exposure to unpredictable chronic mild stress (uCMS) and upon treatment with two antidepressants, fluoxetine and imipramine. Both antidepressants decreased astrocytic complexity immediately after stress exposure. Moreover, we show that astrocytic alterations, particularly an increased number of S100B-positive cells, are observed after recurrent stress exposure. Interestingly, these alterations were prevented at the long-term by either fluoxetine or imipramine treatment.

This article is part of a Special Issue entitled: SI: Brain Metaplasticity

Keywords: Astrocytes, Dentate gyrus, Chronic stress, Recurrent depression, Antidepressants.

INTRODUCTION

Astrocytes are the main type of glial cells and known to play key roles in the central nervous system including maintenance of brain homeostasis, suppliance of nutrition and protection to neurons, synaptic function support, recycling neurotransmitters, promoting connection to the blood vessels, and by stimulating adult hippocampal neurogenesis (Christopherson et al., 2005; Perea and Araque, 2010; Ma et al., 2012; Oliveira et al., 2015; Boku et al., 2018). Typically, astrocytes can be identified through the expression of specific markers such as the glial-fibrillary acid protein (GFAP) and the calcium (Ca^{2+})-binding protein S100B, and due to their star-like morphology (Pekny and Pekna, 2004; Goncalves et al., 2008). In the last decade, a large amount of findings has associated astrogliopathology to the emergence of mood disorders including major depressive disorder (MDD) (Miguel-Hidalgo et al., 2000; Rajkowska, 2000), a recurrent, chronic and devastating neuropsychiatric disorder recently recognized as the leading cause of disability worldwide by the World Health Organization (2017). Both human and preclinical studies have reported numerical and morphological astrocytic changes particularly in the hippocampus, a brain region specially known by its important contribution to cognition and emotional behaviors, which are typically affected in depression (Manji et al., 2000; Cotter et al., 2001; Harrison, 2002; Femenia et al., 2012). Histopathological analysis in *postmortem* brain samples from patients diagnosed with MDD reveal significant reductions in glial cell density as denoted by a decreased number of S100B-expressing cells (Gos et al., 2013) and a significant decreased GFAP

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[1] These authors contributed equally to this work.

Abbreviations: ADs, antidepressants; DG, dentate gyrus; FLX, fluoxetine; GCL, granule cell layer; GFAP, glial-fibrillary acid protein; MDD, major depressive disorder; SAL, saline; SGZ, subgranular zone; uCMS, unpredictable chronic mild stress.

https://doi.org/10.1016/j.neuroscience.2019.10.032
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expression in several brain regions including the hippocampus (Cobb et al., 2016). Furthermore, it has been reported that S100B levels are increased in the serum and cerebral spinal fluid of MDD patients (Arolt et al., 2003; Hetzel et al., 2005; Schroeter et al., 2008; Schroeter and Steiner, 2009; Schroeter et al., 2010) either with a single or recurrent depressive episodes, with different severity of depressive symptoms (assessed with the Hamilton Depression Scale), and can be reduced in response to antidepressants (ADs) (Schroeter et al., 2008; 2010). In agreement, preclinical studies using animals models of depression consistently reveal a decreased expression and number of cells expressing S100B and GFAP (Banasr and Duman, 2008; Liu et al., 2009; Ye et al., 2011; Sun et al., 2012; Li et al., 2013; Tynan et al., 2013; Kim et al., 2018), including in the dentate gyrus (DG) of the hippocampus (Fuchs et al., 2004; Czeh et al., 2007). Interestingly, reduced levels of hippocampal S100B protein expression induced by chronic stress exposure can be reversed by treatment with fluoxetine (Schroeter et al., 2013). Additionally, it has been consistently reported that depression and stress significantly impact on morphometric properties of astrocytes, including in the size and branching (reviewed in (Kim et al., 2018)) either in animal (Czeh et al., 2006; Tynan et al., 2013; Saur et al., 2016)) and human studies (Rajkowska et al., 1999; Torres-Platas et al., 2011). Indeed, in the mature brain, the processes of protoplasmic astrocytes extensively infiltrate into the neuropil and wrap around synapses, actively changing the coverage of synaptic contacts during development, in response to injury and in various physiological conditions, which is known as astrocytic complexity (Hirrlinger et al., 2004; Theodosis et al., 2008; Procko et al., 2011; Allen and Eroglu, 2017). Interestingly, distinct classes of ADs were able to re-establish both numerical and morphological astrocytic alterations (Schroeter et al., 2002; Iwata et al., 2011; Sanacora and Banasr, 2013; Di Benedetto et al., 2016). Together, these studies highlight astrocytic plasticity as an important player for the pathogenesis and treatment of depression. However, taking into account the high rate of recurrence in depression, it is of great importance to understand the longitudinal dynamics and consequent contribution of astrocytic alterations along the course of the disease, including those promoted by ADs, and how they relate to the development of resistance or susceptibility to subsequent depressive episodes (Czeh and Di Benedetto, 2013; Rajkowska and Stockmeier, 2013; Sanacora and Banasr, 2013). Hence, to characterize astrocytic plasticity in the context of recurrent depression, we longitudinally assessed dynamic astrocytic alterations, namely expression of genes, density and morphology, in the hippocampal DG under repeated exposure to unpredictable chronic mild stress (uCMS). To unravel the immediate and long-term astrocytic alterations promoted by AD treatment, subsets of animals were treated with two typical ADs, fluoxetine – a selective inhibitor of serotonin reuptake – and imipramine – a tricyclic agent and a potent inhibitor of serotonin and norepinephrine reuptake. We showed that both AD drugs transiently decrease astrocytic morphological complexity immediately after a first stress exposure. Moreover, astrocytic alterations, particularly an increase in the number of S100B-expressing cells in the hippocampal DG, are observed after a recurrent stress exposure. Interestingly, these alterations were prevented at the long-term through the treatment with either fluoxetine or imipramine.

EXPERIMENTAL PROCEDURES

Animals

Experiments were conducted in adult male (2 months old) Wistar Han rats (Charles River Laboratories, L'Arbresle, France) group housed and kept under standard laboratory conditions at 22 ± 1 °C, 55% relative humidity, 12 h light/dark cycle, food and water *ad libitum*. Rats were randomly divided into four groups, next described in detail. All the procedures were conducted in accordance with EU Directive 2010/63/EU and the Portuguese National Authority for animal experimentation, *Direção-Geral de Alimentação e Veterinária (DGAV)*.

Unpredictable chronic mild stress (uCMS) and drug treatment

Rats were exposed to a pre-validated uCMS protocol for 6 weeks, as previously described (Bessa et al., 2009; Mateus-Pinheiro et al., 2013). This stress paradigm was shown to induce depressive-like behavior, anxiety-like phenotype and cognitive deficits in rats through a random and unpredictable exposure to a wide range of different mild stressors including: confinement to a restricted space for 1 h; placement in a tilted cage (30°) for 3 h; housing on damp bedding for 8 h; removal of bedding material for 3 h; overnight illumination; 15 h of food deprivation followed by exposure to inaccessible food for 1 h; water deprivation for 15 h followed by exposure to an empty bottle for 1 h; exposure to stroboscopic lights during 4 h; replacement of bedding material by cold water (4 °C) for 2 h and reversed light/dark cycle for 48 h, every 7 days. Similarly to previous studies (Bessa et al., 2009; Mateus-Pinheiro et al., 2013), in the last 2 weeks of the protocol, animals were daily injected intraperitoneally with saline (SAL) or with ADs either fluoxetine (FLX; 10 mg.kg^{-1}, Kemprotec, Middlesbrough, UK) or imipramine (IMIP; 10 mg.kg^{-1}; Kemprotec). At the same time, a group of animals not exposed to uCMS, was also injected with saline (CTRL). For the following 4 weeks after uCMS, animals were not subjected to any stressor. Next, to mimic recurrent depression, animals were exposed to a subsequent uCMS protocol (Alves et al., 2017). To avoid habituation to previous stressors, animals were exposed to a slight modified version of the uCMS protocol that included additional stressors, namely removal of sawdust for 3 h, replacement of sawdust with cold water (4 °C) for 2 h and switch of cagemates for 2 h.

Immunostaining procedures

Animals (n = 4–7 *per* group) were deeply anaesthetized with sodium pentobarbital (20%; Eutasil®, Sanofi,

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Gentilly, France) and transcardially perfused with 0.9% saline followed by cold 4% paraformaldehyde (PFA). Brains were removed, post-fixed in 4% PFA, cryoprotected in 30% sucrose overnight, and then embedded in Optimal Cutting Temperature compound (OCT, ThermoScientific, Waltham, MA, USA), snap-frozen and stored at −20 °C. Coronal sections (20 μm) containing the dorsal pole of the hippocampal dentate gyrus (DG) were further stained to assess alterations on astroglial populations. Of notice, tissue samples used in this study were retrieved from a subset of animals used in Alves et al, 2017. Sections were stained with GFAP (1:200; Dako, Glostrup, Denmark) and S100B (1:100; Dako, Glostrup, Denmark). Cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, 1:200; Sigma Aldrich). In addition to the total number of cells *per* DG, the analysis was performed individually for distinct DG subregions as previously (Alves et al., 2018): along its transverse axis, in the suprapyramidal and infrapyramidal blades; granule cell layer (GCL) and the subgranular zone (SGZ), defined as the three deepest rows of granule cells, bordering the *hilus* (Silva et al., 2006; Miller et al., 2013). Additionally, the density of each cell population in the DG was determined by the ratio of the total number of cells and the respective area. Analysis and cell counting were performed using a confocal microscope (Olympus Fluo-ViewTM FV1000, Hamburg, Germany) and each area was determined using an optical microscope (Olympus BX51). Importantly, $GFAP^+$ cells in the DG exhibiting a radial morphology were not included in the analysis as these are cells typically classified as (type-1) neural stem cells (von Bohlen und Halbach, 2011). Observers were blind to the experimental condition of each subject. Cell densities are reported as number of cells *per* mm^2 or percentage of cells of interest in a specific DG subregion.

Morphological analyses

To analyze astrocytic morphology, we applied the previously described open-source tool *Simple Neurite Tracer* (Longair et al., 2011), that was previously reported to enable the tridimensional reconstruction of astrocytic main processes in GFAP-stained sections (Tavares et al., 2017) as this marker specifically stains astrocyte main processes and its expression is tightly related to morphological alterations. After immunostaining, z-stacks of confocal images (magnification: 40×; n = 5–10 astrocytes *per* subregion) were used to determine total processes length (in μ*m*), number of processes, maximum intersection radius (in μ*m*) and number of intersections from the soma.

RT-PCR measurements

For dorsal DG microdissection, animals were firstly anesthetized with pentobarbital (20%; Eutasil®, Sanofi) and transcardially perfused with 0.9% saline. Immediately after dissection, brain tissue was frozen and stored at −80 °C until further analysis.

Total RNA was isolated from the microdissected DGs using the Direct-Zol™ RNA Mini Prep (Zymo Research, CA, USA), according to the manufacturer's instructions.

Total RNA (500 ng) was reverse-transcribed using qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA).

For real-time RT-PCR, oligonucleotide primers for S100 calcium-binding protein b (S100b, sense CAC CGACTGGGCAAAATACT, antisense TCCGAACTTCCA TGTCC), Glial Fibrillary Acidic Protein (GFAP, sense GGACCAGCTTACTACCAACAGTGCC, antisense TGG TTTCATCTTGGAGCTTCTGCCT) and B2M (sense GTGCTTGCCATTCAGAAAACTCC, antisense AGGTG GGTGGAACTGAGACA) were designed using Primer-BLAST software (NCBI). Reactions were performed in an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, LLC, CA, USA) using 5X HOT FIREPol EvaGreen qPCR Mix Plus, ROX (Solis Biodyne, Tartu, Estonia), Target gene expression levels were normalized against the housekeeping gene Beta-2-Microglobulin (B2M). The relative expression was calculated using the DDCt method. Results are presented as fold-change of mRNA levels between the respective experimental groups after normalization to B2M levels.

Statistical analysis

Statistical analysis was performed using Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA). Animals were randomly assigned to the experimental groups. All presented data satisfied normal distribution in Kolmogorov–Smirnov testing. After confirmation of homogeneity of group variances, data was subjected to the appropriate statistical tests. Student's t-test was used for statistical comparisons between experimental groups when appropriate. The comparison between stressed groups was evaluated using One-way analysis of variance. Analysis of variance repeated measures was used to analyze the number of intersections from the soma. Descriptive statistical results are presented as mean ± standard error of the mean (SEM). Differences between groups were determined by Bonferroni's *post-hoc* multiple comparison test and statistical significance was set at $P < 0.05$.

