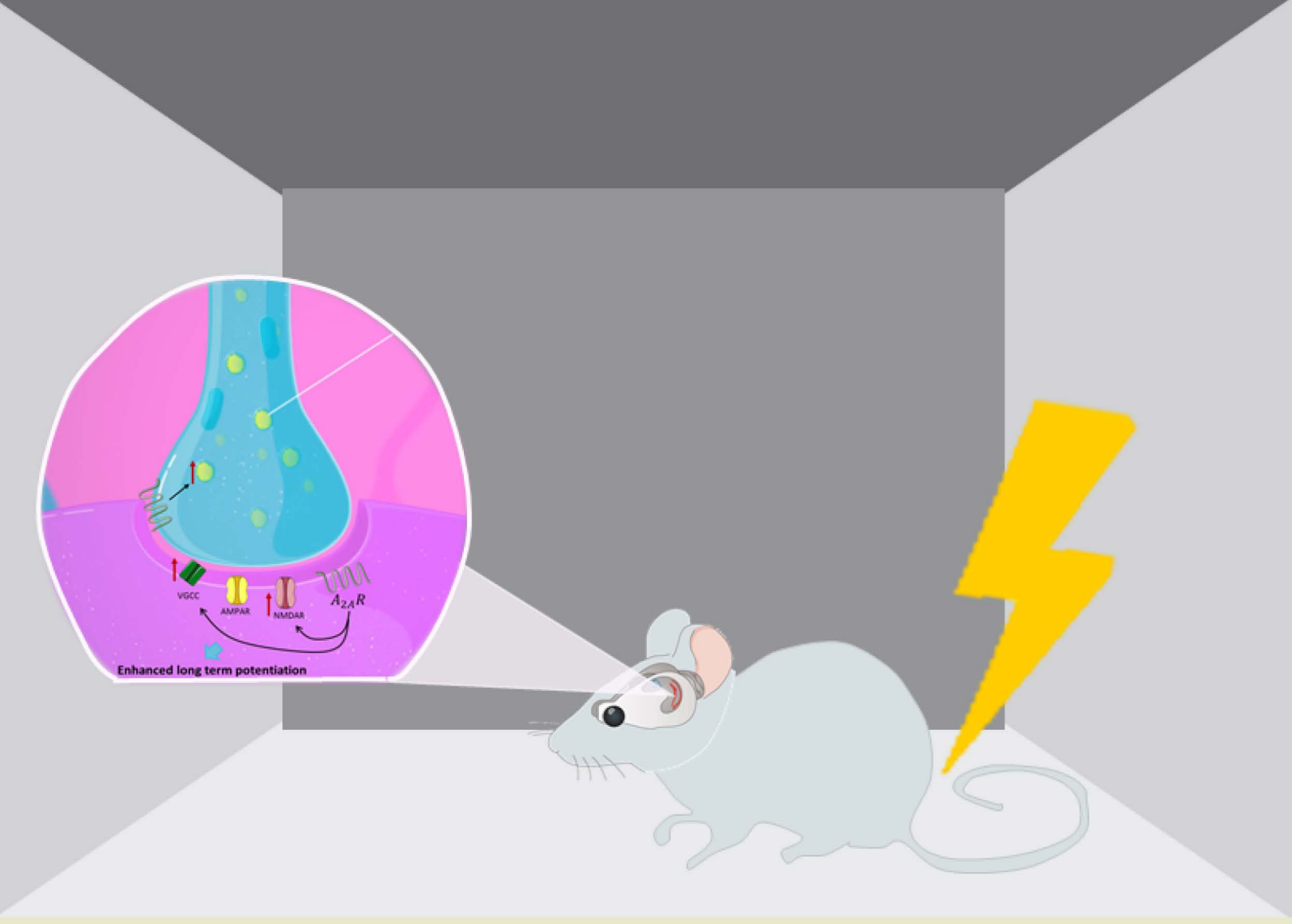


Sara Cristina Lourenço dos Reis

CONTROL OF CONTEXTUAL FEAR MEMORY BY ADENOSINE RECEPTORS

Role in the physiology of the related brain circuitry and implications for fear extinction and post-traumatic stress disorder.

Master dissertation in Cellular and Molecular Biology,
oriented by Ana Patrícia Simões, PhD and Professor Ângelo Tomé, PhD
to be presented to the Life Sciences department of the Faculty of Science and Technology of the University of Coimbra.

June 2017

· U C ·

UNIVERSIDADE DE COIMBRA

UNIVERSIDADE DE COIMBRA

Control of contextual fear memory by adenosine receptors

Role in the physiology of the related brain circuitry and implications for fear extinction and post-traumatic stress disorder.

Author:
Sara Cristina
Lourenço dos Reis

Supervisor:
Ana Patrícia Simões, PhD
Ângelo Tomé, PhD

Thesis submitted in fulfillment of the requirements for the degree of Master in Cellular and Molecular Biology in the

Research group of neuromodulation - Purines
at the Centre for Neuroscience and Cell Biology of the
University of Coimbra

26th of June, 2017

Acknowledgements

First of all I would like to thank my thesis advisor, Ana Patrícia Simões, without whom all this project would have been impossible. I want to express my deep gratitude to all the patience, support, continuously encouragement and guidance during this last year.

I would also like to express my gratefulness to Professor Rodrigo Cunha for giving me the opportunity to do my master thesis in his laboratory and for the useful comments during this process of learning.

I would also like to acknowledge Paula Canas for all the advices and instructions during my master thesis, to Professor Ângelo Tomé for the help in the laboratory of electrophysiology and to all of the group members for the kindness.

I must express my very profound gratitude to my parents and friends for providing me with unfailing support throughout my years of study and through the process of researching and writing this thesis. These accomplishments would not have been possible without all of their support.

Finally, I acknowledge the entities who funded the project: European Regional Development Fund (ERDF) through the COMPETE 2020 programme and Portuguese National Funds via FCT – Fundação para a Ciência e a Tecnologia, I.P., under projects ref POCI-01-0145-FEDER-007440 and research project PTDC/NEU-NMC/4154/2014 - AstroA$_{2A}$R (POCI-01-0145-FEDER-016684); and the entities who provided my scholarship during the last five years: Direção de Serviços de Apoio ao Estudante co-financed with the European social fund in the ambit of the Programa Operacional Capital Humano (PO CH)/ Portugal 2020.

Abstract

Anxiety- and trauma-related disorders such as post-traumatic stress disorder (PTSD) are characterized by pathological fear responses and impaired extinction of aversive memories. In both umans and rodents, caffeine intake in moderate doses correlates negatively with anxiety behavior, depression and cognitive dysfunction. These effects of caffeine are now known to be mediated mainly through the antagonism of adenosine A_{2A} receptors ($A_{2A}R$) in regions of the forebrain. In addition, $A_{2A}R$ in the hippocampus and in the amygdala were shown to modulate synaptic plasticity and control contextual associated fear memory. However, the role of these receptors in the extinction of fear is still unknown.

In the present work, it was implemented and characterized a model of contextual fear conditioning and 'retrieval-extinction' paradigm. Next, the memory trace of contextual, fear and extinction memories was searched through c-Fos immunohistochemistry on brain slices. Finally, it was investigated whether fear and extinction memories altered basal transmission, long-term potentiation (LTP) and depotentiation (metaplasticity) in the dorsal and ventral hippocampus and in the amygdala, through extracellular recordings on brain slices. The role of $A_{2A}R$ on fear extinction was investigated by injecting mice with the selective antagonist, SCH58261 (0.1mg/kg, intraperitoneal), 1 hour before each extinction trial and evaluating the fear response to the conditioning chamber, 24 hours after the last extinction trial. Moreover, it was also investigated if *ex vivo* blockade of $A_{2A}R$ modified basal transmission and plasticity on hippocampal and amygdala slices of naïve, fear conditioned and of mice that went through fear extinction.

The results show that the global blockade of $A_{2A}R$ accelerates the extinction of contextual fear. Moreover, contextual fear conditioning increased activation of the hippocampus and of the amygdala whereas fear recall was associated with activation of other brain regions that orchestrate fear responses, namely the prelimbic area of the medial prefrontal cortex, the hypothalamus and the core region of the nucleus accumbens. Furthermore, contextual fear conditioning caused a stable increase of the LTP in the ventral hippocampus and of the basal excitability in the amygdala. Remarkably, *ex vivo* blockade of $A_{2A}R$, which had no effect in the ventral hippocampal slices from naïve animals, in slices from fear conditioned mice reversed the conditioned-induced exacerbation of the LTP. Moreover, fear extinction also only reversed this effect of contextual fear conditioning in the ventral hippocampus.

These results suggest a gain of function of the $A_{2A}R$ in the ventral hippocampus during the acquisition of contextual fear memories, and provides neurobiological evidence of a role for $A_{2A}R$ on fear extinction reinforcing the view of antagonists of these receptors as novel candidate drugs to manage fear- and anxiety-related disorders such as PTSD.

Key words: Fear, A_{2A} receptor, extinction, memory, PTSD

Resumo

Patologias com sintomas de trauma e ansiedade, como por exemplo, stress pós-traumático (PTSD), são caraterizadas por respostas excessivas de medo e a incapacidade de extinguir memórias aversivas. Tanto em humanos como em roedores, pequenas doses de cafeína têm demonstrado ter efeitos benéficos em comportamentos de ansiedade, depressão e disfunção cognitiva. Já foi demonstrado que estes efeitos da cafeína são mediados, maioritariamente, pelo antagonismo dos receptores de adenosina A_{2A} ($A_{2A}R$) em regiões do prosencéfalo. Estes receptores modulam processos de plasticidade sináptica no hipocampo e na amígdala subjacentes à aquisição e expressão dememórias de medo condicionado. No entanto, nunca foi estudado o impacto destes receptores na extinção de memórias de medo.

No estudo que vai ser apresentado, começou-se por implementar e caraterizar um modelo de medo contextual condicionado, assim como, um paradigma de 'recuperação-extinção' de memórias de medo. De seguida, caraterizou-se o padrão de ativação neuronal neste modelo por imuno-histoquímica da proteína c-Fos. Por fim, através de registos eletrofisiológicos extracelulares, investigou-se de que forma o medo e a extinção de memórias aversivas alterava a transmissão basal, a potenciação de longa duração e a despotenciação no hipocampo dorsal e ventral, e na amígdala. O papel dos receptores A_{2A} foi estudado através da injeção intraperitoneal do antagonista seletivo destes receptores, SCH58261 (0,1mg/kg) 1 hora antes de cada teste de extinção. O efeito deste antagonista foi avaliado através da resposta de medo à caixa de condicionamento, 24 horas depois do último teste de extinção. Foi ainda avaliada se a administração aguda *ex-vivo* do antagonista dos receptores A_{2A} modificava a transmissão basal e os processos de plasticidade em fatias de hipocampo e amigdala de animais control e animais submetidos ao protocolo de medo condicionado e extinção.

Os resultados mostram que o bloqueio geral dos receptores A_{2A} acelera a extinção do medo condicionado ao contexto. O padrão de marcação de c-Fos indica ainda que a aquisição do medo ao contexto aumenta o recrutamento neuronal tanto no hipocampo como na amigdala, mas que a activação dessa memória recruta outras zonas cerebrais, nomeadamente a zona pre-limbica do cortex pre-frontal, o hipotálamo e o núcleo accumbens. Os resultados de eletrofisiolofia mostram que o medo condicionado ao contexto aumenta a amplitude da potenciação de longa duração no hipocampo ventral e a a excitabilidade basal na amigdala. Notoriamente, tanto a extinção como o bloqueio *ex-vivo* dos

receptores A_{2A} normalizou a potenciação de longa duração no hipocampo ventral dos animais condicionados.

Em conclusão, estes resultados sugerem que durante a aquisição do medo condicionado ao contexto existe um ganho de função dos receptores A_{2A} no hipocampo ventral o que poderá explicar o efeito do seu bloqueio na facilitação da extinção desse medo. Assim, antagonistas destes receptores poderão vir a ter um papel no tratamento de doenças relacionadas com a ansiedade como o PTSD.

Palavras chaves: medo, receptores A2A, extinção, memória, PTSD

Table of Contents

List of figures

List of abbreviations

Acquisition group	**ACQ**
Adenosine 5'-diphosphate	**ADP**
Adenosine 5'-monophosphate	**AMP**
Adenosine 5'-triphosphate	**ATP**
Adenosine A_1 receptors	**A_1R**
Adenosine A_{2A} receptors	**A_{2A}R**
Anterior cingulate	**AC**
Basal amygdala	**BA**
Basolateral amygdala	**BLA**
Brain-derived neurotrophic factor	**BDNF**
Camodulin-dependent kinase IV	**CAMKIV**
cAMP responsive element binding protein	**CREB**
Capsular division of CeA	**CeC**
Central nucleus of amygdala	**CeA**
Chronic unpredictable stress	**CUS**
Conditioned stimulus	**CS**
Control group	**CTR**
Cornus Ammonis	**CA**
Cortical-like group of amygdala	**CO**
Dentate gyrus	**DG**
Dopamine D2 receptor	**D2R**
Ecto-5'-nucleotidase	**NT5E/CD73**
Ectonucleoside triphodphate diphosphohydrolase 1	**ENTPD1/CD39**
Elevated plus maze	**EPM**
Extinction group	**EXT**

Forced swim test	**FST**
High performance liquid chromatogaphy, combined with ultraviolet detection	**HPLC-UV**
Immediate eraly genes	**IEG**
Infralimbic	**IL**
Intercalated cell masses	**ICM**
Intercalated cells	**ITC**
Intermediate subdivision of CeA	**CeI**
Inter-trial intervals	**ITI**
Knock – out	**KO**
Lateral amygdala	**LA**
Lateral subdivision of CeA	**CeL**
Long-term depression	**LTD**
Long-term potentiation	**LTP**
L-type Voltage-Gated Calcium channels	**VGCCs**
Medial agranular	**AGm**
medial Prefrontal cortex	**mPFC**
Medial subdivision of CeA	**CeM**
Metabotropic glutamate 5 receptor	**$mGlu_5R$**
Nicotinamide adenine dinucleotide	**NAD**
N-methyl-D-aspartate	**NMDA**
Open-field	**OPF**
Posttraumatic stress disorder	**PTSD**
Prefrontal cortex	**PFC**
Prelimbic	**PL**
Tail suspension test	**TST**
Unconditioned stimulus	**US**
Ventral medial prefrontal cortex	**vmPFC**

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid **AMPA**

1

INTRODUCTION

1.1 THE PURINERGIC SYSTEM

Purines and purine nucleotides are constituents of all living cells as part of the backbone of DNA and pivotal for cell metabolism. Despite their fundamental role in the intracellular milieu, these molecules are also released into the extracellular medium and take part in neurotransmission and in neuromodulation (Dunwiddie and Masino, 2001; Cunha, 2016).

Thus, today it is well established that purines are released from virtually all types of cells, including neurons and glial cells, producing widespread effects on multiple organs by binding to its purinergic receptors located on the cell surface (Burnstock, 2014; Brady and Siegel, 2012). The two principal ligands for the purinergic receptors are adenosine and ATP/ADP.

1.1.1 Adenosine 5'-triphosphate (ATP)

Adenosine 5'-triphosphate (ATP) is composed by an adenine ring that is formed during the purine biosynthesis, the ribose moiety, generated from the pentose phosphate pathway and the triphosphate chain, which is synthesized by independent metabolic pathways. This molecule was first discovered by Dr. Karl Lohamnann in 1929 in muscle and liver extracts. Then, it was described has the key source of energy, since it has the ability to capture free energy from catabolic processes and transfer it to reactions and mechanisms that require energy (Devlin *et al. 2010,* Voet *et al.,2004*).

Later on, in 1959, Doctor Pamela Holton showed that upon stimulation of the rabbit ear nerve there was release of ATP (Holton *et al.,* 1959). Since then, using the luciferin-luciferase assay and the reverse phase high performance liquid chromatography, combined with ultraviolet detection (HPLC-UV) to measure the extracellular levels of ATP, many scientists reported that, indeed, ATP was released from nerve terminals (reviewed in Sperlág and Vizi 1996).

Furthermore, in 1970, George Burnstock and his colleagues, while studying the non-adrenernergic and non-cholinergic nerve transmission in the smooth muscle of the guinea pig *taenia coli*, discovered the "purinergic neurons". These neurons where named "purinergic" because ATP was their principal neurotransmitter (Burnstock *et al.,* 1970; Burnstock, 1972). Later it was shown that ATP was a co-transmitter in the parasympathetic and sympathetic nerves (Burnstock, 1976) and now it is well established that ATP is a co-transmitter in all nerve cells both in the peripheral and in the central nervous system (PNS and CNS, respectively) (Burnstock, 2007).

Extracellularly, ATP can act both as a neurotransmitter and as a neuromodulator, through the activation of its receptors (the P2 receptors) and/or through its dephosphorylation to adenosine, which acts on its own receptors (the P1 receptors). The extracellular catabolism of the ATP occurs mainly through a two-step enzymatic reaction: first the ATP or ADP is converted to AMP by the ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, also known as CD39) and secondly, the AMP is hydrolyzed to adenosine by the ecto-5'-nucleotidase (NT5E, also known as CD73) (Burnstock *et al.,* 2008; Gomes et al.,2010), Figure 1. Thus, both ATP and adenosine fulfill an important role in CNS injury, neurodegenerative and neuropsychiatric disorders (Burnstock *et al.,* 2008; Gomes *et al.,* 2010; Cunha *et al.,* 2016)

1.1.2 Adenosine

Adenosine is one of the most studied purines. The earliest evidence for an extracellular role of adenosine was reported in 1929 by Drury and Szent-Gyorgy where they showed that this molecule could control the heartbeat (Drury and Szent-Gyorgy, 1929). Since then, extracellular adenosine has been established has a major neuromodulator and has been the subject of much research in virtually all biological areas. Adenosine can either be released from cells through mainly (bi-directional) equilibrative nucleoside transporters (ENTs) or, as previously mentioned, through the extracellular catabolism of ATP by the activity of the enzymes CD39 and CD74 (Fredholm *et al.,*2013)

Intracellularly, adenosine levels are tightly coupled to cellular metabolism. In fact, adenosine is the base of nucleic acids, is the backbone of ATP, the energetic currency of cells, and is necessary for the synthesis of nicotinamide adenine dinucleotide (NAD), the major controller of the redox state of cells (Cunha, 2016).

1.1.2.1 General role in the brain

Besides the aforementioned fundamental role in metabolism, adenosine is the prototypic neuromodulator in the nervous system since it does not directly activate neurons but instead modulates the activity of other neurotransmitters thereby controlling neuronal excitability (Sattin *et al.,* 1970). In the brain, this adenosinergic neuromodulatory impact is more evident in excitatory rather than in inhibitory synapses and mainly depends on the balance between the activation of the inhibitory adenosine A_1 and the facilitatory A_{2A} receptors (Cunha, 2008).

1.1.2.2 Receptors in the brain

There are four types of adenosine receptors: A_1, A_{2A}, A_{2B} and A_3. The distribution and relative densities of adenosine receptors is similar in rodents and in humans (Fredholm *et al.,* 2005). In the central nervous system (CNS) the A_1 and A_{2A} receptors are by far the most abundant and therefore the best studied (Fredholm *et al.,* 2005). In fact, these adenosine receptors are pleiotropic and expressed in virtually all cell types in the brain (Fredholm *et al.,* 2005; Cunha, 2016).

The A_1 receptors (A_1R) are widely distributed in the CNS with the highest levels in the cerebral cortex, hippocampus, brain stem, and spinal cord (Reppert *et al.,* 1991). In neurons, these receptors were shown to be predominantly in glutamatergic synapses in both the striatum and in the hippocampus (Rebola *et al.,* 2003; Rebola *et al.,* 2005a). Here, A_1Rs are classically coupled to the G_i group of G proteins that inhibit the adenylyl cyclase therefore the activation of these receptors results in the depression of the excitatory transmission (Dunwiddie *et al.,* 2001). Thus, the activation of these receptors at the presynaptic level inhibits the release of neurotransmitters whereas at postsynaptic sites, A_1R can activate potassium channels leading to the hyperpolarization of the cells (Cunha, 2001). Overall, the inhibitory tonus of adenosine in the brain is attributed to the activation of presynaptic A_1R (Cunha, 2016).

The A_{2A} receptors ($A_{2A}R$) are highly enriched in the striatum, mainly at postsynaptic sites at encephalin containing striatopallidal GABAergic projection neurons, and at 20% lower density in the hippocampus, cortex, amygdala and others brain areas, mainly at excitatory synapses (Fredholm *et al.,* 2005). These receptors were shown to have a limited impact on the control of basal synaptic

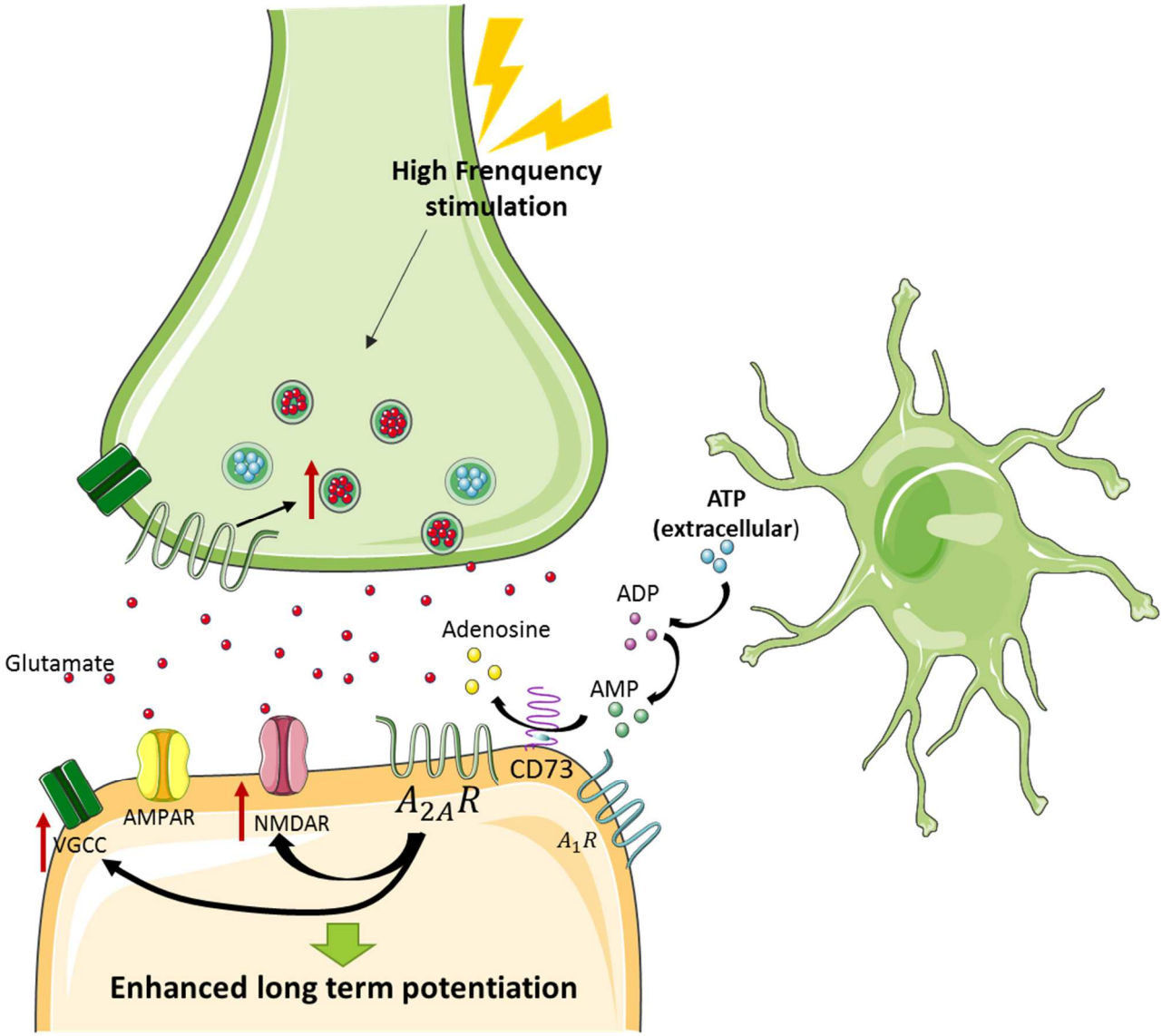


Figure 1 - Modulatory effect of the $A_{2A}R$ in glutamatergic synapses. Upon high frequency stimulation the $A_{2A}R$ have the ability to enhance the release of glutamate and the function of the ionotropic glutamate receptors leading to an increase of the synaptic strength. One of the sources of adenosine in the synaptic cleft is through the release of ATP and its catabolism through an ecto-nucleotidase pathway (Gomes *et al.,* 2010; Cunha *et al.,*2016)

transmission but a pivotal role in the control of synaptic plasticity (Gomes, *et al.,* 2010). In the striatum, $A_{2A}R$ are coupled to the G_{olf} group of G proteins and elsewhere they usually couple to G_S proteins. Thus, typically, activation of these receptors increases the activity of adenylyl cyclase and increases cAMP (Fredholm *et al.,* 2005). Thus, $A_{2A}R$ are considered facilitatory of neurotransmission: at the Presynaptic level, $A_{2A}R$ trigger the release of neurotransmitters such as glutamate and postsynaptically, they can increase NMDA- and $mGluR_5$-mediated currents (Rebola *et al.,* 2008). Furthermore, these adenosine receptors can form heterodimers with dopamine D2 receptors, NMDA receptors, metabotropic

glutamate 5 receptors ($mGlu_5R$) and cannabinoid CB1 receptors, increasing its spectrum of action (reviewed in Chen *et al.,* 2014).

1.1.2.3 Adenosine receptors in memory

The ability of A_1R and of $A_{2A}R$ to modulate the release of neurotransmitters, neuronal excitability and synaptic plasticity has raised the possibility of these receptors having a crucial role in learning and memory.

In fact, over the years, it has been established that $A_{2A}R$ critically impact learning and memory processes and therefore are at the etiology of many cognitive-related disorders (Chen *et al.,* 2014) For instance, using a model of spontaneously hypertensive rats and of aged rats, Prediger and colleagues showed that both caffeine and selective antagonists of $A_{2A}R$, but not of A_1R, reversed deficits in social-related memories (Prediger *et al.,* 2005 a,b). Moreover, mice lacking $A_{2A}R$ had improved spatial recognition memory, confirming that $A_{2A}R$ have a role in short-term memory (Wang *et al.,* 2006).

Furthermore, it was also shown that $A_{2A}R$ have a crucial role in working memory (Chen *et al.,*2014). There have been some controversies in the definition of working memory but it is now accepted that it refers to the temporary storage and management of information required to plan and carry out cognitive tasks such as learning, reasoning, and comprehension (Cowan *et al.,*2008). Impairment of this type of memory is at the core of many brain disorders such as Alzheimer's disease and schizophrenia (Chen *et al.,*2014). Interestingly, in a model of early Alzheimer's disease in rodents antagonists of $A_{2A}R$ had a neuroprotective effect (Cunha *et al.,* 2008; Canas *et al.,* 2009). On the other hand, overexpression of $A_{2A}R$ caused deficits in spatial working memory (Gimenez-Lort *et al.,* 2007) while the genetic deletion of these receptors improved it (Zhou *et al.,* 2009; Wei *et al.,* 2011) and also cognitive flexibility (Wei *et al.,* 2011).

In humans it was also demonstrated that the consume of caffeine (a non-selective antagonist of adenosine receptors, Hamilton *et al.,*2004) improved cognitive performance (Haskell *et al.,*2005) and memory performance (Hameleers *et al.,* 2000; van Boxtel *et al.,* 2003); reduced the risk of developing Alzheimer's disease (Lindsay *et al.,* 2002) and correlated with less cognitive decline in women (Ritchie *et al.,*2007).

In animal models, the beneficial effects of caffeine on LTP and memory have been shown to be due to its antagonism of adenosine $A_{2A}R$ (Costenla *et al.,* 2010 and in Cunha and Agostinho, 2010). In addition to working and social memories, adenosine levels and adenosine receptors were also shown to

impact fear memory (Corodimas *et al.,* 2001; Yee *et al.,* 2009; Wei *et al.,* 2014, Simões *et al.,2016*). The formation and extinction of fear memories have been shown to be NMDA-dependent, since the blockad of NMDA receptors impairs the extinction of fear (Baker *et al.,* 1996; Cox *et al.,* 1994; Liu *et al.,* 2009; Orsini *et al.,* 2012). Since $A_{2A}R$ are able to control NMDA receptors in brain regions of the fear circuitry (Rebola *et al.,* 2008), $A_{2A}R$ may also control plasticity phenomena underlying fear memory.

In fact, our group recently showed that the antagonism of $A_{2A}R$ reduced LTP in the lateral amygdala. Additionally, the selective downregulation of $A_{2A}R$ in the BLA decreased the acquisition of fear behavior (Simões *et al.,* 2016). Interestingly, the deletion of these receptors in the hippocampus did not interfere with fear conditioning; however, when the animals returned to the conditioning chamber one day after training, they displayed low freezing levels (i.e. impaired contextual fear memory) but recalled the sound cue associated with the US (Wei *et al.,* 2013). These results suggest that hippocampal $A_{2A}R$ may modulate the contextual aspects of fear while in the amygdala these receptors interfere with fear learning.

On the other hand, A_1R activation in the hippocampus was shown to interfere negatively with the acquisition of contextual fear (Corodimas *et al.,* 2001). However, the density of A_1R in the amygdala was unaltered after fear conditioning and its blockade did not show any effects on fear memory (Simões *et al.,* 2016).

These pioneering studies highlight the involvement of $A_{2A}R$ in both hippocampal and amygdala circuits to control fear learning and memory and underlying mechanisms (Wei *et al.,* 2013; Simões *et al.,* 2016). However, there are no evidences for a role for these receptors in the extinction of fear memories, which would be of a more translational value for the treatment of anxiety- and trauma- related disorders.

1.1.2.4 Adenosine receptors in mood-related disorders

Due to the previously exposed evidences and the ability of $A_{2A}R$ to integrate both glutamatergic and dopaminergic transmission (Schiffmann *et al.,* 2007), two key players in psychiatric disorders (Schmidt et al.,2005), we propose that these receptors may be a favorable target to manage emotion-related disorders such as PTSD.

Indeed, in rats, overexpression of $A_{2A}R$ is associated with an increase in anhedonia, which is one of the main symptoms of depression (Coelho *et al.,* 2014) and also a symptom in PTSD (Novakovic *et al.,* 2011). Contrarily, caffeine, $A_{2A}R$ antagonists and deletion of $A_{2A}R$ in neurons of the forebrain, were able

to normalize aberrant synaptic plasticity and revert mood and memory alterations caused by chronic unpredictable stress (CUS), Kaster *et al.,* 2015.

Also, antagonists of $A_{2A}R$ were proposed to be anti-depressants in animal models since both the pharmacological blockade and the genetic deletion of these receptors reduced immobility in the classical paradigms to evaluate depressive behavior in rodents, the forced swim test (FST) and the tail suspension test (TST) (El Yacoubi *et al.,* 2001). Nevertheless, these results may be shaded by the doses used of the $A_{2A}R$ selective antagonist, 10mg/kg of SCH58261, which were previously showed to increase locomotor activity in the open field (El Yacoubi *et al.,* 2000). Nevertheless, administration of istradefylline (or KW-6002), also a selective antagonist of $A_{2A}R$ and a medicine used for alleviating the motor symptoms of the Parkinson's disease, again reduced the immobility period in the FST and had a synergistic effect with other well-known antidepressants, paroxetine and fluoxetine (serotonin re-uptake inhibitors) and deprenyl (MAO-B inhibitor) (Yamada *et al.,* 2013). Data from our group also indicate an antidepressant effect of SCH58261 (0.1 mg/kg, administrated intraperitoneally) since it was able to actually revert CUS-induced behavior in the FST without affecting locomotion (Kaster *et al.,* 2015). Moreover, the genetic deletion of $A_{2A}R$ from forebrain neurons in mice reduced anxiety-related behavior measured in the elevated plus maze (EPM) and in the open-field (Wei *et al.,*2014). Noteworthy, these authors also showed that the striatal-selective deletion of $A_{2A}R$ did not influence anxiety-related behavior and actually increased fear conditioning, however when the deletion was extended to all the forebrain (including the cortex, the hippocampus and the amygdala), mice had a less anxious behavior, suggesting that $A_{2A}R$ can oppositely modulate anxiety and fear in a region-specific manner.

In contrast, A_1R knock-out (KO) mice display increased depressive-like behavior (Serchov *et al.,* 2015), and pharmacological blockade of these receptors also increase anxiety (Maximino *et al.,* 2011). These reports suggest that activation of A_1R has an anxiolytic and anti-depressant effect while the activation of $A_{2A}R$ has an anxiogenic and depressive-like effect.

In humans, $A_{2A}R$ have also been implied in mood and anxiety-related disorders. Overall, it has been showed that higher doses of caffeine tend to increase (Loke *et al.,* 1988; Green *et al.,*1996) while low doses tend to decrease anxiety levels (Stern *et al.,* 1989; Haskell *et al.,* 2005). Another line of evidence showed that anxiety levels are associated with polymorphisms of the ADORAA2A locus (locus ofthe gene coding for $A_{2A}R$) in people suffering from panic disorders/anticipatory anxiety (Hamilton *et al.,* 2004; Lam *et al.,* 2005).

Overall, there is good evidence that $A_{2A}R$ have a role on the etiology and pathophysiology of anxiety- and mood-related disorders, both in rodent models and in humans. However, the therapeutic potential of these receptors is still unexplored in most of these models. In this work, we hypothesized that $A_{2A}R$ may also have an impact on fear extinction and therefore constitute a target for the treatment of PTSD.

1.2 FEAR LEARNING AND FEAR EXTINCTION

Fear is a set of innate responses (endocrine, autonomic, cognitive and behavioral) to threatening stimuli. These physiological responses have been retained by natural selection to promote survival (Duvarci *et al.,* 2014). Each day, animals are faced with threatening situations that require defensive behavior or the result might be harm or death. Learned or associative fear is crucial to learn by experience that some stimuli or circumstances predict danger or safety (Mineka *et al.,* 2002). Fear or defensive behavior is known to cause three types of reactions known as flight, freeze or fight responses (Orsini *et al.,* 2012).

Although fear learning is evolutionary advantageous, irrational fear is a major impediment to success, productivity and overall well-being and in extreme cases it can lead to suicide. Overgeneralized fear is one of the main symptoms of anxiety-related disorders such as panic disorder, phobia, and posttraumatic stress disorder (PTSD). However, amongst anxiety disorders, PTSD is the one caused by traumatic events and is characterized by abnormal fear expression, fear generalization and deficient fear extinction (Mahan *et al.,* 2011; Mahan *et al.,* 2012).

Pavlovian fear conditioning has been widely used to investigate the neuronal mechanisms of fear learning (Maren *et al.,* 2001). This paradigm consists in pairing an initial neutral stimulus (conditioned stimulus, CS) such as a chamber, a tone or a light with a noxious unconditioned stimulus (US), usually a foot shock, Figure 2A (Duvarci *et al.,* 2014). As a result of this training the CS acquires the aversive properties of the US and its presentation alone elicit a fear response, which in rodents translates into freezing behavior. There are 3 major types of the fear conditioning test: the cued (classical) fear conditioning; the context fear conditioning and the trace conditioning (Curzon *et al.,* 2009). The difference between the cued and the contextual fear conditioning is that in the first case the CS is usually a tone whereas in the second case the CS is the context chamber (Curzon *et al.,* 2009). The trace conditioning also requires the association of a CS with an US but this association is separated in time, which requires the formation of a temporal relationship between the two stimuli (Runyan *et al.,* 2004).

