PONTIFICIA UNIVERSIDAD CATÓLICA DEL PERÚ

Escuela de Posgrado

Noradrenaline and corticosterone memory enhancement in an object in context task in mice: mPFC activity differs due stress hormones.

Tesis para obtener el grado académico de Magíster en Psicología que presenta:

Francisco Javier Morote Sánchez

Asesor:

Carlos Simón Iberico Alcedo

Lima, 2022

Table of contents

1. Abstract

Memory is an adaptive tool for survival. It is critical to remember events accurately in order to learn and interact with our surroundings. In that regard, noradrenaline and corticosterone stress hormones have been proven to improve memory but also disrupt it depending on the instant that these hormones levels rise in the brain (during information encoding, consolidating or retrieval). A less investigated aspect would be how these two stress hormones influence memory accuracy. Over time, episodic-like memories are transformed into more semantic ones, which become less dependent on the hippocampus and more dependent on cortical regions like the medial pre-frontal cortex (mPFC). The purpose of this study was to investigate the effect of yohimbine (α2-noradrenergic receptor antagonist that increases noradrenaline levels) and corticosterone in memory accuracy in a low-arousing episodic-like memory task (Object in Context), as well as related changes in mice mPFC activity. Male C57BL/6J mice were exposed to a set of two identical objects in one context for 5 min, immediately followed by exposure to another set of two identical objects in a second context. Immediately after the training they received intraperitoneal injection of either yohimbine, corticosterone or vehicle. On the 24-h retention test, mice were placed back into one of the training contexts with two objects, one copy from each set of identical objects used during training. Thus, although both objects were familiar, one of the objects had not previously been encountered in that particular context. Hence, if the animal generated an accurate memory for the association between an object and its context, it would spend significantly more time exploring the object that was not previously experienced in that context. Our findings indicated that both yohimbine and corticosterone enhanced memory accuracy. Moreover, yohimbine increased the number of c-Fos-expressing non-GABAergic cells in cingulate area 1 and prelimbic cortex of mice mPFC. By contrast, corticosterone showed no changes in GABAergic or non-GABAergic activity in the mPFC compared to vehicle. These findings suggest that glucocorticoids-related memory enhancement would be associated to a distinct brain activity that was not addressed in this study.

1. Resumen

La memoria es fundamental para la supervivencia. Recordar un evento con precisión es de gran importancia para aprender e interactuar con nuestro entorno. En ese sentido, se ha observado que las hormonas del estrés noradrenalina y corticosterona pueden mejorar, pero también, deteriorar, la memoria dependiendo del momento de alza hormonal (durante la codificación, consolidación o recuperación de la memoria). Sin embargo, un área menos investigada es cómo estas dos hormonas influyen en la precisión del recuerdo. Con el tiempo, los recuerdos episódicos se transforman en recuerdos más semánticos volviéndose menos dependientes del hipocampo y más dependientes de regiones corticales como la corteza prefrontal medial (CPFm). En este estudio, se quiso investigar el efecto de la yohimbina (antagonista del receptor α2-noradrenérgico que aumenta los niveles de noradrenalina) y la corticosterona en la precisión del recuerdo en una tarea de memoria episódica de baja activación emocional ("Objeto en Contexto"). Asimismo, se quiso observar los cambios en la actividad de la CPFm, asociados a la tarea y al tratamiento farmacológico. Así, ratones macho C57BL/6J fueron expuestos a un set de dos objetos idénticos en un contexto durante 5 minutos, seguido inmediatamente por la exposición a otro set de dos objetos idénticos en un segundo contexto. Después del entrenamiento, los animales recibieron una inyección intraperitoneal de yohimbina, corticosterona o vehículo. En la prueba de recuperación del recuerdo, 24 h después del entrenamiento, se volvieron a colocar a los ratones en uno de los contextos de entrenamiento con una copia de cada set de los objetos idénticos que fueron utilizados previamente. Por lo tanto, aunque ambos objetos eran familiares, uno de ellos no se había encontrado previamente en ese contexto particular. Por consiguiente, si el animal generaba una memoria precisa para la asociación entre un objeto y su contexto, pasaría significativamente más tiempo explorando el objeto que no fue encontrado previamente en ese contexto. Los hallazgos indicaron que tanto la yohimbina como la corticosterona mejoraron la precisión del recuerdo. Adicionalmente, la yohimbina aumentó el número de células no GABAérgicas que expresaron c-Fos en el área cingulada 1 y la corteza prelímbica. Por el contrario, la corticosterona no mostró cambios en la actividad GABAérgica o no GABAérgica en la CPFm en comparación con el vehículo. Estos hallazgos sugieren que la mejora de memoria asociada a los glucocorticoides estaría vinculada a actividad cerebral distinta a la abordada en este estudio.