RESULTS

Antidepressants imipramine and fluoxetine prevent the increased astrocytic density induced by cumulative exposure to stress

To characterize longitudinal dynamic astrocytic alterations in a previously described animal model of recurrent depression (Alves et al., 2017), we assessed changes at two different time-points: (1) at week 6 (*W6*), after exposure to a 6-week uCMS protocol that included 2-weeks of antidepressant (AD) treatment and, (2) at week 16 (*W16*), after re-exposure to a 6-weeks uCMS protocol (Fig. 1A). Assessment of quantitative changes in the density of $S100B^+$ cells in the dorsal dentate gyrus (dDG) (Ogata and Kosaka, 2002), revealed that exposure to a single uCMS protocol did not impact in the number of mature astrocytes in the dDG ($P > 0.1$; Fig. 1B). Similarly, treatment with either fluoxetine (FLX) or imipramine

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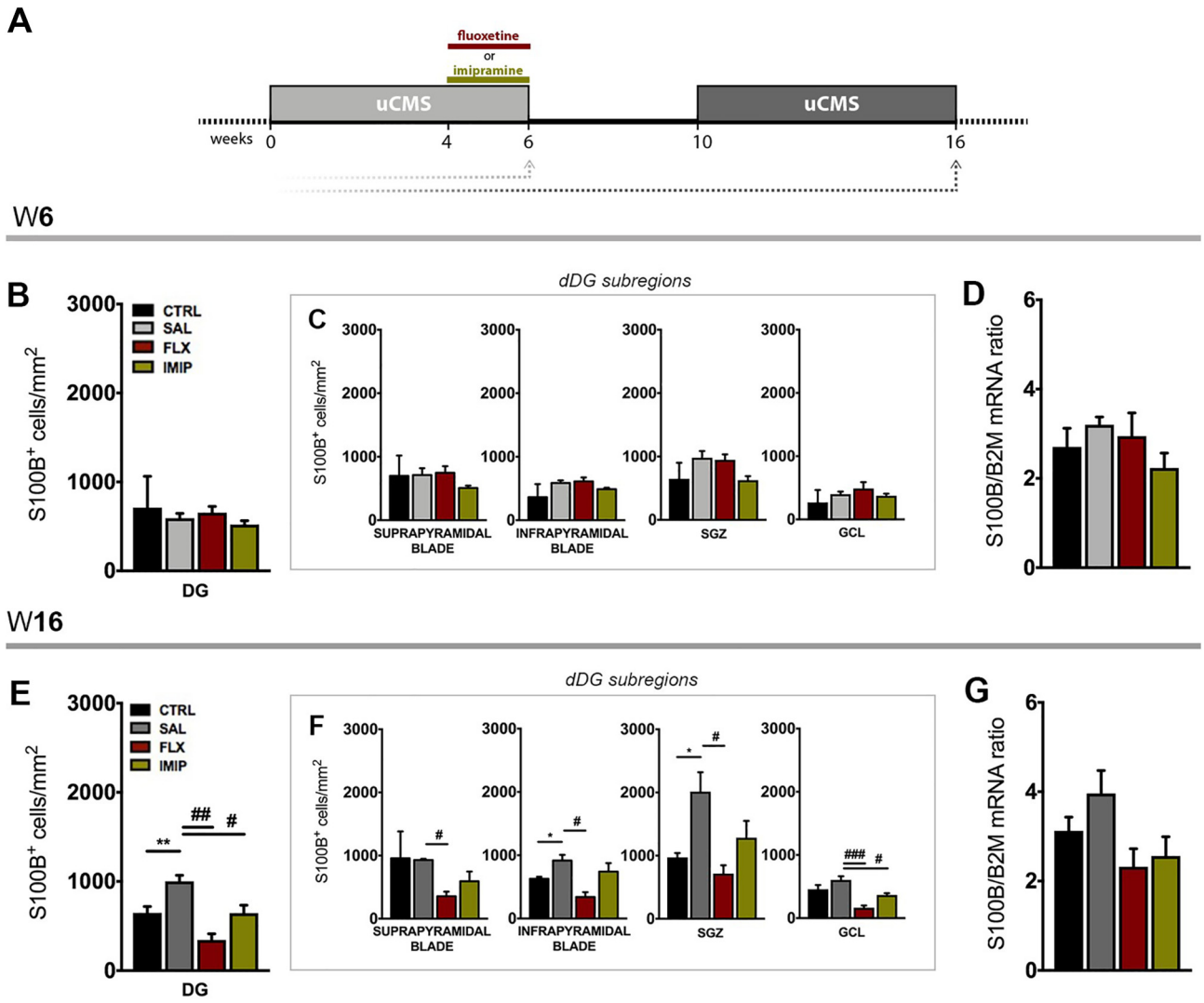

Fig. 1. Longitudinal analysis of the number of mature astrocytes in the dorsal hippocampal dentate gyrus (dDG) in an experimental animal model of recurrent depression. (**A**) Experimental design of the current study. (**B** and **C**) Quantitative analysis of the number of S100b$^+$ cells in the dDG and their subregions after a six-week uCMS protocol that included a treatment with different antidepressants (ADs), fluoxetine and imipramine. (**D**) S100b mRNA expression in the dDG at the experimental week 6 (W6). (**E** and **F**) Quantitative analysis of the number of S100b$^+$ cells in the dDG and their subregions, after re-exposure to uCMS. (**G**) S100b mRNA expression in the dDG at the experimental week 6 (W16). *Denotes the effect of uCMS analyzed by Student's *t*-test. #Denotes the effect of ADs, by comparison of treatment and SAL animals, analyzed by one-way analysis of variance (ANOVA); Data represented as mean ± SEM. *, # P ⩽0.05, **, ## P⩽0.01, ***, ###, P ⩽0.001; *n* = 4–6 *per* group. Abbreviations: uCMS, unpredictable chronic mild stress protocol; S100b, S100 calcium-binding protein B; CTRL, non-stressed animals; SAL, animals repeatedly exposed to uCMS and non-treated; FLX, animals repeatedly exposed to uCMS and treated with fluoxetine; IMIP, animals repeatedly exposed to uCMS and treated with imipramine; dDG, dorsal dentate gyrus, SGZ, subgranular zone; GCL, granule cell layer.

(IMIP) in the last two weeks of uCMS, did not induce significant changes in the density of mature astrocytes ($F_{2,10}$ = 1.14, P > 0.1; Fig. 1B). Moreover, the analysis to dDG subregions [subdivision by the transverse axis into supra- and infrapyramidal poles, subgranular zone (SGZ) and granule cell layer (GCL)] revealed no region-specific changes in the number of S100B$^+$ cells among the different experimental groups (suprapyramidal: P > 0.1, $F_{2,9}$ = 1.83, P > 0.1; infrapyramidal: P = 0.075, $F_{2,10}$ = 1.40, P > 0.1; SGZ: P = 0.09, $F_{2,10}$ = 1.83, P > 0.1; GCL: P > 0.1, $F_{2,10}$ = 0.99, P > 0.1; Fig. 1C). In accordance, relative mRNA expression levels of S100B in the DG were not altered (P > 0.1, $F_{2,12}$ = 1.32, P > 0.1, Fig. 1D). Interestingly, single exposure to uCMS significantly decreased the number of GFAP$^+$ cells in the dDG and all the considered subregions (DG: P = 0.0021; suprapyramidal: P < 0.001; infrapyramidal: P = 0.0089; SGZ: P = 0.0156; GCL: P < 0.001; Fig. 2A–B). These alterations were not reversed by AD treatment with fluoxetine or imipramine. mRNA expression levels of GFAP in the DG were not altered by the initial exposure to uCMS or AD treatment (P > 0.1, $F_{2,12}$ = 3.092, P > 0.1, Fig. 2C).

After the first exposure to uCMS animals were kept at rest for a 4 week period and then re-exposed to uCMS (Alves et al., 2017). After this second stress exposure we observed a significant increase in the number of S100B$^+$ cells in the dDG of non-treated animals

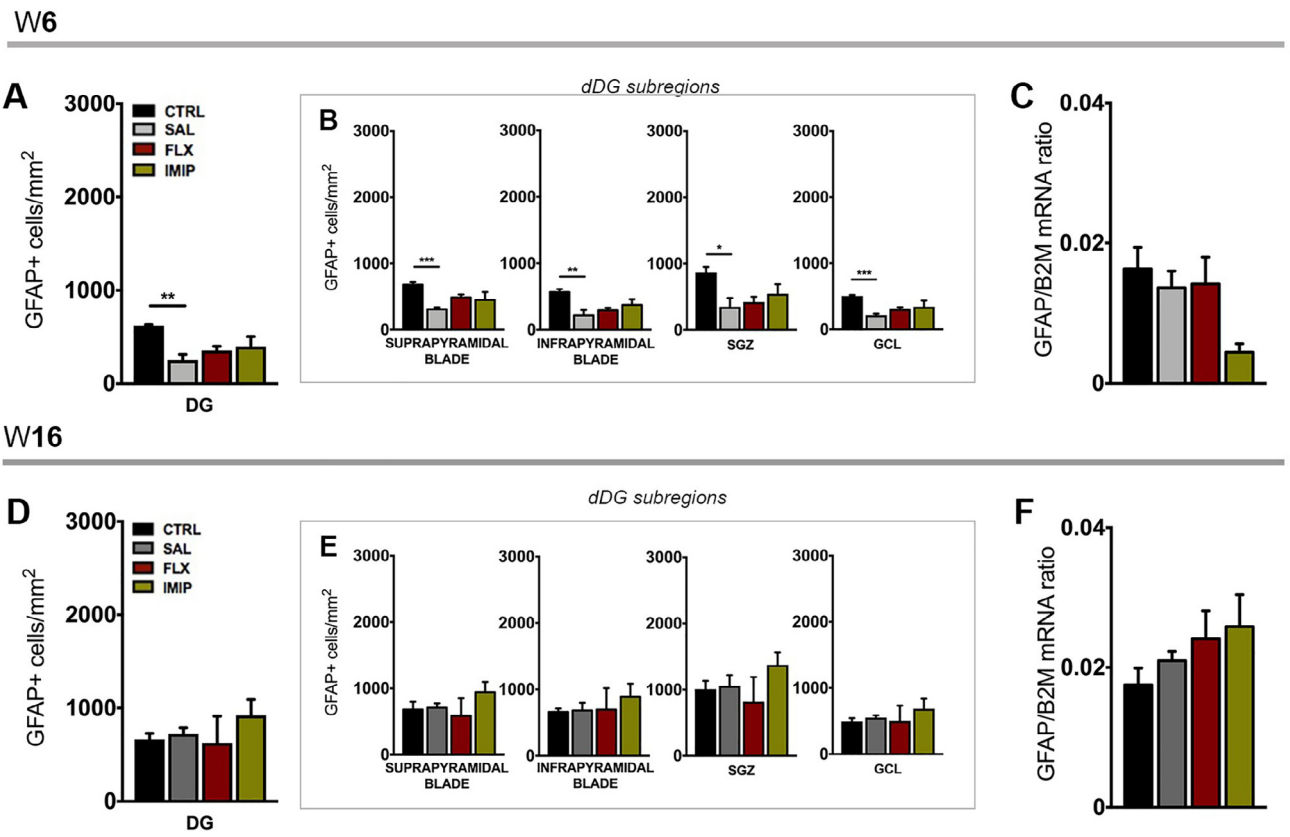

Fig. 2. Longitudinal analysis of the number of GFAP$^+$ cells in the dorsal hippocampal dentate gyrus (dDG) in an experimental animal model of recurrent depression. (**A** and **B**) Quantitative analysis of the number of GFAP$^+$ cells in the dDG and their subregions after a six-week uCMS protocol that included a treatment with different antidepressants (ADs), fluoxetine and imipramine. (**C**) GFAP mRNA expression in the dDG at the experimental week 6 (W6). (**D** and **E**) Quantitative analysis of the number of GFAP$^+$ cells in the dDG and their subregions, after re-exposure to uCMS. (**F**) GFAP mRNA expression in the dDG at the experimental week 6 (W16). *Denotes the effect of uCMS analyzed by Student's t-test. Data represented as mean ± SEM. * $P \leqslant 0.05$, ** $P \leqslant 0.01$, *** $P \leqslant 0.001$; n = 4–6 per group. Abbreviations: GFAP, Glial Fibrillary Acidic Protein; CTRL, non-stressed animals; SAL, animals repeatedly exposed to uCMS and non-treated; FLX, animals repeatedly exposed to uCMS and treated with fluoxetine; IMIP, animals repeatedly exposed to uCMS and treated with imipramine; dDG, dorsal dentate gyrus, SGZ, subgranular zone; GCL, granule cell layer.