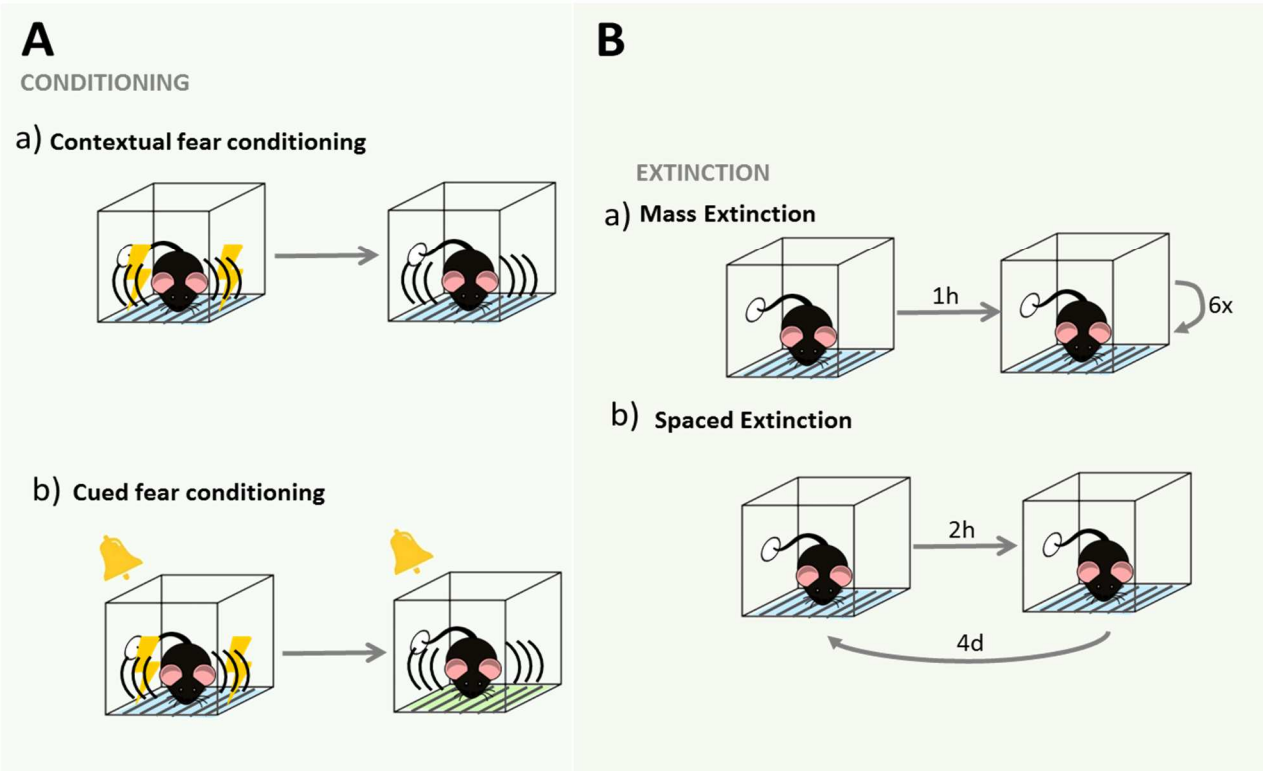


Figure 2 - Fear conditioning paradigm in rodents. This paradigm consists in associating a neutral stimulus (CS), a chamber in the **contextual fear conditioning** (Aa) and a tone in the **cued fear conditioning** (Ab) with a footshock. The extinction of the fear memory can be achieved using two types of protocols: the **mass extinction** (Ba), which consists in exposing the animal to the CS, in the absence of the US, multiple times in the same day, or the **spaced extinction** (Bb) which consists in exposing the animals to the CS alone one or two times per day, for several days (usually 4) in a row.

This paradigm is a powerful tool to investigate the neuronal mechanisms of associative learning (Maren *et al.,* 2001) and to correlate with the dysfunctions that happen in anxiety-related disorders.

Additionally, this paradigm is also useful to study the mechanisms underlying the extinction of fear memories. Spontaneous extinction of conditioned memories was first documented by Pavlov in 1927. The mechanisms through which the brain extinguishes aversive memories have since received particular attention because of their relevance to the treatment of anxiety-related disorders (Dias *et al.,* 2013).

Extinction of associative fear, consist in repeatedly presenting the CS in the absence of the US (LeDoux 2000; Maren 2001), Figure 2B. As a result, by the end of this procedure, the CS no longer elicits fear (Bouton, 2004). In the classical rodent models of fear extinction animals are exposed within 24 hours of the CS-US pairing (the period of memory consolidation) to the CS in the absence of the US, either in multiple trials during one day (massive extinction) or in a single trial during several days (spaced extinction) (Santos *et al.,* 2013),Figure 2 B. It is important to remember that extinction of fear is not the

same as forgetting since forgetting implies the loss of a memory with the passage of time (Orsini and Maren, 2012). In support of this, there are multiple evidences of the recover of conditioned responses after extinction (Bouton *et al.,* 2004; Orsini *et al.,* 2012; McConnel *et al.,* 2015; Izquierdo *et al.,* 2016). Recovery from extinction refers to the re-emerging of the fear response to the CS. This effect includes spontaneous recovering (recovery of the fear response to the CS with the passage of time), renewal (recovery of the fear memory outside the context of extinction), and reinstatement (triggered by the presentation of the US in a different context). These recovery phenomena suggest that extinction does not erase the fear memory or makes the individual unlearn, instead it leads to the formation of a new inhibitory memory that competes with the original one (Orsini and Maren, 2012).

Furthermore, another type of extinction paradigm has recently emerged with better results both in animal models and in humans, the 'retrieval-extinction' paradigm. (Agren *et al.,* 2012; Schiller *et al.,* 2013; Gräff *et al.,* 2014; Johnson and Casey, 2015). These consist in retrieving the fear memory by exposing the subjects to the CS (or a close reminder) in the absence of the US, followed by another exposure to the CS alone within few minutes-hours. Thus, this type of extinction paradigm consists in two trials *per* day, with a small intertrial interval (ITI), during few successive days (Gräff *et al.,* 2014). The success of this type of extinction paradigms relies on their ability to trigger reconsolidation of the fear memory, a period where this memory is more labile and can potentially be erased (Bentz and Schiller, 2015). Thus within the reconsolidation window, previous acquired memories can be updated with new information (Auber *et al.,*2013; Graff *et al.,*2014). Nevertheless, the mechanisms and the functioning of the fear circuitry underlying extinction are still less clear then the mechanisms behind the acquisition of conditioned fear memories.

In the following sections of this subchapter, it will be discussed the current knowledge about the brain areas mediating fear learning and extinction.

1.2.1 The Fear Circuitry

In 1920, Walter Cannon and Philip Bard presented the hypothalamus and its projections as mediators of emotional behavior. Later, in 1937, James Papez extended this emotional circuit to include the structures of the temporal lobe, centered in the hippocampus. In 1949, Paul MacLean revised this circuit to include the medial prefrontal cortex (mPFC) and the amygdala, naming this circuit as the "limbic system" (Orsini *et al.,* 2012). Nowadays, it is well established that these three brain areas

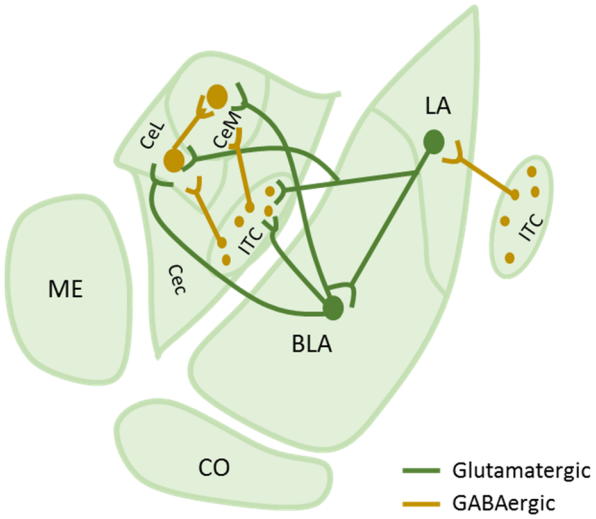


Figure 3 - Intrinsic connectivity in the amygdala. Scheme of the coronal section of the amygdala and of its intranuclear connectivity (green, glutamatergic connections; yellow, GABAergic connections).

(amygdala, hippocampus and mPFC) are the most affected by fear conditioning and stress-related disorders such as PTSD. (Mahan *et al.,* 2013).

1.2.1.1 Amygdala as a crucial player in the fear circuitry

The amygdala seems to play a critical role in the acquisition and extinction of fear memories both in animal models and in humans (Ehrlich *et al.,*2009; Agren *et al.,*2012; Orsini *et al.,*2012; Duvarci *et al.,*2014; Lithari *et al.,*2016). This brain area is an almond-shaped structure constituted by different groups of nuclei located in the medial temporal lobe. Its structure and function are highly conserved across species underlying their importance in the survival of species (Sah *et al.,* 2003). The amygdala or the amygdaloid complex can be divided morphologically and functionally into different subnuclei: the basolateral (BLA), the cortical-like group (CO), the central nucleus (CeA) and the intercalated cell masses (ICM). The groups that have been more studied in the fear circuitry are the BLA, CeA and the ICMs (Figure 3).

The BLA is composed mainly by spine dense glutamatergic neurons (80%), and to a lesser extent by small, stellate and spine sparse GABAergic neurons. The BLA can be divided into the lateral amygdala (LA) and the basal-amygdala (BA) (Spampanato *et al.,* 2011). Moreover, in the BA there are three different populations of excitatory neurons: the 'fear neurons', the 'extinction' neurons and the 'extinction-resistant neurons'. This nomenclature was proposed since some of the neurons (the 'fear neurons') increase their firing during the acquisition and expression of the fear response to the conditioned stimulus (CS), whereas the 'extinction neurons' only fire during the extinction training and

recall (Henry *et al.,* 2008), and the 'extinction-resistant neurons' fire during the acquisition of fear response and continue to be CS responsive after the extinction training. These last cells are proposed to be responsible for the maintenance of the CS-US after the extinction, and might be one of the causes of spontaneous recovery and renewal (Duvarci *et al.,*2014).

The CeA is located in the rostral part of the amygdala boarded with the basolateral complex. This structure consists primarily in GABAergic neurons and has four divisions: the capsular (CeC), the lateral (CeL), the intermediate (CeI) and the medial subdivisions (CeM) (Sah *et al.,* 2008). However, morphologically it can be divided into 2 groups: the CeL neurons that have a smaller soma, multiple primary dendrites that branch freely and have a high density of spines, similar to the medium spiny neurons in the striatum, and the CeM neurons that have a larger soma with few dendritic spines that branch sparsely (Hall *et al.,* 1972). In the CeL two distinct subpopulation of inhibitory GABAergic neurons were identified, the CeL 'on' (that express the protein kinase C delta, PKC-δ) and the CeL 'off'(in which PKC-δ is absent). This differentiation was also based on their response to the fear conditioning. The CeL receives glutamatergic input from various brain structures, while the CeM send the outputs to initiate a fear response.

The ITCs are small densely packed GABAergic cell clusters that connect different neurons according to their position within the amygdala. There are two major agglomerates: the external, in the borders of the amygdala (in the external capsule) and the intermediate, between the BLA and the CeA (Duvarci *et al.,* 2014).

All of these nuclei have strong intranuclear connectivity which is essential to the operation of the amygdala. For instance, the BLA excitatory projections run dorsoventrally and medial-laterally from the LA to the BA and, from to the CeA. These projections between the BLA and the CeA are not reciprocal, and, interestingly, the LA projects exclusively to the CeL while the BA projects to both CeL and CeM (Krettek *et al.,* 1978). The CeL neurons do not project to the BLA, but send GABAergic projections to the CeM, and the CeM reciprocally projects to the CeL, although in a weaker-manner (Ciocchio *et al.,* 2010). Additionally, on their way to the CeA, the principal neurons of the BLA can excite the intercalated cells (ITC) (Royer *et al.,* 1999). This means that the BLA neurons can influence the CeA neurons in two ways: via direct glutamatergic projections and via an indirect pathway that leads to the excitation of the ITC, generating a feedforward inhibition in CeA neurons (Duvarci *et al.,* 2014),Figure 3.

In the classical circuit of fear memory formation the LA nuclei are considered the primary input station of the amygdala since it receives converging visceral and somatosensory projections that allow the association between the conditioned stimulus and the unconditioned stimulus. On the other hand, the CeM nuclei are considered the primary output station of the amygdala since it projects to effector structures that coordinate the autonomic response to fear (such as the brain stem and the hypothalamus) and to structures that coordinate the perception of emotion (such as the anterior cingulate cortex, the orbitofrontal cortex and the prefrontal cortex) (Ciocchio *et al.,* 2010). Thus, the learned fear is regulated by modifying the relative efficacy of the direct *versus* indirect pathways of this intramygdalar microcircuit that either lead to the excitation/disinhibition of CeM, resulting in the expression of the fear response, or to its inhibition, resulting in the suppression of the fear response.

Indeed, based on recent findings from *in vivo* studies showing a distinct pattern of neuronal firing during associative fear learning and expression, it was hypothesized that auditory fear conditioning excites the LA neurons (LeDoux *et al.,* 1990; LeDoux *et al.,* 2000) which then activate the 'fear' neurons in the BA (Herry *et al.,* 2008). Subsequently, the 'fear' neurons in the BA directly activate the CeL 'on' neurons that will inhibit the CeL 'off' leading to a disinhibition of the CeM (Ciocchio *et al.,* 2010). The inhibitory projections of CeM will release the inhibition of the brainstem and of the hypothalamus thereby promoting the autonomic fear response (Flores *et al.,* 2015).

1.2.1.2 Hippocampus and its role in the fear circuitry

Another important and widely investigated structure in the fear learning and extinction is the hippocampus. The hippocampus is a c-shaped structure situated in the caudal part of the brain. This brain region is subdivided into the dentate gyrus (DG), the Cornus Ammonis (CA1, CA2, CA3) and the subiculum and it is surrounded by the entorhinal, parahippocampal and perirhinal cortices (Strien *et al.,* 2009),Figure 4.

Functionally, the hippocampus is divided into dorsal (posterior in humans) and ventral (anterior in humans) hippocampus. The dorsal hippocampus has been implicated in the spatial navigation and memory formation, i.e it mediates cognitive function. On the other hand, the ventral hippocampus is associated with emotional responses, such as anxiety-related behavior (reviewed in Fanselow *et al.,* 2010). These different roles of the two poles of the hippocampus may be due to their different electrophysiological properties, patterns of gene expression and connectivity with cortical and

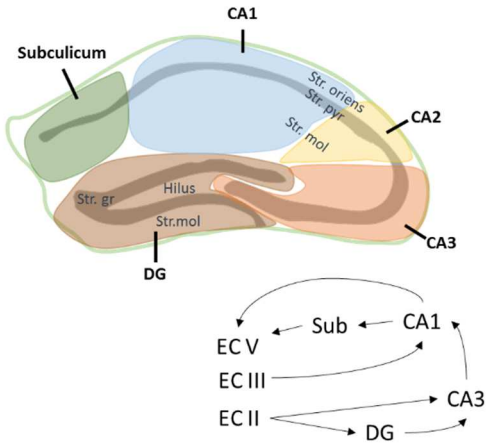


Figure 4 - Anatomy and physiology of the hippocampus. Scheme of the coronal section of the hippocampus. This brain structure has three subregions: the dentate gyrus (DG), the hippocampus proper (CA3, CA2 and CA1) and the subculicum. The cortex that forms the hippocampus can be divided into three layers: the deep layer (comprises a mixture of afferent and efferent fibers and interneurons), the cell layer (composed of principal cells and interneurons), and the superficial layer. The deep layer in the DG is called the hilus, while in the CA regions it is referred to as stratum oriens. The cell layer in the DG is referred to as granule cell layer whereas in the CA regions and in the subculicum it is called the pyramidal cell layer. Finally, the superficial layer is called the molecular layer (the stratum moleculare) in the DG and in the subculicum, whereas in the CA regions this layer can be divided into 2 or 3 sublayers. In CA3 there are 3 sublayers: the stratum lucidum (receives inputs from DG), the stratum radiatum (comprises apical dendrites from neurons of the stratum pyramidale) and the stratum lacunosum-moleculare (comprise apical dendrites and tufts). The layers in CA1 and CA2 are similar to the CA3, with the exception of the stratum lucidum which is missing (Strien *et al.,* 2009).

subcortical areas (Strange *et al.,* 2014). For instance, the ventral part has denser connectivity with the amygdala (Van Groen *et al.,* 1990) while the dorsal region connects strongly with the entorhinal and retrosplenial cortex (Swasson *et al.,* 1977), Figure 5.

The cognitive function of the hippocampus has two major inputs: the medial entorhinal cortex and the lateral entorhinal cortex. The medial entorhinal cortex is thought to create and update spatial representations (Savalli *et al.,* 2008) whereas the lateral entorhinal cortex does not show any robust spatial firing properties but is fundamental to the recognition of objects (Hargreaves *et al.,* 2005). The classical view of how information is processed in the hippocampal circuit is unidirectional, i.e., it starts with a projection from the entorhinal cortex (layers II and III) to the DG. The DG projects to the CA3 via mossy fibers and then CA3 schaffer collaterals transmit information to the CA1. The CA1 will then project to the subculicum, which finally projects back to the entorhinal cortex (layer IV). However, several backprojections within this circuit were found. For instance, CA3 can also project to the hilus and to the molecular layer of the DG (Li *et al.,* 1994), and the CA1 can backproject to the CA3 and to the DG (Cenquizca *et al.,* 2007), Figure 4.

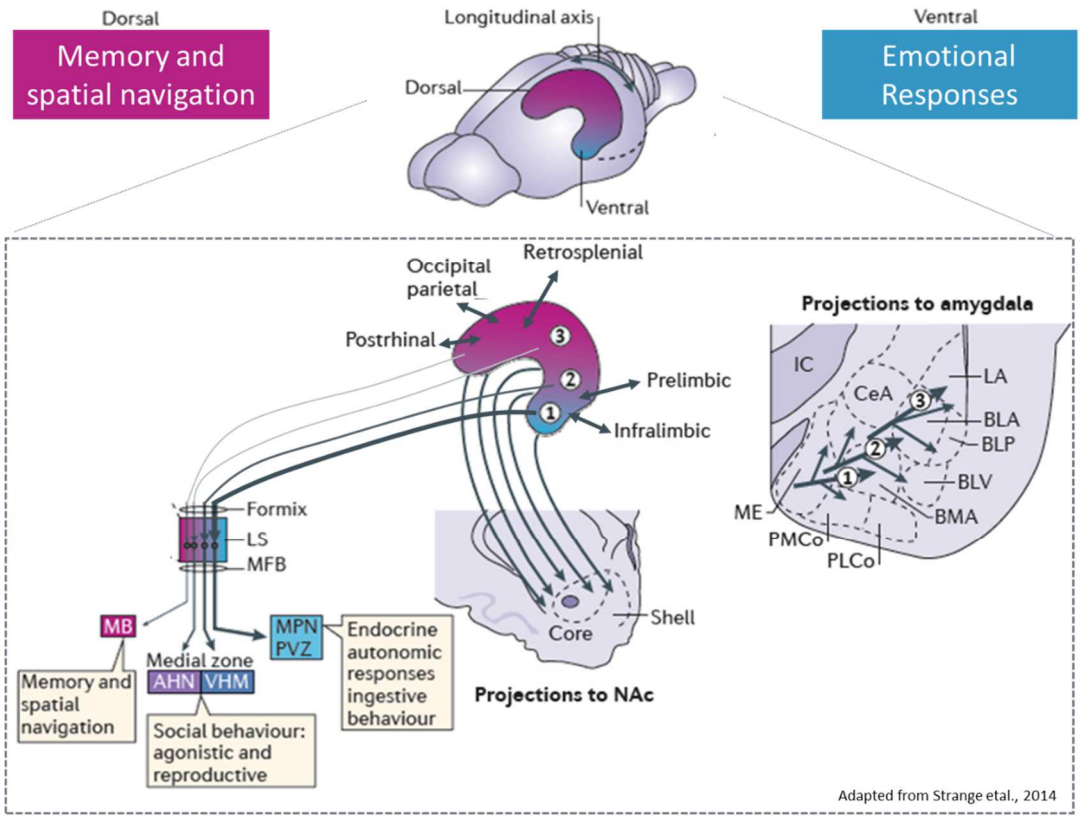


Figure 5 - Extrinsic connectivities of the dorsal and ventral hippocampus. The dorsal hippocampus has been more implicated in memory and spatial navigation manly due to its connections to the shell of the nucleus accumbens (NAc), the lateral septum (LS) and subsenquently to the mammalian body (MB). The ventral hippocampus is more associated to emotional reponses due to its connections to the amygdala (lateral (LA), basolateral (BLA), posterior basolateral (BLP), ventral basolateral (BLV), basomedial (BMA), medial nucleus (ME), posterolateral cortical nucleus (PMCo), posteromedial cortical nucleus (PLCo)), to the hypothalamus (medial preoptic nucleus (MPN) and hypothalamic periventricular zone (PVZ)) and to the core of the NAc. IC: internal capsule, AHN: anterior hypothalamic nucleus; VHM: ventromedial hypothalamic nucleus; MFB: medial forebrain bundle

Due to its characteristics, the hippocampus has shown to have an important role in fear conditioning and memory. For instance, the direct connections between the CA1 region of the ventral hippocampus and the baso-amygdala (BA) BA demonstrated to be required to the contextual fear memory (Xu *et al.*,2016). Furthermore, lesions in the dorsal hippocampus one day after fear conditioning led to a deficit in the contextual acquisition of fear memories, but spared the cued fear response (Phillips *et al.*, 1992; Kim *et al.*, 1992). However if the lesions were performed 7-28 days after the fear conditioning, the contextual fear memories were retained (Kim *et al.*, 1992). Moreover, using optogenetics it was possible to study the real-time contribution of the hippocampal CA1 excitatory neurons in remote fear memory. Using this tool it was found that contextual fear recall, even 9 weeks after contextual fear conditioning, can be reversibly abolished by temporally precise optogenetic inhibition of CA1 neurons, thus suggesting that the hippocampus has a permanent role in the storage of contextual fear memories (Goshen *et al.*, 2011). Overall, these results indicate that when the hippocampus is not available there are

other brain structures that compensate for its loss; however, when the hippocampus is intact, it takes part in the formation of that contextual memory engram and is required for its recall.

1.2.1.3 Medial prefrontal cortex (mPFC) in the fear circuitry

The prefrontal cortex (PFC) of the rat is divided into medial, orbital and lateral parts (Ongur and Price 2000). The medial PFC (mPFC) consists of four main subdivisions: the medial agranular (AGm), the anterior cingulate (AC), the prelimbic (PL) and the infralimbic (IL) cortices (Berendse and Groenewegen 1991). For a long time, this structure has been associated with diverse brain functions including attentional processes, visceromotor activity, decision making, goal directed behavior and working memory (Neafsey 1990; Goldman-Rakic 1994; Petrides1998; Repovs and Baddeley 2006). However, the contribution of the mPFC for aversive memories is a more recent subject.

One of the first studies to examine this role showed that damage to this region did not affect defensive behavior (Divac *et al.,* 1984). In contrast, other studies revealed that lesioning the mPFC increases the reactivity to aversive stimuli (Holson, 1986); however it was also shown that lesions in this brain region impaired fear acquisition (Bissière *et al.,* 2008). These contradictory results may be due to the specific site of the lesion. In fact, selective dorsal PL lesions increased fear acquisition (Morgan *et al.,* 1995) while lesions in the ventral PL decreased the activation of the sympathetic-mediated fear response (Frysztak *et al.,* 1991, 1994).

Despite the contradictory data, which may be related with the different types of fear conditioning and the site of the lesions, the current view on the role of the mPFC in fear memory is that during the acquisition and consolidation of fear there is a heightened response of the excitatory circuit between the PL and the BLA (Giustino *et al.,* 2015).

1.2.1.4 The macrocircuit underlying fear memory formation and fear extinction

It is well established that the acquisition of fear memories requires synaptic plasticity within the amygdala, the hippocampus and the mPFC.

It is described that during the acquisition of cued-conditioned fear, somatosensorial thalamic and cortical inputs conveying information about the CS and the US converge onto the same neurons in the LA. Then, LA will send excitatory projections to the "fear" neurons in the BA and these will excite the "on" GABAergic neurons in the CeL. This leads to the inhibition of the CeL "off" neurons and therefore to the disinhibition of the CeM. Alternatively, the BA neurons may directly excite the CeM. Both situations result in the activation of downstream effector structures, e.g. the brainstem and the hypothalamus, that

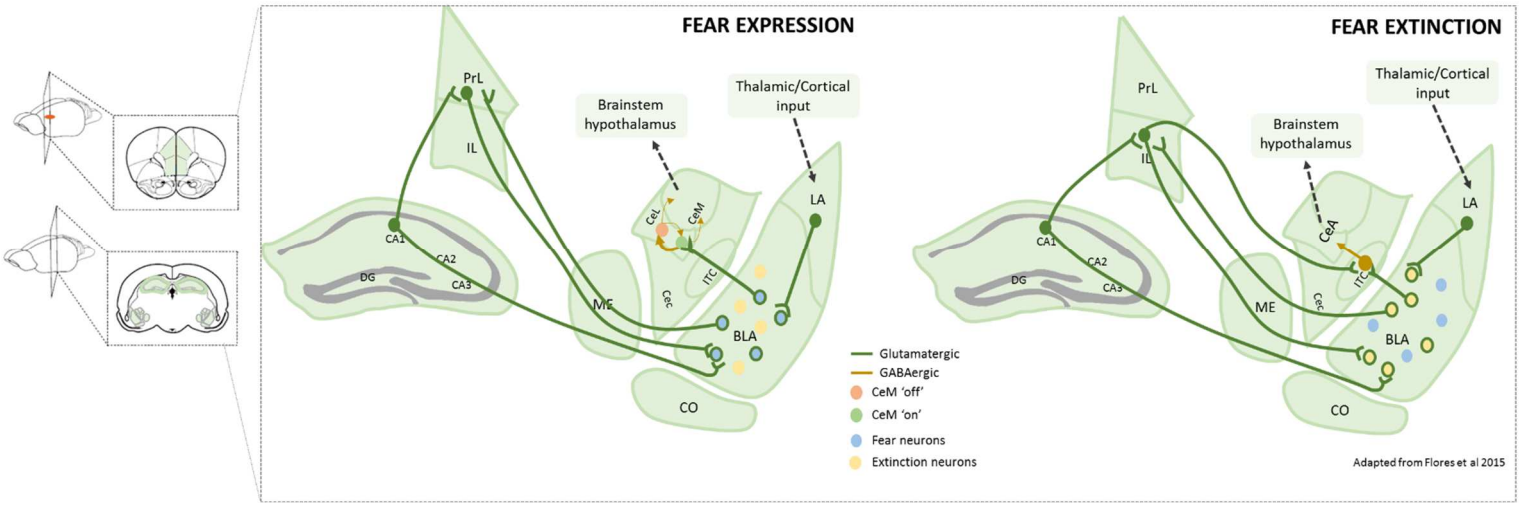


Figure 6 - **Circuitry of fear acquistion and extinction.** The fear acquisition and extinction involve the neuronal circuitry between the hippocampus, the amygdala and the medial prefrontal cortex (mPFC). The critical components in amygdala to fear conditioning are the LA, the BLA, the ITC and the CeA. A) information about the CS-US from the thatlamic/cortical input arrives in the LA and will activate the fear expression through the activation of the 'fear neurons' in the BLA a subsequent disinhibition of the CeA. B) The mechanism of fear extinction is similar to the fear expression, however the LA neurons will activate the 'extinction' neurons in the BLA that will activate the ITC. Adapted from Flores *et al.,* 2015.

will coordinate the fear response. On the other hand, during fear acquisition, the excitatory and reciprocal projections between the BA and the PL region of the mPFC are potentiated. Similarly, the CA1/subculicum region of the hippocampus also exhibits robust excitatory projections to both the PL and the BA, which may be important for the integration of contextual and spatial information during fear conditioning (Orsini *et al.,* 2012; Duvarci *et al.,* 2014; Flores *et al.,* 2015; Giustino *et al.,* 2015), Figure 6.

During extinction the exposition to the context without the aversive stimulus will activate the 'extinction' neurons in the BA. These neurons will activate the ITC that will inhibit the CeA (Paré and Smith, 1993), leading to a decrease of the fear behavior. At the same time, these 'extinction' neurons will activate the IL area of the mPFC that will send projections reciprocally to the BA and, additionally will excite the ITC, contributing to the inhibition of the CeA. Furthermore the hippocampus, will also have reciprocal projections with both amygdala and the IL to control the context-specific expression of fear extinction (Figure 6).

1.2.2 Mechanisms of fear learning and memory

The functioning of this neurocircuitry relies on synaptic plasticity phenomena that when pathologically altered leads to an impaired or aberrant fear expression (Goosens *et al.,* 2003; Chapman *et al.,* 2003).

1.2.2.1 The plasticity and molecular processes of fear learning in the amygdala

The mechanism of synaptic plasticity that is thought to underlie the formation of memories is the long term potentiation (LTP) (Purves *et al.,* 2004). Accordingly, it was shown that during fear acquisition and expression, the excitatory circuit in the amygdala is heightened and occurs a LTP of the synapses from the afferent neurons carrying information about the CS and the US that terminate in principal neurons of the BLA (Orsini *et al.,* 2010; Maren *et al.,* 2014).

In the amygdala, LTP at excitatory synapses was shown to depend on both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and on N-methyl-D-aspartate (NMDA) receptors. These glutamate receptors are required for the depolarization of the postsynaptic membrane and for implementing the synaptic adaptations required for LTP (Purves *et al.,* 2004). During fear acquisition the blockade of these receptors in the BLA prevented LTP and impaired both the acquisition and expression of the fear behavior (Maren *et al.,* 2005; Goosens *et al.,* 2003; Walker *et al.,* 2002). However, Chapman *et al. 2003,* showed that the blockade of NMDA receptors do not completely abolish this LTP, possibly due to the existence of L-Type Voltage-Gated Calcium Channels (VGCCs) that counterbalance the loss of NMDA receptor signaling. These results suggest that there are distinct forms of LTP (NMDA-dependent and NMDA-independent) in the BLA *in vitro* and that a combination of both contributes to the formation of fear memories *in vivo*. Furthermore, Rumpel *et al.,* 2007, showed that fear conditioning drives AMPA receptors to the synapses of the LA neurons, and the blockad of this trafficking leads to impairments in the fear memory formation.

Among the molecules important for amygdala-dependent memory and LTP is the brain-derived neurotrophic factor (BDNF). Thus, BDNF-mediated signaling is essential for the normal acquisition and consolidation of associative fear memory and has been implicated in the pathophysiology of PTSD (Mahan and Ressler, 2012). In rodents, BDNF gene expression and activation (phosphorylation) of its receptor TrkB increase in the amygdala after the pairing of CS-US and intra-amygdala injections of an antagonist of TrkB disrupts fear acquisition (Rattiner *et al.,* 2004).

A downstream molecule to the BDNF pathway, and one that is activated in most synaptic plasticity-related signaling cascades, is the ubiquitous transcription factor 'cAMP response element-binding protein' or CREB. In unstimulated cells, CREB is not phosphorylated and has little or no transcriptional activity. However, phosphorylation (activation) of CREB is responsible for the regulation of protein synthesis, required for LTP and long-term memory, and can be achieved via upstream signaling cascades such as MAPK pathway, or through calcium that will interact with the nuclear

calcium/camodulin-dependant Kinase IV (CAMKIV) (Orsini *et al.,* 2012). The impact of the activation of CREB in the functioning of the amygdala has been shown by Josselyn and colleagues: using herpes simplex virus type 1 vector-mediated gene transfer, these authors overexpressed CREB in the LA of mice and observed an enhancement of the long term fear memory (Josselyn *et al.,* 2001). On the other hand, the blockade of protein synthesis or of CREB activation in the BLA disrupted the memory in a conditioned taste aversion paradigm (Josselyn *et al.,* 2004).

CREB is responsible for the transcription of immediate early genes (IEG) during fear conditioning such as c-Fos (Miyashita *et al.,* 2008). In resting cells, c-Fos is at a very low density but upon neuronal activity there is an increase of its synthesis (Purves *et al.,* 2004). For this reason, c-Fos expression has been widely used to reveal memory engrams and traces (Henry *et al.,* 2014; Knapska *et al.,* 2009; Orsini *et al.,* 2011; Josselyn *et al.,* 2015). The Fos protein has been reported to be increased in the rat brain in response to stressful events (Melia *et al.,* 1994) and, during the acquisition of an auditory fear conditioning there is an increase in the Fos induction in the BLA and in the CeA (Harris *et al.,*2004; Jimenez *et al.,* 2009), but 5h after the conditioning the c-Fos levels returned to preconditioning baseline levels in the BLA (Harris *et al.,*2004). Furthermore, it was demonstrated that the blockade of the synthesis of c-Fos in the amygdala leads to an impairment in the acquisition of fear memories (Radulovic *et al.,* 1998; Yasunobu *et al.,* 2006).

Most studies have focused on the excitatory circuit of the amygdala as the most relevant to the formation of fear memories. In fact, it was shown that the inhibitory system was downregulated in the amygdala during the acquisition and expression of fear, i.e. lower mRNA (Heldt *et al.,* 2007) and extracellular levels of GABA (Stork *et al.,* 2002), and on the other hand, facilitating GABAergic transmission with benzodiazepines impaired the acquisition of fear memory and had an anxiolytic-effect (Dickinson-Anson *et al.,* 1993; Harris and Westbrook, 1995; Pesold and Treit, 1995; Pain *et al.,* 2002). However, recent studies started to shed-light into the relevance of the inhibitory circuits in the amygdala to the normal functioning of the fear circuitry (Ehrlich *et al.,*2009 and on Lee *et al.,* 2013). These studies demonstrate that the inhibitory drive is more than a simple brake of the excitatory transmission and instead, represents a regulatory control over microcircuits that is pivotal for the normal consolidation, expression and extinction of conditioned fear (Zhang *et al.,* 2008; Makkar *et al.,* 2010; Lu *et al.,* 2014). Overall, it is the balance between excitation and inhibition that determines the output from the amygdala (Royer *et al.,* 1999; Ehrlich *et al.,* 2009; Cicchio et al 2010).

1.2.2.2 Plasticity mechanism of fear learning in the hippocampus

Long-term potentiation has long been considered the primary mechanism for hippocampal-dependent learning and memory (Anderson *et al.,* 1967; Bliss *et al.,* 1973; Bliss *et al.,* 1993). As in amygdala, an important molecule for the hippocampus-dependent contextual memory and LTP is the BDNF. Indeed, the BDNF levels were showed to be increased in context fear conditioning but, not in tone fear conditioning (Liu *et al.,* 2004; Takei *et al.,* 2011), emphasizing specific role of the hippocampus in the contextual aspect of fear.

On the other hand, Kim and collegues (1991) showed that intracerebroventricular (ICV) infusions of APV to block the NMDA receptor prevented the acquisition of fear, however, if the animal had already acquired the fear memory this blockade was no longer able to inhibit the expression of fear (Kim *et al.,* 1991).

Additionally, during LTP there is the trafficking of AMPA receptors to the plasma membrane. In order for this exocytosis to occur it is necessary the activation of CREB, to activate the protein synthesis needed to the synthesis and transport of AMPA receptors (Purves *et al.,* 2004). During fear conditioning, there is a significant increase of the levels of pCREB in the hippocampus (Hwang *et al.,* 2010), which suggest that there is a higher recruitment of AMPA receptors to the membrane to strengthen the synaptic transmission. Indeed, it was reported that fear conditioning enhances spontaneous AMPA receptor-mediated synaptic transmission in the CA1 area of the hippocampus (Zhou *et al.,*2009) and that trafficking of the GluR1 subunit of the AMPAR in the hippocampus is required for encoding contextual fear memories (Mitsushima *et al.,* 2011) .

Furthermore, BDNF and TrkB mRNA and protein levels increase in the hippocampus during the consolidation period of contextual fear memory (Takei *et al.,* 2011). In humans, a single nucleotide polymorphism in the BDNF gene (Val66Met) is associated with the incidence of various psychiatric disorders, including depression, schizophrenia and PTSD (Zhang *et al.,*2006; Gonul *et al.,*2011). In particular, this mutation is thought to alter the stability and secretion of BDNF and therefore interferes with memory formation and retrieval (Mahan and Ressler, 2012).

1.2.2.3 The dualities of the medial prefrontal cortex in fear learning

Due to the contradictory outcomes obtained with lesion studies in the mPFC, to clarify these results, eletrophysiological recordings were performed. It was observed that with the presentation of the CS there is a boost of the neural activity in the PL (Burgos-Robles *et al.,* 2009), suggesting a role for this

brain area in the acquisition of fear memories. More recently, it was shown a correlation between the spontaneous firing rate of some neurons in the PL and in the IL with a high and a low-fear state, respectively. Interestingly, the neuronal firing rate returned to basal levels, despite the ongoing freezing behavior (Fitzgerald *et al.,* 2015). Furthermore, it was demonstrated that the inactivation of the prefrontal activity, either through the local application of a GABAA receptor agonist or of an NMDA receptor antagonist, impaired the acquisition but not the expression of fear (Glimartin *et al.,* 2010). Accordingly, using optogenetic methods to explore the plasticity in prefrontal cortex projections, it was shown that during fear learning there is strengthening of the PL excitatory synapses in the BLA which can be attributed to a postsynaptic increase in AMPA-mediated currents (Arruda *et al.,* 2014).

Furthermore, using detection of immediate early genes (IEGs) such as c-Fos, it was shown that gene expression in the mPFC may be context-dependent due to its connections with the hippocampus (Knaspka *et al.,*2009). In line with this, PL and IL exhibited opposite patterns of Fos expression during the renewal and the extinction training in the same context. For instance, PL showed a robust increase of c-Fos during fear renewal while the IL had an increased c-Fos expression during the extinction in the same context (Knaspka *et al.,*2009). These findings suggest that the mPFC may be essential for the integration of contextual cues to process the meaning of the CS. However, it has been also shown that PL and IL exhibit the same levels of c-Fos after the conditioning (Herry *et al.,*2004), suggesting that the PL an IL can fluctuate similarly during the acquisition, extinction and expression of conditional fear.