2. Introduction

2.1 Memory and Stress

Memory is essential for survival in a variety of organisms. The three fundamental stages of memory are encoding, consolidation and retrieval. In that regard, the process by which a short-term memory is converted into a long-lasting one is known as memory consolidation (McGaugh, 2000). Nonetheless, not all experiences are equally well remembered, which raises the question of: why do some memories last for mere seconds while others are preserved throughout our lives? Particularly, emotionally arousing or stressful events are remembered better and more vividly than ordinary events (McGaugh, 2003). This is a highly adaptive mechanism as these are mostly events that are critical for survival (de Quervain et al., 2009; McGaugh, 2003) (e.g., to remember a source of food or a good shelter, or to avoid an area where a predator was encountered in the past).

In the 1970s a study conducted by Gold and van Buskirk (1975) yielded the first evidence for the growing idea that endogenous mechanisms may normally regulate the strength of memory. The authors showed that the stress hormone adrenaline given shortly after training could enhance the memory consolidation process in a one-trial inhibitory avoidance task (Gold & Van Buskirk, 1975). Consequently, the impact of peripherally administered stress hormones on memory was soon investigated. In that respect, several studies evidenced changes in memory formation due to peripheral injection of catecholamines as well as a hormone dose-dependent memory enhancement (Gorelick et al., 1975; Haycock et al., 1977; Walsh & Palfai, 1979). Similarly, other experiments revealed that glucocorticoid hormones induced a dose dependent effect on memory, showing an inverted-U fashion in which moderate doses enhanced memory and lower and higher doses were less effective (Roozendaal, 2000).

Therefore, research related to stress response exhibited the neural substrates that link stress and memory. In this respect, two stress hormone systems are triggered in the presence of a stressful cue. First and swifter, the sympathetic nervous system is activated increasing catecholamines release such as adrenaline and noradrenaline. Then, it comes a longer lasting but slower response which is modulated by the hypothalamic-pituitary-adrenocortical axis (HPA-axis). In the organism, the HPA-axis is in charge of the release of glucocorticoids (Cortisol in humans animals, Corticosterone in non-

human animals). The role of these stress hormones in memory strength has been extensively emphasized in different studies (de Quervain et al., 2017; McIntyre et al., 2002; Okuda et al., 2004; Quirarte et al., 1997; Segal & Cahill, 2009; Villain et al., 2016). These hormones act mainly in regions such as the basolateral amygdala (BLA), hippocampus, entorhinal cortex, insular cortex, and the prefrontal cortex (PFC). Moreover, stress hormones may enhance memory of different type of experiences which are related to distinct neural circuits (Roozendaal, McEwen, et al., 2009; Roozendaal & McGaugh, 2011). For example, noradrenergic stimulation of the BLA modulates expression of Arc protein in the hippocampus and enhances memory consolidation of spatial or contextual information (McIntyre et al., 2005). On the other hand, activation of noradrenergic and dopaminergic mechanisms in the medial prefrontal cortex enhances consolidation of inhibitory avoidance and trace fear conditioning (Liang, 2001; Runyan & Dash, 2004). Considering that stressful events or emotionally arousing experiences are commonly biologically significant, the modulation of memory formation through the stress response is an adaptive and beneficial survival mechanism (de Quervain et al., 2009).