(P = 0.008; Fig. 1E). Interestingly, fluoxetine or imipramine treatment during the first uCMS exposure successfully prevented the effects of the second uCMS re-exposure by significantly decreasing the density of S100B$^+$ cells in the dDG ($F_{2,7}$ = 20.62, P = 0.001; FLX: *post hoc* P = 0.001; IMIP: *post hoc* P = 0.03, Fig. 1E). Importantly, the number of S100B$^+$ cells is slightly decreased in case of treatment with fluoxetine. Among the considered dDG subregions, re-exposure to uCMS significantly increased the number of detected S100B$^+$ cells in the infrapyramidal blade and SGZ (infrapyramidal: P = 0.02; SGZ: P = 0.02; Fig. 1F). Treatment with ADs, particularly fluoxetine, prevented the increased density of mature astrocytes observed upon re-exposure to uCMS (infrapyramidal: $F_{2,7}$ = 8.52, P = 0.01; FLX: *post hoc* P = 0.01; IMIP: *post hoc* $P > 0.1$; SGZ: $F_{2,10}$ = 5.45, P = 0.03; Fig. 1F). No significant alterations were observed in the suprapyramidal blade and GCL (suprapyramidal: $P > 0.1$; GCL: P = 0.08; Fig. 1F). Still, animals previously treated with fluoxetine or imipramine tend to present a lower density of S100B$^+$ cells in the suprapyramidal blade and GCL after re-exposure to uCMS (suprapyramidal: $F_{2,6}$ = 8.51, P = 0.02; FLX: *post hoc* P = 0.02; IMIP: *post hoc* $P > 0.1$; GCL: $F_{2,8}$ = 19.06, $P < 0.001$, FLX: *post hoc* $P < 0.001$; IMIP: *post hoc* P = 0.03; Fig. 1F). Also, S100B mRNA expression levels in the DG were moderately increased in consequence of repeated uCMS exposure, which was not clear in animals previously treated with fluoxetine and imipramine (P = 0.1, $F_{2,14}$ = 3.82, P = 0.048, Fig. 1G). Strikingly, the number of GFAP$^+$ cells in all experimental groups was re-established to the level of control animals upon re-exposure to uCMS ($P > 0.1$; Fig. 2D–E). Similarly, no alterations were observed in the GFAP mRNA levels in the DG ($P > 0.1$, $F_{2,13}$ = 0.852, $P > 0.1$, Fig. 2F). Taken together, these astrocytic changes in the hippocampal DG denote a distinct response to a single or recurrent exposure to chronic stress regarding the number of mature astrocytes. Representative immunostaining image of S100B-stained astrocyte and their regional distribution throughout the hippocampal DG are shown in Fig. 3A–B. Representative

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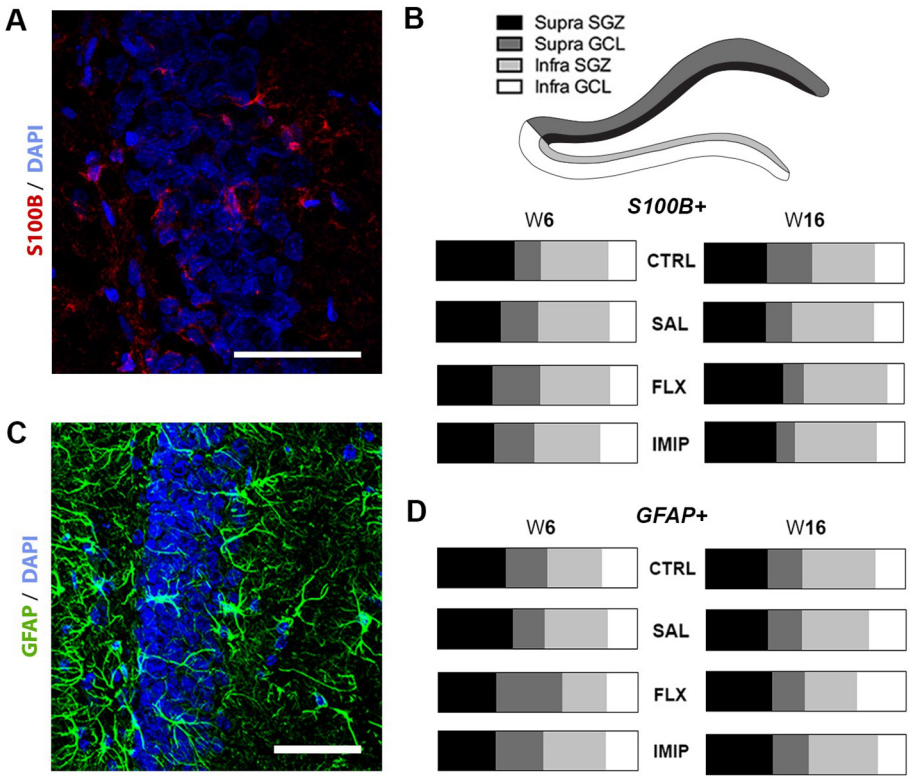

Fig. 3. Cell distribution of GFAP-positive and S100B-positive cells across the dorsal DG subregions and the impact of a single (W6) and recurrent exposure to uCMS. (**A**) Representative images of $S100B^+$ cells. (**B**) Cell distribution of $S100B^+$ cells across the dorsal DG subregions and the impact of a single (W6) and recurrent exposure to uCMS (W16) as well as initial treatment with antidepressants (AD), fluoxetine and imipramine. (**C**) Representative immunostaining of $GFAP^+$ cells in the hippocampal DG. (**D**) Cell distribution of $GFAP^+$ cells across the dorsal DG subregions and the impact of a single (W6) and recurrent exposure to uCMS (W16) as well as initial treatment with antidepressants (AD), fluoxetine and imipramine. Abbreviations: S100B, S100 calcium-binding protein b; uCMS, unpredictable chronic mild stress protocol; CTRL, non-stressed animals; SAL animals exposed to uCMS and injected with saline; FLX, animals exposed to uCMS and injected with fluoxetine; IMIP, animals exposed to uCMS and injected with imipramine; GFAP, Glial Fibrillary Acidic Protein.

immunostaining image of GFAP-stained astrocytes and their regional distribution throughout the hippocampal DG are shown in Fig. 3C–D.

Fluoxetine and imipramine transiently decreased astrocytic complexity

We also assessed changes in astrocytic morphology at the hippocampal DG by the tracings of GFAP-stained astrocytes at the same time-points described for cell density analyses. Our analysis reveals that a single exposure to the uCMS protocol (W6) had no impact on astrocytic morphology, denoted by no changes on *total length*, *number of processes*, *maximum intersection radius* and the *number of intersections from soma* (sholl analysis) ($P > 0.1$; Fig. 4A–D). Similarly, AD treatment with fluoxetine or imipramine within the last two weeks of uCMS did not impact on *total length* and the *number of processes* (*total length*: $F_{2,6} = 3.49$, $P = 0.099$; FLX: *post hoc* $P > 0.1$; IMIP: *post hoc* $P > 0.1$; *number of processes*: $P = 0.08$, $F_{2,6} = 0.21$, $P > 0.1$, FLX: *post hoc* $P > 0.1$; IMIP: *post hoc* $P > 0.1$; Fig. 4A–B). However, treatment with fluoxetine or imipramine significantly decreased *maximum intersection radium* ($F_{2,7} = 7.49$, $P = 0.018$; FLX: *post hoc* $P = 0.042$; IMIP: *post hoc* $P = 0.029$; Fig. 4C). Despite no overall statistical differences on *number of intersections from soma*, astrocytes from animals treated with fluoxetine or imipramine presented a significant decrease in number of intersections at specific distances from the soma. The decreased *maximum intersection radium* and lower *number of intersections from the soma* indicates that chronic AD treatment with fluoxetine and imipramine promoted a reduced complexity of astrocytes in the hippocampal DG. A representative tracing of a GFAP-stained astrocyte from each experimental group at the end of a single uCMS exposure is presented in Fig. 4E. Re-exposure to stress did not promote any particular change on *total length*, *number of processes*, *maximum intersection radius* and the *number of intersections from soma* of astrocytes in the hippocampal DG (W16, $P > 0.1$; Fig. 4F–I). Previous treatment with fluoxetine or imipramine induced no alterations in any of the parameters analyzed after re-exposure to stress. Overall, and despite we could not detect any impact of a single or recurrent exposure to uCMS in the morphology of GFAP-stained cells, treatment with fluoxetine and imipramine promoted a transient decrease of astrocytic complexity in the hippocampal DG. A representative tracing of GFAP-stained astrocyte from each experimental group at the end of the second uCMS exposure is presented on Fig. 4J.

DISCUSSION

In this study we assessed longitudinal changes of different astrocytic parameters (cell number, morphology and expression of genes) in the hippocampal DG of a previously described animal model of recurrent depression (Alves et al., 2017). Using this animal model, we observed earlier that AD treatment with fluoxetine and imipramine evoked a distinct susceptibility to the behavioral effects of repeated exposure to chronic stress. While fluoxetine-treated animals where behaviorally affected by a second uCMS protocol, treatment with imipramine prevented the re-appearance of a depressive-like behavior.

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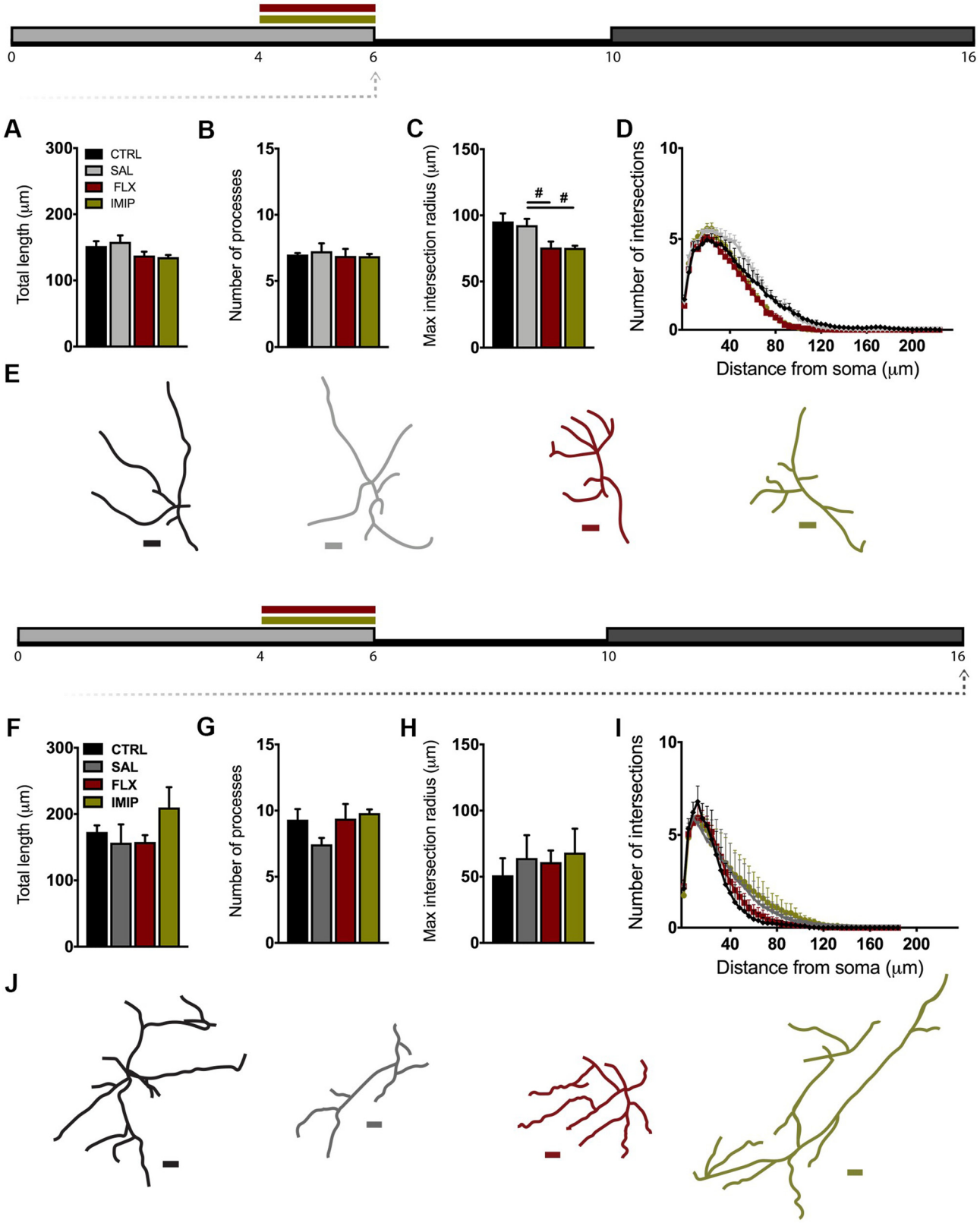

Fig. 4. Longitudinal analysis of the astrocytic morphology in the dorsal hippocampal dentate gyrus (dDG) in an experimental animal model of recurrent depression. (**A**–**E**) Analysis of different morphological paraments of GFAP-stained cells after a six-week uCMS protocol that included a treatment with different antidepressants (ADs), fluoxetine and imipramine and (**F**-**J**) after re-exposure to uCMS. #Denotes the effect of ADs, by comparison of treatment and SAL animals, analyzed by one-way analysis of variance (ANOVA); Data represented as mean ± SEM. # $P \leqslant 0.05$; n = 4–6 *per* group. Abbreviations: CTRL, non-stressed animals; SAL, animals repeatedly exposed to uCMS and non-treated; FLX, animals repeatedly exposed to uCMS and treated with fluoxetine; IMIP, animals repeatedly exposed to uCMS and treated with imipramine.