1.2.3 Mechanisms of fear extinction

1.2.3.1 Amygdala: what is the mechanism to extinguish fear memories?

In the BLA, distinct groups of neurons fire during fear conditioning and fear extinction, thus, these are termed "fear" and "extinction" neurons, respectively (Henry *et al.,* 2008; Duvarci and Pare, 2014).. During fear extinction and expression, the "extinction" neurons in the BA are thought to activate the ITC causing feedforward inhibition of the CeA nuclei which result in a decrease of the fear response (Orsini *et al.,* 2012; Duvarci *et al.,* 2014). However, unlike the formation of fear memories where it is well established that LTP is the mechanism underlying the acquisition and expression of associative fear, the exact synaptic mechanism associated with extinction learning and expression is not as well understood. There are two major hypothesis that are not mutually exclusive: one defends that during extinction learning and expression there is a weakening or depotentiation of the excitatory transmission within the BLA and the other presumes that this weakening of the glutamatergic network is due to a potentiation

of the inhibition onto the excitatory circuit that is promoting the fear response, (McConnell *et al.,* 2015; Maren, 2015).

The first possibility involves the depotentiation or long-term depression (LTD) of the synaptic transmission in the cortico/thalamus excitatory synapses in the LA that was promoting the CS-US association. In support of this hypothesis, Kim *et al.*(2007) conducted *ex vivo* electrophysiological studies in the LA of rats and observed that in animals that underwent fear extinction there was an occlusion of the LTD. In addition, the authors found that the excitability of LA glutamatergic neurons was decreased relative to fear conditioned rats and was equal to the naïve and to the unpaired CS-US groups (Kim *et al.,* 2007).

In support of the second hypothesis, Trouche *et al.* (2013) found that excitatory neurons in the BA that were activated during contextual fear conditioning were less likely to be activated after fear extinction and presented increased levels of perisomatic GAD67 and of parvalbumin, suggestive of enhanced GABAergic transmission onto these neurons. However, there are also other possible mechanisms such as the potentiation/disinhibition of the glutamatergic "extinction" neurons that will lead to the inhibition of the amygdala output nuclei in CeA (reviewed in Duvarci and Pare, 2014) or that extinction learning promotes LTP at the excitatory synapses between the afferent neurons carrying the information about the CS and the cells mediating feedforward inhibition in the BA (Maran, 2015). This last hypothesis involves the activation of the inhibitory ITC in the amygdala. In fact, ITC limit the excitatory transmission between the BLA and the CeA, thereby suppressing the fear responses (Lin *et al.,* 2003 a,b) and the selective elimination of the ITC cells correlates positively with decreased fear extinction (Likhtik *et al.,* 2008). On the other hand, mechanisms of metaplasticity observed in the amygdala might account for the return of the fear memory after extinction (discussed in Maren, 2015).

1.2.3.2 Hippocampus in the extinction of contextual fear memories

As in the acquisition of associative fear memories, the context is also important to the extinction of such memories (Maren *et al.,* 2013). In this process, the hippocampus was shown to control the context-specific learning and expression of extinction (Ji *et al.,* 2007). Thus, inactivation of the rat dorsal hippocampus before extinction training impaired extinction learning and memory and inactivation of the hippocampus after extinction learning disrupted the retrieval of that memory (Corcoran *et al.,* 2005). Moreover, Corcoran *et al.* (2005) also showed that these rats had fear responses to the CS regardless of the context in which it was presented, which indicates that the context-dependent expression of extinction was also disrupted. Additionally, it has been shown that hippocampal place cells (principal

neurons of the hippocampus) remap in response to fear learning (Moita *et al.,* 2004), and this novel representation stabilizes with time (Wang *et al.,* 2012). Likewise, there are hippocampal cells that remap primarily during extinction to form new representations of the context, suggesting that extinction represents new learning. However, at the same time, there are other cells remapping both during conditioning and extinction, demonstrating that extinction may also modify pre-existing memories (Wang *et al.,* 2015).

Confirming these results, analysis of the expression of c-Fos during fear conditioning and extinction showed that different populations of hippocampal principal neurons are activated (Tronson *et al.,* 2009). However, many cells are involved in forming contextual representations during both learning processes (Wang *et al.,* 2015). Future studies are needed to understand if these different subpopulations of cells that appear to fire preferentially during acquisition of fear or during extinction have different connections with the neuronal areas regulating emotion.

At a molecular level, besides the increase in the expression of c-Fos in the hippocampus, an increase in CREB was also observed during fear extinction. Additionally, inhibition of protein synthesis blocks reconsolidation and long-term extinction memory (Mamiya *et al.,* 2009). Furthermore, elimination of BDNF impairs fear extinction by decreasing synaptic plasticity dependent on NMDA receptors (Heldt *et al.,* 2007; Peter *et al.,* 2010). All these data suggests that like fear conditioning, fear extinction requires long-term synaptic plasticity in the hippocampus.

1.2.3.3 Medial prefrontal cortex: is the IL or PL essential to the extinction of fear memories?

One of the earliest observations regarding the neural mechanisms of extinction was when lesions in the ventral medial prefrontal cortex (vmPFC) led to an impaired extinction of conditioned fear (Morgan and LeDoux , 1995). In agreement with this, it was shown that the IL region of the vmPFC can modulate fear expression through descending projections to the amygdala, including projections to the BLA, CeA and ITC (Quirk *et al.,* 2008). Moreover, *In vivo* intracellular recordings showed that the mPFC also regulates the expression of fear by inhibiting the principal cells in LA (Rosenkrans *et al.,* 2003). This regulation is controlled by the mPFC IL neurons through the activation of the ITC (Brinley-Reed *et al.,* 1995; Smith *et al.,* 2000; Quirk *et al.,* 2003). Consistent with this, chemical stimulation of the IL cortex with picrotoxin (a non-competitive GABAA receptor antagonist) results in an increase of c-Fos in the ITC (Berreta *et al.,* 2005). Confirming the hypothesis that increased connectivity between the PFC and the amygdala mediates fear extinction, it was observed an increase of CREB-mediated gene expression

in both the amygdala and the PFC after fear extinction and the blockade of protein synthesis of both these regions impaired the formation of an extinction memory (Mamiya *et al.,* 2009). Additionally, in rats that underwent auditory fear conditioning, infusions of BDNF in the IL decreased the fear response, even without the extinction training (Peter *et al.,* 2010). All these results confirmed the relevance of the mPFC, particularly the IL region, to both the acquisition and consolidation of extinction memories.

As mentioned above, it is important to notice that the belief that the IL and the PL regions of the mPFC exert opposite regulation of fear memories, i.e. the PL promotes fear whereas the IL promotes extinction, and are activated only during fear conditioning or during fear extinction, respectively, is being questioned. Indeed, it was already showned that the PL was also recruited during the suppression of fear and IL during the acquisition of fear (Morgan *et al.,*1995, Herry *et al.,*2004). Nowadays, it is argued that different cell populations along the dorso-ventral axis of the mPFC have overlapping functions with the PL and the IL parts (Giustino *et al.,*2015).

1.3 ROLE OF CONTEXT IN FEAR LEARNING AND EXTINCTION

Memory is not a passive process in which we indiscriminately retain information from our environment. Instead, variables such as the context and prior experiences are important filters to the storage of information and the accuracy with which that retention occurs (Levin *et al.,* 2001). Context, defined as the sensorial recollection and the perception of time serves to define a certain situation thereby framing the memory of an experience (Maren *et al.,* 2013). Context can be the internal (cognitive and hormonal) or the external (environmental and social) backdrop on which psychological processes operate (Spear *et al.,* 1973).

Furthermore, it is described that deficits in contextual processing often lead to inappropriate behavioral responses and may result in paranoia, intrusive thoughts and/or compulsive behaviors. One of the stress-related disorders more representative of a dysfunction in the processing of context is the PTSD (Mahan *et al.,* 2012; Maren *et al.,* 2013).

To understand the mechanisms that lead to the association of a given context to an aversive stimuli, neuroscientists either study the memory of the context during classical cued-fear conditioning paradigms or use pure contextual fear conditioning paradigms in which instead of a specific cue (e.g., a light or a sound), the animals learn to associate an aversive stimuli (e.g., a footshock) to the place where they experience it (e.g., the conditioning or training chamber), Maren *et al.,* 2013.

In order for context learning to occur, the animals must first form a representation of the context (Maren *et al.,* 2013), i.e. they need to have a period of habituation to the conditioning chamber in the absence of the unconditioned stimulus, otherwise the animals will not show context conditioning (Fanselow *et al.,* 1990). After the habituation, the animals receive a footshock to acquire the fear memory. These two learning processes are referred to as "context encoding" and "context conditioning", respectively (Maren *et al.,* 2013). Moreover, it is also known that the extinction of fear memories is context-dependent (Bouton *et al.,*2004).

The engagement of the 'fear neuronal circuitry' underlying contextual fear conditioning is still less defined than its functioning in cued fear conditioning; however, the role of each structure is essentially the same, i.e. the hippocampus is responsible for the context-dependence of the extinction memory(Strange *et al.,*2014) and the amygdala is the place where the association between the CS (context) to the US occurs, mainly due to its strong reciprocal connections with the hippocampus. Indeed it is described that the dorsal hippocampus has the ability to modulate the context-specific firing neurons of the lateral amygdala (Maren *et al.,* 2007) and the ventral hippocampus has projections to the baso-amygdala that are required for the retrieval of contextual fear memory (Xu *et al.,* 2016). Moreover, IEG studies have shown that the activation of the mPFC may be context dependent, possibly as a result of its connections with the hippocampus (Orisini *et al.,* 2012; Giustino *et al.,*2015).

1.4 MOOD- AND ANXIETY - RELATED DISORDERS: POSTTRAUMATIC STRESS DISORDER

Mood- and anxiety-related disorders are characterized by a variety of neuroendocrine, neurotransmitter, and neuroanatomical disruptions (Martin *et al.,*2009). Nowadays, it is well established that the brain areas and neuronal circuitry that contribute to these disorders overlaps with the circuitry of fear (Tovote *et al.,*2015). Indeed, anxiety-related disorders are marked by excessive fear (and avoidance) in situations without real danger (Shin *et al.,*2010). Therefore, many of the symptoms of mood- and anxiety-related disorders are thought to result from an imbalance of the activity of the fear circuitry, especially in the emotional centers, as the amygdala and the ventral hippocampus (Ressler *et al.,*2007; Martin *et al.,*2009; Shin *et al.,*2010). Amongst all the anxiety disorders, posttraumatic stress disorder (PTSD) is the one in which fear becomes more overgeneralized due to a traumatic experience. This disorder is the example of how excessive fear can impair quality of life. Individuals with PTSD have three types of symptoms: re-experiencing, hyperarousal and avoidance. Re-experience of the traumatic events involve nightmares, intrusive recollections, flashbacks, physiological arousal and distress in

response to reminders of the incident. The hyperarousal symptoms include hypervigilance, exaggerated startle and difficulty in sleeping or concentrating. Finally, the avoidance of trauma-associated stimuli can lead to the loss of interest in previously enjoyable activities and deficits in learning and memory (Shin *et al.,* 2010; Mahan *et al.,* 2011).

This pathology is highly heterogeneous due to the variability of trauma types, symptom severity and other existing comorbid disorders (Thomaes *et al.,* 2013). The typical traumatic events that lead to PTSD include abuse during childhood, war and rape (Kessler *et al.,* 1995).

The main therapeutic approaches that are available to PTSD patients are psychological support, anti-depressive medications (Novakovic *et al.,* 2011) and "exposure therapy" (Dias et al. 2013). However, these classical treatments fail in the most severe cases which sometimes culminate in suicide. Thus, the understanding of the neurobiological mechanisms behind the development of PTSD is crucial to develop effective treatments.

Since, the brain circuitry involved in fear processing is preserved amongst mammals (Lang *et al.,* 2000; Loonen *et al.,* 2016), the conditioned fear paradigms in rodents is a powerful tool to gain insight into this pathology (Mahan *et al.,* 2012).

Adenosine, mainly acting through $A_{2A}R$ can control conditioned fear responses (Wei *et al.,* 2013; Simões *et al.,* 2016). Additionally, the prophylactic and therapeutic efficacy of adenosine receptors in stress disorders was already demonstrated. For instance, in rodents, the blockade of $A_{2A}R$ prevented and reverted depressive-like behavior (Yamada *et al.,* 2013; Kaster *et al.,* 2015) while the blockade of A_1 receptors (A_1R) was associated with an increase in depressive-like behavior and in anxiety (Serchov *et al.,* 2015; Maximino *et al.,* 2011). Furthermore, in humans it was demonstrated that low doses of caffeine decrease anxiety levels (Stern *et al.,* 1989; Haskell *et al.,* 2005). Due to the role of adenosine receptors, and especially of $A_{2A}R$, in anxiety- and mood-related disorders, it can be postulated that these receptors may constitute a novel therapeutic target to the treatment of PTSD. Thus, understanding the role of $A_{2A}R$ in PTSD and therefore in the functioning of the fear circuitry is essential.

AIM

Considering the modulatory role of the adenosine A_{2A} receptors ($A_{2A}R$) in glutamatergic synapses in both the hippocampus and in the amygdala and the aberrant functioning of these synapses in fear- and

anxiety-related disorders, which compromise fear extinction, this project aimed to evaluate if targeting $A_{2A}R$ could improve the extinction of fear memories.

To accomplish this aim the project was set with two main objectives:

1) Implementation and characterization of a pure contextual fear conditioning and 'retrieval-extinction' paradigm: evaluation of the brain areas involved in the neurocircuitry and the plasticity processes underlying this model;

2) Evaluation of the impact of $A_{2A}R$ in the extinction of contextual fear memories: behavioral and electrophysiological analysis of the effect of the $A_{2A}R$ antagonist (SCH58261) in the brain regions and synaptic mechanisms determined in the first task.

2

METHODS

Animals: All animal experiments were conducted following the European Union guidelines (86/609/EEC) and approved by the Ethical Committee of the Center for Neuroscience and cell biology. Experiments were performed using 8-10 week-old C57/BL6 males obtained from Charles River (Barcelona, Spain). Mice were housed in an environmentally controlled room (23 ± 2 ºC), 12/12h light-dark cycle (with lights on at 7:00 am) and food and water available *ad libitum*. Animals were given 6 days to acclimatize to the new environment after delivery and all efforts were made to minimize animal suffering and reduce the number of animals used.

Behavior: There are several fear conditioning paradigms described in literature, however, there is not a well-established protocol for contextual fear conditioning. In order to accomplish this,we tested two different protocols as described below.

The first protocol of contextual fear conditioning started with 2 groups, the acquisition group (ACQ) and extinction group (EXT) in a 3 minutes (min) habituation to the conditioning chamber. Twenty-four hours later, the mice from the 2 groups were placed in the chamber for the training session. This training was as described in Santos *et al.,* (2013) and consisted in a 2 min habituation followed by five US presentations (footshock: 2 s, 0.5mA), separated by a variable intertrial interval (ITI; 15–60 seconds). To extinguish the conditioned response (CR; freezing behavior), from day 3 to day 5, mice were re-exposed to the conditioning chamber in the absence of the US for several times. Our protocol was based

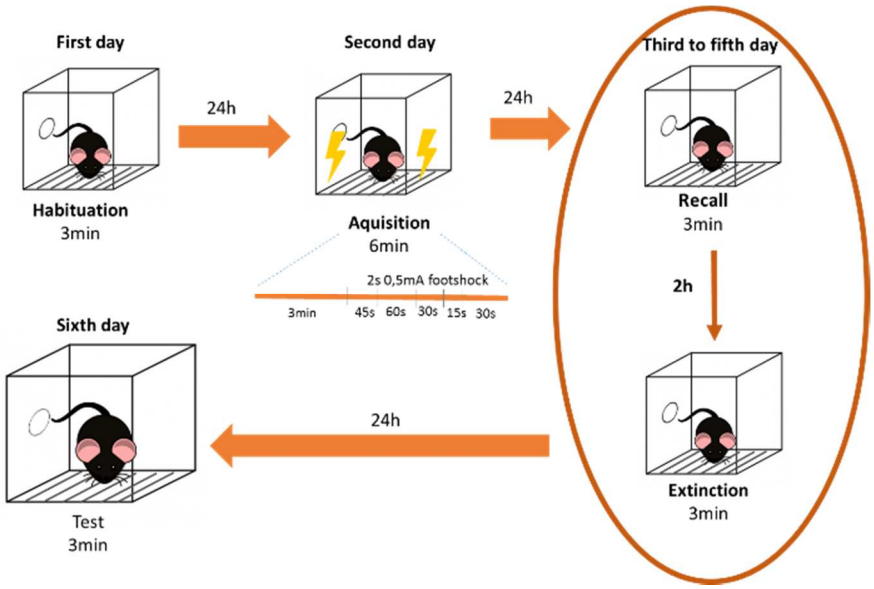


Figure 7 - Schematic representation of the first pure contextual fear conditioning protocol and 'retrieval-extinction' protocol. Based on Santos *et al.*,2013 and on Graff *et al.*,2014

on the 'retrieval-extinction' paradigm described in Gräff *et al.*, 2014. This paradigm consists in the re-exposuring to the same chamber two times *per* day with a 2hours interval. During this period the ACQ group remained in their home cages. In the last day (sixth), all the mice (from the 2 groups) did the extinction (or fear) memory test that consisted in one exposition of 3 min to the training chamber (Santos et al., 2013), Figure 7.

The second protocol was similar to the first except for the training session where instead of the 5 unpredictable US presentation, the mice were expose to 3 US presentations (2s footshocks of 0.8 mA) with a consistent ITI of 28s (Graff *et al*; 2014), Figure 8.

Drug administration: To understand the effect of the $A_{2A}R$ antagonist (SCH 58261) during the extinction of fear memories, a third group of mice were intraperitoneally injected with 0.1mg/kg/day (EXT+SCHip) 1 hour after the recall in the conditioning chamber during the extinction training, from day 3 to 5, Figure 8. A forth group of mice (control, CTR) was also exposed to the conditioning chamber for the same number of times as the ACQ group in order to assess the evolution of the freezing behavior without conditioning.

c-Fos immunohistochemistry: Either 1 hour after the fear conditioning (learning) or 1 hour after the memory test on the sixth day of experiment (retrieval), animals were transcardiacally perfused via the left ventricle with 60mL of ice-cold phosphate-buffered saline 1x (PBS 1x) followed by 90mL of

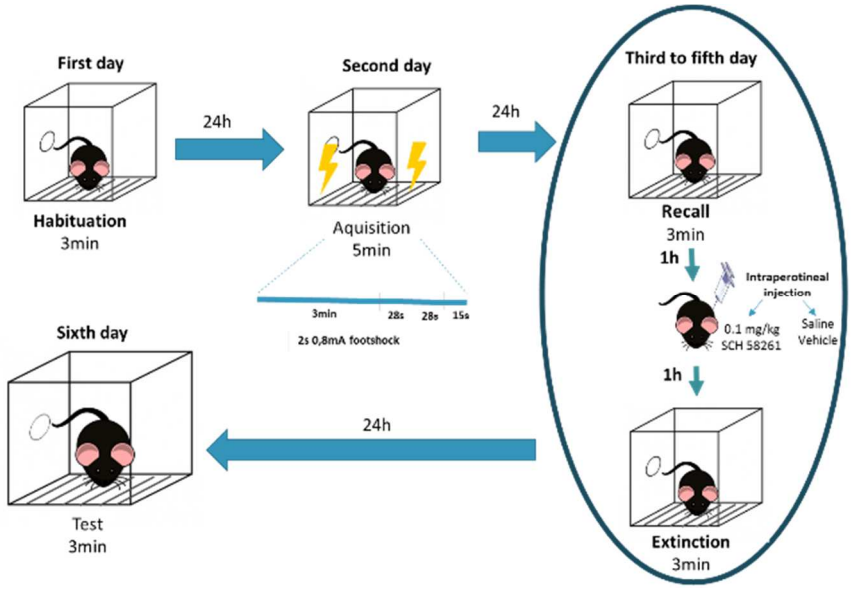


Figure 8 - Schematic representation of the second pure contextual fear conditioning and 'retrieval-extinction' protocol. Based on Graff et al., 2014.

ice-cold 4% paraformaldehyde (in PBS 1x). Brains were then removed and post-fixed in 4% paraformaldehyde during 24h and cryoprotected in 30% sucrose during two days. The brains were then sliced coronally into slices of 50µm thickness in a cryostat (Leica cm3050s) and the brain slices preserved in an anti-freezing solution (composition?) and stored at -20ºC. The slices were then used for c-Fos immunohistochemistry. Regarding the immunohistochemistry, two different protocols were used:

Visible microscopy: the selected brain slices were washed with PBS 1x three times during 5 minutes. Then, to block the endogenous peroxidases, the slices were incubated with 10% methanol+1% H_2O_2 in PBS 1x during 60min at 37ºC without shaking. After washing three more times, the slices were blocked with 10% donkey serum + 0.1% triton in PBS 1x during 2 hours at room temperature, followed by overnight incubation, at 4ºC, with c-Fos primary antibody prepared in the blocking solution. Next, the brain slices were washed 3x with PBS 1x and incubated with the secondary antibody for 2 hours at room temperature in the blocking solution. After washing 3x with PBS 1x, the slices were incubated with the ABC reagent (avidin-biotin complex; Vector Lab, VECTASTAIN® Elite, ABC kit) during 30 min. The sections were subsequently washed with 3x PBS 1x, stained with DAB and then washed again.

Finally, the brain slices were mounted onto gelatin-coated slides and let dry overnight. Slides were then dehydrated in H_2O during 30 seconds and successively submersed in ethanol 70%, 95% and 100%, for 3 minutes each. To eliminate the alcohol, the slides were immersed in xylene for 1min, covered with glass coverslips using DAKO mounting medium and let dry overnight. Slides were viewed and photographed using the microscope axio imager Z2, the polichromatic camera HR3_2, the objective 5x and 20x

(ZEISS) (protocol adapted from Schafe *et al.,* 2000; Davis *et al.,* 2004; Tronson *et al.,* 2009; Peng et al., 2010; Hoffman et al., 2014).

Fluorescent microscopy: free-floating sections were washed two-times in PBS 1x during 10min, followed by a third wash with PBS 1x + 0.3% Triton for 10 minutes. Tissues were then incubated at room temperature with blocking solution (10% donkey serum + 0.3% of Triton + PBS 1x) for 1 hour and immediately transferred into the c-Fos primary antibody solution, previously prepared in blocking solution, for 48h at 4ºC. Afterwards the sections were washed three times with the blocking solution and incubated with the secondary antibody during 2 hours at room temperature. After washing three times with PBS 1x, brain slices were incubated with DAPI (1:5000) during 5 minutes at room temperature and then washed again. The sections were finally mounted onto gelatin-coated slides, coverslipped using DAKO mounting medium and let dry overnight. Slides were viewed and photographed using the microscope Axio Imager Z2, the monochromatic HR3 camera and the objectives 5x and 20x (ZEISS) (protocol adapted from Orsini et al., 2011).

Table 1. Antibodies description

	Antibody	Origin	Provider	Dilution
Primary	c-Fos	Rabbit	Santa Cruz 5C-52	1:500
Secondary	Biotinylated donkey anti-rabbit IgG	Donkey	ThermoScientific, 31462	1:500
	Alexa 488	Donkey	Invitrogen A21206	1:1000
	Alexa594	Donkey	Invitrogen A21207	1:1000

Extracellular electrophysiology: Mice were decapitated after cervical dislocation, the brain was removed and immediately transferred into ice-cold artificial cerebrospinal fluid (aCSF; in mM: NaCl, 124; KCl, 3; NaH2PO4, 1.25; glucose, 10; NaHCO3 26; MgSO4, 1; and CaCl2, 2) gassed with 95% O_2 and 5% CO_2.

Amygdala electrophysiology: horizontal brain slices containing the amygdala (400 μm thickness) were prepared using a vibrating tissues sectioning system (Vibratome 1500, Leica, Wetzlar, Germany). The slices were transferred to a resting chamber with gassed aCSF and allowed to recover for 60 min in

a bath kept at 32 °C. Afterwards, individual slices were transferred to a recording chamber (1 mL capacity) and continuously superfused with gassed aCSF kept at 30.5 °C, at a rate of 3 mL/min . Visual control through a magnifier (World Precision Instruments, Hertfordshire, UK) allowed the correct placement of the electrodes. Test stimuli were delivered via a S44 stimulator (Grass Instruments, West Warwick, RI) every 20 s with rectangular pulses of 0.1 ms (0.05 Hz) through a bipolar concentric tungsten electrode placed at the lateral nuclei of the amygdala (LA).. The amplitude of the population spike (PS) response was used to estimate the synaptic efficacy and was recorded through amicropipette filled with 4 M NaCl (2–4 MΩ resistance) placed at the basal nuclei of the amygdala (BA), Figure 9B.All recordings were obtained with an ISO-80 amplifier (World Precision Instruments, Hertfordshire, UK) and digitized using an ADC-42 board (Pico Technologies, Pelham, NY, USA). The average of three consecutive responses were continuously monitored on a PC-type computer using the WinLTP 1.01 software (

To evaluate basal neurotransmission in the circuit input/output curves (I/O) were first acquired in all slices by continuously increasing the stimulus current applied through the stimulus electrode and measuring the amplitude of the evoked response, starting with a current which elicited no response and terminating when that response stabilized. Based on the I/O curves the following protocols were applied at a stimulus intensity that evoked a signal that was 40% of the maximal. Long-term potentiation (LTP) was induced, after a stable baseline of 20 min, by a high frequency stimulation (HFS) protocol consisting of 5 pulses at 100 Hz delivered with an interval of 30 seconds. The LTP amplitude was quantified by comparing PS amplitudes 10 min before the HFS stimulus and 50-60 min after. Depotentiation was

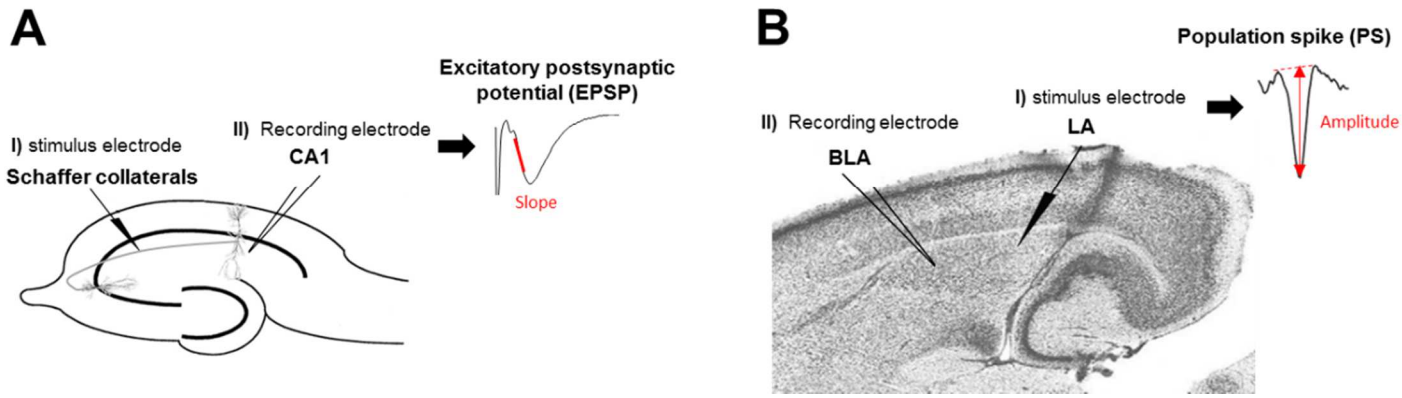


Figure 9 - Extracellular recordings in the hippocampus and in the amygdala. (A) transversal hippocampal slices (400 µm) were prepared and extracellular recordings were performed by placing the stimulus electrode in the schaffer collaterals and the recording electrode in the CA1 region. Field excitatory postsynaptic potentials (fEPSP) were recorded and their slope was continuously measured. (B) For extracellular recordings in the amygdala horizontal brain slices (400 µm) containing the amygdala were prepared and the stimulus electrode was placed in the lateral amygdala (LA) and the recording electrode in the baso-amygdala (BA). Populations spikes (PS) were recorded and their amplitude was continuously measured.

induced by low frequency stimulation (LFS, 900 pulses, 1Hz) 60 min after the HFS protocol. To evaluate the effect of the selective antagonist of A_{2A} receptors, (SCH58261, 50 nM), it was superfused for 20 min before applying the protocols and maintained throughout the experiments. *Hippocampal electrophysiology*: the hippocampus was dissected from the rest of the brain and transversal slices with 400μm thickness were prepared with a McIlwain chopper. After the slicing, the ventral and dorsal hippocampus slices (Figure 10) were transferred to a resting chamber (Massachusetts, USA) with gassed aCSF to recover for 60 min at 32 °C. Then, hippocampal slices were transferred to a recording chamber with the same setup settings previously described. The stimulating electrode was placed on the Schaffer collateral-commissural pathway and the orthodromically-evoked field excitatory postsynaptic potentials (fEPSPs) were recorded at the *stratum radiatum* of the CA1 area, Figure 9A.

Responses were quantified as the initial slope of the averaged fEPSPs. Input-output curves were performed and then it was selected the intensity of the stimulus that evoked a fEPSP of ~ 40% of the maximal slope. LTP was induced using an HFS of a single pulse at 100Hz. Depotentiation was induced

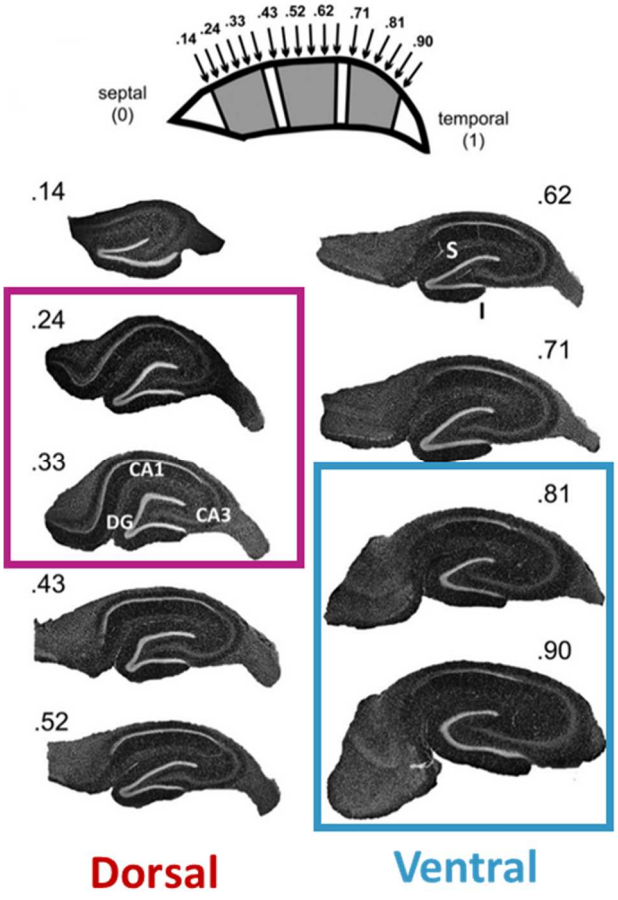


Figure 10 - Hippocampal transversal slices representation. Tranversal sections evenly spaced across the septotemporal axis. The slices .14; .24; .43 represent dorsal hippocampal slices; the .43; .52; .62;.71 are medial slices and the .81 and .90 are the more ventral hippocampal slices. The sections used during the electrophysiological recordings were the more dorsal (red) and the more ventral (blue).

by low frequency stimulation (900pulses, 1Hz) after the HFS protocol. To study the effect of the blockade of A_{2A} receptors, slices were superfused with SCH58261 (50 nM) for 20 min before and throughout the experiments.

Statistical analysis: Results are presented as mean ± SEM from n mice. Behavioral data was analyzed with unpaired Student's *t* test, one-way ANOVA followed by Bonferroni's post hoc test when comparing between more than two conditions and with two-way ANOVA when more than one variable and condition were analyzed (e.g. acquisition *versus* extinction *versus* extinction with SCH58261). Electrophysiological data was compared with either unpaired Student's *t* test or with one-way ANOVA plus Bonferroni's post hoc test except from input-output curves which were analyzed with nonlinear regressions and depotentiation curves that were compared with an hypothetical value of 100%. The significance level was 95%.

3

RESULTS

Optimization of pure contextual fear conditioning and extinction

A major challenge in the treatment of anxiety- and trauma- related disorders is the difficulty in extinguishing fear to certain environments or cues that are reminders of an aversive experience. In the laboratory, the search for novel therapies or molecular targets to effectively extinguish fear takes advantage of fear conditioning and extinction paradigms in rodents (Mahan and Ressler, 2012). Given the impact of adenosine $A_{2A}R$ on fear learning and memory and especially on contextual memories (Wei *et al.*, 2014; Simões *et al.*, 2016), we sought to first test the effect of the pharmacological blockade of $A_{2A}R$ during a protocol of fear extinction, after contextual fear conditioning mice. In order to have a pure contextual fear conditioning protocol, mice must learn to associate an unconditioned stimulus (US; footshock) to a conditioned stimulus that is the context where it receives the shock (CS; which in this case is the fear conditioning chamber). If this association occurs, the next time that the animals are exposed to the conditioning chamber, it will elicit a fear or conditioned response (CR) that positively correlates with the time spent freezing (i.e., total immobilization of the animal except for respiration).Then, to attenuate or extinguish the CR, mice are re-exposed to the conditioning chamber in the absence of the US for several times. Our protocol was based on the 'retrieval-extinction' paradigm described in Gräff *et al.*, 2014 with a few significant alterations: in order to eliminate time as a predictive cue of the shock, we adopted the strategy described in Santos *et al.*, 2013 and applied the footshocks with different inter-trial intervals (ITI) (Figure 11A). Thus, mice were divided into two experimental groups: one that underwent fear conditioning only (the acquisition group, ACQ; blue) and another that underwent both fear conditioning and extinction (the extinction group, EXT; green). The ACQ group was expected to express high freezing behavior in the memory test on the last day of experiment

(Extinction test) while the EXT group was expected to express low freezing behavior in the same test. As expected, all mice had a low freezing during the habituation to the conditioning chamber (Figure 11B: 0 ± 0% freezing, n=10). Moreover, both ACQ and EXT groups expressed high freezing levels 24 h after fear conditioning, i.e. in the acquisition test (Figure 11C-D: 43.545 ± 5.639% freezing, n=10), meaning that all of the animals learned to associate the CS to the US. However, on the last day of experiment, both groups expressed low levels of freezing to the chamber (Figure 11C-D: 12.71 ± 3.127% freezing in the ACQ group and 20.39 ± 3.779% freezing in the EXT group, n=5 *per* group) which indicates that the passage of time was enough to extinguish fear to the chamber. Due to this drawback, we were forced to rethink the protocol. There were two potential problems with the previous protocol: the footshock was too mild and a fixed ITI may be essential for contextual representation in animals. Thus, in the following experiments we used a protocol that was closer to the one used by Gräff and colleagues (2014): instead of 5 unpredictable presentations of the US, with an intensity of 0.5 mA, the mice were expose to 3 presentations of the US, 2s footshock with an intensity of 0.8 mA, at a ITI of 28 s (Figure 12A). With this protocol, in a similar way to the first protocol, mice learned to associate the US to the CS since at the acquisition test all the animals expressed a high freezing behavior (Figure 12B-C: 49.14 ± 4.277% freezing, n=13). However, on the last day of the experiment, at the extinction test, the ACQ group freezed significantly more than the EXT group (Figure 12C-D: 39.29 ± 3.677% freezing in the ACQ group and 9.429 ± 1.856% freezing in the EXT group, n=13, *per* group, $p<0.001$). Moreover, the EXT group decreased their freezing in 39.72 ± 4.663% from the acquisition test to the extinction test (Figure 12C-D). Importantly, we also confirmed that a second exposure to the conditioning chamber did not change freezing behavior *per se*, in a group of mice (control group, CTR; grey) that were habituated to the chamber and returned to it for the test on the last day (Figure 12C: 0 ± 0% freezing during habituation and 0 ± 0% freezing during the memory test on the last day). Thus, this last paradigm of contextual fear conditioning allows a successful learning and extinction of fear memories.

Pharmacological blockade of $A_{2A}R$ accelerates extinction learning.