The amygdala is a brain region crucially involved in the modulation of emotionally arousing memories (Cahill & McGaugh, 1998). This is achieved by directing the activity of other brain regions (McGaugh, 2000). In the early 60s, it was shown that low intensity current stimulation in the rats amygdaloid nucleus after training could lead to an impairment of an aversive memory (Goddard, 1964). In that regard, case studies of patients with Urbach-Wiethe disease yielded more evidence of the role of the amygdala in the memory of emotionally arousing events. This disorder is characterized by a loss of the amygdaloid complex bilaterally. Interestingly, it has been observed no memory enhancement of an emotionally arousing fragment of a story in these patients (Cahill et al., 1995). It was previously said that shortly after a stress response noradrenaline is released in the basolateral amygdala (BLA). Importantly, the memory enhancement effect of noradrenaline is lost when propranolol (a β-adrenoceptor blocker) is injected into the amygdala (Liang et al., 1986). Besides, the amygdala has an important role in the effects of glucocorticoids on memory (Roozendaal, 2000). That is, an enhancement of retention performance in an inhibitory avoidance task was observed after infusion of a glucocorticoid receptor (GR) agonist into the BLA (Roozendaal & McGaugh, 1997). Nonetheless, via interacting with the

noradrenergic system and the BLA, glucocorticoids may impair memory retrieval and working memory (Roozendaal et al., 2006).

Importantly, amygdala interacts with other brain regions such as dorsal striatum, hippocampus and the mPFC to form a memory (McDonald & White, 2013; Roozendaal, McReynolds, et al., 2009). In that regard, it was observed that the memory-enhancing effect of an immediate post-training infusion of the GR agonist RU 28362 into the mPFC depends on an increased phosphorylation of extracellular signal-regulated kinase 1/2 (pErk1/2) in the BLA. Similarly, the memory enhancement induced by intra-BLA administration of RU 28362 requires elevated pErk1/2 levels in the mPFC (Roozendaal, McReynolds, et al., 2009). These findings point out a precise tuning of stress hormones interactions inside the amygdala, as well as the importance of the amygdala interaction with other brain regions in the process of memory formation.

2.2 Noradrenaline and Memory

As mentioned above, the autonomic nervous system activates as a response to stressful stimulus. Respectively, the sympathetic subdivision of the autonomic nervous system responds quickly to stressful stimuli by releasing catecholamines from the adrenal medulla. Since adrenaline is a polar molecule, it is unable to cross the blood-brain barrier in rodents and humans (Weil-Malherbe et al., 1959). The blood-brain barrier is composed of capillary endothelial cells and pericytes that prevent catecholamines from directly penetrate into the brain (Hardebo & Owman, 1980; Loizou, 1970). Thus, catecholamines work by binding to adrenoceptors on the vagus nerve to break through the brain barrier. As a result, noradrenergic cell groups in the Locus Coeruleus (LC) and the nucleus of solitary tract (NTS) are activated, altering brain function. The LC releases noradrenaline into the brain after activation, raising catecholamine levels (McGaugh, 2000). The alpha-adrenergic and beta-adrenergic G protein-coupled receptors (GPCRs) are the principal targets of noradrenaline binding in the brain. These receptors are found in the cell membranes and are found throughout the brain (Trzaskowski et al., 2012).

The relevance of noradrenaline in memory consolidation has been demonstrated numerous times. In an inhibitory avoidance task, rats that were systemically treated with noradrenaline after training exhibited a stronger and longer-lasting memory (Gold & van Buskirk, 1978; Gold & Van Buskirk, 1975). Moreover, the beta-adrenoceptor

antagonist propranolol disrupts the memory consolidation process (Lonergan et al., 2013; Villain et al., 2016). These discoveries appear to be persistent across species. In people, viewing photos while listening to an emotionally arousing story resulted in an improved remembering of those images. Nonetheless, the memory-enhancing effects of the emotionally arousing stimuli vanished when the participants received a beta-adrenoceptor antagonist (Cahill et al., 1994).

Importantly, the β-blocker propranolol not only inhibits the memory-enhancing impact in people while seeing an emotional picture, but it is also linked to a decrease in amygdala activity (van Stegeren et al., 2007). Furthermore, microdialysis experiments revealed that noradrenaline is released in the amygdala following a stressful experience, such as a footshock, and that influences memory formation (Galvez et al., 1996). Additionally, immediately after training, microinfusing noradrenaline directly into the amygdala improved memory performance in a water maze task in a dose-dependent manner. When propranolol was administered, however, retention was dramatically reduced (Hatfield & McGaugh, 1999). Different studies have since yielded evidence for noradrenaline dose-dependent memory enhancing effect during consolidation (McGaugh, 2000; McIntyre et al., 2002; Segal & Cahill, 2009). Besides, it has been suggested the use of a beta-adrenergic receptor blocker as a potential treatment for PTSD, where it might be employed to impair the fear-memory retrieval process (Parsons & Ressler, 2013). All of these studies show that noradrenaline plays a critical role in memory enhancement following emotionally arousing events.