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In the current study, we were particularly focused on the analysis of the typical astrocytic markers S100B and GFAP in the hippocampal DG after a single and recurrent stress exposure (Raponi et al., 2007). Firstly, we observed that a single stress exposure did not alter the number of $S100B^+$ cells in the hippocampal DG. Nevertheless, uCMS promoted a robust decrease in the number of GFAP-expressing cells, consistent with previous studies using other stress models (Gosselin et al., 2009; Li et al., 2013; Bender et al., 2016) and in depressed patients (Webster et al., 2001; Bowley et al., 2002). These observations endorse the view that exposure to chronic stress affects astrocytic populations in a differential manner (Sofroniew, 2009; Sofroniew and Vinters, 2010; Bender et al., 2016). Indeed, GFAP is a typical astrocytic marker that yet is not present in all astrocytes (Kimelberg, 2004; Sofroniew and Vinters, 2010; Kettenmann and Verkhratsky, 2011), and its expression varies among brain regions (Sofroniew, 2009; Sofroniew and Vinters, 2010). Moreover, in animals exposed to stress and treated with fluoxetine or imipramine, we could not detect changes in the number of $S100B^+$ cells but a slight increase in the number of $GFAP^+$ cells was observed, as previously described (Boldrini et al., 2009; Zhou et al., 2016). We further show that a subsequent exposure to the uCMS protocol significantly increases the number of S100B expressing cells and, therefore, possibly promoting reactive astrogliosis. This phenomenon is associated with a wide spectrum of astrocytic changes that may include gain or loss of function that can either produce a neuroprotective or harmful effect, depending on the intensity of the injury (Sofroniew, 2009; Sofroniew and Vinters, 2010), and has been previously described in neuropsychiatric disorders (Verkhratsky et al., 2014).

In this study, cumulative stress exposure is shown to impact on the number of mature astrocytes in the hippocampal DG. Contrastingly, treatment with fluoxetine or imipramine during a first stress exposure prevented the increase in the number of $S100B^+$ cells in the DG promoted by recurrent uCMS exposure suggesting a plastic astrocytic capacity to alter its population and a protective role of these drugs to revert numerical alterations evoked by recurrent stress exposure. Here, we can speculate that preventing the increased number of $S100B^+$ cells on the stress re-exposed groups by AD treatment, particularly in animals treated with imipramine, might be contributing to the beneficial resistance to the deleterious effects of stress on behavior, specifically on these experimental groups, as previously reported for this animal model (Alves et al., 2017). In contrast, the slight decrease in the number of $S100B^+$ cells in animals previously treated with fluoxetine could be associated to an increased behavioral susceptibility to recurrent depression.

An increase in the number of $S100B^+$ cells induced by stress has been previously described in brain regions such as the prefrontal cortex (Braun et al., 2009). However, to our knowledge, this study is the first to report it in the hippocampus. Likely, the impact on the number of S100B-expressing cells may depend on the severity of the insult. In agreement, and despite no significant alterations, we also observed that recurrent stress exposure tends to increase S100B gene expression levels. In fact, previous studies have shown a stress-induced increase in S100B expression levels (Schroeter et al., 2008; Schroeter et al., 2011; Wang et al., 2016). Considering that S100B is known to promote the secretion of pro-inflammatory cytokines (Li et al., 2000; Dowlati et al., 2010), recurrent stress may promote a neuroinflammatory effect in activated glial cells in the hippocampus through an elevation of S100B expression levels (Goncalves et al., 2013; Wang et al., 2016). Additional studies should tackle possible changes on inflammatory cytokines driven by repeated stress exposure or in the context of recurrent depression. Others have previously reported a stress-induced increase in the number of $BrdU^+S100B^+$ cells in the DG (Veena et al., 2009) which may also account for such increase in the total number of $S100B^+$ cells. Interestingly, a previous study from our lab has shown that upon exposure to a single uCMS protocol, treatment with fluoxetine, but not imipramine is able to decrease the expression of IL-6 and TNF signaling-related pro-inflammatory molecules (Patricio et al., 2015) further suggesting a differential profile of these two ADs in what relates to inflammation and possibly astroglial effects. Furthermore, treatment with fluoxetine or imipramine tend to promote a differential impact on the number of GFAP-expressing cells, in comparison to the population of $S100B^+$ cells, suggesting a distinct action or temporal effect of ADs on astrocytes in the hippocampus.

The dynamic changes of astrocytes are not limited to changes in the number, astrogliosis and gene expression alterations. Astrocytes possess large cell bodies and extensive processes and can suffer significant changes in their morphology that ultimately impact on their function. It is well documented that smaller or atrophic astrocytes may have altered interaction with neurons leading to disrupted synaptic plasticity. Critical disruptions at the synapse include inability to mediate glutamate homeostasis, reduced tone of signaling molecules and trophic factors, altered glucose metabolism and reduced network connectivity (Pekny et al., 2016; Wang et al., 2017; Kim et al., 2018). Previous reports have described that chronic stress induces astrocytic atrophy, by reducing the length, branching and volume of the processes, suggesting their contribution to the reduction in hippocampal volume with increasing duration of MDD (Diniz et al., 2012; Tynan et al., 2013; Ardalan et al., 2017). In the present study and using a longitudinal analysis of astrocytic morphology (from $GFAP^+$ cells) in an animal model of recurrent depression we did not observe significant changes in the morphology of astrocytes in the hippocampal DG, evaluated after a single and recurrent exposure to uCMS. As this morphological analysis is dependent on GFAP expression, evidence is corroborated by the absence of stress-induced changes in GFAP mRNA levels in the dorsal DG.

Interestingly, and in accordance to previous studies (Cabras et al., 2010; Kusakawa et al., 2010; Sanacora and Banasr, 2013; Wang et al., 2017), treatment with fluoxetine and imipramine promoted a transient decreased astrocytic complexity after a single exposure to stress

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as these alterations were no longer observed after stress re-exposure. Importantly, in previous studies we showed that the therapeutic actions of fluoxetine or imipramine after a single stress exposure are associated to their impact on the generation of neurons and astrocytes in the DG (Mateus-Pinheiro et al., 2013) while in this animal model of recurrent depression, these ADs promote a distinct behavioral response to re-exposure to stress (Alves et al., 2017). In particular, fluoxetine treatment within the first uCMS exposure was shown to increase susceptibility to the deleterious effects of recurrent stress including on anxiety-like behavior and cognition. Contrastingly, imipramine-treated animals displayed behavioral resilience to repeated stress effects. In light of the observations in this study, we speculate that the profile of astrocytic plastic alterations (combination of cell densities and morphological changes) promoted by these ADs may contribute distinctively to the behavioral response to recurrent stress exposure. In particular, the decrease in the number of mature astrocytes in animals treated with fluoxetine, after re-exposure to stress, can represent an important trigger to increased susceptibility to chronic stress leading to recurrent episodes of depression. It is plausible to suggest that such distinct astrocytic impact promoted by fluoxetine and imipramine may, at least partially rely on a mechanism dependent of the noradrenergic system. Thus, other studies including the usage of a more noradrenergic selective drug over imipramine or eventually the specific blockage of the noradrenergic system in imipramine-treated animals may represent an important follow up experiment to dissect the differential impact of these ADs in the context of recurrent stress.

In concordance, studies showed that a decreased number and morphology of astrocytes significantly impair neurophysiology and function, particularly in cognitive tasks (Lima et al., 2014). Still, additional studies using for instance previously described models of astrocytic dysfunction (Lima et al., 2014; Sardinha et al., 2017) are needed to directly correlate numerical and morphologic changes in astrocytes to the behavioral consequences of recurrent stress. In this study we show that the number of astrocytes, namely S100B-positive cells, are increased in the hippocampal DG after recurrent stress exposure. Interestingly, imipramine or fluoxetine prevent alterations in astrocytic numbers induced by stress re-exposure. In our view, this study adds relevant information about the dynamic astrocytic changes in the context of recurrent depression, and the particular impact of treatment with different classes of ADs.

FUNDING

ARMS, NDA, PP, AMP, ELC, MM and LP received fellowships from the Portuguese Foundation for Science and Technology (FCT). This work was funded by FCT (IF/01079/2014).

This article has been developed under the scope of the project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER).

This work has been funded by FEDER funds, through the Competitiveness Factors Operational Programme (COMPETE), and by National funds, through the Foundation for Science and Technology (FCT), under the scope of the project POCI-01-0145-FEDER-007038.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Allen NJ, Eroglu C (2017) Cell biology of astrocyte-synapse interactions. Neuron 96:697–708.

Alves ND, Correia JS, Patricio P, Mateus-Pinheiro A, Machado-Santos AR, Loureiro-Campos E, Morais M, Bessa JM, Sousa N, Pinto L (2017) Adult hippocampal neuroplasticity triggers susceptibility to recurrent depression. Transl Psychiatry 7.

Alves ND, Patricio P, Correia JS, Mateus-Pinheiro A, Machado-Santos AR, Loureiro-Campos E, Morais M, Bessa JM, Sousa N, Pinto L (2018) Chronic stress targets adult neurogenesis preferentially in the suprapyramidal blade of the rat dorsal dentate gyrus. Brain Struct Funct 223:415–428.

Ardalan M, Rafati AH, Nyengaard JR, Wegener G (2017) Rapid antidepressant effect of ketamine correlates with astroglial plasticity in the hippocampus. Br J Pharmacol 174:483–492.

Arolt V, Peters M, Erfurth A, Wiesmann M, Missler U, Rudolf S, Kirchner H, Rothermundt M (2003) S100B and response to treatment in major depression: a pilot study. Eur Neuropsychopharmacol 13:235–239.

Banasr M, Duman RS (2008) Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biol Psychiatry 64:863–870.

Bender CL, Calfa GD, Molina VA (2016) Astrocyte plasticity induced by emotional stress: A new partner in psychiatric physiopathology? Prog Neuropsychopharmacol Biol Psychiatry 65:68–77.

Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, Almeida OF, Sousa N (2009) The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. Mol Psychiatry 14(764–73):739.

Boku S, Nakagawa S, Toda H, Hishimoto A (2018) Neural basis of major depressive disorder: beyond monoamine hypothesis. Psychiatry Clin Neurosci 72:3–12.

Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, Arango V (2009) Antidepressants increase neural progenitor cells in the human hippocampus. Neuropsychopharmacology 34:2376–2389.

Bowley MP, Drevets WC, Ongur D, Price JL (2002) Low glial numbers in the amygdala in major depressive disorder. Biol Psychiatry 52:404–412.

Braun K, Antemano R, Helmeke C, Buchner M, Poeggel G (2009) Juvenile separation stress induces rapid region- and layer-specific changes in S100ss- and glial fibrillary acidic protein-immunoreactivity in astrocytes of the rodent medial prefrontal cortex. Neuroscience 160:629–638.

Cabras S, Saba F, Reali C, Scorciapino ML, Sirigu A, Talani G, Biggio G, Sogos V (2010) Antidepressant imipramine induces human astrocytes to differentiate into cells with neuronal phenotype. Int J Neuropsychopharmacol 13:603–615.

Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA (2005)

Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 120:421–433.