To understand the role of the $A_{2A}R$ in fear extinction, we added a new group of mice that were intraperitoneally injected with the selective antagonist of $A_{2A}R$, SCH58261 (0,1mg/kg), (EXT+SCHip; pink). The antagonist was administrated 1 h after the recall sessions (R1-R3) of the extinction protocol (i.e. 1 h before the extinction sessions, E1-E3) in a dose that does not alter locomotion in rodents (Kaster *et al.*, 2015; Canas *et al.*, 2009). As shown in Figure 3B, mice injected with SCH58261 had a

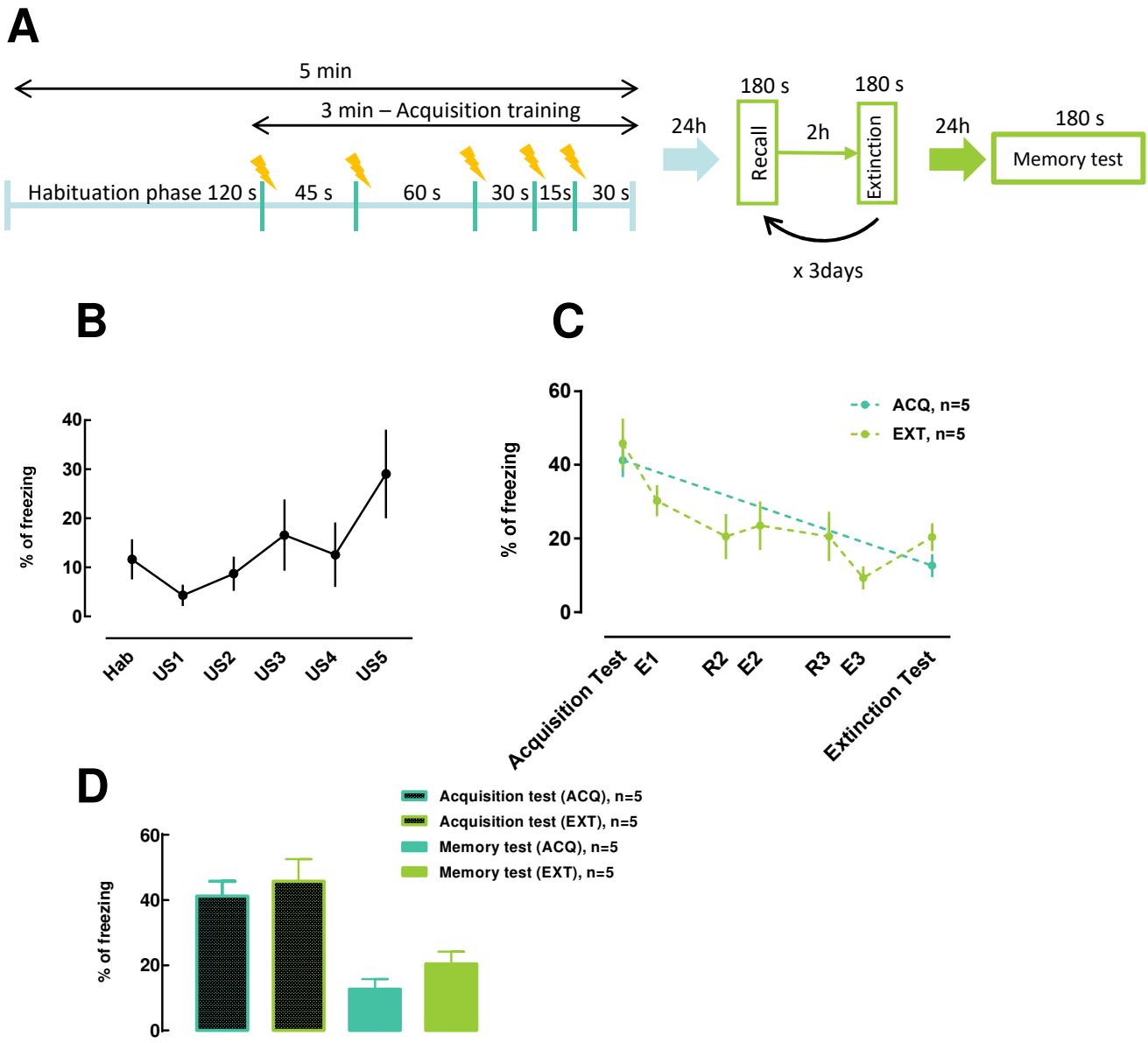


Figure 11 - Pure contextual fear conditioning, first paradigm. **(A)** Schematic representation of the pure contextual fear conditioning paradig, based on the 'between session' extinction paradigm described in Santos et al. 2013. **(B)** Fear conditioning learning curve: 5 trials (US1-5) of pairing the context (the conditioning chamber or conditioned stimulus, CS) with a footshock (0.5 mA, 2 s; unconditioned stimulus, US) during 3 min (the footshocks were separated in time according to the scheme above the graphic; percentage of freezing was quantified for 15 s after each footshock); **(C)** Aquisition test (or recall 1, R1) 24 h after the CS-US pairings: mice were re-exposed to the CS (conditioning chamber or context) for 3 min and their freezing response was quantified throughout the experiment. The animals of the acquisition group (ACQ) returned to their home cages whereas the animals of the extinction group (EXT) were exposed for a second time to the CS, 2 h after the aquisition test (extinction 1, E1) before returning to their home cages. On the following day, 24 h after the acquisition test, mice belonging to the extinction group were again exposed to the CS for a recall session (R2, freezing behavior quantified during the 3 min of test) and again 2 h later (extinction 2, E2). This procedure was repeated for one more day and only for the group of mice undergoing extinction. On the third day, both the ACQ and the EXT groups were exposed to the conditioning chamber for the last time, during 3 min, to evaluate fear memory (extinction test). **(D)** Bar graph comparing the average freezing of all mice during the acquisition test (green, open bar) with the average freezing of the ACQ group (blue, full bar) and of the EXT group (green, full bar) during the extinction test. Data are mean ± SEM of n= 5 mice for each experimental group. *p < 0.05, unpaired Student t-test

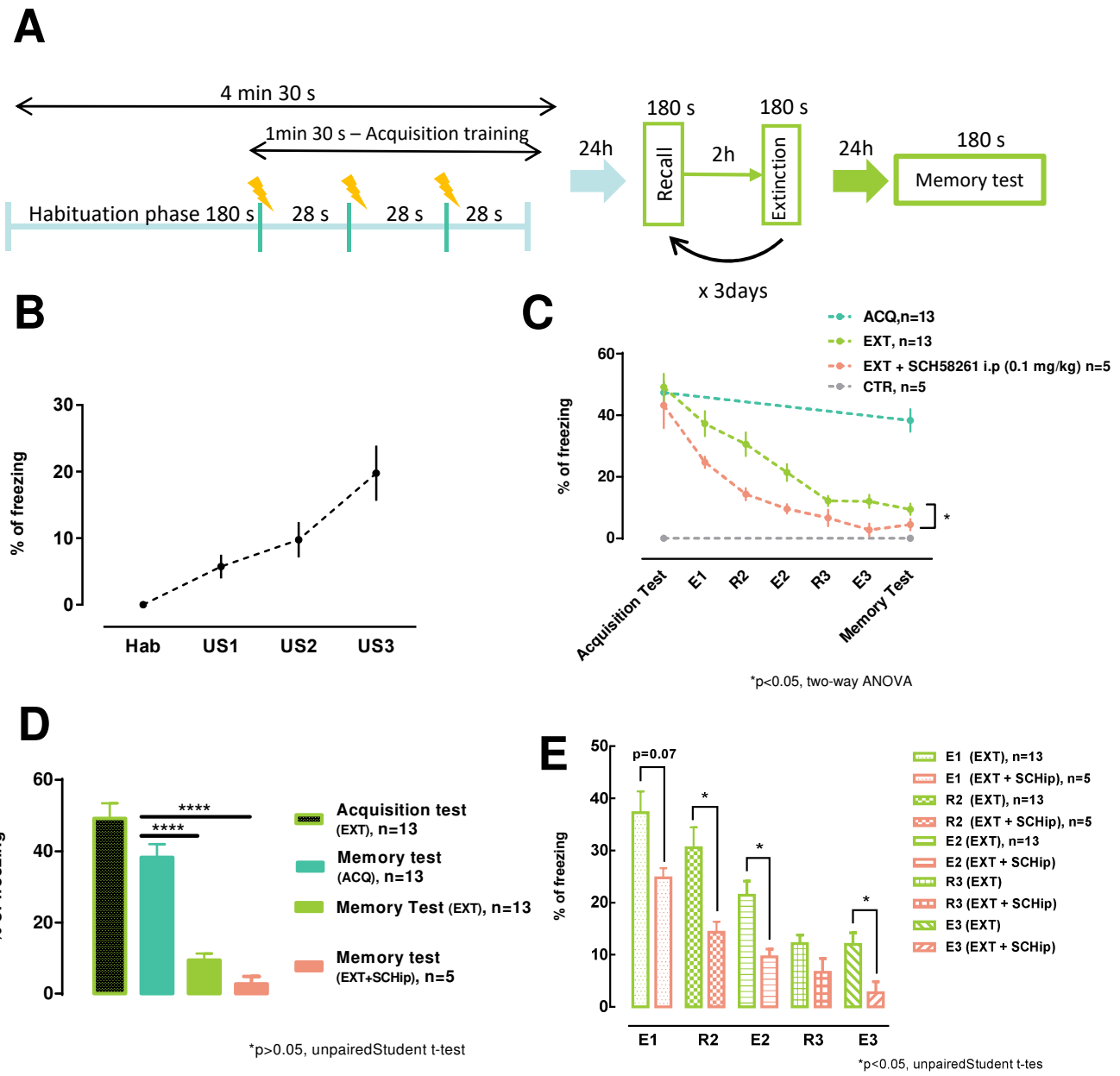


Figure 12 - Contextual fear conditioning, second paradigm. (**A**) Schematic representation of the contextual fear conditioning paradig, based on the 'between session' extinction paradigm described in Santos *et al.*, 2013 and Graff *et al.*, 2014. (**B**) Fear conditioning learning curve: 3 trials (US1-3) of pairing the context (the conditioning chamber or conditioned stimulus, CS) with a footshock (0.8 mA, 2 s; unconditioned stimulus, US) during 1min30s (the footshocks were separated in time according to the scheme above the graphic; percentage of freezing was quantified for 15 s after each footshock); (**C**) Aquisition test (or recall 1, R1) 24 h after the CS-US pairings: mice were re-exposed to the CS (conditioning chamber or context) for 3 min and their freezing response was quantified throughout the experiment. The animals of the acquisition group (ACQ) returned to their home cages whereas the animals of the extinction group (EXT) and extinction group intraperitoneally injected with SCH58261 (0,1mg/kg) (EXT-SCHip) were exposed for a second time to the CS, 2 h after the aquisition test (extinction 1, E1) before returning to their home cages. On the following day, 24 h after the acquisition test, mice belonging to the extinction group were again exposed to the CS for a recall session (R2, freezing behavior quantified during the 3 min of test) and again 2 h later (extinction 2, E2). This procedure was repeated for one more day and only for the group of mice undergoing extinction. On the third day, the ACQ, the EXT and the EXT+SCHip groups were exposed to the conditioning chamber for the last time, during 3 min, to evaluate fear memory (extinction test),*p < 0.05, ANOVA two-way (**D**) Bar graph comparing the average freezing of all mice during the acquisition test (green, open bar) with the average freezing of the ACQ group (blue, full bar), of the EXT group (green, full bar) and the EXT+SCHip group (pink, full bar) during the extinction test. Data are mean ± SEM of n= 5-13 mice for each experimental group, *p < 0.05, unpaired Student t-test. (**E**) Bar graph comparing the average freezing of mice of EXT and EXT+SCHip during the Recal 1, 2 and 3 and the Extinction 1, 2 and 3. Data are mean ± SEM of n=5-13 mice for each experimental group, *p < 0.05, unpaired Student t-test

significantly different extinction curve comparing to the mice injected with vehicle (EXT) (Figure 12C, $p<0.05$ ANOVA two-way). Moreover, when analyzing each of the endpoints of the extinction curve, it was observed that the effect of SCH58261 was very fast since the animals in EXT+SCHip already had a tendency to freeze less then mice in the EXT group at E1 ($p=0.07$, Student's *t* test) and these two experimental groups were different in all of the endpoints thereafter (from R2 to E3) with the exception of the R3 (Figure 12E). Nevertheless, both the animals in the EXT and in the EXT+SCHip groups extinguished fear to the context in the 3 days of the protocol since both groups froze less than 15% of the time in the extinction memory test, on the last day of the experiment (Figure 12C-D: 9.429 ± 1.856% freezing in the EXT group and 2.728 ± 2.142% freezing in the EXT+SCHip group, n=5-13).

c-Fos immunohistochemistry

In order to investigate the brain areas involved in the neurocircuitry of contextual fear conditioning we performed immunohistochemistry analysis of a classical marker for neuronal activation, the transcription factor c-Fos. Thus, 1 h after fear acquisition or exposure to the conditioning chamber, the ACQ and the CTR groups, respectively, were transcardiacally perfused with a fixative solution of paraformaldehyde. Researchers usually use biotinylated antibodies together with DAB staining and visible microscopy to amplify the signal-to-noise ratio of c-Fos immunostaining (Schafe *et al.*, 2000; Davis *et al.*, 2004; Tronson *et al.*, 2009; Peng *et al.*, 2010; Santos *et al.*, 2013; Hoffman *et al.*, 2014). However, in the near future, we intend to perform double labeling of neurons with c-Fos and a glutamatergic/GABAergic neuronal markers. Thus, c-Fos immunofluorescence was also used and the results compared with the staining obtained using visible microscopy,Figure 13. As shown in Figure 13B the enzymatic method to label c-Fos revealed a marked neuronal activation throughout the hippocampus (in all regions of the tripartite excitatory synaptic loop: DG, CA3 and CA1) and surrounding cortical areas in the ACQ group comparing to the CTR group. Moreover, this c-Fos immunostaining was also more intense in the amygdala (mainly in the LA and BLA nuclei) and surrounding cortical areas of the ACQ mice comparing to the CTR mice (Figure 13B). Similarly, the fluorescent-immunostaining of c-Fos revealed the same increase in neuronal activation in the hippocampus and in the amygdala of the ACQ group comparing to the CTR group (Figure 13C). However, comparing the two types of immunostaining, a different pattern of neuronal activation is revealed and especially, the amygdala of the CTR group is clearly more stained in the fluorescent images then in the DAB-stained images (Figure 13B-C). This difference might be due to two main reasons: (1)

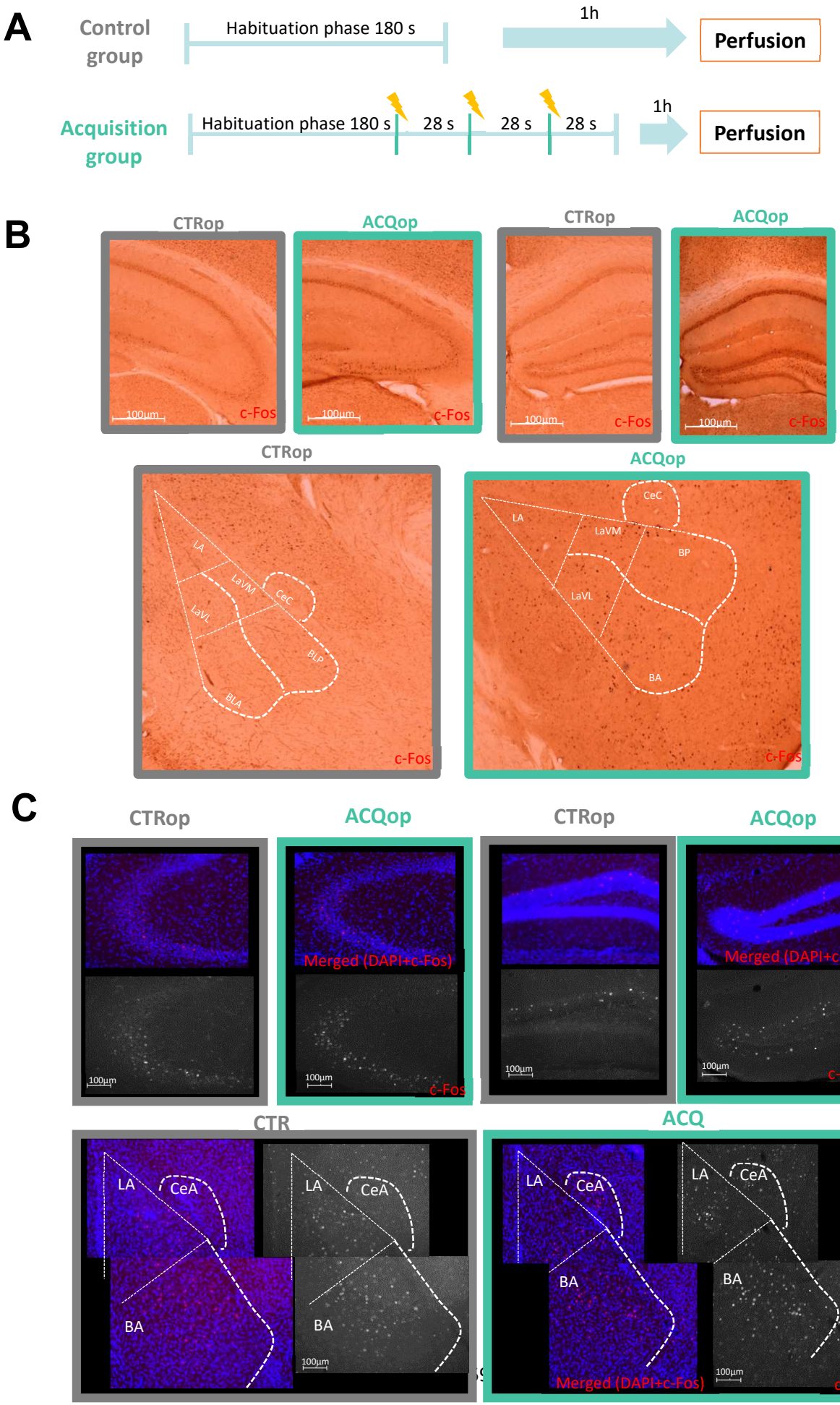
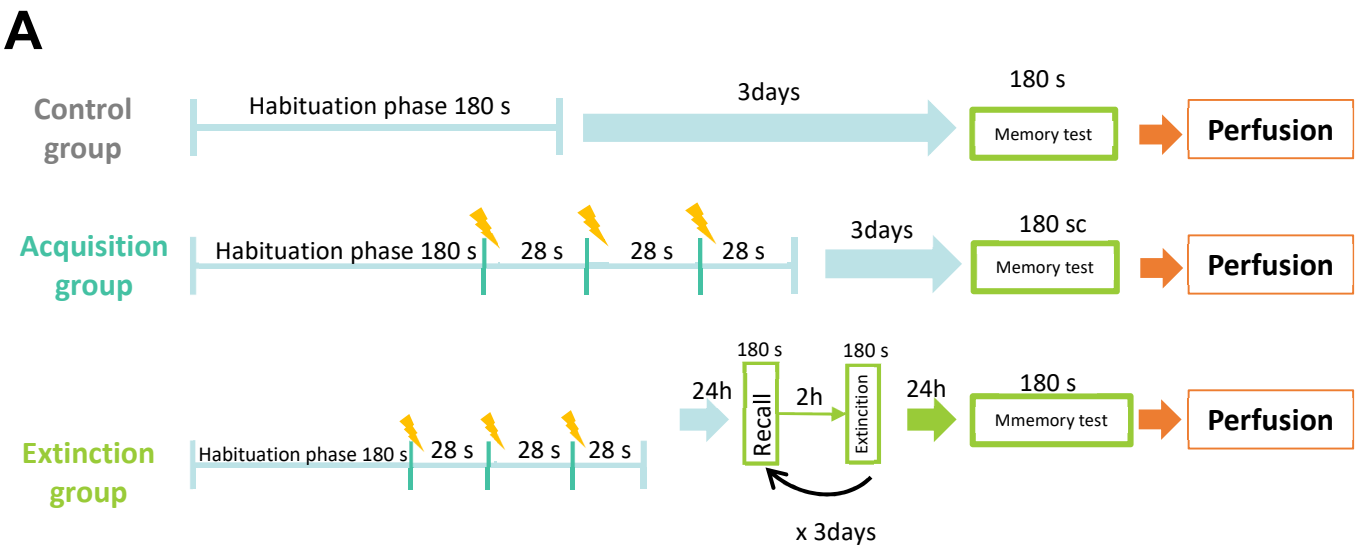

A
Control group
Habituation phase 180 s
1h
Perfusion
Acquisition group
Habituation phase 180 s
28 s
28 s
28 s
1h
Perfusion
B
CTRop
ACQop
CTRop
ACQop
100µm
c-Fos
CTRop
ACQop
LA
LaVM
CeC
LaVL
BLP
BLA
BP
BA
C
CTRop
ACQop
CTRop
ACQop
Merged (DAPI+c-Fos)
CTR
ACQ
LA
CeA
BA

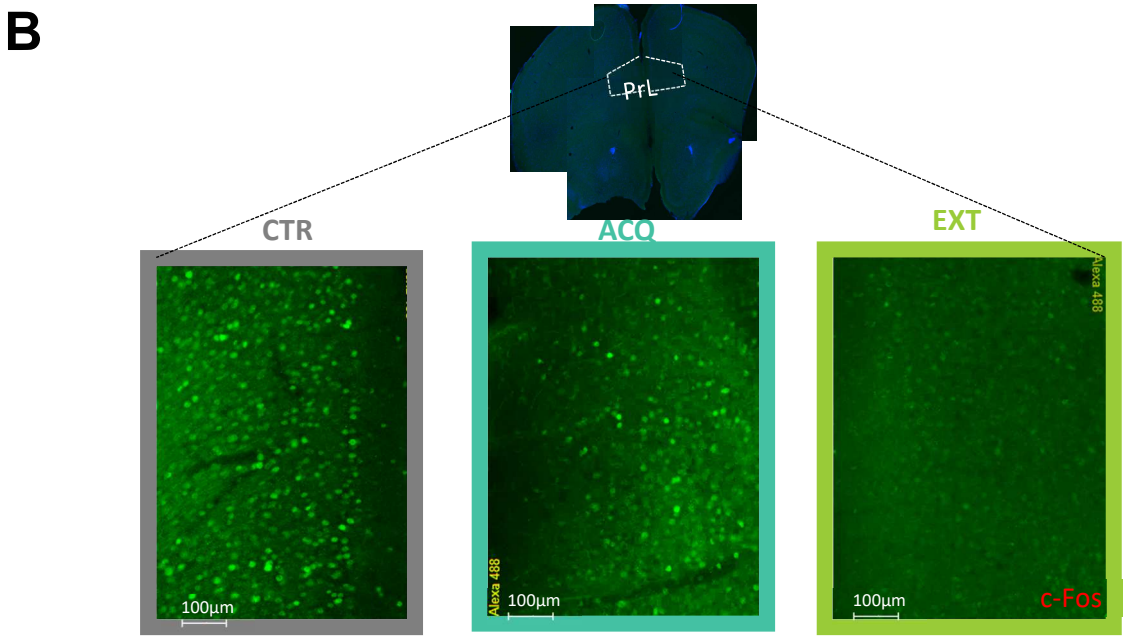
Figure 13 - Pattern of neuronal activation upon contextual fear conditioning in the hippocampus and in the amygdala. (**A**) Schematic representation of contextual fear conditioning paradigm with the timepoints of sacrifice. Representative photomicrographs of c-Fos immunohistochemistry (**B**) using the enzymatic method and (**C**) the fluorescence method, to show the level of neuronal activation in both the hippocampus and the amygdala, 1 h after contextual fear conditioning (acquisition group, **ACQop**) and after contextual learning (control or naïve group, **CTRop**). Note the increase in c-Fos positive neurons in all of the trisynaptic circuit of the hippocampus (DG, CA1 and CA3), throughout the amygdala (especially in the LA, LaVL, LaVM and BLA) and also in cortical areas surrounding the amygdala and the hippocampus, of the ACQ mice. Cornu Ammonis areas 1 and 3 (CA1 and CA3, respectively); dentate gyrys (DG); lateral nuclei (LA); lateral ventrolateral and ventromedial nuclei (LaVL and LaVM, respectively); baso anterior nuclei (BA); baso posterior nuclei (BP); central nuclei (CeA).

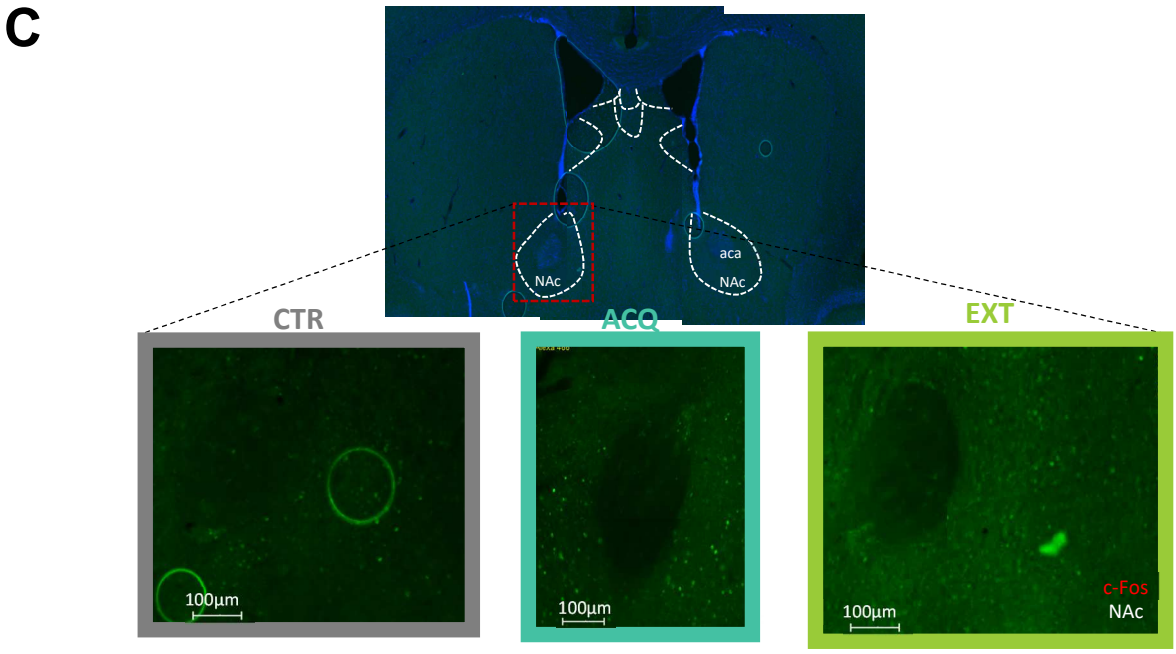
the slices have slightly different coordinates; (2) Fluorescent labeling yields images with a lower signal-to-noise ratio.

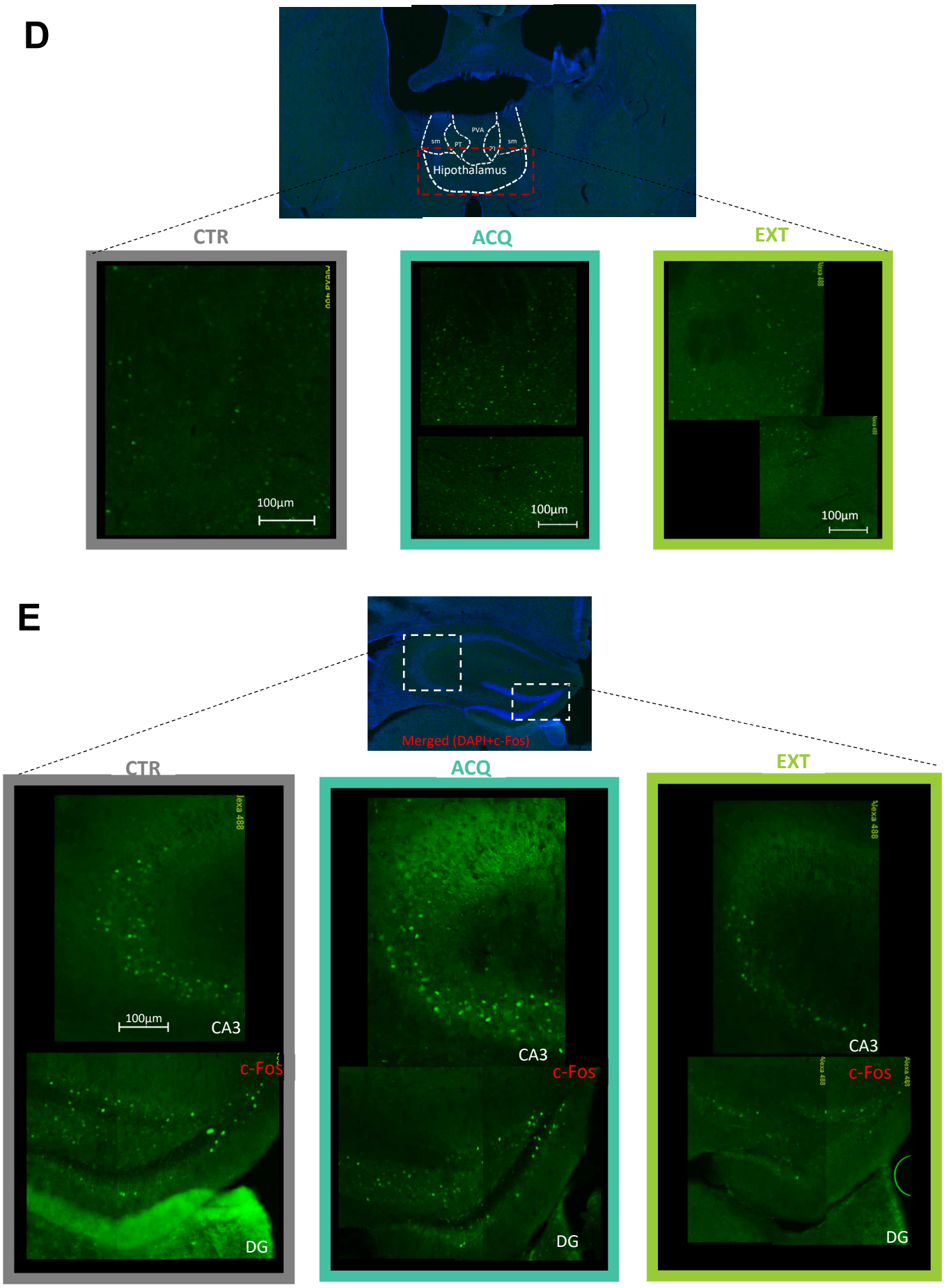
Pattern of neuronal activation of contextual, fear and extinction memories.

In the search for a memory trace left in the brain upon fear, extinction or simply context recall, we performed fluorescent immunohistochemistry for c-Fos in three experimental groups: CTR, ACQ and EXT. All three groups of mice were re-exposed to the conditioning chamber on the last day of the behavioral protocol, for memory recall. One hour after this last test, animals were perfused with paraformaldehyde, according to the scheme in the Figure 14A. In Figure 14 are the representative images from regions in the brain showing marked activation (i.e. c-Fos immunolabelling) in the ACQ group. The prelimbic region of the mPFC had considerable neuronal activation in the ACQ group and almost no neuronal activation in the EXT group (Figure 14B, center and right images, respectively). However, the CTR group also showed marked neuronal activation in this region (Figure 14B, left image). The core region of the nucleus accumbens (NAc), which mediates Pavlovian instrumental transfer (a process through which the conditioned stimuli modifies operant behavior) (Corbit *et al.*, 2016), was also more activated in the ACQ then in the EXT group (Figure 14C, center and right images, respectively), but also relative to CTR mice (Figure 14C, left image). Moreover, the hypothalamus, a brain region with a neuroendocrine function and known to be a "stress sensor" that coordinates the physical response to fear (Romanov et al., 2017), is also more activated in the ACQ group than in the CTR and EXT groups (Figure 14D). However, the dorsal and ventral hippocampus showed the same levels of neuronal activation in the CTR group as in the ACQ group (Figure 14E-F, left and center images), even though both regions had much less c-Fos labelled neurons in the EXT group (Figure 14E-F, right images). Surprisingly, the amygdala nuclei had little c-Fos labelling except for few nuclei in the BLA and CeA region of the CTR group (Figure 14G, right images).


A
Control group
Habituation phase 180 s
3days
180 s
Memory test
Perfusion
Acquisition group
Habituation phase 180 s
28 s
28 s
28 s
3days
180 sc
Memory test
Perfusion
Extinction group
Habituation phase 180 s
28 s
28 s
28 s
24h
180 s
Recall
2h
180 s
Extincition
x 3days
24h
180 s
Mmemory test
Perfusion


B
PrL
CTR
ACQ
EXT
100µm
100µm
100µm
c-Fos


C
NAc
aca
NAc
CTR
ACQ
EXT
100µm
100µm
100µm
c-Fos
NAc


D
PVA
sm
PT
sm
Hipothalamus
CTR
ACQ
EXT
100µm
100µm
100µm
E
Merged (DAPI+c-Fos)
CTR
ACQ
EXT
100µm
CA3
c-Fos
DG
CA3
c-Fos
DG
CA3
c-Fos
DG

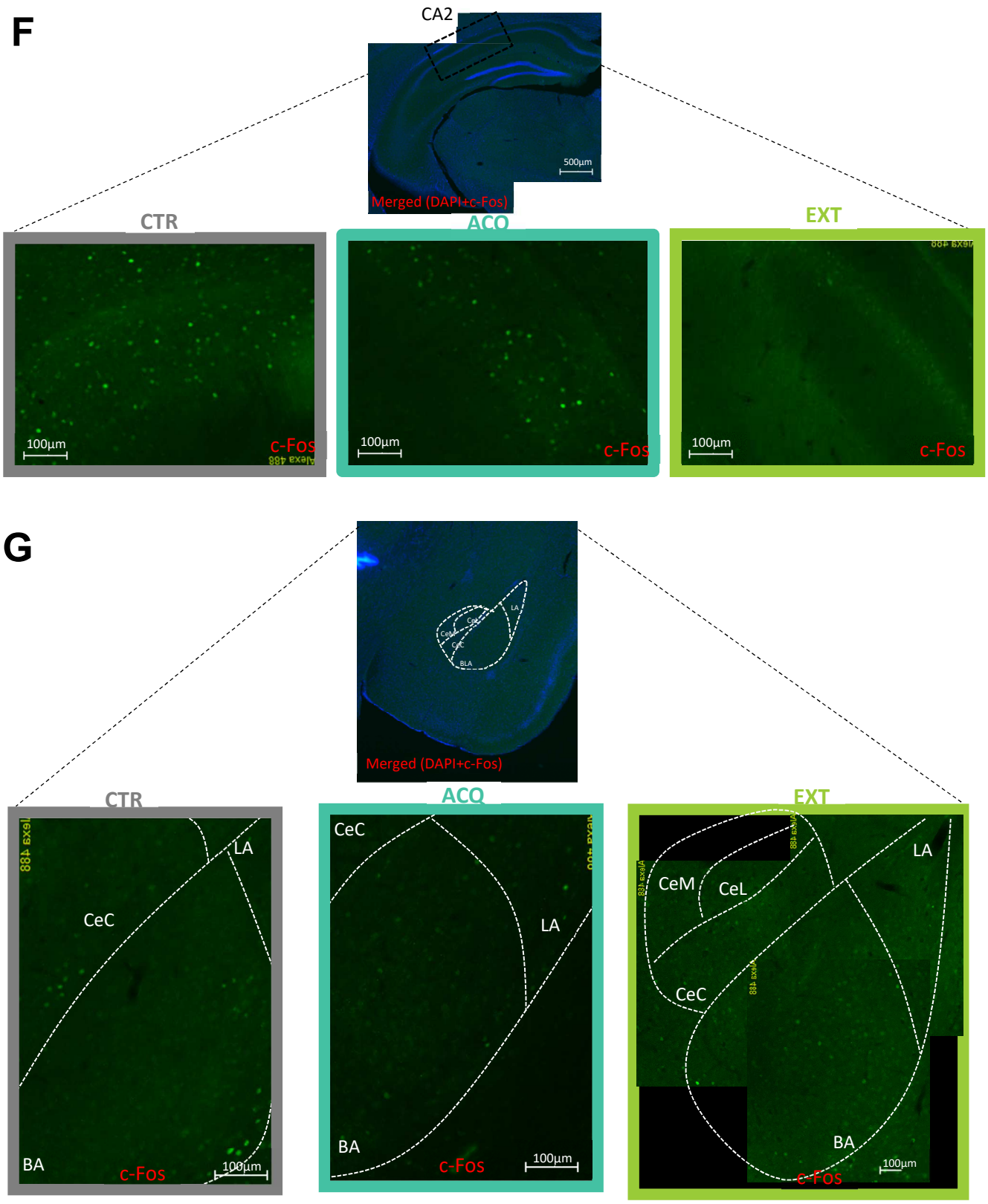


Figure 14 - Pattern of neuronal activation of contextual, fear and extinction memories. Memory trace or state of alertness? (**A**) Schematic representation of contextual fear conditioning and extinction paradigm with the timepoints of sacrifice. Representative photomicrographs of c-Fos immunohistochemistry (**B**) in prelimbic area (PL) of the medial prefrontal cortex (mPFC), (**C**) in the core of the nucelus accumbens (NAc), (**D**) in the hypothalamus, in the (**E**) dorsal and (**F**) ventral hippocampus and in (**G**) amygdala to show the level of neuronal activation in these strucutures, 1 h after the memory test on the last day. Control group, CTR (images framed in gray); acquisition group, ACQ (images framed in blue) and extinction group, EXT (images framed in green). In the nucleus accumbens and in the hypothalamus, the ACQ had more c-Fos positive neurons than the CTR and the EXT. In the PL, in the dorsal and ventral hippocampus both the CTR and the ACQ group had a higher c-Fos activation comparing to the EXT group and, in contrast, the amygdala had little c-Fos activation. Cornu Ammonis areas 2 and 3 (CA2 and CA3, respectively); dentate gyrys (DG); lateral nuclei (LA); lateral amygdala, baso-amygdala (BA); central nuclei (CeA) constitute by the capsular (CeC), lateral (CeI) and medial (CeM) subdivisions.