2.3 Glucocorticoids and Memory

The activation of the HPA-axis is responsible for the late response to stress. The hypothalamus releases corticotropin-releasing factor (CRF), which causes the anterior pituitary to release adrenocorticotropic hormone (ACTH), which then causes the adrenal cortex to release glucocorticoids (CORT) (Ulrich-Lai & Herman, 2009). Glucocorticoids, unlike catecholamines, are lipophilic, meaning they can pass the blood-brain barrier and enter into the brain. When glucocorticoids reach the brain, they bind to two types of receptors: glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) (Reul & Kloet, 1985). Both the expression levels of the two receptors across the brain and their affinity to CORT appear to be different. GRs are found throughout the brain, whereas MRs are located in larger concentrations in the limbic

system, specifically in the hippocampus and the central amygdala (Sapolsky et al., 1983). Importantly, GRs and MRs are also found in the mPFC (Diorio et al., 1993; McEwen et al., 1986; Reis et al., 2016). De Kloet postulated that these two receptors have a dual function and may play separate roles during a stress response. It has been claimed that MRs are more involved in stress regulation and HPA-axis negative feedback, whereas GRs are linked to memory consolidation process (De Kloet et al., 2005).

In fact, only GR antagonists have shown an effect on memory consolidation when both MRs and GRs were blocked during a water maze task. In the water-maze, MR antagonists were found to change predominantly searching and scaping behavior (Oitzl & De Kloet, 1992; Roozendaal, Portillo-Marquez, et al., 1996). Moreover, it has been observed that GRs penetrate the cytoplasm and form a complex with various proteins, including the heat-shock protein 90 (hsp90) (Sanchez et al., 1986). The receptor then is translocated to the nucleus where it forms a homodimer that will interact with DNA and other proteins, causing changes in gene expression (Chandler et al., 1983). Glucocorticoids can have slow genomic effects by changing gene expression but it has also been shown that they have a non-genomic effect. Glucocorticoids rapid non-genomic effects are largely linked to behavioral changes, pathological phenomena and pharmaceutical effects (Makara & Haller, 2001). Therefore, glucocorticoids act across inter and intracellular processes in both a slow genomic and a rapid non-genomic fashion (De Kloet et al., 2005; De Kloet et al., 2008). In the inhibitory avoidance task glucocorticoids were found to have a dose-dependent effect on memory consolidation (Kovács et al., 1977).

The inverted U-shape of the dose-dependent correlation of corticosterone with memory consolidation was further investigated. The term inverted U-shape is referred to the graph that emerges with the x-axis being the different doses in an increasing line and the y-axis their efficacy. Thus, the lower and higher doses are not as effective as the moderate doses when the line of the graph follows an inverted U-shape pattern. Furthermore, it was also observed that the dose dependence efficacy of glucocorticoids varies based on the aversiveness of the task (Roozendaall, Bohus, et al., 1996). Experiments with rats in the Morris water-maze task revealed that removing the adrenal glands of the animals impaired their memory, but the phenotype could be recovered

with a systemic injection of dexamethasone (glucocorticoid) after training (Roozendaal, Bohus, et al., 1996). This data suggests that the task, dose, and endogenous glucocorticoid release all play a role in memory consolidation (Roozendaal, 2000).

It has already been mentioned that the noradrenergic and glucocorticoid systems in the amygdala appear to interact. That is, glucocorticoids memory-enhancing effects appear to be dependent on noradrenergic activity (de Quervain et al., 2017; Okuda et al., 2004; Quirarte et al., 1997). When all groups of animals were given glucocorticoids, the memory of long habituated animals or animals given β-adrenoceptor blockers differed from the memory of animals given placebo, demonstrating that noradrenergic activation is required to see memory changes due to the glucocorticoid system (Roozendaal et al., 2006). However, a rise in glucocorticoids could impair memory retrieval either through systemic injections or hippocampal infusions of GR agonists (McGaugh & Roozendaal, 2002). These results point out the importance of glucocorticoids in memory consolidation, as well as their fine interplay with the noradrenergic system.