Cobb JA, O'Neill K, Milner J, Mahajan GJ, Lawrence TJ, May WL, Miguel-Hidalgo J, Rajkowska G, Stockmeier CA (2016) Density of GFAP-immunoreactive astrocytes is decreased in left hippocampi in major depressive disorder. Neuroscience 316:209–220.

Cotter DR, Pariante CM, Everall IP (2001) Glial cell abnormalities in major psychiatric disorders: the evidence and implications. Brain Res Bull 55:585–595.

Czeh B, Di Benedetto B (2013) Antidepressants act directly on astrocytes: evidences and functional consequences. Eur Neuropsychopharmacol 23:171–185.

Czeh B, Muller-Keuker JI, Rygula R, Abumaria N, Hiemke C, Domenici E, Fuchs E (2007) Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. Neuropsychopharmacology 32:1490–1503.

Czeh B, Simon M, Schmelting B, Hiemke C, Fuchs E (2006) Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology 31:1616–1626.

Di Benedetto B, Malik VA, Begum S, Jablonowski L, Gomez-Gonzalez GB, Neumann ID, Rupprecht R (2016) Fluoxetine requires the endfeet protein aquaporin-4 to enhance plasticity of astrocyte processes. Front Cell Neurosci 10:8.

Diniz DG, Raiol Fôro C, Kronka M, Bento Torres Neto J, Vasconcelos P, Wanderley C, Picanço Diniz C (2012) Aging, environmental enrichment, object recognition and astrocyte plasticity in dentate gyrus.

Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctot KL (2010) A meta-analysis of cytokines in major depression. Biol Psychiatry 67:446–457.

Femenia T, Gomez-Galan M, Lindskog M, Magara S (2012) Dysfunctional hippocampal activity affects emotion and cognition in mood disorders. Brain Res 1476:58–70.

Fuchs E, Czeh B, Kole MH, Michaelis T, Lucassen PJ (2004) Alterations of neuroplasticity in depression: the hippocampus and beyond. Eur Neuropsychopharmacol 14(Suppl 5):S481–S490.

Goncalves CA, Leite MC, Nardin P (2008) Biological and methodological features of the measurement of S100B, a putative marker of brain injury. Clin Biochem 41:755–763.

Goncalves FM, Freitas AE, Peres TV, Rieger DK, Ben J, Maestri M, Costa AP, Tramontina AC, Goncalves CA, Rodrigues AL, Nagano CS, Teixeira EH, Nascimento KS, Cavada BS, Leal RB (2013) Vatairea macrocarpa lectin (VML) induces depressive-like behavior and expression of neuroinflammatory markers in mice. Neurochem Res 38:2375–2384.

Gos T, Schroeter ML, Lessel W, Bernstein HG, Dobrowolny H, Schiltz K, Bogerts B, Steiner J (2013) S100B-immunopositive astrocytes and oligodendrocytes in the hippocampus are differentially afflicted in unipolar and bipolar depression: a postmortem study. J Psychiatr Res 47:1694–1699.

Gosselin RD, Gibney S, O'Malley D, Dinan TG, Cryan JF (2009) Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression. Neuroscience 159:915–925.

Harrison PJ (2002) The neuropathology of primary mood disorder. Brain 125:1428–1449.

Hetzel G, Moeller O, Evers S, Erfurth A, Ponath G, Arolt V, Rothermundt M (2005) The astroglial protein S100B and visually evoked event-related potentials before and after antidepressant treatment. Psychopharmacology (Berl) 178:161–166.

Hirrlinger J, Hulsmann S, Kirchhoff F (2004) Astroglial processes show spontaneous motility at active synaptic terminals in situ. Eur J Neurosci 20:2235–2239.

Iwata M, Shirayama Y, Ishida H, Hazama GI, Nakagome K (2011) Hippocampal astrocytes are necessary for antidepressant treatment of learned helplessness rats. Hippocampus 21:877–884.

Kettenmann H, Verkhratsky A (2011) Neuroglia–living nerve glue. Fortschr Neurol Psychiatr 79:588–597.

Kim R, Healey KL, Sepulveda-Orengo MT, Reissner KJ (2018) Astroglial correlates of neuropsychiatric disease: From astrocytopathy to astrogliosis. Prog Neuropsychopharmacol Biol Psychiatry 87:126–146.

Kimelberg HK (2004) The problem of astrocyte identity. Neurochem Int 45:191–202.

Kusakawa S, Nakamura K, Miyamoto Y, Sanbe A, Torii T, Yamauchi J, Tanoue A (2010) Fluoxetine promotes gliogenesis during neural differentiation in mouse embryonic stem cells. J Neurosci Res 88:3479–3487.

Li LF, Yang J, Ma SP, Qu R (2013) Magnolol treatment reversed the glial pathology in an unpredictable chronic mild stress-induced rat model of depression. Eur J Pharmacol 711:42–49.

Li Y, Barger SW, Liu L, Mrak RE, Griffin WS (2000) S100beta induction of the proinflammatory cytokine interleukin-6 in neurons. J Neurochem 74:143–150.

Lima A, Sardinha VM, Oliveira AF, Reis M, Mota C, Silva MA, Marques F, Cerqueira JJ, Pinto L, Sousa N, Oliveira JF (2014) Astrocyte pathology in the prefrontal cortex impairs the cognitive function of rats. Mol Psychiatry 19:834–841.

Liu Q, Li B, Zhu HY, Wang YQ, Yu J, Wu GC (2009) Clomipramine treatment reversed the glial pathology in a chronic unpredictable stress-induced rat model of depression. Eur Neuropsychopharmacol 19:796–805.

Longair MH, Baker DA, Armstrong JD (2011) Simple Neurite Tracer: open source software for reconstruction, visualization and analysis of neuronal processes. Bioinformatics 27:2453–2454.

Ma S, Kwon HJ, Huang Z (2012) A functional requirement for astroglia in promoting blood vessel development in the early postnatal brain. PLoS One 7 e48001.

Manji HK, Moore GJ, Rajkowska G, Chen G (2000) Neuroplasticity and cellular resilience in mood disorders. Mol Psychiatry 5:578–593.

Mateus-Pinheiro A, Pinto L, Bessa JM, Morais M, Alves ND, Monteiro S, Patricio P, Almeida OF, Sousa N (2013) Sustained remission from depressive-like behavior depends on hippocampal neurogenesis. Transl Psychiatry 3 e210.

Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA, Rajkowska G (2000) Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. Biol Psychiatry 48:861–873.

Miller JA, Nathanson J, Franjic D, Shim S, Dalley RA, Shapouri S, Smith KA, Sunkin SM, Bernard A, Bennett JL, Lee CK, Hawrylycz MJ, Jones AR, Amaral DG, Sestan N, Gage FH, Lein ES (2013) Conserved molecular signatures of neurogenesis in the hippocampal subgranular zone of rodents and primates. Development 140:4633–4644.

Ogata K, Kosaka T (2002) Structural and quantitative analysis of astrocytes in the mouse hippocampus. Neuroscience 113:221–233.

Oliveira JF, Sardinha VM, Guerra-Gomes S, Araque A, Sousa N (2015) Do stars govern our actions? Astrocyte involvement in rodent behavior. Trends Neurosci 38:535–549.

Patricio P, Mateus-Pinheiro A, Irmler M, Alves ND, Machado-Santos AR, Morais M, Correia JS, Korostynski M, Piechota M, Stoffel R, Beckers J, Bessa JM, Almeida OF, Sousa N, Pinto L (2015) Differential and converging molecular mechanisms of antidepressants' action in the hippocampal dentate gyrus. Neuropsychopharmacology 40:338–349.

Pekny M, Pekna M (2004) Astrocyte intermediate filaments in CNS pathologies and regeneration. J Pathol 204:428–437.

Pekny M, Pekna M, Messing A, Steinhauser C, Lee JM, Parpura V, Hol EM, Sofroniew MV, Verkhratsky A (2016) Astrocytes: a central element in neurological diseases. Acta Neuropathol 131:323–345.

Perea G, Araque A (2010) GLIA modulates synaptic transmission. Brain Res Rev 63:93–102.

Procko C, Lu Y, Shaham S (2011) Glia delimit shape changes of sensory neuron receptive endings in C. elegans. Development 138:1371–1381.

Please cite this article in press as: Machado-Santos AR et al. Astrocytic plasticity at the dorsal dentate gyrus on an animal model of recurrent depression. Neuroscience (2019), https://doi.org/10.1016/j.neuroscience.2019.10.032

Rajkowska G (2000) Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. Biol Psychiatry 48:766–777.

Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, Overholser JC, Roth BL, Stockmeier CA (1999) Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry 45:1085–1098.

Rajkowska G, Stockmeier CA (2013) Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. Curr Drug Targets 14:1225–1236.

Raponi E, Agenes F, Delphin C, Assard N, Baudier J, Legraverend C, Deloulme JC (2007) S100B expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage. Glia 55:165–177.

Sanacora G, Banasr M (2013) From pathophysiology to novel antidepressant drugs: glial contributions to the pathology and treatment of mood disorders. Biol Psychiatry 73:1172–1179.

Sardinha VM, Guerra-Gomes S, Caetano I, Tavares G, Martins M, Reis JS, Correia JS, Teixeira-Castro A, Pinto L, Sousa N, Oliveira JF (2017) Astrocytic signaling supports hippocampal-prefrontal theta synchronization and cognitive function. Glia 65:1944–1960.

Saur L, Baptista PP, Bagatini PB, Neves LT, de Oliveira RM, Vaz SP, Ferreira K, Machado SA, Mestriner RG, Xavier LL (2016) Experimental post-traumatic stress disorder decreases astrocyte density and changes astrocytic polarity in the CA1 hippocampus of male rats. Neurochem Res 41:892–904.

Schroeter ML, Abdul-Khaliq H, Diefenbacher A, Blasig IE (2002) S100B is increased in mood disorders and may be reduced by antidepressive treatment. Neuroreport 13:1675–1678.

Schroeter ML, Abdul-Khaliq H, Krebs M, Diefenbacher A, Blasig IE (2008) Serum markers support disease-specific glial pathology in major depression. J Affect Disord 111:271–280.

Schroeter ML, Abdul-Khaliq H, Sacher J, Steiner J, Blasig IE, Mueller K (2010) Mood disorders are glial disorders: evidence from in vivo studies. Cardiovasc Psychiatry Neurol 2010 780645.

Schroeter ML, Sacher J, Steiner J, Schoenknecht P, Mueller K (2013) Serum S100B represents a new biomarker for mood disorders. Curr Drug Targets 14:1237–1248.

Schroeter ML, Steiner J (2009) Elevated serum levels of the glial marker protein S100B are not specific for schizophrenia or mood disorders. Mol Psychiatry 14:235–237.

Schroeter ML, Steiner J, Mueller K (2011) Glial pathology is modified by age in mood disorders–a systematic meta-analysis of serum S100B in vivo studies. J Affect Disord 134:32–38.

Silva R, Lu J, Wu Y, Martins L, Almeida OF, Sousa N (2006) Mapping cellular gains and losses in the postnatal dentate gyrus: implications for psychiatric disorders. Exp Neurol 200:321–331.

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638–647.

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7–35.

Sun JD, Liu Y, Yuan YH, Li J, Chen NH (2012) Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. Neuropsychopharmacology 37:1305–1320.

Tavares G, Martins M, Correia JS, Sardinha VM, Guerra-Gomes S, das Neves SP, Marques F, Sousa N, Oliveira JF (2017) Employing an open-source tool to assess astrocyte tridimensional structure. Brain Struct Funct 222:1989–1999.

Theodosis DT, Poulain DA, Oliet SH (2008) Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. Physiol Rev 88:983–1008.

Torres-Platas SG, Hercher C, Davoli MA, Maussion G, Labonte B, Turecki G, Mechawar N (2011) Astrocytic hypertrophy in anterior cingulate white matter of depressed suicides. Neuropsychopharmacology 36:2650–2658.

Tynan RJ, Beynon SB, Hinwood M, Johnson SJ, Nilsson M, Woods JJ, Walker FR (2013) Chronic stress-induced disruption of the astrocyte network is driven by structural atrophy and not loss of astrocytes. Acta Neuropathol 126:75–91.

Veena J, Srikumar BN, Raju TR, Shankaranarayana Rao BS (2009) Exposure to enriched environment restores the survival and differentiation of new born cells in the hippocampus and ameliorates depressive symptoms in chronically stressed rats. Neurosci Lett 455:178–182.

Verkhratsky A, Rodriguez JJ, Steardo L (2014) Astrogliopathology: a central element of neuropsychiatric diseases? Neuroscientist 20:576–588.

von Bohlen und Halbach O (2011) Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus. Cell Tissue Res 345:1–19.