$A_{2A}R$ control synaptic plasticity in the amygdala and in the dorsal hippocampus but not in the ventral hippocampus of naïve mice

Next we performed *ex vivo* extracellular electrophysiological studies at the CA3-CA1 pathway of the hippocampus (dorsal and ventral) and in the amygdala (at the lateral to the basolateral nuclei pathway) of naïve mice and evaluated the acute effect of the selective antagonist of $A_{2A}R$. These are brain regions known to be involved in the acquisition of conditioned contextual fear (Izquierdo *et al.*, 2016) and also in the extinction of this fear using 'retrieval-extinction' protocols (Schiller *et al.*, 2013).

When comparing basal neuronal recruitment or neuronal excitability in the dorsal *versus* ventral hippocampus of naïve mice, we observed no differences, i.e. the input-output (IO) curves were similar in the two poles of the hippocampus (Figure 15A and D). However, LTP had a higher amplitude in the dorsal when comparing to the ventral hippocampus (64.70±943.% increase from baseline of the dorsal *versus* 38.29±4.059% in the ventral, n=5-6, $p<0.05$, Student's t test), Figure 15C and F). Moreover, SH58261 (50 nM) decreased the amplitude of the LTP in the dorsal hippocampus (37.62±4.278% with SCH58261 *versus* 64.70±9.543% in the control, n=5-6, $p<0.05$, Student's t test), Figure 15B-C, whereas it was devoid of effect in the ventral hippocampus (37.07±7.990% with SCH58261 *versus* 38.29±4.059% in the control, n=5), Figure 15E-F.

In the intra-amygdalar LA-BLA pathway, LTP had a magnitude of 59.06±9.333% above baseline (n=3) in slices from naïve mice and the blockade of $A_{2A}R$ with SCH58261 tended to decrease the amplitude of LTP (15.53±11.31% in the presence of SCH58261, n=2), whereas IO curves were unaltered (Figure 15G-I).

Fear extinction reverted contextual fear conditioning-induced increase of LTP in the ventral hippocampus and decreased the effects of fear conditioning on basal transmission in the amygdala

The same type of electrophysiological recordings were performed in slices from mice that underwent fear conditioning alone or fear conditioning *plus* fear extinction with or without the administration of the $A_{2A}R$ antagonist.

Here, we observed that contextual fear conditioning or extinction did not alter IO curves nor the LTP measured in the dorsal hippocampus, Figure 16A-C. However, only in the slices from the EXT group was possible to depotentiate LTP with a low frequency stimulation (LFS) (-8.083 ±1.973% of baseline in EXT, $p<0.05$ comparing to the hypothetical value of 0, n=5), Figure 18A and E. Importantly, in dorsal hippocampal slices from EXT+SCHip mice, LTP was not depotentiated (Figure 18D-E).

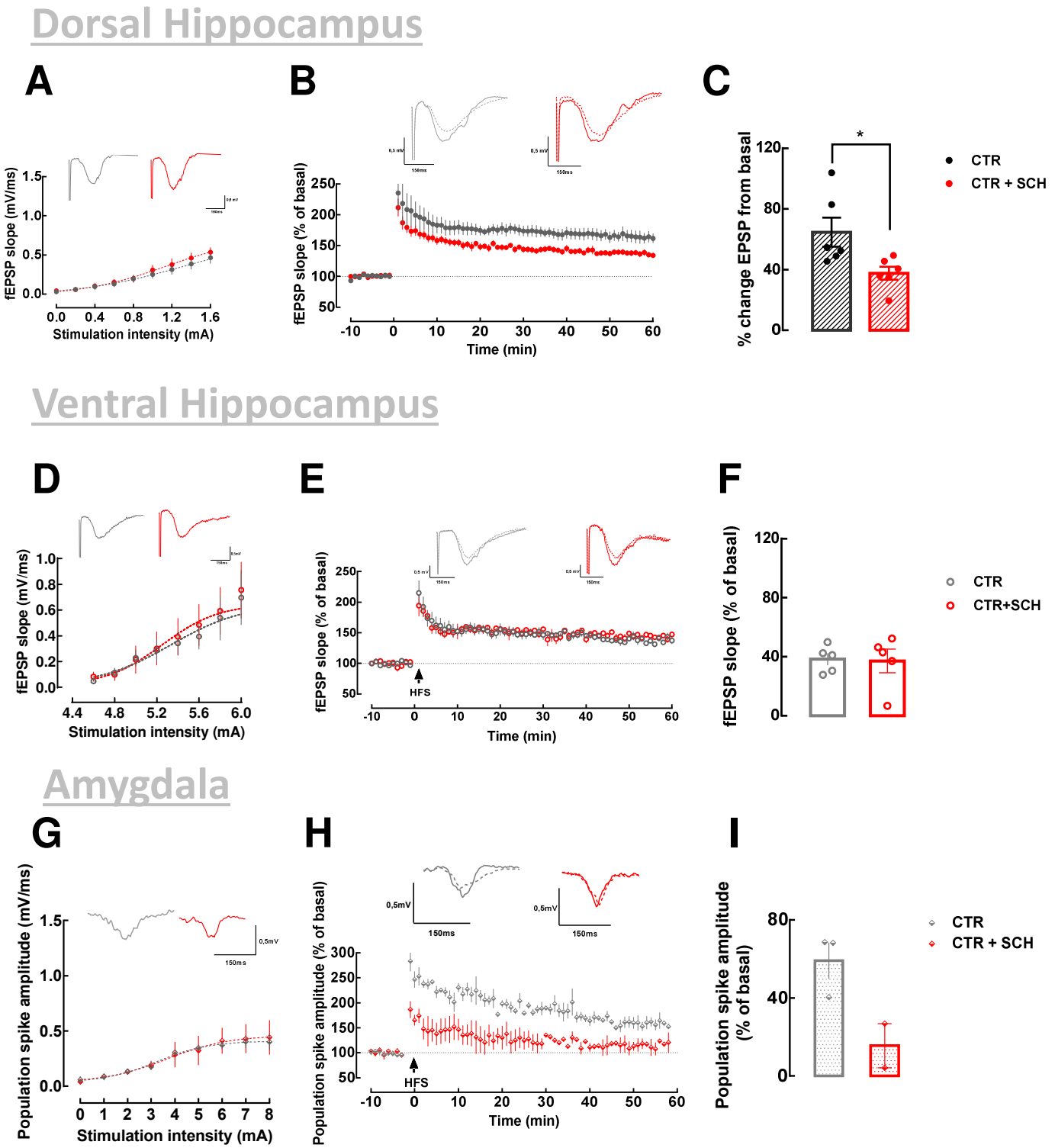


Figure 15 - $A_{2A}R$ control synaptic plasticity in the amygdala and in the dorsal hippocampus, but not in the ventral hippocampus, in naive mice. In extracellular recordings of the field excitatory postsynaptic potentials (fEPSP) in the CA1 region triggered by stimulation of the schaffer colaterals in the dorsal hippocampus, the selective $A_{2A}R$ antagonist, SCH58261 (50nM) (**CTR+SCH**) (**A**) did not change the input-output curves (I/O) but, (**B**) decreased the long-term potentiation (LTP) of the fEPSPs triggered by a high-frequency stimulation (HFS; 100Hz, 1s). In the ventral hippocampus, SCH58261 did not change (**D**) the I/O curve nor (**E**) the amplitude of LTP. In the amygdala, the extracellular recordings of the amplitude of the population spike (PS) in the BA nuclei were triggered by the stimulation of the LA. SCH58261 (**G**) did not change the I/O curves but (H) decreased the LTP triggered by HFS (5 pulses of 100Hz delivered with an interval of 30 seconds). **(C,F,I) bar graphs of the respective LTP**.Data are mean±SEM of n= 3-5 mice per group. *p<0.05 unpaired Student's *t*-test. Additionally, (A, D, G) have the representative responses of the maximal fEPSP slope/PS amplitude and (B, E, H) have the pair of super-imposed fEPSP/PS responses before the train (baseline) and 60 min after HFS.

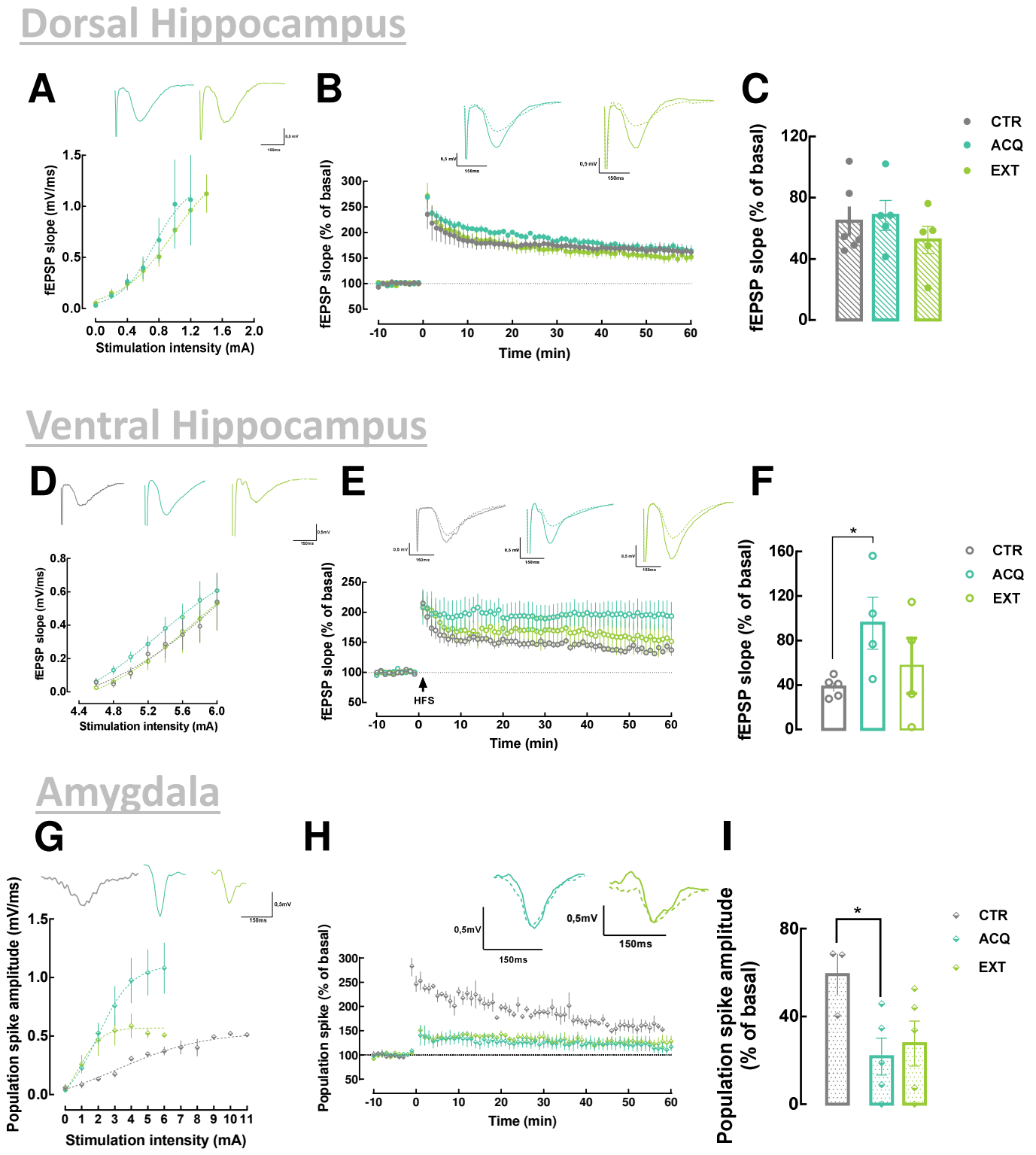


Figure 16 - Fear extinction reverts contextual fear conditioning-induced increase of LTP in the ventral hippocampus and of basal transmission in the amygdala. In extracellular recordings of the field excitatory postsynaptic potentials (fEPSPs) in the CA1 region triggered by stimulation of the schaffer colaterals, in the dorsal hippocampus, there was no differences between the control (CTR), acquisition group (ACQ) and the extinction group (EXT) neither (**A**) in the input-output curves (I/O) nor (**B**) in the LTP of the fEPSP triggered by high-frequency stimulation (HFS; 100Hz, 1s). In the ventral hippocampus, there were no differences between the ACQ, the EXT and the control groups (CTR) in (**D**) the I/O curves but (**E**) there was an increase of the amplitude of the LTP in the ACQ group comparing to the CTR and EXT groups. In the amygdala, the extracellular recordings of the population spikes (PS) in the BA regions were triggered by the stimulation of the LA nuclei and there was (**G**) an increase of the I/O curves in the ACQ group comparing to the CTR and EXT groups. However, in contrast, there was a (**H**) decrease of the LTP triggered by a HFS train (5 pulses of 100Hz delivered with an interval of 30 seconds) in both ACQ and EXT group comparing to the CTR group. (**C,F,I**) Data are mean±SEM of n= 3-5 mice per group. *$p < 0.05$, unpaired Student's *t*-test. Additionally (A, D, G) have the representative responses of the maximal fEPSP slope/PS amplitude and (B, E, H) have the pair of super-imposed fEPSP/PS responses before the train (baseline) and 60 min after HFS.

On the other hand, in the ventral hippocampus, the ACQ group had LTPs with significantly higher amplitude comparing to those obtained in slices from the CTR group (95.68±23.49% above baseline in ACQ *versus* 38.29±4.059% in CTR, n=5, $p<0.05$, Student's t test), Figure 16E-F. Moreover, in the EXT group LTPs were equal to the CTR (40.23±15.01% in EXT *versus* 38.29±4.059% in CTR, n=5), Figure16E-F. Also, treatment with SCH58621 (ip) did not significantly alter the LTP when comparing to the EXT (without SCH58261), Figure 19F and H. Another noteworthy result was the fact that again only LTP in the EXT group had a tendency to depotentiate (-6.536±2.470% in EXT, p=0.0572 comparing to the hypothetical value of 0%, n=5) and interestingly, in the group of EXT+SCHip, instead of a depotentiation, LFS caused a further increase in the LTP (20.43±7.557 % of in EXT+SCHip, $p<0.05$ comparing to the hypothetical value of 0%, n=5), Figure 20E.

In the amygdala, the IO curves were considerably higher in slices from ACQ mice when comparing to both the CTR and the EXT mice ($p<0.05$, nonlinear regression), even though the curves of the EXT were still higher than the CTR ($p<0.05$, Student's t test), Figure 16G. Moreover, the LTPs of ACQ and EXT groups were significantly lower than in the CTR group (21.69±8.344% in ACQ and 27.69±10.26% in EXT *versus* 59.06±9.333% in CTR, n=3-5, $p<0.05$, unpaired Student *t-test*, Figure 16H-I. Also, the LTP in the EXT with SCH58261 (ip) group was not different from the EXT group (Figure 21F). Once again, when comparing the results from depotentiation after LTP, only the EXT and the EXT+SCHip groups had a significant depotentiation (-18.77±2.322% in EXT and -28.97±5.225% in EXT+SCHip, $p<0.05$ comparing to the hypothetical value of 0%, n=5), Figure 22E.

Ex vivo blockade of $A_{2A}R$ reverted the effects of contextual fear conditioning on the LTP of the ventral hippocampus

The acute superfusion of brain slices from ACQ mice with SCH58261 (50 nM), showed that the acute *ex vivo* blockade of $A_{2A}R$ was able to revert the increase in the amplitude of the LTP caused by fear conditioning (95.68±23.49% in ACQ *versus* 28.92±12.07% in ACQ+SCH, n=5, $p<0.05$, Student's t test), Figure 19D and H. On the other hand, superfusion of SCH58261 did not normalize the IO curves in the amygdala of ACQ mice, Figure 21A . Interestingly, as in naïve animals, the $A_{2A}R$ antagonist did not have any effect in the LTP amplitude of the EXT group (57.30±24.99% in EXT *versus* 40.23.92±15.01% in EXT+SCH, n=5, $p<0.05$, Student's t test) Figure 19E.

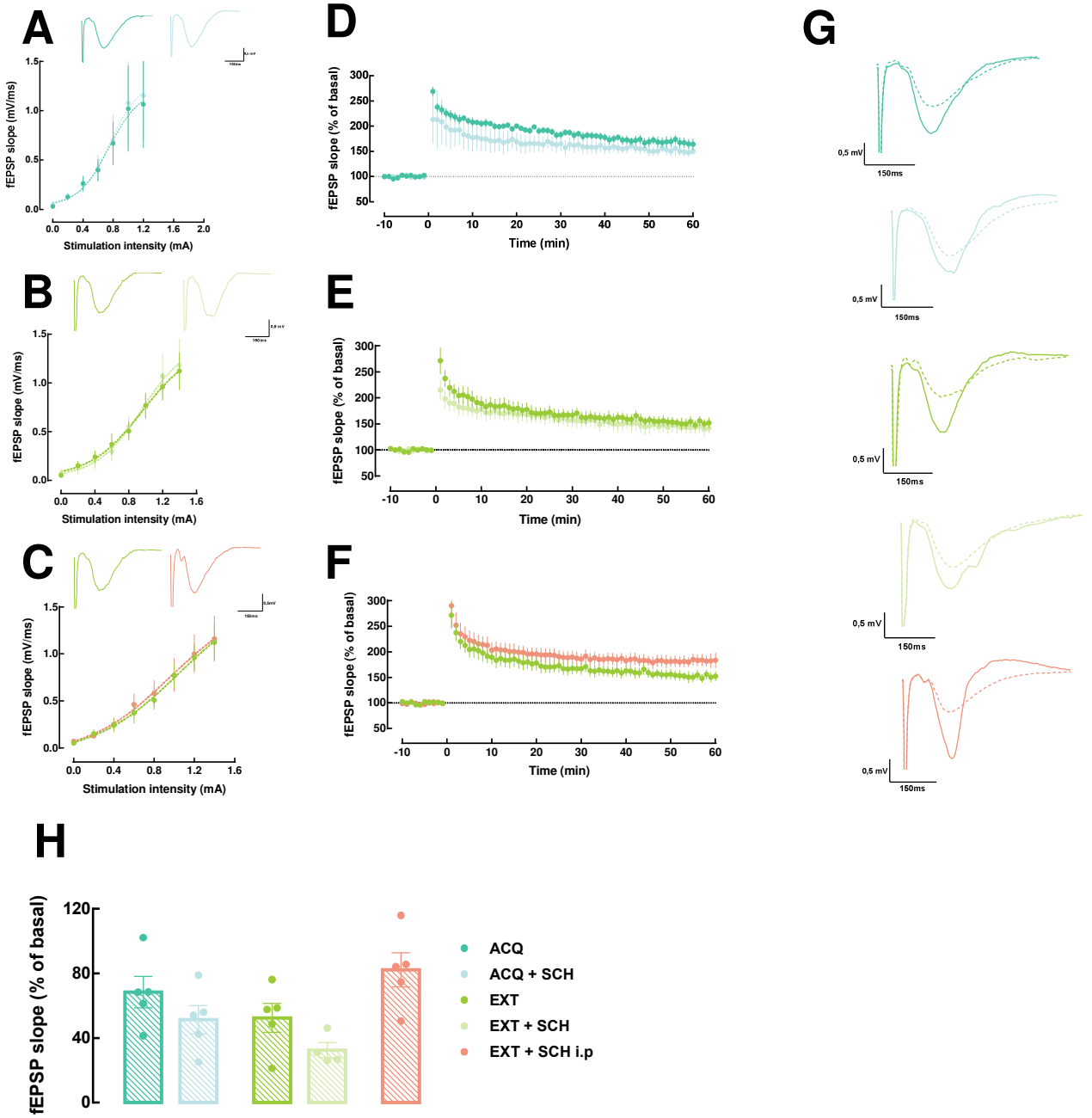


Figure 17 - *Ex-vivo* blockade of the A_{2A} receptor had a tendency to decrease the amplitude of the LTP in the dorsal hippocampus of both the acquisition and extinction groups. In extracellular recordings of the field excitatory postsynaptic potentials (fEPSP) in the CA1 region triggered by stimulation of the schaffer colaterals, the selective A_{2A}R antagonist, SCH58261 (50nM), (**A**) did not change the input-output curves neither in the ACQ group (ACQ+SCH) nor (**B**) in the EXT (EXT+SCH) groups but (**D-E**) had a tendency to decrease the long-term potentiation (LTP) of the fEPSP triggered by a high-frequency stimulation (HFS; 100Hz, 1s). The group injected with SCH58261intraperitoneally (EXT+SCHip; 0,1mg/kg) during extinction had no differences in the I/O curves nor in the amplitude of the LTP comparing to the EXT group. (**H**) **Bar graph comparing the LTPs between the different groups.** Data are mean±SEM of n=5 mice *per* group. *$p < 0.05$, unpaired Student's *t*-test. Additionally, (**A-C**) have the representative responses of the maximal fEPSP slope of the I/O and (**G**) have the pair of superimposed fEPSP responses before the train (baseline) and 60 min after HFS.

Dorsal Hippocampus

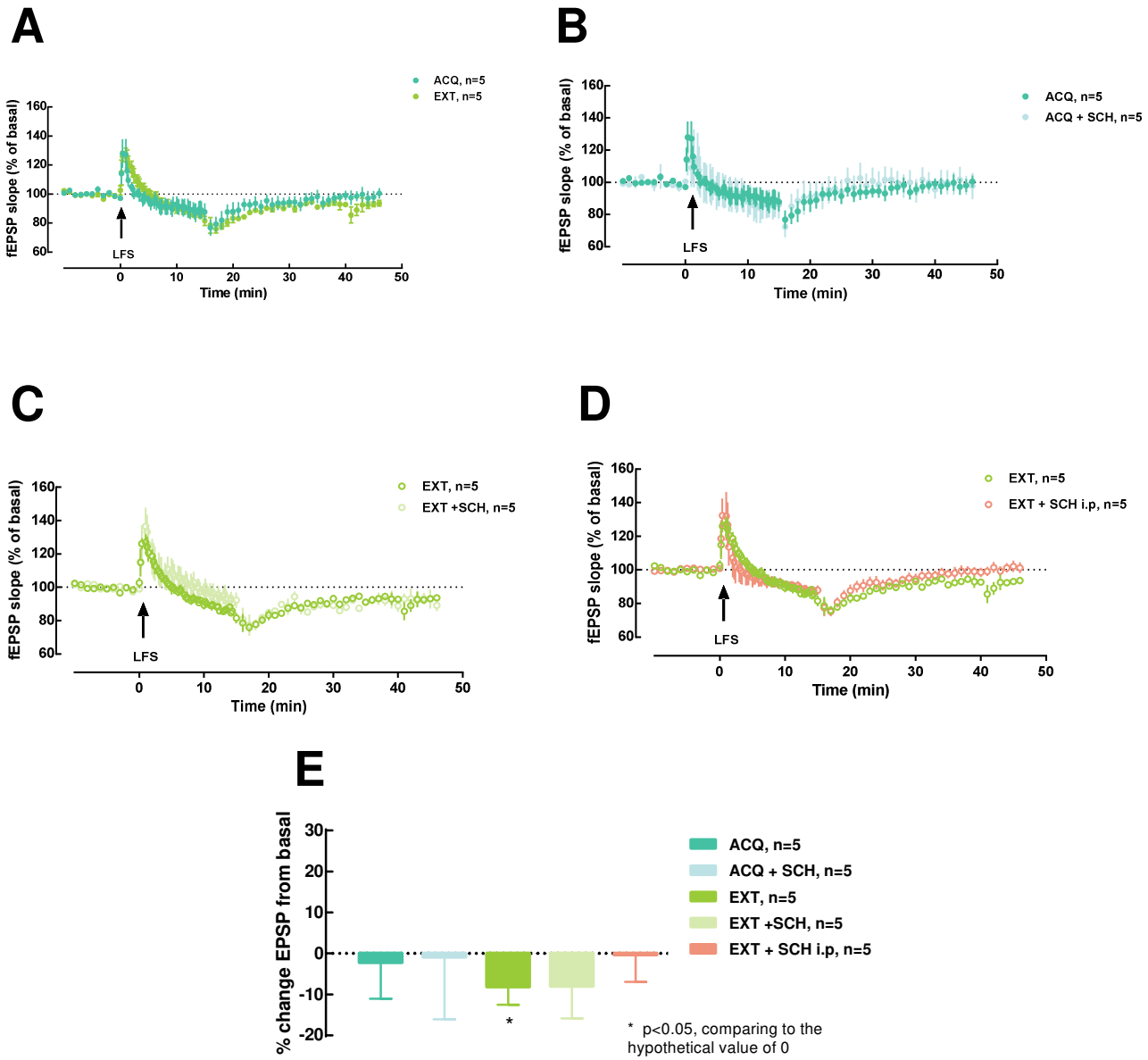


Figure 18 - Low frequency stimulation caused depotentiation in the dorsal hippocampus only in the extinction groups. In extracellular recordings of the field excitatory postsynaptic potentials (fEPSPs) in the CA1 region triggered by stimulation of the Schaffer collaterals (**A**) only the EXT group depotentiated the fEPSPs triggered by a low frequency stimulation (LFS; 900pulses, 1Hz). (**B-D**) The selective $A_{2A}R$ antagonist, SCH58261 had no effect on depotentiation from the LTP in any of the experimental groups. (**E**) Data are mean±SEM of n=5 mice *per* group. *$p < 0.05$, comparing to the hypothetical value of 0.

Ventral Hippocampus

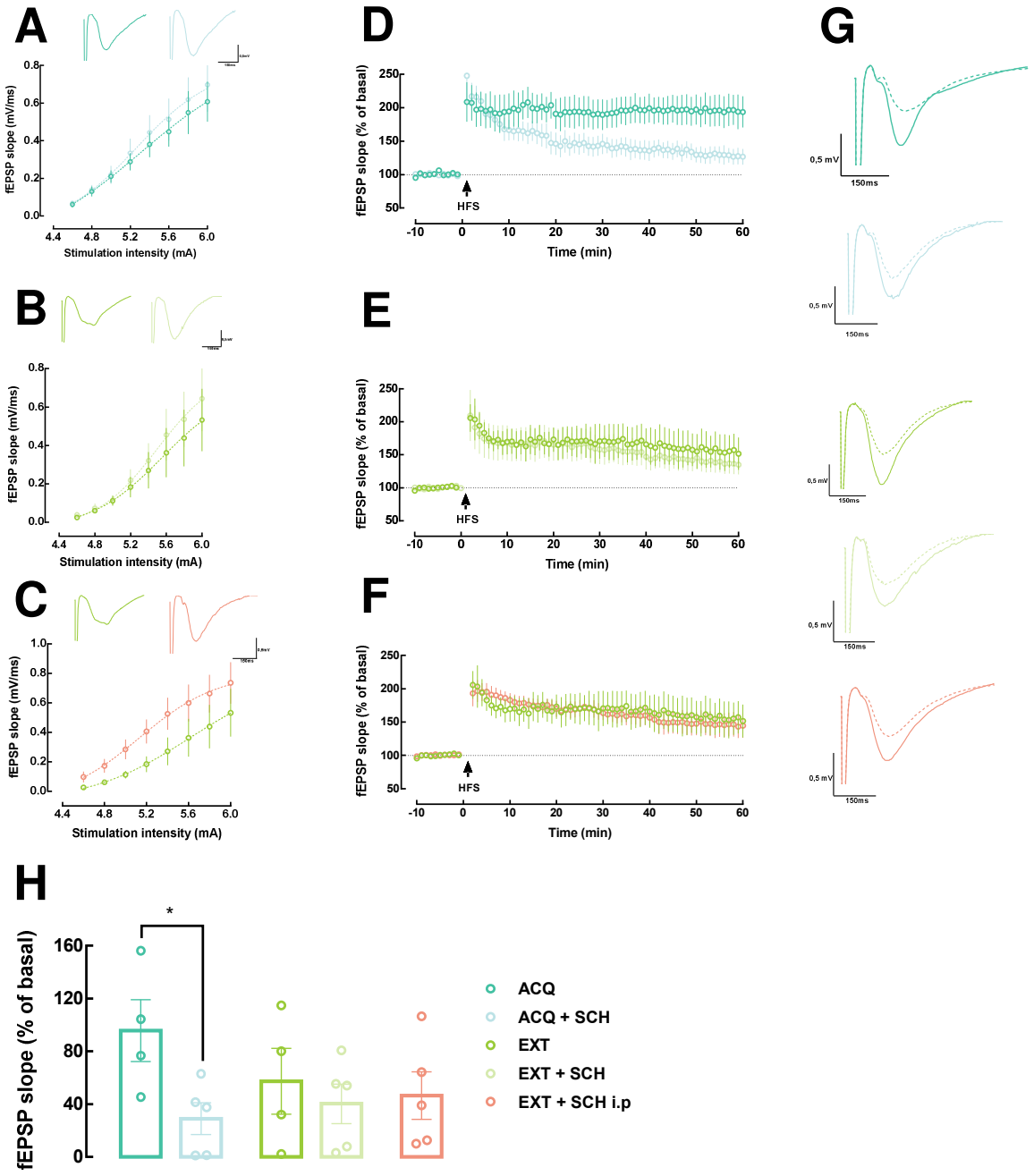


Figure 19 - Ex vivo blockade of $A_{2A}R$ reverted the effects of contextual fear conditioning on the LTP of the ventral hippocampus. In extracellular recordings of the field exceitatory postsynaptic potential (fEPSP) in the CA1 region triggered by stimulation of the schaffer colaterals, the selective $A_{2A}R$ antagonist, SCH58261 (50nM), (**A**) did not change the input-output curves neither in the ACQ group (ACQ+SCH) nor (**B**) the EXT (EXT+SCH), but, (**D**) significantly decreased, the long-term potentiation (LTP) of the fEPSP triggered by a high-frequency stimulation (HFS; 100Hz, 1s) of the ACQ group. No differences were observed in the EXT+SCH and EXT+SCHip in comparison to the EXT group. (**H**) **Bar graph comparing the LTPs between the different groups.** Data are mean±SEM of 5 mice per group. *p < 0.05, unpaired Student's *t*-test. Additionally, (**A-C**) have the representative responses of the maximal fEPSP slope of the I/O and (**G**) have the pair of super-imposed fEPSP responses before the train (baseline) and 60 min after HFS.

Ventral Hippocampus

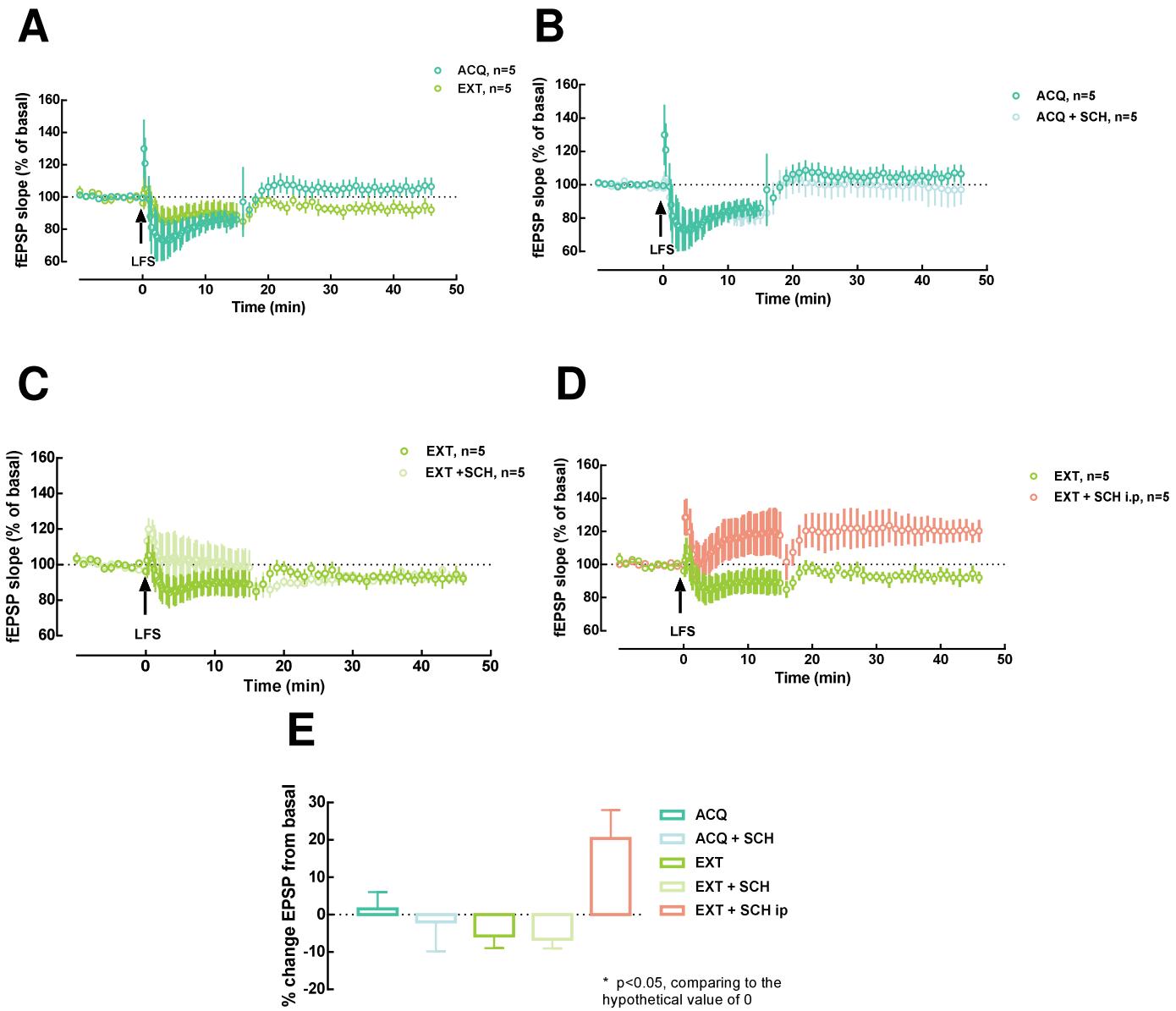


Figure 20 – Low frequency stimulation potentiated the synapses of the ventral hippocampus in mice injected with SCH58261 during the extinction process. In extracellular recordings of the field excitatory postsynaptic potential (fEPSP) in the CA1 region triggered by stimulation of the schaffer colaterals, (**A**) the **EXT** had a tendency to depotentiate the fEPSP triggered by a low frequency stimulation (LFS; 900pulses, 1Hz). (**B-C**) Ex-vivo blockade of $A_{2A}R$ had no effect, but, in contrast (D) the **EXT+SCHip** group had a tendency to potentiate after the LFS. (**E**) Data are mean±SEM of 5 mice per group. *p < 0.05, comparing to the hypothetical value of 0%.