2.4 Aspects of Memory: Strength and Accuracy

Human research reveals that even though memories from emotionally arousing situations are enhanced, they are often inaccurate (Morgan et al., 2004; Sharot et al., 2004). Despite the fact that participants are confident in predicting their memory accuracy, this prediction they do is not objective (Hoscheidt et al., 2014; Levine et al., 2020; Rimmele et al., 2011; Talarico & Rubin, 2003). Moreover, it has been noticed that the amygdala is active during emotional "remembering," indicating that respondents were depending on amygdala arousal effects to assess and recall emotional experiences (Sharot et al., 2004). Nonetheless, the contrary has also been claimed, namely increased emotional memory accuracy (Segal et al., 2012).

A recently study confirms that giving rats the noradrenergic stimulant yohimbine improves memory strength and accuracy in an inhibitory avoidance discriminating task (Roozendaal & Mirone, 2020). The administration of corticosterone, on the other hand, had the contrary effect with memory appearing to be generalized yet still strengthened. In the inhibitory avoidance discriminating task, the animal is first presented to two boxes one with a light compartment and the other with a dark compartment. One box is

designated as the shock box while the other is designated as the non-shock box. The animal receives an electric jolt in the shock box as it moves from the light to the dark compartment, which is safer for the animal. Then, during the retention test the animal is exposed to these two boxes, as well as to a novel box. The difference in time spent by the animal until it enters the dark apparatus in the shock box relative to the non-shock box is used to determine memory accuracy. That is to say, if the animal has an accurate memory, it should elude enter to the dark compartment in the shock box and not in the non-shock box or the novel box. The authors observed that animals given yohimbine had greater retention latencies only in the dark compartments of the shock box, but not in the non-shock box or the novel box. Instead, animals given corticosterone were unable to distinguish between the shock and non-shock boxes and displayed long retention latencies in both type of boxes. Data from the same research lab has previously shown that infusing noradrenaline into the BLA improves memory accuracy and that this process "interferes" with systems consolidation by keeping the memory hippocampal dependent (Atucha et al., 2017).

2.5 The Medial Pre-Frontal Cortex and Memory

It has been shown that over time episodic-like memories are transformed into more semantic ones, which do not require hippocampal activation anymore to be recalled but only of cortical regions; this process is known as systems consolidation (Winocur & Moscovitch, 2011). In that regard, the role of the mPFC in memory of rodents has been extensively studied (Euston et al., 2012; Morici et al., 2015; Peters et al., 2013). The dorsolateral region of the human prefrontal cortex is considered functionally homologous to the mPFC in rodents (Farovik et al., 2008; Kolb & Tees, 1990; Preuss, 1995; Uylings et al., 2003). However, the homology between the human dorsolateral prefrontal cortex and rodent mPFC is still on debate (Granon & Poucet, 2000; Jobson et al., 2021; Laubach et al., 2018; Öngür & Price, 2000; Uylings & van Eden, 1991). The mPFC subdivision includes the prelimbic (PrL), infralimbic (IL) and ventral anterior cingulate cortices. These sub-structures have reciprocal connections with the perirhinal and entorhinal cortices, the hippocampus, and with the agranular insular cortex (Morici et al., 2015). In addition, mice PFC polysynaptic route crosses through various structures, especially, nucleus acumbens (NAc) and ventral tegmental area (VTA), creating the hippocampus-NAc-VTA-PFC circuit. In the latter circuit, VTA

and hippocampus influence each other. On the other hand, in the hippocampus-BLA-PFC circuit, the PFC may affect the hippocampus through the amygdala. In this circuit, the amygdala is linked bilaterally with the prefrontal cortex and hippocampus. Besides, the PFC may affect the function of the hippocampus through the Nucleus Reuniens (RE) of the thalamus due to the PFC-RE- hippocampus circuit (Figure 1) (Zangbar et al., 2020). Many of the same functions ascribed to the human dorsolateral prefrontal cortex are also associated to the mPFC in rodents, despite the differences in size and complexity (Kolb, 1984; Uylings et al., 2003; Uylings & van Eden, 1991).