Wang CH, Gu JY, Zhang XL, Dong J, Yang J, Zhang YL, Ning QF, Shan XW, Li Y (2016) Venlafaxine ameliorates the depression-like behaviors and hippocampal S100B expression in a rat depression model. Behav Brain Funct 12:34.

Wang Q, Jie W, Liu JH, Yang JM, Gao TM (2017) An astroglial basis of major depressive disorder? An overview. Glia 65:1227–1250.

Webster MJ, Knable MB, Johnston-Wilson N, Nagata K, Inagaki M, Yolken RH (2001) Immunohistochemical localization of phosphorylated glial fibrillary acidic protein in the prefrontal cortex and hippocampus from patients with schizophrenia, bipolar disorder, and depression. Brain Behav Immun 15:388–400.

World Health Organization (2017) Depression and Other Common Mental Disorders: Global Health Estimates. http://www.who.int/iris/handle/10665/254610.

Ye Y, Wang G, Wang H, Wang X (2011) Brain-derived neurotrophic factor (BDNF) infusion restored astrocytic plasticity in the hippocampus of a rat model of depression. Neurosci Lett 503:15–19.

Zhou QG, Lee D, Ro EJ, Suh H (2016) Regional-specific effect of fluoxetine on rapidly dividing progenitors along the dorsoventral axis of the hippocampus. Sci Rep 6:35572.

(Received 4 May 2019, Accepted 16 October 2019)
(Available online xxxx)

Chapter 5

General Discussion

Although many studies have been focusing in the overall comprehension of depression's pathophysiology during the last years, depression is still one of the world's major public health concerns (WHO, 2017). Indeed, the clinical impact and outcomes are usually very slow to achieve, which creates the need for a continuous investigation in this field.

In the recent literature, we have seen the emergence of different hypothesis, including the involvement of adult neural plasticity, in the onset and treatment of depressive episodes. Moreover, we have also witnessed the shift of the research focus from neurons to glial cells, which are now recognized as equally affected in the disruption of brain neural plasticity that occurs in a depressive episode. However, astroglial plasticity is still poorly characterized in the course of this disorder and upon treatment with different ADs.

In this thesis, we have focused on astrocytic alterations – both in terms of generation of new astrocytes and plasticity of resident astrocytes - in the hippocampal DG in the longitudinal course of depression and upon AD treatment.

We decided to focus our studies on the hippocampus, as it is considered a structural target for dysregulation in depression (Campbell & Macqueen, 2004; MacQueen & Frodl, 2011). Moreover, astroglial plasticity events in the hippocampal DG, including alterations in processes length and density of astrocytes, as well as astrogliogenesis, were hypothesized to mediate some of the behavioral outcomes observed in depression onset and remission (Rajkowska & Miguel-Hidalgo, 2007; Banasr & Duman, 2008; Oliveira *et al.*, 2015; Kim *et al.*, 2018). Therefore, to explore these different mechanisms in this disease context, we used an animal model of depression – the unpredictable chronic mild stress (uCMS) model – to gain more insights on the pathways and astrocytic alterations that occur after chronic stress exposure, a major precipitation factor of depression, after ADs treatment, and in a recurrent episode of depression (after exposing the animals to a second uCMS protocol). Additionally, *in vitro* studies to assess the astrocytic fate of neural precursor cells from the hippocampal DG upon administration of ADs' active metabolites (norfluoxetine or desipramine) were performed, thus complementing our *in vivo* analyses.

Two main studies were developed: In the first one, we aimed to longitudinally evaluate the short-term and long-term memory alterations that occur in an animal model of depression and after treatment with two different ADs, and to associate those impairments with astroglial plasticity changes. AD treatments included one representative of TCAs (imipramine) and one of SSRIs (fluoxetine), with the main purpose of understanding if different classes of currently used ADs would re-establish basal hippocampal astrocytic plasticity via the same mechanisms or if they use differential cellular/molecular strategies to improve cognitive behavior after a depressive-like episode.

In the second study, we aimed to reveal plastic astrocytic changes in the context of recurrent depression. For that, we longitudinally assessed dynamic astrocytic alterations - gene expression, cell densities and morphologic alterations - in the hippocampal DG of an animal model of recurrent depression (after repeated exposure to uCMS), upon treatment with two ADs, fluoxetine and imipramine.

In this discussion, I will first focus on each of these two works separately, showing their main implications to the field. Furthermore, I will highlight some aspects and major hurdles that may have been limiting the understanding of the astrocytic and astrogliogenesis roles in the context of depression.

DIFFERENTIAL ACTION OF TWO DIFFERENT ADS IN ASTROCYTIC PLASTICITY AFTER EXPOSURE TO A STRESS-INDUCED DEPRESSIVE-LIKE EPISODE

In the adult rodent hippocampus, newborn neurons and glial cells are generated from neural stem cells (NSCs) from the subgranular zone (SGZ) of the dentate gyrus (DG), a layer of cells located between the granule cell layer (GCL) and the hilus (Ming & Song, 2011; Patrício *et al.*, 2013). Before the integration of these new cells in the circuitry and along the maturation process, adult-born cells undergo a critical period of selection, with the survival of a small fraction able to integrate and establish future functional connections (Kuhn *et al.*, 2005). Considering the functional segregation along the septotemporal axis of the hippocampus, which suggests an association between the dorsal pole to cognitive control and ventral pole to emotional behavior (Fanselow & Dong, 2010; Tanti & Belzung, 2013), it is expected that adult-born cells generated in the hippocampal DG may present different properties and function, depending on the location in which they integrate. Furthermore, as we aimed to study their association to cognitive-related alterations in an animal model of depression, we focused our studies in the dorsal DG.

The importance of post-natal neuroplasticity in the context of MDD is further supported by the well documented morphogenic (Bessa *et al.*, 2009a; Patricio *et al*, 2015) and pro-cytogenic effects of ADs (Boldrini *et al.*, 2009; Mateus-Pinheiro *et al.*, 2013) that are daily prescribed in the clinics. These facts support the hypothesis that the therapeutic effect of these drugs may be exerted, at least to some extent, by "reshaping" cell connections and boosting the generation of new cells in the adult brain. These exciting observations challenged the classic conceptions of depression, thus raising many unanswered questions: is post-natal structural neuroplasticity indeed causally implicated in the precipitation of depression?; or are the volume alterations, the dendritic remodeling and the reduced generation of new cells a mere epiphenomenon? If newborn cells and dendrites are in fact important for proper brain function, are these processes decisive for the long-term recovery from a depressive episode? What about the mechanisms of action of ADs? Do different classes of ADs rely in similar neuroplastic substrates? These questions have

been addressed by a number of studies, many of them contributing to a better understanding of the role of post-natal neuroplasticity both as pathological trigger and therapeutic target in the context of MDD (D'Sa & Duman, 2002;Pittenger & Duman, 2008; Serafini, 2012; Castrén & Hen, 2013; Mateus-Pinheiro, Patrício, *et al.*, 2013; Ota & Duman, 2013; Pilar-Cuéllar *et al.*, 2013; Lucassen *et al.*, 2014).

Nevertheless, most of the evidence gathered so far in this context focus on neuronal cells in disregard of glial cells. In fact, it is now well recognized that glial cells, namely astrocytes, undergo several plastic alterations both in the healthy and depressed brain (Manji *et al.*, 2000; Rajkowska & Miguel-Hidalgo, 2007; Bélair *et al.*, 2010; Ben Achour & Pascual, 2010). Therefore, understanding the importance of astroglioplasticity in the adult brain may contribute to assess its potential role in the pathophysiology and treatment of depression.

In our study, we could longitudinally understand the effects of chronic stress on cognitive behavior, both on short-term and long-term memory. According to our results, stress exposure could immediately impact both short and long-term memory, although it was fully rescued 4 weeks after (at time-point 2, 10 weeks of the experimental protocol). It is important to mention that the hippocampus is the region mainly responsible for long-term object recognition, whereas the short-term memory is a cognitive function that mainly involves prefrontal cortex (PFC) activity (Reger *et al.*, 2009). These observations might suggest that resident hippocampal cell populations are immediately affected by uCMS exposure, not being able to establish projections with the PFC, under stress conditions, thus affecting both short and long-term memory at tp1. An additional argument is that both the hippocampus and PFC cells are affected by uCMS, thus also leading to disruption of the normal connectivity between both regions and impairments in short- and long-term memories. However, it seems that 4 weeks after stress-exposure, these cognitive impairments are fully recovered. In this regard, several studies already reported altered cell numbers and morphological changes in the hippocampus and PFC of stressed animals (Cerqueira, Taipa, *et al.*, 2007; Bessa *et al.*, 2009). Specifically, those alterations may lead to an increased difficulty in the communication between these two regions (Cerqueira, Mailliet, *et al.*, 2007; Oliveira *et al.*, 2013) and can explain our results. However, it is still important to clarify which is the cell type (or cell types) essential for the regulation of these connectivity impairments between the hippocampus and PFC under depressive-like conditions that lead to such marked cognitive dysregulations. So far, gathered studies have been focusing on the disruption of neurons in disease contexts, correlating with cognitive impairments.

However, and considering the crucial role of astrocytes already vastly demonstrated in contributing to synaptic plasticity and normal neuronal function, it is important to understand how these specific cells

are modulating and impairing the cognitive domain. In fact, some studies have already shown the crucial role of astrocytes in the cognitive domain regulation (Lima *et al.*, 2014).

In an astrocytic perspective, we can think of a poor support provided by astrocytes to the resident and newborn neurons that can be causing all these connectivity alterations on both short- and long-term memory. Indeed, we can see a decrease in the number of astrocytes immediately after stress exposure (tp 1). Interestingly, Imipramine administration, but not fluoxetine, rescued cognitive impairments induced by stress exposure, which suggests that Imipramine might be fast acting on the reestablishment of the neural circuits connecting the hippocampus and the PFC, rescuing the cellular and molecular changes induced by stress-induced depression. Corroborating our results, Imipramine was already shown to enable cognitive improvements in different animal models of disease, either in depression or traumatic brain injury (Han *et al.*, 2011; Riga *et al.*, 2017).

Furthermore, when we proceeded to astrogliogenesis analysis, the long-term perspective of this animal model (time-point 2) holds the most interesting results. Indeed, new generated cells in the adult neurogenic regions take between 4 to 8 weeks to fully differentiate and establish new contacts with the neighbour network cells. Thus, at this time-point, it is plausible to assume that new astrocytes are already differentiated and integrated in the neuronal network, becoming more important in the contribution to the normal function of the system. Therefore, the astrogliogenic process becomes an important factor in this long-term perspective of depression (tp2), as previously described by our group (Mateus-Pinheiro *et al.*, 2013).

Indeed, stress induces a decrease in the density of new astrocytes, shown by a less density of GFAP and BrdU – double positive cells in the hippocampal DG. It is also important to mention that, on these astrogliogenesis analyses at long-term, BrdU administration was performed during 5 days, and the proliferation/differentiation results reflect the proliferative status of the cell population immediately after the end of the uCMS protocol, as well as after the conclusion of the AD treatment, including the 3 initial days of the recovery period. Interestingly, imipramine, but not fluoxetine, promoted the generation of new astrocytes in the hippocampal DG, eliciting a strong pro-astrogliogenic effect in this brain region. We hypothesize that the increased number of newly generated astrocytes caused by imipramine administration can be a rescue in response to the decreased number of these newly generated cells reached after stress exposure, compensating in this way the lost functions. The same results were confirmed with our *in vitro* results, with norfluoxetine administration to primary hippocampal cell cultures decreasing the number of GFAP-expressing cells, while desipramine increased its numbers.

Curiously, imipramine treatment did not elicit any alteration regarding astrocytic morphology, contrarily to fluoxetine. In fact, fluoxetine treatment not only induced morphological alterations in resident astrocytes (like mentioned above), but also on newly generated astrocytes. This AD was not able to rescue the reduced density of newborn astrocytes caused by stress exposure, acting on their cellular morphology by promoting an increased astrocytic length.

This morphological action can be seen as reactive astrogliosis, a process by which astrocytes respond to CNS injury and disease states. Indeed, and based on several studies, reactive astrogliosis has recently been shown to be always accompanied by varying degrees of cellular hypertrophy (Liu et al., 2017). In a functional view, reactive astrocytes can absorb glutamate from the synaptic cleft, not only reducing excitotoxicity but also providing cells with the substances required for neuronal metabolism (Cheng et al., 2019). Consequently, we may think that astrocytes can be morphologically and functionally activated by fluoxetine treatment to cope with the increased neuronal production that occurs with this AD, thus responding to the network changes and assuming a protective role. However, it would be important to conduct detailed molecular analysis on fluoxetine-treated animals in order to prove that this AD is inducing a reactive astrogliosis state in this animal model.