Amygdala

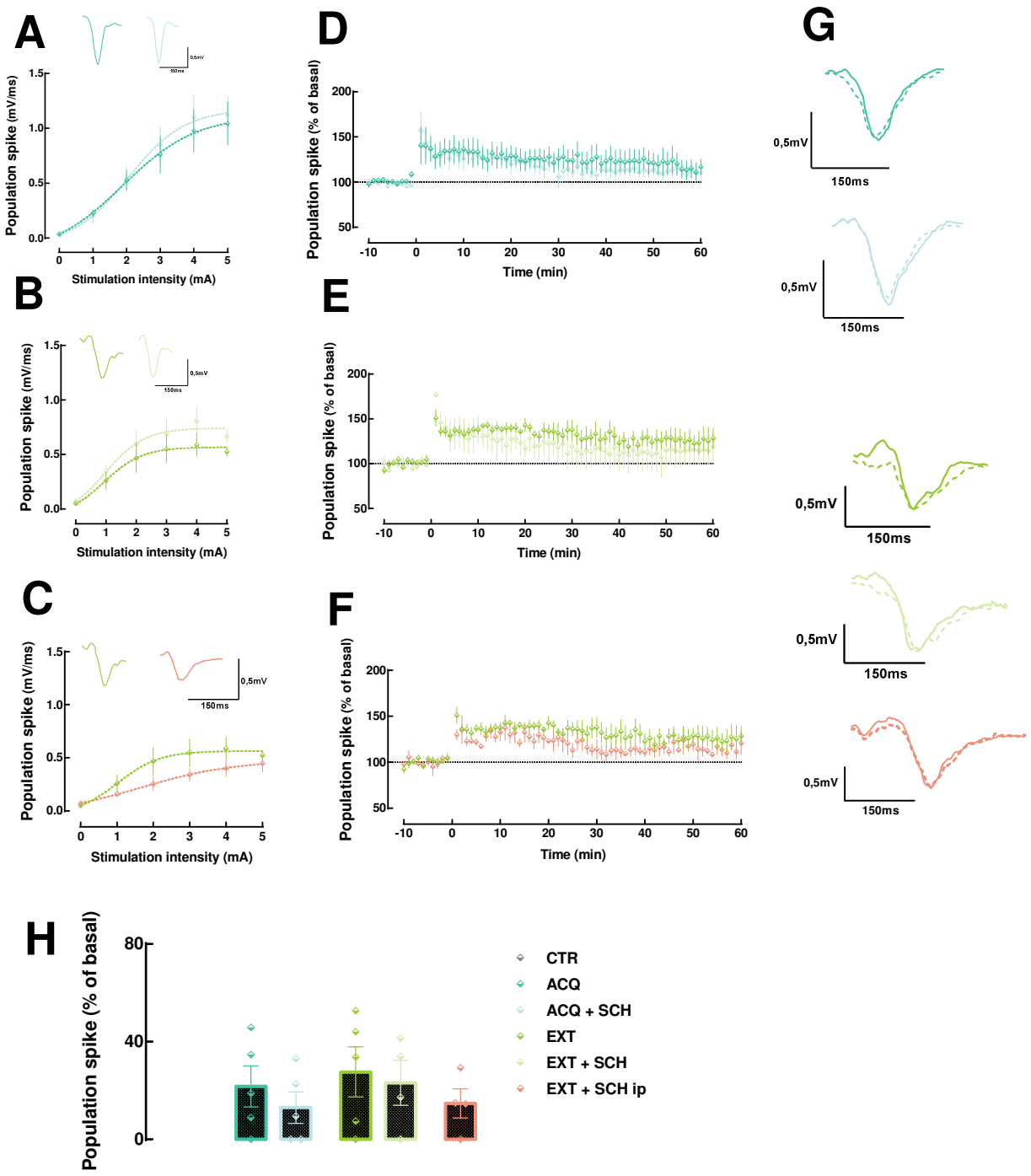


Figure 21 - Ex vivo blockade of the A_{2A} receptor did not alter neither the input-output cuves nor the long term potentiation both in the ACQ and EXT group in amygdala . In extracellular recordings of the population spike (PS) in the BA region triggered by stimulation of the LA region, the selective $A_{2A}R$ antagonist, SCH58261 (50nM), **(A-B)** did not change the input-output curves nor **(D-E)** the long-term potentiation (LTP) of the PS triggered by a high-frequency stimulation train of 5 pulses delivered with an interval of 30s. The slices of animals injected with SCH58261 (EXT+SCHip; 0,1mg/kg), also did not had any differences neither in the I/O nor in the LTP magnitude comparing to the EXT group. **(H) Bar graph comparing the LTPs between the different groups.** Data are mean±SEM of 5 mice per group. *p < 0.05, unpaired Student's *t*-test Additionally, **(A-C)** have the representative responses of the maximal PS amplitude of the I/O and **(G)** have the pair of super-imposed PS responses before the train (baseline) and 60 min after HFS.

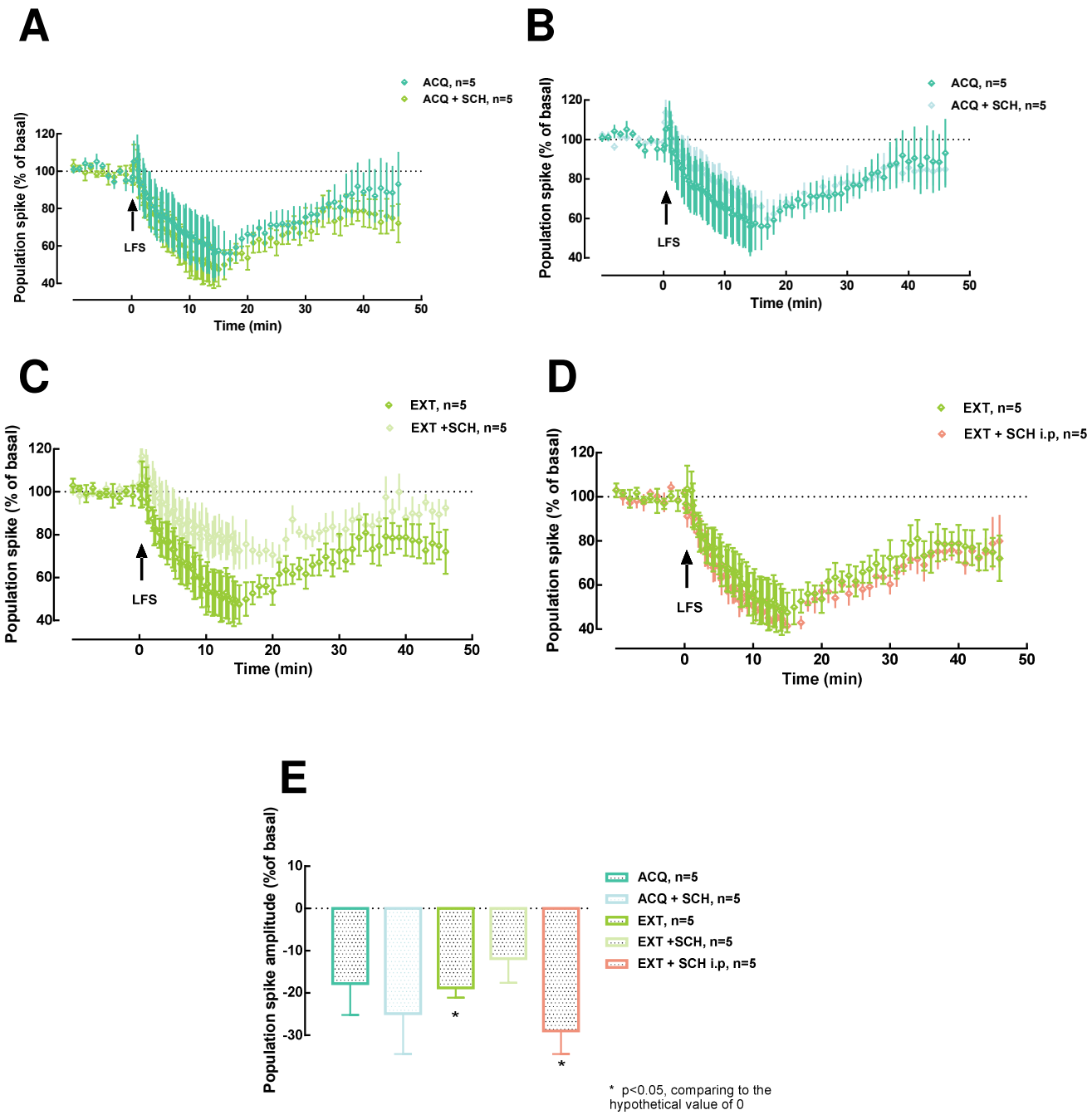


Figure 22 – Low frequency stimulation caused depotentiation in the extinction group and in the extinction group injected with SCH58261 in amygdala. Upon extracellular recordings of the population spike (PS) in the BA region triggered by stimulation of the LA region, (**A**) the EXT significantly depotentiated the PS triggered by a low frequency stimulation (LFS; 900pulses, 1Hz). (**B-C**) Ex-vivo blockage of $A_{2A}R$ had no effect, but, in contrast (D) the EXT+SCHip group significantly depotentiate after the LFS. (**E**) Data are mean±SEM of 5 mice per group. *p < 0.05, comparing to the hypothetical value of 0%.

4

DISCUSSION

We started our project by adapting a protocol of pure contextual fear conditioning from Santos and colleagues (2013) in which the time between the multiple presentations of the unconditioned stimulus (footshock, US) was variable (different intertrial intervals, ITI). We decided to use the same unpredictable ITI so that animals could only rely on the context to predict the shocks. In addition, we chose a 'retrieval-extinction' paradigm as in Gräff *et al.*, 2014, because this type of paradigm has been shown to be more effective at extinguishing aversive memories both in animals and in humans, and to cause less spontaneous recovery (Urcelay *et al.*, 2009; Agren *et al.*, 2012; Schiller et al., 2013; Gräff *et al.*, 2014; Johnson and Casey, 2015). The problem regarding the aforementioned paradigm was that fear stressed mice that did not undergo an active extinction, lost the fear memory with the passage of time (Figure 11C-D). This may have happened due to the footshocks being too mild (0.5 mA) for a fear conditioning paradigm that lacks a specific cue, and/or because the time predictability of the US may be essential for contextual representation in animals. Therefore, we tested another *contextual fear-conditioning paradigm* where during the training session, the US (now with an intensity of 0.8 mA and presented only 3 times) was delivered at a constant interval. With this protocol the mice learned to associate the conditioning chamber (conditioned stimulus, CS) to the aversive US, as typified by the learning curve of the animals, i.e. the increasing freezing behavior at each US presentation (Figure 12B) and the freezing behavior 24 hours later, during the fear memory test (Figure 12C-D). Importantly, unlike what had previously happened in the first protocol, this time the mice maintained high freezing levels to the conditioning chamber until the last day of experiment (Figure 12C-D). Furthermore, the 'retrieval-extinction' protocol used is thought to be more efficient because it opens a window for memory reconsolidation that allows previous acquired memories to be updated with new information (Auber *et*

al.,2013; Graff *et al.*,2014). Using this strategy, the mice successfully decreased the fear response (freezing behavior) over time (Figure 12C-E), validating our model of extinction.

To understand how the $A_{2A}R$ modulate the extinction of fear memories, we intraperitoneally injected the mice with the $A_{2A}R$ antagonist, SCH58261, 1h before each extinction trial (EXT+SCHi.p group) (Figure 8). We observed that these animals displayed a faster extinction of the conditioned response (i.e. freezing behavior) to the training chamber than the EXT group (Figure 12C-D), which suggests that the $A_{2A}R$ antagonist accelerates the extinction of fear memories. Although these results seem promising, there are a few controls to be made to validate this hypothesis. For instance, it is described in the literature that the SCH58261 also modulates anxiety (Kaster et al.,2015; Caetano et al.,2016; López-Cruz et al.,2017) and, in higher concentrations, locomotion (Yacoubi et al.,2000). Thus, to confirm that SCH58261 is modulating the extinction of fear memories and not anxiety it is necessary to do an elevated plus maze (EPM) to evaluate the anxiolytic/anxiogenic effect of this antagonist, and an open-field (OPF) to determine if the dose used of the antagonist affects locomotion. Furthermore, to test if the $A_{2A}R$ antagonist is actively modulating the extinction of fear memories, it would be interesting to intraperitoneally inject the animals with the antagonist after the acquisition training and evaluate whether it can *per se* extinguish fear memories. These controls will allow us to understand if in our model the blockade of $A_{2A}R$is indeed modulating extinction learning.

In the literature there is a panoply of studies characterizing the neuronal circuitry that underlies cued-fear conditioning and extinction (Hall *et al.*, 2001; Herry *et al.*, 2004; Herry *et al.*,2008; Knapska *et al.*, 2009; Cicchio *et al.*,2010; Hoffman *et al.*, 2014); however there is scarce information about the recruitment of this same circuitry during contextual-fear conditioning and extinction (Izquierdo *et al.*,2016). To characterize the pattern of neuronal activation involved in our contextual fear conditioning paradigm we took advantage of c-Fos expression, which is an early neuronal activity-regulated gene that has been widely used to unveil memory engrams and traces (Henry et al., 2014; Knapska et al., 2009; Orsini et al., 2011; Josselyn et al., 2015). There are two possible protocols for labelling c-Fos through immunohistochemistry: the enzymatic method and the immunofluorescence method. As expected, both labelling methods revealed a marked increase in neuronal activation in the hippocampus (DG, CA3) and in the amygdala (mostly in LA and BLA nuclei but also in CeA) of fear conditioned mice (ACQop) when comparing with naïve mice (CTRop) that had simply been exposed to the conditioning chamber (Figure 13B and 13C). However, differences were more evident in the DAB stained images since it yielded a better signal-to-noise ratio (Figure 13B). Nevertheless, the immunofluorescence

method allows performing co-localization with glutamatergic/GABAergic markers, which is an asset to distinguish the recruitment of different neuronal populations (Calu *et al.*, 2013). Therefore, next we used immunofluorescence labelling of c-Fos to investigate the pattern of neuronal activation in the brain of the different experimental groups triggered by memory recall, on the last day of experiment. Thus, we compared between the group that was only exposed to the chamber without receiving footshocks (Control; CTR), the group that underwent fear conditioning (Acquisition group; ACQ) and the group that was fear conditioned followed by extinction training (Extinction group; EXT). Even though a quantitative analysis of the c-Fos labelling was not performed, here we showed representative images of the brain areas with distinct patterns of neuronal activation between the three experimental groups (Figure 14).

Since the hippocampus is essential to the perception of physical environment but also to associate context to an emotional experience (Maren et al., 2013; Izquierdo et al., 2016), it was anticipated that this brain region would be activated in all of the experimental groups. Thus, we started our analysis in the dorsal hippocampus. The dorsal hippocampus has been associated to cognitive functions, such as working memory and reference memory mainly due to is connections to cortical areas and a high density of place cells (Fanselow et al., 2012; Strange et al., 2014). These place cells have been implicated in the formation, consolidation and storage of a spatial representation of the context. Moreover, lesioning these cells leads to deficits in contextual fear memory (Fanselow et al., 2000), evidencing the importance of the dorsal hippocampus in this type of memory. In fact, our data shows that the dorsal hippocampus, and in particular the DG and CA3 regions, were highly activated in both the CTR and ACQ groups whereas the EXT group had considerably less c-Fos labelling (Figure 14E). These results contrast with those obtained immediately after fear conditioning (ACQop) or after the second exposure to the conditioning chamber (CTRop), in which the ACQ group had clearly more c-Fos labelling in the dorsal hippocampus than the CTR group (Figure 3). One possible explanation for these contradictory results is the distinct endpoint of the experiments. In the first experiment, CTR animals were exposed two days in a row to the conditioned chamber, and sacrificed 1h after the second exposition (Figure 13A) whereas in the second experiment, the CTR were exposed to the chamber in the first day (habituation day) and in the sixth day (memory test) (Figure 14A). This interval of 4 days may have been sufficient for CTR mice forgetting the conditioning chamber, since in this group the context was never associated to a strong stimulus. In this case, the novelty of the context is likely to trigger neuronal activation of the dorsal hippocampus in the same way that fear conditioning does (Keeley et al., 2006; Cho et al., 2017). Another possible reason

for the lack of differences between the CTR and the ACQ group is that the fear memory engram of the hippocampus might have started to dissipate with the passage of time, as memory becomes gradually dependent on other parts of the brain, i.e. neocortical areas (Cowansage et al., 2014; Kitamura et al., 2017).

On the other hand, neuronal activation in the dorsal hippocampus of the EXT group was scarce (Figure 14E) which may mean that in fact, c-Fos induction by context exposure in the hippocampus may actually be more related to the state of arousal of the animals than to a specific memory engram (Cho et al., 2017). In fact, the EXT group was exposed to the conditioning chamber everyday and 2 times *per* day until sacrifice whereas both the CTR and the ACQ groups were only exposed for 3 times to the conditioning chamber (Figure 14A). This last hypothesis is also supported by the same patterns of neuronal activation found in the ventral hippocampus (Figure 14F). One possible solution to overcome this confounding factor is to label the activated neurons that are actually part of a memory engram taking advantage of TetTagged mice. These animals express a transgene that combines elements of the tetracycline system with the cfos promoter, allowing the tagging of neurons that are recruited only during the learning of a specific task (Reijmers et al., 2009).

Another brain region analyzed was the amygdala. The amygdala is a core player in fear memory formation, consolidation and extinction (Orsini et al.,2012; Maren,2015). In previous reports, it was shown that upon tone fear-conditioning there is an increase of c-Fos expression in the amygdala (Henry et al., 2004; Knapska et al.,2009; Orsini et al., 2011; Jin et al.,2014). This also happens during contextual fear conditioning (Santos et al., 2013; Kitamura et al., 2017). Moreover, Santos et al.(2013) showed that the central nucleus of the amygdala (CeA) and intercalated cells masses (ITC) were key regions activated during contextual fear conditioning. Also, Kitamura et al. (2017) found that the BLA nuclei of the amygdala were activated both at contextual fear learning and at fear recall. Thus, surprisingly, we did not observe significant c-Fos labelling except for few neurons in the BLA and CeA regions of the CTR group and in the BLA of the EXT group (Figure 14G). Considering that the amygdala was highly activated during contextual fear acquisition (Figure 13B and C), this may mean that the amygdala is less engaged during fear memory recall and is mostly recruited during the learning, at least in our paradigm. Nevertheless, a more detailed analysis is required to drive any conclusions from these results.

We also analyzed the recruitment of the medial prefrontal cortex (mPFC). The mPFC is associated with diverse functions including attentional processes, decision making and working memory (Neafsey 1990; Goldman-Rakic 1994; Petrides1998; Repovs and Baddeley 2006). For that reason it is

expected to have neuronal activation in this brain region when an animal is exploring a new environment. Furthermore, recent studies have consistently shown that the activation of the prelimbic area of the mPFC is required for conditioned fear expression and recall (Giustino et al.,2015). Accordingly, we also observed a high neuronal activation in the mPFC in the CTR and in the ACQ groups and no activation in the EXT group (Figure 14B). On the other hand, the infralimbic area (IL) of the mPFC has been shown to be recruited during fear extinction learning and recall (Giustino et al.,2015). However, we did not observe any neuronal activation in this area in the EXT group nor in the CTR and ACQ groups (data not shown).

We also analyzed other areas of the brain that are equally important for an appropriate fear response, such as the hypothalamus and the nucleus accumbens (NAc), and asked whether these would be differentially activated according to the memory of the mice. The Hypothalamus is a pivotal structure in the coordination of the stress response (Smith,2006). It receives and integrates internal and external signals, including stressful stimuli and triggers the appropriate responses (Smith, 2006)During stressful situations, the hippocampus and the amygdala directly activate the hypothalamus that, in turn, activates the sympathetic system through the hypothalamic-pituitary-adrenal axis thereby triggering the body response to stress (Jacobson et al., 1991; Smith, 2006; Romanov et al., 2017). Since fear is a major stressor, both fear acquisition and recall are expected to drive neuronal activation in the hypothalamus (Troglic et al., 2011; Furlong et al.,2016). In agreement with this, our results also showed a higher level of c-Fos labelling in the hypothalamus of the ACQ group comparing to the CTR and EXT groups (Figure 4D).

Finally, our results also show that the core of the nucleus accumbens (NAc) is more activated in the ACQ mice than in the CTR and EXT mice (Figure 14C). Accordingly, the nucleus accumbens has been implicated in fear conditioning since it receives inputs from the BLA nuclei of the amygdala (Janak et al.,2015), the subiculum of the dorsal and ventral hippocampus (Fanselow et al., 2000) and from the mPFC (Tzschentke and Schmidt, 2000). Moreover, since it projects to the basal ganglia, globus pallidus and substantia nigra (Zahm, 2000), it was implied in Pavlovian-instrumental transfer, i.e. the process whereby a conditioned stimulus modifies operant behavior (Cartoni et al., 2013). In fact, the relevance of these nuclei for context-shock associations has been previously demonstrated (Westbrook et al., 1997) and the activation of NAc during fear conditioning paradigms was also shown (Veening et al., 2009).

Overall, this pilot study suggests that fear and extinction recall may induce different patterns of neuronal activation throughout the brain (Figure 14). However, as previously discussed, some of these patterns of c-Fos labelling may be more related to the level of arousal of the animals during the test rather than to a memory trace (Cho et al., 2017). Thus, perhaps it is best to adopt a different strategy to specifically label the neurons belonging to context, fear and extinction memory engrams before proceeding with a detailed analysis of brain sections.

Long-term synaptic plasticity is considered to be the neuronal mechanism whereby memories are formed, stored and retrieved. These involve the potentiation or depotentiation of synapses in defined neuronal circuitries (Bliss *et al.,*2011*)*. In a variety of psychiatric disorders these mechanisms are impaired, exacerbated or simply unbalanced, which leads to an aberrant connectivity between the different brain regions (Mahan et al.,2012; Orsini et al.,2012; Maren et al., 2014). Since the hippocampus (dorsal and ventral) and the amygdala are the two brain regions mostly implied in the acquisition and retrieval of contextual fear memories (Izquierdo et al., 2016), we performed *ex vivo* electrophysiological recordings of basal transmission (input-output curves, IO) and of long-term synaptic plasticity (LTP and depotentiation) in the CA3-CA1 synapses of the dorsal and ventral hippocampus and in the LA-BLA synapses of the amygdala. These recordings were performed on brain slices from naïve mice (CTR), from contextual fear conditioned mice (ACQ), from mice that underwent both fear conditioning and fear extinction (ACQ+EXT) and from mice that were injected with SCH58261 (0.1 mg/kg, ip) before fear extinction (EXT+SCHip). Moreover, the effect of blocking $A_{2A}R$ on these mechanisms was tested through slice superfusion with SCH58261 (50 nM).

Even though the dorsal hippocampus is required for contextual fear conditioning and retrieval (Matus-Amat et al., 2004), we did not observe any significant change in the IO curves or in the LTP between the 4 experimental groups (Figure 16A-C and Figure 17C-H). Nevertheless, these results are in agreement with previous data from a chronic unpredictable stress (CUS) model in which the authors showed that after a tetanic stimulation there were no differences in the magnitude of the LTP in the dorsal hippocampus between control and stressed rats (Pinto et al., 2015). However, Maggio and Seagal (2011) showed that 1 day after acute stress in adult rats,-LTP in the dorsal hippocampus was decreased but if the eletrophysiological recordings were performed 1 week after the stress protocol this effect of stress was no longer observed. These results of Maggio and Seagal (2011) highlight the importance of the endpoint at which the *ex vivo* electrophysiological analyses are performed in the effects observed. Thus, it is important to emphasize that our data relative to the ACQ and EXT groups were obtained

during an interval of 4-8 days after the contextual fear conditioning, which may explain the lack of differences found between the groups. In addition, we evaluated mechanisms of metaplasticity. This process evaluates the ability of modified synapses to further alter their strength. Thus, it does not directly affect synaptic efficacy but instead leads to changes in the magnitude of the synaptic plasticity, in other words, it is the "plasticity of plasticity" (Citri et al., 2008). Thus, 60 minutes into the LTP it was applied a low frequency stimulation (LFS) protocol to induce depotentiation, i.e. to reverse LTP (Bear et al., 1996). In the dorsal hippocampus, depotentiation was only observed in slices from the EXT group (Figure 18C and 18E) which suggests that the dorsal hippocampal circuits of this group are more prone to be altered, which may facilitate new learning and memory. Unfortunately, this protocol was not performed on slices from naïve mice and therefore a comparison with a system that was not previously modified by an aversive experience cannot be made. Curiously, superfusion of slices with SCH58261 did not alter depotentiation in all of the groups (Figure 18) but the EXT+SCHip group did not display depotentiation unlike the EXT group (Figure 18D and 18E) which suggests that the i.p. injection of the $A_{2A}R$ before extinction somehow modified the synapses in the dorsal hippocampus.

On the other hand, despite the variable times after the last memory test at which animals were sacrificed, the ventral hippocampal slices from the ACQ group had significantly higher LTPs comparing to slices from naïve mice (Figure 16E-F). Importantly, all of the other groups displayed LTPs that were equal to the CTR group (Figure 16E-F and Figure 19F) Supporting our results, Maggio et al.(2011) and Pinto et al.(2015), also observed an increase of the amplitude of the LTP in the ventral hippocampus of stressed rats. These data suggest that aversive experiences induce stable and long-lasting modifications in the synaptic strength of ventral hippocampal circuits. Interestingly, SCH58261, applied acutely on slices, was able to modify the LTP only in the dorsal hippocampus (and not in the ventral) of naïve mice (Figure 15B and E) but was still able to normalize the LTP at the ventral hippocampal slices from the ACQ group (Figure 19D and H). These results suggest a gain of function of the $A_{2A}R$ during the acquisition and consolidation of contextual fear memories in the ventral hippocampus, leading to modifications in the neuronal network and subsequently to an increase of synaptic plasticity. A possible mechanism underlying this gain of function of $A_{2A}R$ is through neurogenesis triggered by contextual fear conditioning. Indeed, it was shown that a genetic model of impaired neurogenesis in the hippocampus lead to deficient contextual memory (Mateus-Pinheiro et al., 2016). Furthermore Pinto et al. (2015) showed that chronic unpredictable stress enlarges the volume of the ventral hippocampus concomitantly with an increase in the length of the CA3 apical dendrites. Additionally, Reverse et al., 2009, showed that

deficits in hippocampal neurogenesis increased anxiety-related behaviors. These results evidenced the relevance of adult neurogenesis in memory and in anxiety-related disorders. Likewise, overexpression of $A_{2A}R$ leads to memory impairments (Giménez-Llort et al., 2007) and depressive-like behaviors (Coelho et al., 2014). Thus, it is possible that during contextual fear conditioning there is an increase in neurogenesis that is accompanied by a boost of $A_{2A}R$ activity. This suggestion could explain the gain of function of the $A_{2A}R$ during contextual fear conditioning but, to test the veracity of this hypothesis, further studies are required.

On the other hand, none of the experimental groups displayed depotentiation from the LTP in the ventral hippocampus (Figure 20A-E). Also, superfusion of ventral hippocampal slices with SCH58261 did not modify depotentiation in any of these groups (Figure 20A-C). Noteworthy, in the group of animals injected with SCH58261 (EXT+SCHip) the LFS caused potentiation instead of depotentiation (Figure 20D and E). Although it is unexpected, this result is not odd. For the past years a series of reports have been questioning if the "HFS-LTP, LFS-LTD" dichotomy truly captures the entire spectrum and direction of synaptic modifications. In fact, Thomas et al., 1996 showed that under the activation of the β-adrenergic receptor, LFS could elicit LTP using a 5Hz stimulation protocol. Moreover, Lanté et al., 2006, demonstrated that in physiological conditions, a low frequency protocol of 1Hz for 5min can trigger a slow-onset long-term potentiation, $mGlu_5R$-dependent, at the CA1 synapses (Lanté et al., 2006 a,b). Concomitantly, Habib and Dringenberg, 2009, showed that LFS in the commissural and medial septal afferents induced LTP in the CA1 region that was NMDAR-dependent. These differences in the mechanism underlying LFS-LTP indicate the existence of several forms of LFS-induced synaptic potentiation, however, there is not a clear understanding neither of the cellular and molecular mechanism mediating these forms of plasticity nor of its physiological relevance.

Finally, electrophysiological recordings were performed at the LA-BLA pathway of the amygdala. To the best of our knowledge, there is no previous data demonstrating the effects of the contextual fear conditioning on this pathway. Most studies have focused in the long-term synaptic modification of afferent inputs to the amygdala, since these pathways are critical for auditory (or cued) fear conditioning (Rogan et al., 1997McKernan et al., 1997; Stevens, 1998, Maren, 1999; Tsvetkov et al.,2002 a,b). Thus, it has been shown that the auditory fear conditioning occludes the LTP in the cortico-amygdala pathway (that conveys input from the auditory cortex to the LA) (Tsevetkov et al., 2002a), and that leads to a synaptic enhancement in the thalamo-amygdala pathway (whereby auditory information reaches the LA through the auditory thalamus) (Rogan et al., 1997; McKernan et al., 1997). In contextual fear

conditioning the hippocampus has been shown to have a more prominent influence. Indeed, the dorsal hippocampus has the ability to modulate the context-specific firing of neurons in the lateral amygdala (Maren et al., 2007) and the ventral hippocampus has projections to the baso-amygdala that are required for contextual fear memory retrieval (Xu et al., 2016). Since we did not have the tools to study the hippocampal-amygdala pathway and since the internuclear excitatory connections in the amygdala are also essential to the acquisition and extinction of fear memories (Duvarci et al., 2014; Radley et al.,2015), we decided to do extracellular recording in this intra-amygdalar (LA→BLA) pathway. Due to the scarce number of studies in this pathway, it was necessary to optimize a protocol of high frequency stimulation (HFS) that would induce a robust LTP. The only protocol that was able to produce a consistent and stable potentiation was one consisting in 5 trains of HFS (100Hz, 30s of inter-train interval). Using this protocol, we observed a decrease of the amplitude of the LTP in the amygdala of both ACQ and EXT mice when compared to the CTR (Figure 16H-I) suggesting changes in the synaptic strength of these synapses in animals that underwent contextual fear conditioning. Interestingly, the IO curves in the amygdala of the ACQ group were significantly higher than the curves of the EXT and CTR groups (Figure 16G), suggesting increased neuronal excitability within this microcircuit. In a similar way that was observed for the ventral hippocampus, this result also has a parallel with those obtained in models of stress. According to previous studies, during stress there is an increase of the spontaneous firing of neurons in the BLA (Zhang et al.,2012), an increase in the dendritic arborization of BLA pyramidal neurons (Vyas et al., 2002) and a robust increase in the spine density of the BLA spiny neurons (Mitra et al., 2005), pointing towards a long lasting effect of stress in the basal excitatory activity of principal neurons in the BLA. Furthermore, in human imaging studies it was demonstrated that individuals that suffer from depression and anxiety disorders have increased amygdalar activity and volume and this hyperactivity of the amygdala may be a key contributor to many neuropsychiatric disorders, such as anxiety and PTSD (Radley et al., 2015). Knowing this, we propose that the observed increase of the basal transmission in the ACQ may be due to the formation of new dendritic spines in the BLA which is suggested to be a locus of storage for fear memories (Mitra et al.,2005; Christoffel et al.,2011); however further studies are necessary to corroborate this hypothesis. Nevertheless, *ex vivo*, SCH58261 was not able to modify the I/O curves of the ACQ group (Figure 21A) nor the LTP of any of the groups (Figure 21D-H) with the exception of the naïve mice (Figure 15H).

On the other hand, in terms of metaplasticity, only the LTPs in slices from the EXT group were able to depotentiate from LTP upon LFS, which could mean that the neuronal circuit in this group is more flexible.

Despite the lack of evidence, given that it is in the amygdala that extinction learning is initially encoded, it would be interesting to perform*in vivo* extracellular recordings using chronic recording eletrodes in the BLA and compare the contextual-evoked spike firing in control mice *versus* mice that were intraperitoneally injected with the $A_{2A}R$ antagonist (detailed method described in Herry et al.,2008 and Ciocchi et al.,2010).

5

CONCLUSION

In the present study, it was shown that the global pharmacological blockade of adenosine A_{2A} receptors ($A_{2A}R$) during contextual fear extinction accelerated this process. Furthermore, our results suggest that this happens through $A_{2A}R$-mediated modulation of long-term synaptic plasticity (LTP) in the ventral hippocampus since: 1) contextual fear conditioning increases the amplitude of LTP in the ventral hippocampus; 2) fear extinction normalizes the amplitude of LTP in this brain region and 3) superfusion of slices from the ventral hippocampus of fear conditioned mice with the selective antagonist of $A_{2A}R$ (SCH58261) also normalizes LTP.

BIBLIOGRAPHY

Agren, T., J. Engman, A. Frick, J. Bjorkstrand, E. M. Larsson, T. Furmark and M. Fredrikson (2012). "Disruption of reconsolidation erases a fear memory trace in the human amygdala." Science 337(6101): 1550-1552.

Albrechet-Souza, L., M. C. Carvalho and M. L. Brandao (2013). "D(1)-like receptors in the nucleus accumbens shell regulate the expression of contextual fear conditioning and activity of the anterior cingulate cortex in rats." Int J Neuropsychopharmacol 16(5): 1045-1057.

Andersen, P. and T. Lomo (1967). "Control of hippocampal output by afferent volley frequency." Prog Brain Res 27: 400-412.

Atkins, C. M., J. C. Selcher, J. J. Petraitis, J. M. Trzaskos and J. D. Sweatt (1998). "The MAPK cascade is required for mammalian associative learning." Nat Neurosci 1(7): 602-609.

Augusto, E., M. Matos, J. Sevigny, A. El-Tayeb, M. S. Bynoe, C. E. Muller, R. A. Cunha and J. F. Chen (2013). "Ecto-5'-nucleotidase (CD73)-mediated formation of adenosine is critical for the striatal adenosine A2A receptor functions." J Neurosci 33(28): 11390-11399.

Bari, A., T. Niu, J. P. Langevin and I. Fried (2014). "Limbic neuromodulation: implications for addiction, posttraumatic stress disorder, and memory." Neurosurg Clin N Am 25(1): 137-145.

Bauer, E. P., G. E. Schafe and J. E. LeDoux (2002). "NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala." J Neurosci 22(12): 5239-5249.

Bear, M. F. and W. C. Abraham (1996). "Long-term depression in hippocampus." Annu Rev Neurosci 19: 437-462.

Bender, R. A., L. Zhou, R. Vierk, N. Brandt, A. Keller, C. E. Gee, M. K. Schafer and G. M. Rune (2017). "Sex-Dependent Regulation of Aromatase-Mediated Synaptic Plasticity in the Basolateral Amygdala." J Neurosci 37(6): 1532-1545.

Bentz, D. and D. Schiller (2015). "Threat processing: models and mechanisms." Wiley Interdiscip Rev Cogn Sci 6(5): 427-439.

Bliss, T. V. and G. L. Collingridge (1993). "A synaptic model of memory: long-term potentiation in the hippocampus." Nature 361(6407): 31-39.

Bliss, T. V. and S. F. Cooke (2011). "Long-term potentiation and long-term depression: a clinical perspective." Clinics (Sao Paulo) 66 Suppl 1: 3-17.

Blumenfeld, R. S. and C. Ranganath (2006). "Dorsolateral prefrontal cortex promotes long-term memory formation through its role in working memory organization." J Neurosci 26(3): 916-925.

Botton, P. H., D. Pochmann, A. S. Rocha, F. Nunes, A. S. Almeida, D. M. Marques and L. O. Porciuncula (2017). "Aged mice receiving caffeine since adulthood show distinct patterns of anxiety-related behavior." Physiol Behav 170: 47-53.

Burgos-Robles, A., I. Vidal-Gonzalez and G. J. Quirk (2009). "Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure." J Neurosci 29(26): 8474-8482.

Burnstock, G. (1972). "Purinergic nerves." Pharmacol Rev 24(3): 509-581.

Burnstock, G. (1976). "Do some nerve cells release more than one transmitter?" Neuroscience 1(4): 239-248.

Burnstock, G. (2007). "Physiology and pathophysiology of purinergic neurotransmission." Physiol Rev 87(2): 659-797.

Burnstock, G. (2008). "Purinergic signalling and disorders of the central nervous system." Nat Rev Drug Discov 7(7): 575-590.

Burnstock, G., G. Campbell, D. Satchell and A. Smythe (1970). "Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut." Br J Pharmacol 40(4): 668-688.

Caetano, L., H. Pinheiro, P. Patricio, A. Mateus-Pinheiro, N. D. Alves, B. Coimbra, F. I. Baptista, S. N. Henriques, C. Cunha, A. R. Santos, S. G. Ferreira, V. M. Sardinha, J. F. Oliveira, A. F. Ambrosio, N. Sousa, R. A. Cunha, A. J. Rodrigues, L. Pinto and C. A. Gomes (2017). "Adenosine A2A receptor regulation of microglia morphological remodeling-gender bias in physiology and in a model of chronic anxiety." Mol Psychiatry 22(7): 1035-1043.

Canas, P. M., L. O. Porciuncula, G. M. Cunha, C. G. Silva, N. J. Machado, J. M. Oliveira, C. R. Oliveira and R. A. Cunha (2009). "Adenosine A2A receptor blockade prevents synaptotoxicity and memory dysfunction caused by beta-amyloid peptides via p38 mitogen-activated protein kinase pathway." J Neurosci 29(47): 14741-14751.

Careaga, M. B., C. E. Girardi and D. Suchecki (2016). "Understanding posttraumatic stress disorder through fear conditioning, extinction and reconsolidation." Neurosci Biobehav Rev 71: 48-57.

Carola, V., F. D'Olimpio, E. Brunamonti, F. Mangia and P. Renzi (2002). "Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice." Behav Brain Res 134(1-2): 49-57.

Cenquizca, L. A. and L. W. Swanson (2007). "Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex." Brain Res Rev 56(1): 1-26.

Chagoya de Sanchez, V. (1995). "Circadian variations of adenosine and of its metabolism. Could adenosine be a molecular oscillator for circadian rhythms?" Can J Physiol Pharmacol 73(3): 339-355.

Chapman, P. F. and L. L. Bellavance (1992). "Induction of long-term potentiation in the basolateral amygdala does not depend on NMDA receptor activation." Synapse 11(4): 310-318.

Chapman, P. F., M. F. Ramsay, W. Krezel and S. G. Knevett (2003). "Synaptic plasticity in the amygdala: comparisons with hippocampus." Ann N Y Acad Sci 985: 114-124.