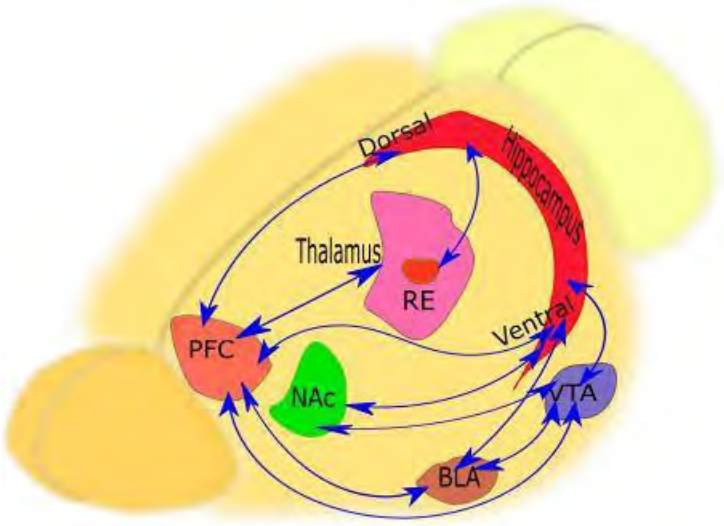

Figure 1: Mice brain polisynaptic routes (Zangbar et al., 2020). Hip, Hippocampus; NAc, Nucleus Accumbance; VTA, Ventral Tegmental Area; PFC, Prefrontal Cortex; RE, Nucleus Reuniens; BLA, Basolateral Amygdala.

The mPFC is involved in response inhibition, representation of egocentric space, temporal order, stress response, behavioral flexibility and attention among other processes (Kesner & Churchwell, 2011). Novel object recognition tasks permitted the study of episodic-like memory and specifically, the role of the mPFC with little confounds from other mPFC related functions (Ameen-Ali et al., 2015). Different lesion studies connect the mPFC with the correct resolution of tasks that assess location memory, temporal order memory or spatial memory, as well as more recent electrophysiological data (Morici et al., 2015; Nelson et al., 2011; Weible et al., 2012). For example, in one study, rats with lesions in the mPFC were assessed for temporal memory object recognition. The authors observed that these rats preferred to explore the "oldest" object over the "newest" for the different time points assessed. Thus, lesions to

the mPFC affected the animal's ability to solve the task pointing out that mPFC is involved in recency discrimination (TMOR) (Mitchell & Laiacona, 1998).

Other studies support these findings. Using variants of the task described above combined with different pharmacological manipulations, a mPFC role in recency discrimination has been repeatedly declared in rodents (Barker et al., 2007; Dere et al., 2005a, 2005b, 2007; Dere et al., 2006; DeVito & Eichenbaum, 2011; Hannesson et al., 2004). For instance, Barker et al. (2007) found that lesion to the mPFC affect an Object-in-place (OiP) task, providing evidence that another function of the mPFC is the association of the object with the context and its place inside the context. However, the same groups of animals showed no deficits in an Object location (OL) task neither they showed deficits in the spontaneous object recognition (SOR) task. These results suggested an important role of the mPFC in cases in which integration of object and spatial location information is needed. Nonetheless, one of the disadvantages of mPFC lesion models in the study of recognition memory is the difficulty to dissect the specific memory phase(s) during which this structure is involved. Temporary local manipulations of mPFC can be used to modify the activity of this structure during different memory phases such as encoding, consolidation, retrieval and reconsolidation (Morici et al., 2015).

Interactions between the mPFC and other structures sustain the idea that a network in which the mPFC might be playing a top-down role is required for acquisition of a memory (Barker & Warburton, 2015). To date, it is extensively considered that, for memories to last more than a few hours, memory processing involves long-lasting, activity-dependent changes in synaptic strength within the neural networks activated during learning. This is expected to be mediated by molecular mechanisms underlying functional and structural remodeling of network connectivity, which in part is dependent on protein synthesis. This protein-synthesis-dependent phase constitutes what we know as memory consolidation (Davis et al., 2010; Gonzalez et al., 2013).