Corroborating this view that fluoxetine is not acting through astrogliogenesis processes, several studies reported that the recovery induced by fluoxetine treatment seems to be more related with neurogenesis rather than with gliogenesis (Wang *et al.*, 2008; Xi *et al.*, 2011; Mateus-Pinheiro, Patrício, *et al.*, 2013). However, it is also important to notice here, that treatment with fluoxetine (contrarily to imipramine) was not effective in rescuing the cognitive impairments that emerged immediately after stress exposure, either on short-term and long-term memory. Therefore, we might assume that this AD, by only acting through astrocytic morphology alterations and increased neurogenesis, is not able to exert its effect in this behavioral domain in such a short timeframe.

Additionally, a study from our lab, from Alves and colleagues, focused on the ability of both imipramine and fluoxetine in counteracting the effects of stress re-exposure, showing distinct patterns of ADs action. It is important to mention that all the experiments timeline followed the same strategy as the current study, only adding a second hit of stress. On that study, it was observed that while imipramine treatment could re-establish hippocampal neurogenesis and neuronal dendritic arborization, contributing to resilience to recurrent depressive-like behavior, stress re-exposure in fluoxetine-treated animals resulted in an overproduction of adult-born neurons along with neuronal atrophy of granule neurons, accounting for an increased susceptibility to recurrent behavioral changes that are typical of depression (Alves *et al.*, 2017). This potent pro-neurogenic capacity of fluoxetine in the adult hippocampal DG in basal conditions,

but also its ability to revert stress-induced reduction of the neurogenic process were largely described (Malberg *et al.*, 2000; Santarelli *et al.*, 2003; Mateus-Pinheiro, Patrício, *et al.*, 2013). This data, although focusing on neuronal cells, can somehow complement our results. Indeed, if we add to those results the fact that imipramine is also acting on the production of new astrocytes, it seems that this AD can have a faster action on depressive episodes possibly because it acts on both neurons and astrocytes, while fluoxetine seems to act more specifically in neurons.

HIPPOCAMPAL ASTROGLIAL PLASTICITY ON AN ANIMAL MODEL OF RECURRENT DEPRESSION

Discussing now our results presented on Chapter IV, it is currently known that chronic stress exposure leads to persistent morphological and behavioral scars on both animals and humans (Swaab *et al.*, 2005; Mateus-Pinheiro, Patrício, *et al.*, 2013; Oliveira *et al.*, 2013; Sousa, 2016). In this context, it is reasonable to hypothesize that the severity of the first depressive episode and further impact on cytogenic and neuronal remodelling processes may define the rate and extent of relapse and recurrence of a subsequent depressive episode. Indeed, in the first 5 years after recovery, around 50-70% of depressed patients suffer recurrent episodes (Burcusa & Iacono, 2007; Baldessarini *et al.*, 2015), so it becomes crucial to understand the neurobiological mechanisms behind recurrent depression. It is also of the upmost importance to study the impact of treatment with typical ADs and find strong indicators of susceptibility to experiment recurrent depression.

Importantly, using an animal model of recurrent exposure to uCMS protocol, we previously observed that treatment with imipramine and fluoxetine induced a distinct susceptibility to the behavioral effects of repeated exposure to chronic stress, with only imipramine preventing the re-appearance of a depressive-like behavior (Alves *et al.*, 2017). Therefore, and considering that most of the studies in the context of recurrent depression have been focusing in neurons, with this current study we aimed at understanding how recurrent stress and treatment with two different ADs could differentially impact astroglial plasticity.

Considering our results, we observed that exposure to chronic stress affects astrocytic populations in a differential manner, as already reported (Sofroniew, 2009; Sofroniew & Vinters, 2010; Bender *et al.*, 2016), being S100B+ cells not altered after a single exposure to uCMS, while GFAP+ cells decreased their density, in the hippocampal DG (Gosselin *et al.*, 2009; Li *et al.,* 2013; Bender *et al.*, 2016).

Furthermore, treatment with both ADs did not impact on mature astrocytes, although a small increase in the GFAP+ cells density was observed, as previously reported (Boldrini *et al.*, 2009; Zhou *et al.*, 2016). Indeed, some studies already showed that stress can differently impact neuronal cells, although few studies have been conducted on astrocytes. In this vein, a study from our lab showed for the first time

that adult-born neurons are preferentially located in the suprapyramidal blade and suggest a regional-specific impact of chronic stress in this blade with potential repercussions for its functional significance (Alves *et al.*, 2018).

However, re-exposure to the stress protocol induced a significantly increase in mature astrocytic population, which can be a consequence of reactive astrogliosis, therefore conditioning the functional properties of these cells. These alterations can produce a neuroprotective or harmful effect, according to the intensity of the injury (Sofroniew, 2009; Sofroniew & Vinters, 2010), which were already described in neuropsychiatric disorders (Verkhratsky *et al.*, 2014).

Interestingly, treatment with both ADs prevented the increase in the number of mature astrocytes (S100B+ cells) in the hippocampal DG, promoted by recurrent uCMS exposure, which suggests a plastic astrocytic capacity to change its population and a protective role of both drugs to revert numerical alterations induced by recurrent stress exposure. Here, we can speculate that preventing the increased number of S100B+ cells on the stress re-exposed groups by treatment with ADs, particularly in animals treated with imipramine, might be contributing to the beneficial resistance to the deleterious effects of stress on behavior, specifically on this experimental group, as previously reported for this animal model (Alves *et al.*, 2017). Contrarily, the slight decrease in the number of S100B+ cells in animals previously treated with fluoxetine could be associated to an increased behavioural susceptibility to recurrent depression.

Moreover, and taking into account that S100β is known to promote the secretion of proinflammatory cytokines (Li *et al.*, 2000; Dowlati *et al.,* 2010), recurrent stress may be promoting a neuroinflammatory effect in hippocampal DG astrocytes, through an elevation of S100β expression levels (Goncalves *et al.*, 2013; Wang *et al.*, 2016) on the double stress group. Interestingly, a previous study from our group in the context of a single exposure to stress, showed that treatment with fluoxetine, but not with imipramine, could decrease the expression of IL-6 and TNF signalling-related proinflammatory molecules (Patricio *et al.*, 2015). This study specifically suggests a differential profile of these two ADs in what relates to inflammation and possibly astroglial effects.

As alterations in astrocytic cell bodies and their processes can have an impact on cellular function, we also wanted to understand the dynamic morphological alterations in the recurrent depressive context. Indeed, astrocytic atrophy may alter the interaction of astrocytes with neurons, leading to disrupted synaptic plasticity, according to the tripartite synapse concept. In detail, disruptions at the synapse can include the inability to mediate glutamate homeostasis, reduced tone of signalling molecules and trophic factors, altered glucose metabolism and decreased network connectivity (Pekny *et al.,* 2016; Wang *et al.*,

2017; Kim *et al.,* 2018). Those disruptions, such as decreased number and morphology of astrocytes, can significantly impair neurophysiology and function, particularly in cognitive tasks (Lima *et al.*, 2014). Moreover, astrocytic atrophy induced by chronic stress was implicated in the reduction of hippocampal volume and with increasing duration of MDD (Diniz *et al.*, 2012; Tynan *et al.*, 2013; Ardalan *et al.*, 2017).

Here, we did not observe significant changes in the morphology of astrocytes in the hippocampal DG, evaluated after a single and recurrent exposure to uCMS, although treatment with fluoxetine and imipramine promoted a transient decrease in astrocytic complexity after a single exposure to stress, which is in accordance to previous studies (Cabras *et al.*, 2010; Kusakawa *et al.*, 2010; Sanacora & Banasr, 2013; Wang *et al.*, 2017). Considering those results, we speculate that the profile of astrocytic plastic alterations promoted by these ADs may contribute to the distinct behavioral response to recurrent stress exposure. In particular, the decrease in the number of mature astrocytes in animals treated with fluoxetine, after re-exposure to stress, can represent an important trigger to increased susceptibility to chronic stress, leading to recurrent episodes of depression (reported in Alves *et al.* 2017). However, other studies including the use of a more noradrenergic selective drug over imipramine, or eventually the specific blockage of the noradrenergic system in imipramine-treated animals can stand as important follow up experiments to specifically understand the differential impact of these ADs in the context of recurrent stress exposure.

Overall, evidences presented in this work provide a further characterization of the mechanisms of adult astrocytic plasticity within the hippocampal DG and novel insights regarding their involvement in the development, treatment and recurrence of depression. Furthermore, both studies showed that different classes of ADs can have differential actions in astrocytic plasticity, either immediately after stress, or after recovery and even recurrence. We foresee that the knowledge raised with this work will also pave the way for further studies to specifically understand the role of astrocytic plasticity in the context of depression, which can contribute to the discovery of new targeted therapies.

However, and although some studies have emerged in the last few years providing new animal models to better study astrocytic plastic mechanisms (reviewed in Kery *et al.*, 2020), there are still no available tools that enable the specific ablation of newborn astrocytes. In fact, this work would benefit from those tools, thus allowing us to make a direct correlation between astrogliogenesis in the hippocampal DG and cognitive behaviour, in depressive-like animals. Those tools are crucial and needed to have specific evidences of astrogliogenesis functions in the disease context.

Furthermore, much of the astroglioplasticity research still relies on mammalian model systems and on current genetic understanding of astrocytes, although emerging technologies may further allow to

monitor astrocytic activity in humans (Edison *et al.*, 2018). These technologies, integrated with clinical genetic strategies, may lead to targeted treatment of astrocyte-mediated impairments in neurodegenerative disorders such as MDD. Overall, there is an untapped opportunity in astrocytes that may be crucial for future clinical studies in the context of depression.

References

Alves, N.D., Correia, J.S., Patrício, P., Mateus-Pinheiro, A., Machado-Santos, A.R., Loureiro-Campos, E., Morais, M., Bessa, J.M., Sousa, N., & Pinto, L. (2017) Adult hippocampal neuroplasticity triggers susceptibility to recurrent depression. *Transl. Psychiatry*, **7**, e1058–e1058.

Alves, N.D., Patrício, P., Correia, J.S., Mateus-Pinheiro, A., Machado-Santos, A.R., Loureiro-Campos, E., Morais, M., Bessa, J.M., Sousa, N., & Pinto, L. (2018) Chronic stress targets adult neurogenesis preferentially in the suprapyramidal blade of the rat dorsal dentate gyrus. *Brain Struct. Funct.*, **223**, 415–428.

Ardalan, M., Rafati, A.H., Nyengaard, J.R., Wegener, G. (2017) Rapid antidepressant effect of ketamine correlates with astroglial plasticity in the hippocampus. *Br J Pharmacol.,* **174**, 483–492.

Baldessarini, R. J., Lau, W. K., Sim, J., Sum, M. Y., & Sim, K. (2015) Duration of initial antidepressant treatment and subsequent relapse of major depression. *Journal of Clinical Psychop.*, **35**, 75–76.

Banasr, M. & Duman, R.S. (2008) Glial Loss in the Prefrontal Cortex Is Sufficient to Induce Depressive-like Behaviors. *Biol. Psychiatry*, **64**, 863–870.

Bélair, E.-L., Vallée, J., & Robitaille, R. (2010) In vivo long-term synaptic plasticity of glial cells. *J. Physiol.*, **588**, 1039–1056.

Ben Achour, S. & Pascual, O. (2010) Glia: The many ways to modulate synaptic plasticity. *Neurochem. Int.*, **57**, 440–445.

Bessa, J.M., Ferreira, D., Melo, I., Marques, F., Cerqueira, J.J., Palha, J.A., Almeida, O.F.X., & Sousa, N. (2009) The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Mol. Psychiatry*, **14**, 764–773, 739.

Boldrini, M., Underwood, M.D., Hen, R., Rosoklija, G.B., Dwork, A.J., John Mann, J., & Arango, V. (2009) Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology*, **34**, 2376–2389.

Burcusa, S.L. & Iacono, W.G. (2007) Risk for recurrence in depression. *Clin. Psychol. Rev.*, **27**, 959–985.

Cabras, S., Saba, F., Reali, C., Scorciapino, M.L., Sirigu, A., Talani, G., Biggio, G., & Sogos, V. (2010) Antidepressant imipramine induces human astrocytes to differentiate into cells with neuronal phenotype. *Int. J. Neuropsychopharmacol.*, **13**, 603–615.