Chen, A., W. W. Hu, X. L. Jiang, M. Potegal and H. Li (2017). "Molecular mechanisms of group I metabotropic glutamate receptor mediated LTP and LTD in basolateral amygdala in vitro." Psychopharmacology (Berl) 234(4): 681-694.

Chen, J. F., H. K. Eltzschig and B. B. Fredholm (2013). "Adenosine receptors as drug targets--what are the challenges?" Nat Rev Drug Discov 12(4): 265-286.

Chen, J.-F. (2014). Chapter Twelve - Adenosine Receptor Control of Cognition in Normal and Disease. International Review of Neurobiology. M. Akihisa, Academic Press. Volume 119: 257-307.

Chhatwal, J. P., L. Stanek-Rattiner, M. Davis and K. J. Ressler (2006). "Amygdala BDNF signaling is required for consolidation but not encoding of extinction." Nat Neurosci 9(7): 870-872.

Cho, J. H., S. D. Rendall and J. M. Gray (2017). "Brain-wide maps of Fos expression during fear learning and recall." Learn Mem 24(4): 169-181.

Christoffel, D. J., S. A. Golden and S. J. Russo (2011). "Structural and synaptic plasticity in stress-related disorders." Rev Neurosci 22(5): 535-549.

Ciocchi, S., C. Herry, F. Grenier, S. B. Wolff, J. J. Letzkus, I. Vlachos, I. Ehrlich, R. Sprengel, K. Deisseroth, M. B. Stadler, C. Muller and A. Luthi (2010). "Encoding of conditioned fear in central amygdala inhibitory circuits." Nature 468(7321): 277-282.

Citri, A. and R. C. Malenka (2008). "Synaptic plasticity: multiple forms, functions, and mechanisms." Neuropsychopharmacology 33(1): 18-41.

Clem, R. L. and R. L. Huganir (2010). "Calcium-permeable AMPA receptor dynamics mediate fear memory erasure." Science 330(6007): 1108-1112.

Coelho, J. E., P. Alves, P. M. Canas, J. S. Valadas, T. Shmidt, V. L. Batalha, D. G. Ferreira, J. A. Ribeiro, M. Bader, R. A. Cunha, F. S. do Couto and L. V. Lopes (2014). "Overexpression of Adenosine A2A Receptors in Rats: Effects on Depression, Locomotion, and Anxiety." Front Psychiatry 5: 67.

Cognato, G. P., P. M. Agostinho, J. Hockemeyer, C. E. Muller, D. O. Souza and R. A. Cunha (2010). "Caffeine and an adenosine A(2A) receptor antagonist prevent memory impairment and synaptotoxicity in adult rats triggered by a convulsive episode in early life." J Neurochem 112(2): 453-462.

Cohen, H., T. Liu, N. Kozlovsky, Z. Kaplan, J. Zohar and A. A. Mathe (2012). "The neuropeptide Y (NPY)-ergic system is associated with behavioral resilience to stress exposure in an animal model of post-traumatic stress disorder." Neuropsychopharmacology 37(2): 350-363.

Colgan, S. P., H. K. Eltzschig, T. Eckle and L. F. Thompson (2006). "Physiological roles for ecto-5'-nucleotidase (CD73)." Purinergic Signal 2(2): 351-360.

Corbit, L. H., S. C. Fischbach and P. H. Janak (2016). "Nucleus accumbens core and shell are differentially involved in general and outcome-specific forms of Pavlovian-instrumental transfer with alcohol and sucrose rewards." Eur J Neurosci 43(9): 1229-1236.

Corcoran, K. A., T. J. Desmond, K. A. Frey and S. Maren (2005). "Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction." J Neurosci 25(39): 8978-8987.

Costa, M. S., A. P. Ardais, G. T. Fioreze, S. Mioranzza, P. H. Botton, L. V. Portela, D. O. Souza and L. O. Porciuncula (2012). "Treadmill running frequency on anxiety and hippocampal adenosine receptors density in adult and middle-aged rats." Prog Neuropsychopharmacol Biol Psychiatry 36(1): 198-204.

Costenla, A. R., A. de Mendonca and J. A. Ribeiro (1999). "Adenosine modulates synaptic plasticity in hippocampal slices from aged rats." Brain Res 851(1-2): 228-234.

Costenla, A. R., M. J. Diogenes, P. M. Canas, R. J. Rodrigues, C. Nogueira, J. Maroco, P. M. Agostinho, J. A. Ribeiro, R. A. Cunha and A. de Mendonca (2011). "Enhanced role of adenosine A(2A) receptors in the modulation of LTP in the rat hippocampus upon ageing." Eur J Neurosci 34(1): 12-21.

Costenla, A. R., R. A. Cunha and A. de Mendonca (2010). "Caffeine, adenosine receptors, and synaptic plasticity." J Alzheimers Dis 20 Suppl 1: S25-34.

Cowan, N. (2008). "What are the differences between long-term, short-term, and working memory?" Prog Brain Res 169: 323-338.

Cox, J. and R. F. Westbrook (1994). "The NMDA receptor antagonist MK-801 blocks acquisition and extinction of conditioned hypoalgesic responses in the rat." Q J Exp Psychol B 47(2): 187-210.

Cunha, R. A. (2001). "Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors." Neurochem Int 38(2): 107-125.

Cunha, R. A. (2008). "Different cellular sources and different roles of adenosine: A1 receptor-mediated inhibition through astrocytic-driven volume transmission and synapse-restricted A2A receptor-mediated facilitation of plasticity." Neurochem Int 52(1-2): 65-72.

Cunha, R. A. (2016). "How does adenosine control neuronal dysfunction and neurodegeneration?" J Neurochem 139(6): 1019-1055.

Cunha, R. A. and P. M. Agostinho (2010). "Chronic caffeine consumption prevents memory disturbance in different animal models of memory decline." J Alzheimers Dis 20 Suppl 1: S95-116.

Cunha, R. A., P. Correia-de-Sa, A. M. Sebastiao and J. A. Ribeiro (1996). "Preferential activation of excitatory adenosine receptors at rat hippocampal and neuromuscular synapses by adenosine formed from released adenine nucleotides." Br J Pharmacol 119(2): 253-260.

Cunha, R. A., S. Ferre, J. M. Vaugeois and J. F. Chen (2008). "Potential therapeutic interest of adenosine A2A receptors in psychiatric disorders." Curr Pharm Des 14(15): 1512-1524.

Dale Purves, G. J. A., David Fitzpatrick, Lawrence C Katz, Anthony-Samuel LaMantia, James O McNamara, and S Mark Williams. (2004). Neuroscience. Sunderland, MA,USA, Sinauer Association Inc.

Davies, M. F., J. Tsui, J. A. Flannery, X. Li, T. M. DeLorey and B. B. Hoffman (2004). "Activation of alpha2 adrenergic receptors suppresses fear conditioning: expression of c-Fos and phosphorylated CREB in mouse amygdala." Neuropsychopharmacology 29(2): 229-239.

De Mendonça, A. and J. A. Ribeiro (2001). "Adenosine and synaptic plasticity." Drug Development Research 52(1-2): 283-290.

Deguchi, Y., F. Donato, I. Galimberti, E. Cabuy and P. Caroni (2011). "Temporally matched subpopulations of selectively interconnected principal neurons in the hippocampus." Nat Neurosci 14(4): 495-504.

DeMet, E., M. K. Stein, C. Tran, A. Chicz-DeMet, C. Sangdahl and J. Nelson (1989). "Caffeine taste test for panic disorder: adenosine receptor supersensitivity." Psychiatry Res 30(3): 231-242.

Deng, W., J. B. Aimone and F. H. Gage (2010). "New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory?" Nat Rev Neurosci 11(5): 339-350.

Deppermann, S., H. Storchak, A. J. Fallgatter and A. C. Ehlis (2014). "Stress-induced neuroplasticity: (mal)adaptation to adverse life events in patients with PTSD--a critical overview." Neuroscience 283: 166-177.

Diamond, D. M., T. V. Dunwiddie and G. M. Rose (1988). "Characteristics of hippocampal primed burst potentiation in vitro and in the awake rat." J Neurosci 8(11): 4079-4088.

Dias, R. B., J. A. Ribeiro and A. M. Sebastiao (2012). "Enhancement of AMPA currents and GluR1 membrane expression through PKA-coupled adenosine A(2A) receptors." Hippocampus 22(2): 276-291.

Dickinson-Anson, H. and J. L. McGaugh (1993). "Midazolam administered into the amygdala impairs retention of an inhibitory avoidance task." Behav Neural Biol 60(1): 84-87.

Dityatev, A. E. and V. Y. Bolshakov (2005). "Amygdala, long-term potentiation, and fear conditioning." Neuroscientist 11(1): 75-88.

Do-Monte, F. H., K. Quinones-Laracuente and G. J. Quirk (2015). "A temporal shift in the circuits mediating retrieval of fear memory." Nature 519(7544): 460-463.

Drury, A. N. and A. Szent-Gyorgyi (1929). "The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart." J Physiol 68(3): 213-237.

Dunwiddie, T. V. and S. A. Masino (2001). "The role and regulation of adenosine in the central nervous system." Annu Rev Neurosci 24: 31-55.

Duvarci, S. and D. Pare (2014). "Amygdala microcircuits controlling learned fear." Neuron 82(5): 966-980.

Dziubina, A., K. Szmyd, M. Zygmunt, J. Sapa, M. Dudek, B. Filipek, A. Drabczynska, M. Zaluski, K. Pytka and K. Kiec-Kononowicz (2016). "Evaluation of antidepressant-like and anxiolytic-like activity of purinedione-derivatives with affinity for adenosine A2A receptors in mice." Pharmacol Rep 68(6): 1285-1292.

Ehrlich, I., Y. Humeau, F. Grenier, S. Ciocchi, C. Herry and A. Luthi (2009). "Amygdala inhibitory circuits and the control of fear memory." Neuron 62(6): 757-771.

Eisenberg, M., T. Kobilo, D. E. Berman and Y. Dudai (2003). "Stability of retrieved memory: inverse correlation with trace dominance." Science 301(5636): 1102-1104.

El Yacoubi, M., C. Ledent, M. Parmentier, J. Costentin and J. Vaugeois (2000). "SCH 58261 and ZM 241385 differentially prevent the motor effects of CGS 21680 in mice: evidence for a functional 'atypical' adenosine A(2A) receptor." Eur J Pharmacol 401(1): 63-77.

El Yacoubi, M., C. Ledent, M. Parmentier, R. Bertorelli, E. Ongini, J. Costentin and J. M. Vaugeois (2001). "Adenosine A2A receptor antagonists are potential antidepressants: evidence based on pharmacology and A2A receptor knockout mice." Br J Pharmacol 134(1): 68-77.

Fadok, J. P., S. Krabbe, M. Markovic, J. Courtin, C. Xu, L. Massi, P. Botta, K. Bylund, C. Muller, A. Kovacevic, P. Tovote and A. Luthi (2017). "A competitive inhibitory circuit for selection of active and passive fear responses." Nature 542(7639): 96-100.

Fanselow, M. S. (2000). "Contextual fear, gestalt memories, and the hippocampus." Behav Brain Res 110(1-2): 73-81.

Fanselow, M. S. (2010). "From contextual fear to a dynamic view of memory systems." Trends Cogn Sci 14(1): 7-15.

Fanselow, M. S. and H. W. Dong (2010). "Are the dorsal and ventral hippocampus functionally distinct structures?" Neuron 65(1): 7-19.

Flavell, C. R., E. A. Lambert, B. D. Winters and T. W. Bredy (2013). "Mechanisms governing the reactivation-dependent destabilization of memories and their role in extinction." Front Behav Neurosci 7: 214.

Flores, A., R. Saravia, R. Maldonado and F. Berrendero (2015). "Orexins and fear: implications for the treatment of anxiety disorders." Trends Neurosci 38(9): 550-559.

Fournier, N. M. and R. S. Duman (2013). "Illuminating hippocampal control of fear memory and anxiety." Neuron 77(5): 803-806.

Fredholm, B. B., J. F. Chen, R. A. Cunha, P. Svenningsson and J. M. Vaugeois (2005). "Adenosine and brain function." Int Rev Neurobiol 63: 191-270.

Frysztak, R. J. and E. J. Neafsey (1991). "The effect of medial frontal cortex lesions on respiration, "freezing," and ultrasonic vocalizations during conditioned emotional responses in rats." Cereb Cortex 1(5): 418-425.

Furlong, T. M., R. Richardson and G. P. McNally (2016). "Habituation and extinction of fear recruit overlapping forebrain structures." Neurobiol Learn Mem 128: 7-16.

Furness, J. B. (2000). "Types of neurons in the enteric nervous system." J Auton Nerv Syst 81(1-3): 87-96.

Gale, G. D., S. G. Anagnostaras, B. P. Godsil, S. Mitchell, T. Nozawa, J. R. Sage, B. Wiltgen and M. S. Fanselow (2004). "Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats." J Neurosci 24(15): 3810-3815.

Gao, Y., H. Han, R. Xu, J. Cao, J. Luo and L. Xu (2008). "Effects of prolonged exposure to context following contextual fear conditioning on synaptic properties in rat hippocampal slices." Neurosci Res 61(4): 385-389.

George, S. A., M. Rodriguez-Santiago, J. Riley, J. L. Abelson, S. B. Floresco and I. Liberzon (2015). "Alterations in cognitive flexibility in a rat model of post-traumatic stress disorder." Behav Brain Res 286: 256-264.

Geuze, E., B. N. van Berckel, A. A. Lammertsma, R. Boellaard, C. S. de Kloet, E. Vermetten and H. G. Westenberg (2008). "Reduced GABAA benzodiazepine receptor binding in veterans with post-traumatic stress disorder." Mol Psychiatry 13(1): 74-83, 73.

Gewirtz, J. C., K. A. McNish and M. Davis (2000). "Is the hippocampus necessary for contextual fear conditioning?" Behav Brain Res 110(1-2): 83-95.

Gillespie, C. F., B. Bradley, K. Mercer, A. K. Smith, K. Conneely, M. Gapen, T. Weiss, A. C. Schwartz, J. F. Cubells and K. J. Ressler (2009). "Trauma exposure and stress-related disorders in inner city primary care patients." Gen Hosp Psychiatry 31(6): 505-514.

Gilmartin, M. R. and F. J. Helmstetter (2010). "Trace and contextual fear conditioning require neural activity and NMDA receptor-dependent transmission in the medial prefrontal cortex." Learn Mem 17(6): 289-296.

Giustino, T. F. and S. Maren (2015). "The Role of the Medial Prefrontal Cortex in the Conditioning and Extinction of Fear." Front Behav Neurosci 9: 298.

Gomes, C. V., M. P. Kaster, A. R. Tome, P. M. Agostinho and R. A. Cunha (2011). "Adenosine receptors and brain diseases: neuroprotection and neurodegeneration." Biochim Biophys Acta 1808(5): 1380-1399.

Gonul, A. S., O. Kitis, M. C. Eker, O. D. Eker, E. Ozan and K. Coburn (2011). "Association of the brain-derived neurotrophic factor Val66Met polymorphism with hippocampus volumes in drug-free depressed patients." World J Biol Psychiatry 12(2): 110-118.

Goosens, K. A. and S. Maren (2003). "Pretraining NMDA receptor blockade in the basolateral complex, but not the central nucleus, of the amygdala prevents savings of conditional fear." Behav Neurosci 117(4): 738-750.

Goshen, I., M. Brodsky, R. Prakash, J. Wallace, V. Gradinaru, C. Ramakrishnan and K. Deisseroth (2011). "Dynamics of retrieval strategies for remote memories." Cell 147(3): 678-689.

Gould, T. D., D. T. Dao and C. E. Kovacsics (2009). "Mood and Anxiety Related Phenotypes in Mice." Neuromethods 42: 1-20.

Graff, J., N. F. Joseph, M. E. Horn, A. Samiei, J. Meng, J. Seo, D. Rei, A. W. Bero, T. X. Phan, F. Wagner, E. Holson, J. Xu, J. Sun, R. L. Neve, R. H. Mach, S. J. Haggarty and L. H. Tsai (2014). "Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories." Cell 156(1-2): 261-276.

Green, P. J. and J. Suls (1996). "The effects of caffeine on ambulatory blood pressure, heart rate, and mood in coffee drinkers." J Behav Med 19(2): 111-128.

Gruene, T., K. Flick, S. Rendall, J. H. Cho, J. Gray and R. Shansky (2016). "Activity-dependent structural plasticity after aversive experiences in amygdala and auditory cortex pyramidal neurons." Neuroscience 328: 157-164.

Guire, E. S., M. C. Oh, T. R. Soderling and V. A. Derkach (2008). "Recruitment of calcium-permeable AMPA receptors during synaptic potentiation is regulated by CaM-kinase I." J Neurosci 28(23): 6000-6009.

Habib, D. and H. C. Dringenberg (2009). "Alternating low frequency stimulation of medial septal and commissural fibers induces NMDA-dependent, long-lasting potentiation of hippocampal synapses in urethane-anesthetized rats." Hippocampus 19(3): 299-307.

Habib, D. and H. C. Dringenberg (2010). "Low-frequency-induced synaptic potentiation: a paradigm shift in the field of memory-related plasticity mechanisms?" Hippocampus 20(1): 29-35.

Habib, D., C. K. Tsui, L. G. Rosen and H. C. Dringenberg (2014). "Occlusion of low-frequency-induced, heterosynaptic long-term potentiation in the rat hippocampus in vivo following spatial training." Cereb Cortex 24(11): 3090-3096.

Hafting, T., M. Fyhn, S. Molden, M. B. Moser and E. I. Moser (2005). "Microstructure of a spatial map in the entorhinal cortex." Nature 436(7052): 801-806.

Hall, E. (1972). "The amygdala of the cat: a Golgi study." Z Zellforsch Mikrosk Anat 134(4): 439-458.

Hall, J., K. L. Thomas and B. J. Everitt (2001). "Fear memory retrieval induces CREB phosphorylation and Fos expression within the amygdala." Eur J Neurosci 13(7): 1453-1458.

Hamilton, S. P., S. L. Slager, A. B. De Leon, G. A. Heiman, D. F. Klein, S. E. Hodge, M. M. Weissman, A. J. Fyer and J. A. Knowles (2004). "Evidence for genetic linkage between a polymorphism in the adenosine 2A receptor and panic disorder." Neuropsychopharmacology 29(3): 558-565.

Harris, J. A. (1998). "Using c-fos as a neural marker of pain." Brain Res Bull 45(1): 1-8.

Harris, J. A. and R. F. Westbrook (1995). "Effects of benzodiazepine microinjection into the amygdala or periaqueductal gray on the expression of conditioned fear and hypoalgesia in rats." Behav Neurosci 109(2): 295-304.

Haskell, C. F., D. O. Kennedy, K. A. Wesnes and A. B. Scholey (2005). "Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine." Psychopharmacology (Berl) 179(4): 813-825.

Heldt, S. A., L. Stanek, J. P. Chhatwal and K. J. Ressler (2007). "Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories." Mol Psychiatry 12(7): 656-670.

Herry, C. and N. Mons (2004). "Resistance to extinction is associated with impaired immediate early gene induction in medial prefrontal cortex and amygdala." Eur J Neurosci 20(3): 781-790.

Herry, C., S. Ciocchi, V. Senn, L. Demmou, C. Muller and A. Luthi (2008). "Switching on and off fear by distinct neuronal circuits." Nature 454(7204): 600-606.

Hobin, J. A., K. A. Goosens and S. Maren (2003). "Context-dependent neuronal activity in the lateral amygdala represents fear memories after extinction." J Neurosci 23(23): 8410-8416.

Hoffman, A. N., N. G. Lorson, F. Sanabria, M. Foster Olive and C. D. Conrad (2014). "Chronic stress disrupts fear extinction and enhances amygdala and hippocampal Fos expression in an animal model of post-traumatic stress disorder." Neurobiol Learn Mem 112: 139-147.

Hong, I., J. Kim, J. Lee, S. Park, B. Song, J. Kim, B. An, K. Park, H. W. Lee, S. Lee, H. Kim, S. H. Park, K. D. Eom, S. Lee and S. Choi (2011). "Reversible plasticity of fear memory-encoding amygdala synaptic circuits even after fear memory consolidation." PLoS One 6(9): e24260.

Hughes, R. N., N. J. Hancock, G. A. Henwood and S. A. Rapley (2014). "Evidence for anxiolytic effects of acute caffeine on anxiety-related behavior in male and female rats tested with and without bright light." Behav Brain Res 271: 7-15.

Hwang, Y. K., J. C. Song, S. H. Han, J. Cho, D. R. Smith, M. Gallagher and J. S. Han (2010). "Differences in hippocampal CREB phosphorylation in trace fear conditioning of two inbred mouse strains." Brain Res 1345: 156-163.

Introini-Collison, I. B., C. Castellano and J. L. McGaugh (1994). "Interaction of GABAergic and beta-noradrenergic drugs in the regulation of memory storage." Behav Neural Biol 61(2): 150-155.

Izquierdo, I., C. R. Furini and J. C. Myskiw (2016). "Fear Memory." Physiol Rev 96(2): 695-750.

Izumi, T., T. Inoue, A. Kato, Y. Kitaichi, S. Nakagawa and T. Koyama (2008). "Changes in amygdala neural activity that occur with the extinction of context-dependent conditioned fear stress." Pharmacol Biochem Behav 90(3): 297-304.

Jacobson, K. A. and Z. G. Gao (2006). "Adenosine receptors as therapeutic targets." Nat Rev Drug Discov 5(3): 247-264.

Janak, P. H. and K. M. Tye (2015). "From circuits to behaviour in the amygdala." Nature 517(7534): 284-292.

Ji, J. and S. Maren (2007). "Hippocampal involvement in contextual modulation of fear extinction." Hippocampus 17(9): 749-758.

Jimenez, S. A. and S. Maren (2009). "Nuclear disconnection within the amygdala reveals a direct pathway to fear." Learn Mem 16(12): 766-768.

Jin, J. and S. Maren (2015). "Fear renewal preferentially activates ventral hippocampal neurons projecting to both amygdala and prefrontal cortex in rats." Sci Rep 5: 8388.

Johnson, D. C. and B. J. Casey (2015). "Extinction during memory reconsolidation blocks recovery of fear in adolescents." Sci Rep 5: 8863.

Josselyn, S. A., C. Shi, W. A. Carlezon, Jr., R. L. Neve, E. J. Nestler and M. Davis (2001). "Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala." J Neurosci 21(7): 2404-2412.

Josselyn, S. A., S. Kida and A. J. Silva (2004). "Inducible repression of CREB function disrupts amygdala-dependent memory." Neurobiol Learn Mem 82(2): 159-163.

Josselyn, S. A., S. Kohler and P. W. Frankland (2015). "Finding the engram." Nat Rev Neurosci 16(9): 521-534.

Kaster, M. P., N. J. Machado, H. B. Silva, A. Nunes, A. P. Ardais, M. Santana, Y. Baqi, C. E. Muller, A. L. Rodrigues, L. O. Porciuncula, J. F. Chen, A. R. Tome, P. Agostinho, P. M. Canas and R. A. Cunha (2015). "Caffeine acts through neuronal adenosine A2A receptors to prevent mood and memory dysfunction triggered by chronic stress." Proc Natl Acad Sci U S A 112(25): 7833-7838.

Katayama, T., E. Jodo, Y. Suzuki, K. Y. Hoshino, S. Takeuchi and Y. Kayama (2009). "Phencyclidine affects firing activity of basolateral amygdala neurons related to social behavior in rats." Neuroscience 159(1): 335-343.

Kemp, A. and D. Manahan-Vaughan (2007). "Hippocampal long-term depression: master or minion in declarative memory processes?" Trends Neurosci 30(3): 111-118.

Kerr, D. S. and W. C. Abraham (1996). "LTD: many means to how many ends?" Hippocampus 6(1): 30-34.

Keshavarzi, S., R. K. Sullivan, D. J. Ianno and P. Sah (2014). "Functional properties and projections of neurons in the medial amygdala." J Neurosci 34(26): 8699-8715.

Kheirbek, M. A., L. J. Drew, N. S. Burghardt, D. O. Costantini, L. Tannenholz, S. E. Ahmari, H. Zeng, A. A. Fenton and R. Hen (2013). "Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus." Neuron 77(5): 955-968.

Kim, J. J. and M. S. Fanselow (1992). "Modality-specific retrograde amnesia of fear." Science 256(5057): 675-677.

Kim, J. J., J. P. DeCola, J. Landeira-Fernandez and M. S. Fanselow (1991). "N-methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning." Behav Neurosci 105(1): 126-133.

Kim, J., S. Lee, K. Park, I. Hong, B. Song, G. Son, H. Park, W. R. Kim, E. Park, H. K. Choe, H. Kim, C. Lee, W. Sun, K. Kim, K. S. Shin and S. Choi (2007). "Amygdala depotentiation and fear extinction." Proc Natl Acad Sci U S A 104(52): 20955-20960.

Kitamura, T., S. K. Ogawa, D. S. Roy, T. Okuyama, M. D. Morrissey, L. M. Smith, R. L. Redondo and S. Tonegawa (2017). "Engrams and circuits crucial for systems consolidation of a memory." Science 356(6333): 73-78.

Klenowski, P. M., M. J. Fogarty, A. Belmer, P. G. Noakes, M. C. Bellingham and S. E. Bartlett (2015). "Structural and functional characterization of dendritic arbors and GABAergic synaptic inputs on interneurons and principal cells in the rat basolateral amygdala." J Neurophysiol 114(2): 942-957.

Knapska, E. and S. Maren (2009). "Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear." Learn Mem 16(8): 486-493.

Knapska, E., M. Macias, M. Mikosz, A. Nowak, D. Owczarek, M. Wawrzyniak, M. Pieprzyk, I. A. Cymerman, T. Werka, M. Sheng, S. Maren, J. Jaworski and L. Kaczmarek (2012). "Functional anatomy of neural circuits regulating fear and extinction." Proc Natl Acad Sci U S A 109(42): 17093-17098.

Krettek, J. E. and J. L. Price (1978). "A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections." J Comp Neurol 178(2): 255-280.

Krugel, U. (2016). "Purinergic receptors in psychiatric disorders." Neuropharmacology 104: 212-225.

Kwon, O. B., J. H. Lee, H. J. Kim, S. Lee, S. Lee, M. J. Jeong, S. J. Kim, H. J. Jo, B. Ko, S. Chang, S. K. Park, Y. B. Choi, C. H. Bailey, E. R. Kandel and J. H. Kim (2015). "Dopamine Regulation of Amygdala Inhibitory Circuits for Expression of Learned Fear." Neuron 88(2): 378-389.

Lalumiere, R. T. (2014). "Optogenetic dissection of amygdala functioning." Front Behav Neurosci 8: 107.

Lam, P., C. J. Hong and S. J. Tsai (2005). "Association study of A2a adenosine receptor genetic polymorphism in panic disorder." Neurosci Lett 378(2): 98-101.

Lange, M. D., M. Doengi, J. Lesting, H. C. Pape and K. Jungling (2012). "Heterosynaptic long-term potentiation at interneuron-principal neuron synapses in the amygdala requires nitric oxide signalling." J Physiol 590(1): 131-143.

Lante, F., M. C. de Jesus Ferreira, J. Guiramand, M. Recasens and M. Vignes (2006). "Low-frequency stimulation induces a new form of LTP, metabotropic glutamate (mGlu5) receptor- and PKA-dependent, in the CA1 area of the rat hippocampus." Hippocampus 16(4): 345-360.

Lebois, L. A., J. D. Wolff and K. J. Ressler (2016). "Neuroimaging genetic approaches to Posttraumatic Stress Disorder." Exp Neurol 284(Pt B): 141-152.

Lebow, M. A. and A. Chen (2016). "Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders." Mol Psychiatry 21(4): 450-463.

Ledent, C., J. M. Vaugeois, S. N. Schiffmann, T. Pedrazzini, M. El Yacoubi, J. J. Vanderhaeghen, J. Costentin, J. K. Heath, G. Vassart and M. Parmentier (1997). "Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor." Nature 388(6643): 674-678.

LeDoux, J. (2007). "The amygdala." Curr Biol 17(20): R868-874.

LeDoux, J. E. (2000). "Emotion circuits in the brain." Annu Rev Neurosci 23: 155-184.

LeDoux, J. E., P. Cicchetti, A. Xagoraris and L. M. Romanski (1990). "The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning." J Neurosci 10(4): 1062-1069.

Lee, K. S., M. Reddington, P. Schubert and G. Kreutzberg (1983). "Regulation of the strength of adenosine modulation in the hippocampus by a differential distribution of the density of A1 receptors." Brain Res 260(1): 156-159.

Lei, Z., B. Liu and J. H. Wang (2016). "Reward memory relieves anxiety-related behavior through synaptic strengthening and protein kinase C in dentate gyrus." Hippocampus 26(4): 502-516.

Li, H., M. A. Penzo, H. Taniguchi, C. D. Kopec, Z. J. Huang and B. Li (2013). "Experience-dependent modification of a central amygdala fear circuit." Nat Neurosci 16(3): 332-339.

Li, P., D. Rial, P. M. Canas, J. H. Yoo, W. Li, X. Zhou, Y. Wang, G. J. van Westen, M. P. Payen, E. Augusto, N. Goncalves, A. R. Tome, Z. Li, Z. Wu, X. Hou, Y. Zhou, P. I. A, E. S. Boyden, R. A. Cunha, J. Qu and J. F. Chen (2015). "Optogenetic activation of intracellular adenosine A2A receptor signaling in the hippocampus is sufficient to trigger CREB phosphorylation and impair memory." Mol Psychiatry 20(11): 1481.

Li, X. G., P. Somogyi, A. Ylinen and G. Buzsaki (1994). "The hippocampal CA3 network: an in vivo intracellular labeling study." J Comp Neurol 339(2): 181-208.

Likhtik, E., D. Popa, J. Apergis-Schoute, G. A. Fidacaro and D. Pare (2008). "Amygdala intercalated neurons are required for expression of fear extinction." Nature 454(7204): 642-645.

Lin, C. H., S. H. Yeh, T. H. Leu, W. C. Chang, S. T. Wang and P. W. Gean (2003). "Identification of calcineurin as a key signal in the extinction of fear memory." J Neurosci 23(5): 1574-1579.

Lindsay, J., D. Laurin, R. Verreault, R. Hebert, B. Helliwell, G. B. Hill and I. McDowell (2002). "Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging." Am J Epidemiol 156(5): 445-453.

Lithari, C., S. Moratti and N. Weisz (2016). "Limbic areas are functionally decoupled and visual cortex takes a more central role during fear conditioning in humans." Sci Rep 6: 29220.

Liu, I. Y., W. E. Lyons, L. A. Mamounas and R. F. Thompson (2004). "Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning." J Neurosci 24(36): 7958-7963.

Liu, J. L., M. Li, X. R. Dang, Z. H. Wang, Z. R. Rao, S. X. Wu, Y. Q. Li and W. Wang (2009). "A NMDA receptor antagonist, MK-801 impairs consolidating extinction of auditory conditioned fear responses in a Pavlovian model." PLoS One 4(10): e7548.

Liu, J., L. Zhao, Y. Xue, J. Shi, L. Suo, Y. Luo, B. Chai, C. Yang, Q. Fang, Y. Zhang, Y. Bao, C. L. Pickens and L. Lu (2014). "An unconditioned stimulus retrieval extinction procedure to prevent the return of fear memory." Biol Psychiatry 76(11): 895-901.

Liu, W., Q. X. Peng, X. L. Lin, C. H. Luo, M. J. Jiang, Z. X. Mo and K. K. Yung (2014). "Effect of rhynchophylline on the expression of p-CREB and sc-Fos in triatum and hippocampal CA1 area of methamphetamine-induced conditioned place preference rats." Fitoterapia 92: 16-22.

Liu, X., S. Ramirez, P. T. Pang, C. B. Puryear, A. Govindarajan, K. Deisseroth and S. Tonegawa (2012). "Optogenetic stimulation of a hippocampal engram activates fear memory recall." Nature 484(7394): 381-385.

Loke, W. H. (1988). "Effects of caffeine on mood and memory." Physiol Behav 44(3): 367-372.

Long Ii, R. P., V. Aroniadou-Anderjaska, E. M. Prager, V. I. Pidoplichko, T. H. Figueiredo and M. F. Braga (2016). "Repeated Isoflurane Exposures Impair Long-Term Potentiation and Increase Basal GABAergic Activity in the Basolateral Amygdala." Neural Plast 2016: 8524560.

Loonen, A. J. and S. A. Ivanova (2016). "Circuits Regulating Pleasure and Happiness: The Evolution of the Amygdalar-Hippocampal-Habenular Connectivity in Vertebrates." Front Neurosci 10: 539.

Lopes, L. V., A. M. Sebastiao and J. A. Ribeiro (2011). "Adenosine and related drugs in brain diseases: present and future in clinical trials." Curr Top Med Chem 11(8): 1087-1101.

Lopes, L. V., L. Halldner, N. Rebola, B. Johansson, C. Ledent, J. F. Chen, B. B. Fredholm and R. A. Cunha (2004). "Binding of the prototypical adenosine A(2A) receptor agonist CGS 21680 to the cerebral cortex of adenosine A(1) and A(2A) receptor knockout mice." Br J Pharmacol 141(6): 1006-1014.

Lopez-Cruz, L., M. Carbo-Gas, M. Pardo, P. Bayarri, O. Valverde, C. Ledent, J. D. Salamone and M. Correa (2017). "Adenosine A2A receptor deletion affects social behaviors and anxiety in mice: Involvement of anterior cingulate cortex and amygdala." Behav Brain Res 321: 8-17.

Loureiro, M., L. Lecourtier, M. Engeln, J. Lopez, B. Cosquer, K. Geiger, C. Kelche, J. C. Cassel and A. Pereira de Vasconcelos (2012). "The ventral hippocampus is necessary for expressing a spatial memory." Brain Struct Funct 217(1): 93-106.

Lu, Y., X. D. Sun, F. Q. Hou, L. L. Bi, D. M. Yin, F. Liu, Y. J. Chen, J. C. Bean, H. F. Jiao, X. Liu, B. M. Li, W. C. Xiong, T. M. Gao and L. Mei (2014). "Maintenance of GABAergic activity by neuregulin 1-ErbB4 in amygdala for fear memory." Neuron 84(4): 835-846.

Lynn, M. and S. Maille (2017). "Acute Stress Shapes Synaptic Inhibition within an Amygdala Microcircuit." J Neurosci 37(3): 474-476.

Machado, N. J., A. P. Simoes, H. B. Silva, A. P. Ardais, M. P. Kaster, P. Garcao, D. I. Rodrigues, D. Pochmann, A. I. Santos, I. M. Araujo, L. O. Porciuncula, A. R. Tome, A. Kofalvi, J. M. Vaugeois, P. Agostinho, M. El Yacoubi, R. A. Cunha and C. A. Gomes (2017). "Caffeine Reverts Memory But Not Mood Impairment in a Depression-Prone Mouse Strain with Up-Regulated Adenosine A2A Receptor in Hippocampal Glutamate Synapses." Mol Neurobiol 54(2): 1552-1563.

Maggio, N. and M. Segal (2011). "Persistent changes in ability to express long-term potentiation/depression in the rat hippocampus after juvenile/adult stress." Biol Psychiatry 69(8): 748-753.

Mahan, A. L. and K. J. Ressler (2012). "Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder." Trends Neurosci 35(1): 24-35.

Mahanty, N. K. and P. Sah (1998). "Calcium-permeable AMPA receptors mediate long-term potentiation in interneurons in the amygdala." Nature 394(6694): 683-687.

Makkar, S. R., S. Q. Zhang and J. Cranney (2010). "Behavioral and neural analysis of GABA in the acquisition, consolidation, reconsolidation, and extinction of fear memory." Neuropsychopharmacology 35(8): 1625-1652.

Mamiya, N., H. Fukushima, A. Suzuki, Z. Matsuyama, S. Homma, P. W. Frankland and S. Kida (2009). "Brain region-specific gene expression activation required for reconsolidation and extinction of contextual fear memory." J Neurosci 29(2): 402-413.

Manahan-Vaughan, D. (2005). Hippocampal Long-Term Depression as a Declarative Memory Mechanism. Synaptic Plasticity and Transsynaptic Signaling. P. K. Stanton, Bramham, Clive, Scharfman, Helen E. , Springer US: 305-319.

Maren, S. (1996). "Synaptic transmission and plasticity in the amygdala. An emerging physiology of fear conditioning circuits." Mol Neurobiol 13(1): 1-22.

Maren, S. (1998). "Effects of 7-nitroindazole, a neuronal nitric oxide synthase (nNOS) inhibitor, on locomotor activity and contextual fear conditioning in rats." Brain Res 804(1): 155-158.

Maren, S. (1999). "Long-term potentiation in the amygdala: a mechanism for emotional learning and memory." Trends Neurosci 22(12): 561-567.

Maren, S. (2001). "Neurobiology of Pavlovian fear conditioning." Annu Rev Neurosci 24: 897-931.

Maren, S. (2005). "Synaptic mechanisms of associative memory in the amygdala." Neuron 47(6): 783-786.