Campbell, S. & Macqueen, G. (2004) The role of the hippocampus in the pathophysiology of major depression. *J. Psychiatry Neurosci.*, **29**, 417–426.

Castrén, E. & Hen, R. (2013) Neuronal plasticity and antidepressant actions. *Trends Neurosci.*, **36**, 259–267.

Cerqueira, J.J., Mailliet, F., Almeida, O.F.X., Jay, T.M., & Sousa, N. (2007) The prefrontal cortex as a key target of the maladaptive response to stress. *J. Neurosci.*, **27**, 2781–2787.

Cerqueira, J.J., Taipa, R., Uylings, H.B.M., Almeida, O.F.X., & Sousa, N. (2007) Specific configuration of dendritic degeneration in pyramidal neurons of the medial prefrontal cortex induced by differing corticosteroid regimens. *Cereb. Cortex*, **17**, 1998–2006.

Diniz, D.G., Raiol Fôro, C., Kronka, M., Bento Torres Neto, J., Vasconcelos, P., Wanderley, C., Picanço Diniz, C. (2012) Environmental impoverishment and aging alter object recognition, spatial learning, and dentate gyrus astrocytes. *Europ. Journal of Neurosc.*, **32**, 509–519.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctot, K.L. (2010) A meta-analysis of cytokines in major depression. *Biol Psychiatry*, **67**, 446–457.

D'Sa, C. & Duman, R.S. (2002) Antidepressants and neuroplasticity. *Bipolar Disord.*, **4**, 183–194.

Edison, P., Donat, C.K., Sastre, M. (2018) In vivo imaging of glial activation in Alzheimer's disease. *Front Neurol.*, **9**, 625.

Fanselow, M.S. & Dong, H.W. (2010) Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? *Neuron*, **65**, 7–19.

Goncalves, F.M., Freitas, A.E., Peres, T.V., Rieger, D.K., Ben, J., Maestri, M., Costa, A.P., Tramontina, A.C., Goncalves, C.A., Rodrigues, A.L., Nagano, C.S., Teixeira, E.H., Nascimento, K.S., Cavada, B.S., Leal, R.B. (2013) Vatairea macrocarpa lectin (VML) induces depressive-like behavior and expression of neuroinflammatory markers in mice. *Neurochem Res*, **38**, 2375–2384.

Gosselin, R.D., Gibney, S., O'Malley, D., Dinan, T.G., Cryan, J.F. (2009) Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression. *Neuroscience*, **159**, 915–925.

Han, X., Tong, J., Zhang, J., Farahvar, A., Wang, E., Yang, J., Samadani, U., Smith, D.H., & Huang, J.H. (2011) Imipramine Treatment Improves Cognitive Outcome Associated with Enhanced Hippocampal Neurogenesis after Traumatic Brain Injury in Mice. *J. Neurotrauma*, **28**, 995–1007.

Kery, R., Chen, A., & Kirschen, G. (2020) Genetic targeting of astrocytes to combat neurodegenerative disease. *Neural Regeneration Research.*, **15**, 199-211.

Kim, R., Healey, K.L., Sepulveda-Orengo, M.T., & Reissner, K.J. (2018) Astroglial correlates of neuropsychiatric disease: From astrocytopathy to astrogliosis. *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, **87**, 126–146.

Kuhn, H.G., Biebl, M., Wilhelm, D., Li, M., Friedlander, R.M., & Winkler, J. (2005) Increased generation of granule cells in adult Bcl-2-overexpressing mice: a role for cell death during continued hippocampal neurogenesis. *Eur. J. Neurosci.*, **22**, 1907–1915.

Kusakawa, S., Nakamura, K., Miyamoto, Y., Sanbe, A., Torii, T., Yamauchi, J., & Tanoue, A. (2010) Fluoxetine promotes gliogenesis during neural differentiation in mouse embryonic stem cells. *J. Neurosci. Res.*, **88**, 3479–3487.

Li, Y., Barger, S.W., Liu, L., Mrak, R.E., Griffin, W.S. (2000) S100beta induction of the proinflammatory cytokine interleukin-6 in neurons. *J Neurochem*, **74**, 143–150.

Lima, A., Sardinha, V.M., Oliveira, A.F., Reis, M., Mota, C., Silva, M.A., Marques, F., Cerqueira, J.J., Pinto, L., Sousa, N., Oliveira, J.F. (2014) Astrocyte pathology in the prefrontal cortex impairs the cognitive function of rats. *Mol Psychiatry*, **19**, 834–841.

Liu, Q., Li, B., Zhu, H. Y., Wang, Y. Q., Yu, J., & Wu, G. C. (2009) Clomipramine treatment reversed the glial pathology in a chronic unpredictable stress-induced rat model of depression. *Eur. Neuropsyc.,* **19**, 796–805.

Lucassen, P.J., Pruessner, J., Sousa, N., Almeida, O.F.X., Van Dam, A.M., Rajkowska, G., Swaab, D.F., & Czéh, B. (2014) Neuropathology of stress. *Acta Neuropathol.*, **127**, 109–135.

MacQueen, G. & Frodl, T. (2011) The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? *Mol. Psychiatry*, **16**, 252–264.

Malberg, J.E., Eisch, A.J., Nestler, E.J., & Duman, R.S. (2000) Chronic Antidepressant Treatment Increases Neurogenesis in Adult Rat Hippocampus. *J. Neurosci.*, **20**, 9104–9110.

Manji, H.K., Moore, G.J., Rajkowska, G., & Chen, G. (2000) Neuroplasticity and cellular resilience in mood disorders. *Mol. Psychiatry*, **5**, 578–593.

Mateus-Pinheiro, A., Patrício, P., Bessa, J.M., Sousa, N., & Pinto, L. (2013) Cell genesis and dendritic plasticity: a neuroplastic pas de deux in the onset and remission from depression. *Mol. Psychiatry*, **18**, 748–750.

Mateus-Pinheiro, A., Pinto, L., Bessa, J.M., Morais, M., Alves, N.D., Monteiro, S., Patrício, P., Almeida, O.F.X., & Sousa, N. (2013) Sustained remission from depressive-like behavior depends on hippocampal neurogenesis. *Transl. Psychiatry*, **3**, e210–e210.

Ming, G. & Song, H. (2011) Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions. *Neuron*, **70**, 687–702.

Oliveira, J.F., Dias, N.S., Correia, M., Gama-Pereira, F., Sardinha, V.M., Lima, A., Oliveira, A.F., Jacinto, L.R., Ferreira, D.S., Silva, A.M., Reis, J.S., Cerqueira, J.J., & Sousa, N. (2013) Chronic stress disrupts neural coherence between cortico-limbic structures. *Front. Neural Circuits*, **7**, 10.

Oliveira, J.F., Sardinha, V.M., Guerra-Gomes, S., Araque, A., & Sousa, N. (2015) Do stars govern our actions? Astrocyte involvement in rodent behavior. *Trends Neurosci.*, **38**, 535–549.

Ota, K.T. & Duman, R.S. (2013) Environmental and pharmacological modulations of cellular plasticity: role in the pathophysiology and treatment of depression. *Neurobiol. Dis.*, **57**, 28–37.

Patrício, P., Mateus-Pinheiro, A., Sousa, N., & Pinto, L. (2013) Re-cycling paradigms: cell cycle regulation in adult hippocampal neurogenesis and implications for depression. *Mol. Neurobiol.*, **48**, 84–96.

Patricio, P., Mateus-Pinheiro, A., Irmler, M., Alves, N.D., Machado-Santos, A.R., Morais, M., Correia, J.S., Korostynski, M., Piechota, M., Stoffel, R., Beckers, J., Bessa, J.M., Almeida, O.F., Sousa, N., Pinto, L. (2015) Differential and converging molecular mechanisms of antidepressants' action in the hippocampal dentate gyrus. *Neuropsychop.,* **40**, 338–349.

Pekny, M., Pekna, M., Messing, A., Steinhauser, C., Lee, J.M., Parpura, V., Hol, E.M., Sofroniew, M.V., Verkhratsky, A. (2016) Astrocytes: a central element in neurological diseases. *Acta Neuropathol.*, **131**, 323–345.

Pilar-Cuéllar, F., Vidal, R., Díaz, A., Castro, E., dos Anjos, S., Pascual-Brazo, J., Linge, R., Vargas, V., Blanco, H., Martínez-Villayandre, B., Pazos, Á., & Valdizán, E.M. (2013) Neural Plasticity and Proliferation in the Generation of Antidepressant Effects: Hippocampal Implication. *Neural Plast.*, **2013**, 1–21.

Pittenger, C. & Duman, R.S. (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology*, **33**, 88–109.

Rajkowska, G. & Miguel-Hidalgo, J. (2007) Gliogenesis and Glial Pathology in Depression. *CNS Neurol. Disord. - Drug Targets*, **6**, 219–233.

Reger, M.L., Hovda, D.A., & Giza, C.C. (2009) Ontogeny of Rat Recognition Memory measured by the novel object recognition task. *Dev. Psychobiol.*, **51**, 672–678.

Riga, D., Kramvis, I., Koskinen, M.K., van Bokhoven, P., van der Harst, J.E., Heistek, T.S., Jaap Timmerman, A., van Nierop, P., van der Schors, R.C., Pieneman, A.W., de Weger, A., van Mourik, Y., Schoffelmeer, A.N.M., Mansvelder, H.D., Meredith, R.M., Hoogendijk, W.J.G., Smit, A.B., & Spijker, S. (2017) Hippocampal extracellular matrix alterations contribute to cognitive impairment associated with a chronic depressive-like state in rats. *Sci. Transl. Med.*, **9**, eaai8753.

Sanacora, G. & Banasr, M. (2013) From pathophysiology to novel antidepressant drugs: glial contributions to the pathology and treatment of mood disorders. *Biol. Psychiatry*, **73**, 1172–1179.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., & Hen, R. (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*, **301**, 805–809.

Serafini, G. (2012) Neuroplasticity and major depression, the role of modern antidepressant drugs. *World J. psychiatry*, **2**, 49–57.

Sofroniew, M.V. (2009) Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci,* **32**, 638–647.

Sofroniew, M.V., Vinters, H.V. (2010) Astrocytes: biology and pathology. *Acta Neuropathol* , **119**, 7–35.

Sousa, N. (2016) The dynamics of the stress neuromatrix. *Mol. Psychiatry*, **21**, 302–312.

Swaab, D.F., Bao, A.-M., & Lucassen, P.J. (2005) The stress system in the human brain in depression and neurodegeneration. *Ageing Res. Rev.*, **4**, 141–194.

Tanti, A. & Belzung, C. (2013) Neurogenesis along the septo-temporal axis of the hippocampus: Are depression and the action of antidepressants region-specific? *Neuroscience*, **252**, 234–252.

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 Neuropathol.*, **126**, 75–91.

Verkhratsky, A., Rodriguez, J.J., Steardo, L. (2014) Astrogliopathology: a central element of neuropsychiatric diseases? *Neuroscientist*, **20**, 576–588.

Wang, J.-W., David, D.J., Monckton, J.E., Battaglia, F., & Hen, R. (2008) Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *J. Neurosci.*, **28**, 1374–1384.

Wang, C.H., Gu, J.Y., Zhang, X.L., Dong, J., Yang, J., Zhang, Y.L., Ning, Q.F., Shan, X.W., Li, Y. (2016) Venlafaxine ameliorates the depressionlike behaviors and hippocampal S100B expression in a rat depression model. *Behav Brain Funct*, **12** ,34.

Wang, Q., Jie, W., Liu, J.-H., Yang, J.-M., & Gao, T.-M. (2017) An astroglial basis of major depressive disorder? An overview. *Glia*, **65**, 1227–1250.

Wu, M. V., Sahay, A., Duman, R.S., & Hen, R. (2015) Functional Differentiation of Adult-Born Neurons along the Septotemporal Axis of the Dentate Gyrus: Figure 1. *Cold Spring Harb. Perspect. Biol.*, **7**, a018978.

Xi, G., Zhang, X., Zhang, L., Sui, Y., Hui, J., Liu, S., Wang, Y., Li, L., & Zhang, Z. (2011) Fluoxetine attenuates the inhibitory effect of glucocorticoid hormones on neurogenesis in vitro via a two-pore domain potassium channel, TREK-1. *Psychopharmacology (Berl).*, **214**, 747–759.

Zhou, Q.G., Lee, D., Ro, E.J., Suh, H. (2016) Regional-specific effect of fluoxetine on rapidly dividing progenitors along the dorsoventral axis of the hippocampus. *Sci Rep,* **6**, 35572.