Maren, S. (2015). "Out with the old and in with the new: Synaptic mechanisms of extinction in the amygdala." Brain Res 1621: 231-238.

Maren, S. and G. J. Quirk (2004). "Neuronal signalling of fear memory." Nat Rev Neurosci 5(11): 844-852.

Maren, S. and J. A. Hobin (2007). "Hippocampal regulation of context-dependent neuronal activity in the lateral amygdala." Learn Mem 14(4): 318-324.

Maren, S., K. L. Phan and I. Liberzon (2013). "The contextual brain: implications for fear conditioning, extinction and psychopathology." Nat Rev Neurosci 14(6): 417-428.

Marschner, A., R. Kalisch, B. Vervliet, D. Vansteenwegen and C. Buchel (2008). "Dissociable roles for the hippocampus and the amygdala in human cued versus context fear conditioning." J Neurosci 28(36): 9030-9036.

Martin, E. I., K. J. Ressler, E. Binder and C. B. Nemeroff (2009). "The neurobiology of anxiety disorders: brain imaging, genetics, and psychoneuroendocrinology." Psychiatr Clin North Am 32(3): 549-575.

Massey, P. V. and Z. I. Bashir (2007). "Long-term depression: multiple forms and implications for brain function." Trends Neurosci 30(4): 176-184.

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

Mateus-Pinheiro, A., N. D. Alves, P. Patricio, A. R. Machado-Santos, E. Loureiro-Campos, J. M. Silva, V. M. Sardinha, J. Reis, H. Schorle, J. F. Oliveira, J. Ninkovic, N. Sousa and L. Pinto (2016). "AP2gamma controls adult hippocampal neurogenesis and modulates cognitive, but not anxiety or depressive-like behavior." Mol Psychiatry.

McConnell, B. L. and R. R. Miller (2014). "Associative Accounts of Recovery-from-Extinction Effects." Learn Motiv 46: 1-15.

McCullough, K. M., F. G. Morrison and K. J. Ressler (2016). "Bridging the Gap: Towards a cell-type specific understanding of neural circuits underlying fear behaviors." Neurobiol Learn Mem 135: 27-39.

McDonald, A. J., F. Mascagni and L. Guo (1996). "Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat." Neuroscience 71(1): 55-75.

McEwen, B. S. and R. M. Sapolsky (1995). "Stress and cognitive function." Curr Opin Neurobiol 5(2): 205-216.

McKernan, M. G. and P. Shinnick-Gallagher (1997). "Fear conditioning induces a lasting potentiation of synaptic currents in vitro." Nature 390(6660): 607-611.

Mears, D. and H. B. Pollard (2016). "Network science and the human brain: Using graph theory to understand the brain and one of its hubs, the amygdala, in health and disease." J Neurosci Res 94(6): 590-605.

Melia, K. R., A. E. Ryabinin, R. Schroeder, F. E. Bloom and M. C. Wilson (1994). "Induction and habituation of immediate early gene expression in rat brain by acute and repeated restraint stress." J Neurosci 14(10): 5929-5938.

Merlo, E., A. L. Milton, Z. Y. Goozee, D. E. Theobald and B. J. Everitt (2014). "Reconsolidation and extinction are dissociable and mutually exclusive processes: behavioral and molecular evidence." J Neurosci 34(7): 2422-2431.

Miller, J. M., E. L. Kinnally, R. T. Ogden, M. A. Oquendo, J. J. Mann and R. V. Parsey (2009). "Reported childhood abuse is associated with low serotonin transporter binding in vivo in major depressive disorder." Synapse 63(7): 565-573.

Minor, T. R., W. C. Chang and J. L. Winslow (1994). "Stress and adenosine: I. Effect of methylxanthine and amphetamine stimulants on learned helplessness in rats." Behav Neurosci 108(2): 254-264.

Mitra, R., S. Jadhav, B. S. McEwen, A. Vyas and S. Chattarji (2005). "Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala." Proc Natl Acad Sci U S A 102(26): 9371-9376.

Mitsushima, D., K. Ishihara, A. Sano, H. W. Kessels and T. Takahashi (2011). "Contextual learning requires synaptic AMPA receptor delivery in the hippocampus." Proc Natl Acad Sci U S A 108(30): 12503-12508.

Miyashita, T., S. Kubik, G. Lewandowski and J. F. Guzowski (2008). "Networks of neurons, networks of genes: an integrated view of memory consolidation." Neurobiol Learn Mem 89(3): 269-284.

Moita, M. A., S. Rosis, Y. Zhou, J. E. LeDoux and H. T. Blair (2004). "Putting fear in its place: remapping of hippocampal place cells during fear conditioning." J Neurosci 24(31): 7015-7023.

Monfils, M. H., K. K. Cowansage, E. Klann and J. E. LeDoux (2009). "Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories." Science 324(5929): 951-955.

Morais, M., P. A. Santos, A. Mateus-Pinheiro, P. Patricio, L. Pinto, N. Sousa, P. Pedroso, S. Almeida, A. Filipe and J. M. Bessa (2014). "The effects of chronic stress on hippocampal adult neurogenesis and dendritic plasticity are reversed by selective MAO-A inhibition." J Psychopharmacol 28(12): 1178-1183.

Morgan, M. A. and J. E. LeDoux (1995). "Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats." Behav Neurosci 109(4): 681-688.

Moser, E. I. (2011). "The multi-laned hippocampus." Nat Neurosci 14(4): 407-408.

Moser, E. I., Y. Roudi, M. P. Witter, C. Kentros, T. Bonhoeffer and M. B. Moser (2014). "Grid cells and cortical representation." Nat Rev Neurosci 15(7): 466-481.

Nabavi, S., R. Fox, C. D. Proulx, J. Y. Lin, R. Y. Tsien and R. Malinow (2014). "Engineering a memory with LTD and LTP." Nature 511(7509): 348-352.

Neafsey, E. J. (1990). "Prefrontal cortical control of the autonomic nervous system: anatomical and physiological observations." Prog Brain Res 85: 147-165; discussion 165-146.

Nicolelis, M. A., D. Dimitrov, J. M. Carmena, R. Crist, G. Lehew, J. D. Kralik and S. P. Wise (2003). "Chronic, multisite, multielectrode recordings in macaque monkeys." Proc Natl Acad Sci U S A 100(19): 11041-11046.

Novakovic, V., L. Sher, K. A. Lapidus, J. Mindes, A. G. J and R. Yehuda (2011). "Brain stimulation in posttraumatic stress disorder." Eur J Psychotraumatol 2.

Nugent, F. S., E. C. Penick and J. A. Kauer (2007). "Opioids block long-term potentiation of inhibitory synapses." Nature 446(7139): 1086-1090.

Ongur, D. and J. L. Price (2000). "The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans." Cereb Cortex 10(3): 206-219.

Orsini, C. A. and S. Maren (2012). "Neural and cellular mechanisms of fear and extinction memory formation." Neurosci Biobehav Rev 36(7): 1773-1802.

Orsini, C. A., J. H. Kim, E. Knapska and S. Maren (2011). "Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction." J Neurosci 31(47): 17269-17277.

Pain, L., A. Launoy, N. Fouquet and P. Oberling (2002). "Mechanisms of action of midazolam on expression of contextual fear in rats." Br J Anaesth 89(4): 614-621.

Pariante, C. M. and A. H. Miller (2001). "Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment." Biol Psychiatry 49(5): 391-404.

Patenaude, C., C. A. Chapman, S. Bertrand, P. Congar and J. C. Lacaille (2003). "GABAB receptor- and metabotropic glutamate receptor-dependent cooperative long-term potentiation of rat hippocampal GABAA synaptic transmission." J Physiol 553(Pt 1): 155-167.

Peng, S., Y. Zhang, B. Ren, J. Zhang and H. Wang (2011). "Effect of ketamine administration on memory consolidation, p-CREB and c-fos expression in the hippocampal slices of minor rats." Mol Biol Rep 38(4): 2401-2407.

Pesold, C. and D. Treit (1995). "The central and basolateral amygdala differentially mediate the anxiolytic effects of benzodiazepines." Brain Res 671(2): 213-221.

Peters, J., L. M. Dieppa-Perea, L. M. Melendez and G. J. Quirk (2010). "Induction of fear extinction with hippocampal-infralimbic BDNF." Science 328(5983): 1288-1290.

Petrides, M. (1996). "Specialized systems for the processing of mnemonic information within the primate frontal cortex." Philos Trans R Soc Lond B Biol Sci 351(1346): 1455-1461; discussion 1461-1452.

Phillips, R. G. and J. E. LeDoux (1992). "Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning." Behavioral neuroscience 106(2): 274-285.

Pinto, V., J. C. Costa, P. Morgado, C. Mota, A. Miranda, F. V. Bravo, T. G. Oliveira, J. J. Cerqueira and N. Sousa (2015). "Differential impact of chronic stress along the hippocampal dorsal-ventral axis." Brain Structure and Function 220(2): 1205-1212.

Ploski, J. E., K. W. Park, J. Ping, M. S. Monsey and G. E. Schafe (2010). "Identification of plasticity-associated genes regulated by Pavlovian fear conditioning in the lateral amygdala." Journal of Neurochemistry 112(3): 636-650.

Poucet, B., C. Thinus-Blanc and R. U. Muller (1994). "Place cells in the ventral hippocampus of rats." Neuroreport 5(16): 2045-2048.

Poulos, A. M., N. Mehta, B. Lu, D. Amir, B. Livingston, A. Santarelli, I. Zhuravka and M. S. Fanselow (2016). "Conditioning- and time-dependent increases in context fear and generalization." Learn Mem 23(7): 379-385.

Pratt, D. V. J. G. V. C. W. (2004). Fundamentals of Biochemistry: Life at the molecular level, John Wiley & Sons, Inc.

Prediger, R. D. S., D. Fernandes and R. N. Takahashi (2005). "Blockade of adenosine A2A receptors reverses short-term social memory impairments in spontaneously hypertensive rats." Behavioural Brain Research 159(2): 197-205.

Prediger, R. D., D. Fernandes and R. N. Takahashi (2005). "Blockade of adenosine A2A receptors reverses short-term social memory impairments in spontaneously hypertensive rats." Behav Brain Res 159(2): 197-205.

Prediger, R. D., L. C. Batista and R. N. Takahashi (2005). "Caffeine reverses age-related deficits in olfactory discrimination and social recognition memory in rats. Involvement of adenosine A1 and A2A receptors." Neurobiol Aging 26(6): 957-964.

Quirk, G. J. and D. Mueller (2008). "Neural mechanisms of extinction learning and retrieval." Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33(1): 56-72.

Radley, J., D. Morilak, V. Viau and S. Campeau (2015). "Chronic stress and brain plasticity: Mechanisms underlying adaptive and maladaptive changes and implications for stress-related CNS disorders." Neurosci Biobehav Rev 58: 79-91.

Radulovic, J., J. Kammermeier and J. Spiess (1998). "Relationship between fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure." J Neurosci 18(18): 7452-7461.

Radwanska, K., E. Nikolaev, E. Knapska and L. Kaczmarek (2002). "Differential response of two subdivisions of lateral amygdala to aversive conditioning as revealed by c-Fos and P-ERK mapping." Neuroreport 13(17): 2241-2246.

Ramirez, F., J. M. Moscarello, J. E. LeDoux and R. M. Sears (2015). "Active Avoidance Requires a Serial Basal Amygdala to Nucleus Accumbens Shell Circuit." Journal of Neuroscience 35(8): 3470-3477.

Rashid, A. J., C. Yan, V. Mercaldo, H.-l. L. Hsiang, S. Park, C. J. Cole, A. D. Cristofaro, J. Yu, C. Ramakrishnan, S. Y. Lee, K. Deisseroth, P. W. Frankland and S. A. Josselyn (2016). "Competition between engrams influences fear memory formation and recall." Science 353(6297): 383-388.

Rattiner, L. M., M. Davis, C. T. French and K. J. Ressler (2004). "Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning." J Neurosci 24(20): 4796-4806.

Rauch, S. L., L. M. Shin and E. A. Phelps (2006). "Neurocircuitry Models of Posttraumatic Stress Disorder and Extinction: Human Neuroimaging Research-Past, Present, and Future." Biological Psychiatry 60(4): 376-382.

Rebola, N., A. M. Sebastiao, A. de Mendonca, C. R. Oliveira, J. A. Ribeiro and R. A. Cunha (2003). "Enhanced adenosine A2A receptor facilitation of synaptic transmission in the hippocampus of aged rats." J Neurophysiol 90(2): 1295-1303.

Rebola, N., P. M. Canas, C. R. Oliveira and R. A. Cunha (2005). "Different synaptic and subsynaptic localization of adenosine A2A receptors in the hippocampus and striatum of the rat." Neuroscience 132(4): 893-903.

Rebola, N., R. J. Rodrigues, L. V. Lopes, P. J. Richardson, C. R. Oliveira and R. A. Cunha (2005). "Adenosine A1 and A2A receptors are co-expressed in pyramidal neurons and co-localized in glutamatergic nerve terminals of the rat hippocampus." Neuroscience 133(1): 79-83.

Reijmers, L. G. and M. Mayford (2009). "Genetic control of active neural circuits." Frontiers in molecular neuroscience 2(December): 1-8.

Repovs, G. and A. Baddeley (2006). "The multi-component model of working memory: explorations in experimental cognitive psychology." Neuroscience 139(1): 5-21.

Reppert, S. M., D. R. Weaver, J. H. Stehle and S. A. Rivkees (1991). "Molecular cloning and characterization of a rat A1-adenosine receptor that is widely expressed in brain and spinal cord." Mol Endocrinol 5(8): 1037-1048.

Ressler, K. J. and H. S. Mayberg (2007). "Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic." Nature neuroscience 10(9): 1116-1124.

Revest, J. M., D. Dupret, M. Koehl, C. Funk-Reiter, N. Grosjean, P. V. Piazza and D. N. Abrous (2009). "Adult hippocampal neurogenesis is involved in anxiety-related behaviors." Molecular Psychiatry 14(10): 959-967.

Ribeiro, J. A. and A. M. Sebastio (2010). "Caffeine and adenosine." Journal of Alzheimer's Disease 20(SUPPL.1).

Ribeiro, J. A., A. M. Sebastião and A. De Mendonça (2002). "Adenosine receptors in the nervous system: Pathophysiological implications." Progress in Neurobiology 68(6): 377-392.

Richter-Levin, G. and I. Akirav (2001). "Amygdala-hippocampus dynamic interaction in relation to memory." Molecular neurobiology 22: 11-20.

Ritchie, K., I. Carriere, A. de Mendonca, F. Portet, J. F. Dartigues, O. Rouaud, P. Barberger-Gateau and M. L. Ancelin (2007). "The neuroprotective effects of caffeine: a prospective population study (the Three City Study)." Neurology 69(6): 536-545.

Rivera-Oliver, M. and M. Diaz-Rios (2014). "Using caffeine and other adenosine receptor antagonists and agonists as therapeutic tools against neurodegenerative diseases: a review." Life Sci 101(1-2): 1-9.

Rodrigues, T. M., A. Jerónimo-Santos, A. M. Sebastião and M. J. Diógenes (2014). "Adenosine A2A Receptors as novel upstream regulators of BDNF-mediated attenuation of hippocampal Long-Term Depression (LTD)." Neuropharmacology 79: 389-398.

Rogan, M. T., U. V. Staubli and J. E. LeDoux (1997). "AMPA receptor facilitation accelerates fear learning without altering the level of conditioned fear acquired." J Neurosci 17(15): 5928-5935.

Rogan, M. T., U. V. Stäubli and J. E. LeDoux (1997). "Fear conditioning induces associative long-term potentiation in the amygdala." Nature 390(6660): 604-607.

Rogerson, T., B. Jayaprakash, D. J. Cai, Y. Sano, Y.-S. Lee, Y. Zhou, P. Bekal, K. Deisseroth and A. J. Silva (2016). "Molecular and Cellular Mechanisms for Trapping and Activating Emotional Memories." Plos One 11(8): e0161655-e0161655.

Romanov, R. A., A. Alpar, T. Hokfelt and T. Harkany (2017). "Molecular diversity of corticotropin-releasing hormone mRNA-containing neurons in the hypothalamus." J Endocrinol 232(3): R161-R172.

Rosenkranz, J. A. and A. A. Grace (2001). "Dopamine attenuates prefrontal cortical suppression of sensory inputs to the basolateral amygdala of rats." J Neurosci 21(11): 4090-4103.

Rosenkranz, J. A., E. R. Venheim and M. Padival (2010). "Chronic stress causes amygdala hyperexcitability in rodents." Biol Psychiatry 67(12): 1128-1136.

Rosenkranz, J. A., H. Moore and A. A. Grace (2003). "The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli." The Journal of neuroscience 23(35): 11054-11064.

Royer, S., M. Martina and D. Pare (1999). "An inhibitory interface gates impulse traffic between the input and output stations of the amygdala." J Neurosci 19(23): 10575-10583.

Rumpel, S., J. Ledoux, A. Zador and R. Malinow (2007). "Postsynaptic Receptor Trafficking Underlying a Form of Associative Learning Postsynaptic Receptor Trafficking Underlying a Form of Associative Learning." Science (New York, N.Y.) 83(2005): 83-88.

Runyan, J. D., A. N. Moore and P. K. Dash (2004). "A role for prefrontal cortex in memory storage for trace fear conditioning." J Neurosci 24(6): 1288-1295.

Sah, P., E. S. Faber, M. Lopez De Armentia and J. Power (2003). "The amygdaloid complex: anatomy and physiology." Physiol Rev 83(3): 803-834.

Sah, P., R. F. Westbrook and A. Luthi (2008). "Fear conditioning and long-term potentiation in the amygdala: What really is the connection?" Annals of the New York Academy of Sciences 1129: 88-95.

Sanders, M. J., B. J. Wiltgen and M. S. Fanselow (2003). "The place of the hippocampus in fear conditioning." European Journal of Pharmacology 463(1-3): 217-223.

Sangha, S. (2015). "Plasticity of Fear and Safety Neurons of the Amygdala in Response to Fear Extinction." Frontiers in behavioral neuroscience 9(December): 354-354.

Santos, M., D. D'Amico, O. Spadoni, A. Amador-Arjona, O. Stork and M. Dierssen (2013). "Hippocampal Hyperexcitability Underlies Enhanced Fear Memories in TgNTRK3, a Panic Disorder Mouse Model." Journal of Neuroscience 33(38): 15259-15271.

Sattin, A. and T. W. Rall (1970). "The effect of adenosine and adenine nucleotides on the cyclic adenosine 3', 5'-phosphate content of guinea pig cerebral cortex slices." Mol Pharmacol 6(1): 13-23.

Sauer, A., R. Jofra Hernandez, V. Bianchi, F. Fumagalli, P. L. Poliani, E. Riboni, C. Dallatomasina, C. A. Forcellini, N. Carriglio, A. Tabucchi, F. Carlucci, C. Azzari, C. Baldoli, S. Canale, M. Sessa, P. D'Adamo and A. Aiuti (2012). "Alterations in the brain adenosine metabolism cause behavioral and neurological alterations in adadeficient mice and patients." Journal of Clinical Immunology 32(November 2016): S179-S179.

Schafe, G. E., C. M. Atkins, M. W. Swank, E. P. Bauer, J. D. Sweatt and J. E. LeDoux (2000). "Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning." J Neurosci 20(21): 8177-8187.

Schafe, G. E., E. P. Bauer, S. Rosis, C. R. Farb, S. M. Rodrigues and J. E. Ledoux (2005). "Memory consolidation of Pavlovian fear conditioning requires nitric oxide signaling in the lateral amygdala." European Journal of Neuroscience 22(1): 201-211.

Schiffmann, S. N., G. Fisone, R. Moresco, R. A. Cunha and S. Ferre (2007). "Adenosine A2A receptors and basal ganglia physiology." Prog Neurobiol 83(5): 277-292.

Schiller, D., J. W. Kanen, J. E. LeDoux, M.-H. Monfils and E. a. Phelps (2013). "Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement." Proceedings of the National Academy of Sciences of the United States of America 110(50): 20040-20045.

Schmidt, M. V., W. C. Abraham, M. Maroun, O. Stork and G. Richter-Levin (2013). "Stress-induced metaplasticity: From synapses to behavior." Neuroscience 250: 112-120.

Schmidt, W. and M. Reith (2005). Dopamine and Glutamate in Psychiatric Disorders, Humana Press.

Scoville, W. B. and B. Milner (2000). "Loss of recent memory after bilateral hippocampal lesions. 1957." The Journal of neuropsychiatry and clinical neurosciences 12(1): 103-113.

Segal, M., G. Richter-Levin and N. Maggio (2010). "Stress-induced dynamic routing of hippocampal connectivity: A hypothesis." Hippocampus 20(12): 1332-1338.

Shen, H.-Y. and J.-F. Chen (2009). "Adenosine A2A Receptors in Psychopharmacology: Modulators of Behavior, Mood and Cognition." Current Neuropharmacology 7(3): 195-206.

Shin, L. M. and I. Liberzon (2010). "The neurocircuitry of fear, stress, and anxiety disorders." Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 35(1): 169-191.

Sierra-Mercado, D., N. Padilla-Coreano and G. J. Quirk (2011). "Dissociable Roles of Prelimbic and Infralimbic Cortices, Ventral Hippocampus, and Basolateral Amygdala in the Expression and Extinction of Conditioned Fear." Neuropsychopharmacology 36(2): 529-538.

Simões, A. P., N. J. Machado, N. Gonçalves, M. P. Kaster, A. T. Simões, A. Nunes, L. P. de Almeida, K. A. Goosens, D. Rial and R. A. Cunha (2016). "Adenosine A2A Receptors in the Amygdala Control Synaptic Plasticity and Contextual Fear Memory." Neuropsychopharmacology(November 2015).

Smith, Y., J. F. Pare and D. Pare (2000). "Differential innervation of parvalbumin-immunoreactive interneurons of the basolateral amygdaloid complex by cortical and intrinsic inputs." J Comp Neurol 416(4): 496-508.

Solstad, T., E. I. Moser and G. T. Einevoll (2006). "From grid cells to place cells: a mathematical model." Hippocampus 16(12): 1026-1031.

Souza, R. R. and A. P. Carobrez (2016). "Acquisition and expression of fear memories are distinctly modulated along the dorsolateral periaqueductal gray axis of rats exposed to predator odor." Behavioural brain research 315: 160-167.

Spampanato, J., J. Polepalli and P. Sah (2011). "Interneurons in the basolateral amygdala." Neuropharmacology 60(5): 765-773.

Squire, L. R. (1992). "Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans." Psychol Rev 99(2): 195-231.

Stern, K. N., L. D. Chait and C. E. Johanson (1989). "Reinforcing and subjective effects of caffeine in normal human volunteers." Psychopharmacology 98(1): 81-88.

Stevens, C. F. (1998). "A million dollar question: does LTP = memory?" Neuron 20(1): 1-2.

Stork, O., F. Y. Ji and K. Obata (2002). "Reduction of extracellular GABA in the mouse amygdala during and following confrontation with a conditioned fear stimulus." Neurosci Lett 327(2): 138-142.

Strange, B. a., M. P. Witter, E. S. Lein and E. I. Moser (2014). "Functional organization of the hippocampal longitudinal axis." Nature Reviews Neuroscience 15(10): 655-669.

Swanson, L. W. and W. M. Cowan (1977). "An Autoradiographic Study of the Organization of the Efferent Connections of the Hippocampal\rFormation in the Rat." J. Comp. Neur. 172: 49-84.

Swanson, R. a., M. T. Morton, G. Tsao-Wu, R. A. Savalos, C. Davidson and F. R. Sharp (1990). "A semiautomated method for measuring brain infarct volume." Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 10(2): 290-293.

Sweatt, J. D. (2016). "Neural plasticity and behavior - sixty years of conceptual advances." Journal of Neurochemistry 139: 179-199.

Szabo, A., J. Somogyi, B. Cauli, B. Lambolez, P. Somogyi and K. P. Lamsa (2012). "Calcium-permeable AMPA receptors provide a common mechanism for LTP in glutamatergic synapses of distinct hippocampal interneuron types." The Journal of neuroscience : the official journal of the Society for Neuroscience 32(19): 6511-6516.

Szinyei, C., R. T. Narayanan and H. C. Pape (2007). "Plasticity of inhibitory synaptic network interactions in the lateral amygdala upon fear conditioning in mice." European Journal of Neuroscience 25(4): 1205-1211.

Takei, S., S. Morinobu, S. Yamamoto, M. Fuchikami, T. Matsumoto and S. Yamawaki (2011). "Enhanced hippocampal BDNF/TrkB signaling in response to fear conditioning in an animal model of posttraumatic stress disorder." J Psychiatr Res 45(4): 460-468.

Tan, J.-W., T.-T. Duan, Q.-X. Zhou, Z.-Y. Ding, L. Jing, J. Cao, L.-P. Wang, R.-R. Mao and L. Xu (2015). "Impaired contextual fear extinction and hippocampal synaptic plasticity in adult rats induced by prenatal morphine exposure." Addiction biology 20(4): 652-662.

Tedesco, V., R. F. Roquet, J. DeMis, C. Chiamulera and M. H. Monfils (2014). "Extinction, applied after retrieval of auditory fear memory, selectively increases zinc-finger protein 268 and phosphorylated ribosomal protein S6 expression in prefrontal cortex and lateral amygdala." Neurobiology of Learning and Memory 115(October): 78-85.

Thomaes, K., E. Dorrepaal, N. Draijer, M. B. de Ruiter, B. M. Elzinga, Z. Sjoerds, A. J. van Balkom, J. H. Smit and D. J. Veltman (2013). "Increased anterior cingulate cortex and hippocampus activation in Complex PTSD during encoding of negative words." Social Cognitive and Affective Neuroscience 8(2): 190-200.

Thomas, G. M. and R. L. Huganir (2004). "MAPK cascade signalling and synaptic plasticity." Nat Rev Neurosci 5(3): 173-183.

Thomas, M. J., T. D. Moody, M. Makhinson and T. J. O'Dell (1996). "Activity-dependent beta-adrenergic modulation of low frequency stimulation induced LTP in the hippocampal CA1 region." Neuron 17(3): 475-482.

Tovote, P., J. P. Fadok and A. Luthi (2015). "Neuronal circuits for fear and anxiety." Nat Rev Neurosci 16(6): 317-331.

Trogrlic, L., Y. M. Wilson, a. G. Newman and M. Murphy (2011). "Context fear learning specifically activates distinct populations of neurons in amygdala and hypothalamus." Learning & Memory 18(10): 678-687.

Tronson, N. C., C. Schrick, Y. F. Guzman, K. H. Huh, D. P. Srivastava, P. Penzes, A. L. Guedea, C. Gao and J. Radulovic (2009). "Segregated populations of hippocampal principal CA1 neurons mediating conditioning and extinction of contextual fear." J Neurosci 29(11): 3387-3394.

Tronson, N. C., S. L. Wiseman, R. L. Neve, E. J. Nestler, P. Olausson and J. R. Taylor (2012). "Distinctive roles for amygdalar CREB in reconsolidation and extinction of fear memory." Learn Mem 19(5): 178-181.

Trouche, S., J. M. Sasaki, T. Tu and L. G. Reijmers (2013). "Fear extinction causes target-specific remodeling of perisomatic inhibitory synapses." Neuron 80(4): 1054-1065.

Tsvetkov, E., W. A. Carlezon, F. M. Benes, E. R. Kandel and V. Y. Bolshakov (2002). "Fear conditioning occludes LTP-induced presynaptic enhancement of synaptic transmission in the cortical pathway to the lateral amygdala." Neuron 34(2): 289-300.

Van Boxtel, M. P. J., J. A. J. Schmitt, H. Bosma and J. Jolles (2003). "The effects of habitual caffeine use on cognitive change: A longitudinal perspective." Pharmacology Biochemistry and Behavior 75(4): 921-927.

Van Groen, T. and J. M. Wyss (1990). "Extrinsic projections from area CA1 of the rat hippocampus: Olfactory, cortical, subcortical, and bilateral hippocampal formation projections." Journal of Comparative Neurology 302(3): 515-528.

van Strien, N. M., N. L. M. Cappaert and M. P. Witter (2009). "The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network." Nature reviews. Neuroscience 10(4): 272-282.

Varela, P., J. C. Escosteguy-Neto, C. T. Coelho, L. E. Mello, D. X. da Silveira and J. G. Santos-Junior (2014). "Chronic light deprivation inhibits appetitive associative learning induced by ethanol and its respective c-Fos and pCREB expression." The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP) 17: 1815-1830.

Veening, J. G., K. B. E. Böcker, P. M. Verdouw, B. Olivier, R. De Jongh and L. Groenink (2009). "Activation of the septohippocampal system differentiates anxiety from fear in startle paradigms." Neuroscience 163(4): 1046-1060.

Vertes, R. P. (2004). "Differential projections of the infralimbic and prelimbic cortex in the rat." Synapse 51(1): 32-58.

Viana da Silva, S., M. G. Haberl, P. Zhang, P. Bethge, C. Lemos, N. Gonçalves, A. Gorlewicz, M. Malezieux, F. Q. Gonçalves, N. Grosjean, C. Blanchet, A. Frick, U. V. Nägerl, R. A. Cunha and C. Mulle

(2016). "Early synaptic deficits in the APP/PS1 mouse model of Alzheimer's disease involve neuronal adenosine A2A receptors." Nature Communications 7(June): 11915-11915.

Victoria, N. C., E. Marron Fernandez de Velasco, O. Ostrovskaya, S. Metzger, Z. Xia, L. Kotecki, M. A. Benneyworth, A. N. Zink, K. A. Martemyanov and K. Wickman (2016). "G Protein-Gated K+ Channel Ablation in Forebrain Pyramidal Neurons Selectively Impairs Fear Learning." Biological Psychiatry 80(10): 796-806.

Vyas, A., R. Mitra, B. S. Shankaranarayana Rao and S. Chattarji (2002). "Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons." J Neurosci 22(15): 6810-6818.

Wagner, J. J. and B. E. Alger (1996). "Homosynaptic LTD and depotentiation: Do they differ in name only?" Hippocampus 6(1): 24-29.

Walker, D. L. and M. Davis (2002). "The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction." Pharmacology Biochemistry and Behavior 71(3): 379-392.

Wang, D. D., J. Li, L. P. Yu, M. N. Wu, L. N. Sun and J. S. Qi (2016). "Desipramine improves depression-like behavior and working memory by up-regulating p-CREB in Alzheimer's disease associated mice." J Integr Neurosci 15(2): 247-260.

Wang, J. H., Y. Y. Ma and M. van den Buuse (2006). "Improved spatial recognition memory in mice lacking adenosine A2A receptors." Exp Neurol 199(2): 438-445.

Wang, M. E., E. G. Wann, R. K. Yuan, M. M. Ramos Alvarez, S. M. Stead and I. A. Muzzio (2012). "Long-term stabilization of place cell remapping produced by a fearful experience." J Neurosci 32(45): 15802-15814.

Wang, M. E., R. K. Yuan, A. T. Keinath, M. M. Ramos Alvarez and I. A. Muzzio (2015). "Extinction of Learned Fear Induces Hippocampal Place Cell Remapping." J Neurosci 35(24): 9122-9136.

Wei, C. J., E. Augusto, C. A. Gomes, P. Singer, Y. Wang, D. Boison, R. A. Cunha, B. K. Yee and J. F. Chen (2014). "Regulation of fear responses by striatal and extrastriatal adenosine A 2A receptors in forebrain." Biological Psychiatry 75(11): 855-863.

Wei, C. J., P. Singer, J. Coelho, D. Boison, J. Feldon, B. K. Yee and J.-F. Chen (2011). "Selective inactivation of adenosine A(2A) receptors in striatal neurons enhances working memory and reversal learning." Learning {&} Memory 18(7): 459-474.

Wei, C. J., W. Li and J. F. Chen (2011). "Normal and abnormal functions of adenosine receptors in the central nervous system revealed by genetic knockout studies." Biochimica et Biophysica Acta - Biomembranes 1808(5): 1358-1379.

Westbrook, R. F., a. J. Good and M. J. Kiernan (1997). "Microinjection of morphine into the nucleus accumbens impairs contextual learning in rats." Behavioral Neuroscience 111(5): 996-1013.

Wilson, Y. M. and M. Murphy (2009). "A discrete population of neurons in the lateral amygdala is specifically activated by contextual fear conditioning." Learning & memory (Cold Spring Harbor, N.Y.) 16(6): 357-361.

Witter, M. P. (2007). "The perforant path: projections from the entorhinal cortex to the dentate gyrus." Prog Brain Res 163: 43-61.

Wolff, S. B. E., J. Gründemann, P. Tovote, S. Krabbe, G. A. Jacobson, C. Müller, C. Herry, I. Ehrlich, R. W. Friedrich, J. J. Letzkus and A. Lüthi (2014). "Amygdala interneuron subtypes control fear learning through disinhibition." Nature 509(7501): 453-458.

Wood, T., D. Osredkar, M. Puchades, E. Maes, M. Falck, T. Flatebø, L. Walløe, H. Sabir and M. Thoresen (2016). "Treatment temperature and insult severity influence the neuroprotective effects of therapeutic hypothermia." Scientific Reports 6(October 2015): 23430-23430.

Words, K. E. Y. (1999). "Habitual Caffeine Consumption and its Relation to Memory, Attention, Planning Capacity and Psychomotor Performance across Multiple Age Groups." Human Psychopharmacology: 462-470.

Wscieklica, T., M. S. C. F. Silva, J. A. Lemes, L. Melo-Thomas, I. C. Céspedes and M. B. Viana (2017). "Deep brain stimulation of the dorsal raphe inhibits avoidance and escape reactions and activates forebrain regions related to the modulation of anxiety/panic." Behavioural Brain Research 321: 193-200.

Xu, C., S. Krabbe, J. Grundemann, P. Botta, J. P. Fadok, F. Osakada, D. Saur, B. F. Grewe, M. J. Schnitzer, E. M. Callaway and A. Luthi (2016). "Distinct Hippocampal Pathways Mediate Dissociable Roles of Context in Memory Retrieval." Cell 167(4): 961-972.e916.

Yamada, K., M. Kobayashi and T. Kanda (2014). "Involvement of adenosine A2A receptors in depression and anxiety." Int Rev Neurobiol 119: 373-393.

Yamada, K., M. Kobayashi, A. Mori, P. Jenner and T. Kanda (2013). "Antidepressant-like activity of the adenosine A(2A) receptor antagonist, istradefylline (KW-6002), in the forced swim test and the tail suspension test in rodents." Pharmacol Biochem Behav 114-115: 23-30.

Yang, S. and C. L. Cox (2007). "Modulation of inhibitory activity by nitric oxide in the thalamus." J Neurophysiol 97(5): 3386-3395.

Yasoshima, Y., N. Sako, E. Senba and T. Yamamoto (2006). "Acute suppression, but not chronic genetic deficiency, of c-fos gene expression impairs long-term memory in aversive taste learning." Proceedings of the National Academy of Sciences of the United States of America 103(18): 7106-7111.

Yee, B. K., P. Singer, J. F. Chen, J. Feldon and D. Boison (2007). "Transgenic overexpression of adenosine kinase in brain leads to multiple learning impairments and altered sensitivity to psychomimetic drugs." Eur J Neurosci 26(11): 3237-3252.

Young, M. B., R. Andero, K. J. Ressler and L. L. Howell (2015). "3,4-Methylenedioxymethamphetamine facilitates fear extinction learning." Translational psychiatry 5(9): e634-e634.

Young, S. L., D. L. Bohenek and M. S. Fanselow (1994). "NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: immunization against amnesia by context preexposure." Behav Neurosci 108(1): 19-29.

Zhang, H. T., J. H. Xue, Z. W. Zhang, H. B. Kong, A. J. Liu, S. C. Li and D. G. Xu (2015). "Cold-inducible RNA-binding protein inhibits neuron apoptosis through the suppression of mitochondrial apoptosis." Brain Research 1622: 474-483.

Zhang, W. and J. A. Rosenkranz (2012). "Repeated restraint stress increases basolateral amygdala neuronal activity in an age-dependent manner." Neuroscience 226: 459-474.

Zhou, K. W., X. M. Zheng, Z. W. Yang, L. Zhang and H. D. Chen (2009). "Overexpression of CIRP may reduce testicular damage induced by cryptorchidism." Clinical and Investigative Medicine 32(2): 103-111.

Zhou, M., L. Conboy, C. Sandi, M. Joels and H. J. Krugers (2009). "Fear conditioning enhances spontaneous AMPA receptor-mediated synaptic transmission in mouse hippocampal CA1 area." Eur J Neurosci 30(8): 1559-1564.

Zovkic, I. B. and J. D. Sweatt (2013). "Epigenetic mechanisms in learned fear: implications for PTSD." Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 38(1): 77-93.

Zushida, K., M. Sakurai, K. Wada and M. Sekiguchi (2007). "Facilitation of extinction learning for contextual fear memory by PEPA: a potentiator of AMPA receptors." The Journal of neuroscience : the official journal of the Society for Neuroscience 27(1): 158-166.