Some studies have aimed to understand the consolidation process in the mPFC during memory recognition. For example, Akirav & Maroun (2006) worked with a SOR task to assess the role of ventromedial prefrontal cortex (vmPFC) during consolidation. Immediately after the sample phase the infusion of anisomycin (a protein synthesis

inhibitor) disrupted the long term memory of object recognition without affecting the short term memory, which was measured three hours after acquisition, suggesting that vmPFC is involved in long-term memory consolidation in this task (Akirav & Maroun, 2006). Pharmacological manipulations studies support the idea of a mPFC role in the consolidation of recognition memory. Interestingly two electrophysiological studies also support this function. Using mice as a model, Weible et al. (2009) reported electrophysiological correlates of individual anterior cingulate cortex (ACC) neurons to object–place associations following short delays. As a follow up study, they modified a SOR task to assess if the ACC played also a role in consolidated recognition memory. Two groups of animals were habituated to the task, the first group had only a single session to explore both objects while the second group had been largely familiarized to both objects over the course of many days. The hypothesis was that a stronger memory of the object-location association would be observed due to familiarization to the task and that this strength would be correlated with activity in the ACC. Indeed, they found that mice preferred to explore locations of the arena where an object had previously been, and that ACC neurons also activated in that location, indicating the memory of the object/place association. For animals extensively familiarized to both objects over the course of many days those responses to absent objects were clearer. Moreover, 30 days after the last training session the correlation was still evident when mice were exposed to the absent-object arena, suggesting that ACC neurons are required in long term recognition memory of object place (Weible et al., 2012).

2.6 Object-in-Context as a low arousing episodic-like memory task

To date, the influence of noradrenaline and glucocorticoids on memory quality has mainly been studied in emotionally arousing episodic-like tasks (i.e. the inhibitory avoidance discrimination task) (Atucha et al., 2017; Roozendaal, McReynolds, et al., 2009). Despite the fact that in a former experiment it has been observed that infusing noradrenaline into the BLA improved memory accuracy in the Object-in-Context (OiC) task in a dose-dependent manner (Barsegyan et al., 2014), it is still uncertain if glucocorticoids would have a similar impact on the accuracy of memory in an episodic-like low-arousing memory task. To assess this, the OiC task is used in this study. This task was developed to evaluate the memory of an object, a location and the context at the same time (Dix & Aggleton, 1999). It has been employed as an episodic-like

memory task and already the fornix (the hippocampus major output track) appears to be a brain region of interest (Eacott & Norman, 2004) in interaction with the mPFC (DeVito & Eichenbaum, 2010). In rodents, the OiC task appears to be a good instrument for measuring episodic-like memory in low arousing conditions.

3. Aim

The Behavioral Neuroscience lab of the Donders Institute has found evidence for the opposite effects of the two stress hormones in memory accuracy. Specifically, the noradrenergic stimulant yohimbine enhanced memory accuracy and corticosterone induced memory generalization in a high arousing task in rats (Roozendaal & Mirone, 2020). The present study investigates the impact of corticosterone and yohimbine on memory accuracy in mice performing a low-arousing OiC task. The primary question is whether differentiation on memory accuracy also becomes evident in a low-arousing condition by pharmacological stimulation of stress hormones and how this is reflected in mPFC activity. To accomplish this, this study is split into a behavioral and a molecular part.

The OiC task is utilized in the behavioral part of the study to determine the effects of corticosterone and yohimbine on memory accuracy in a low-arousing episodic-like condition. For the molecular part, immunohistochemistry is used to assess the differences in the cell populations that become activated or inhibited during the post-learning consolidation period one hour after the training phase. The mPFC regions analyzed are cingulate cortex area 1 (Cg1), PrL, and IL, and their cortical layers, in response to the administration of either yohimbine or corticosterone immediately after the training period.

Hypothesis

The present study hypothesizes that yohimbine will improve memory accuracy but corticosterone will likely cause generalization of memory. This effect is expected to be mirrored in the neural substrates as well. Essentially, it is expected that noradrenaline increases neuronal activity within the mPFC, whereas corticosterone should produce the contrary effect, eventually increasing inhibitory mechanisms.

4. Materials and Methods

4.1 Subjects

Male wild type C57BL/6J mice (n = 141, 10 weeks old, 24-26 gram of body weight) were used. 113 mice were part of the behavioral experiments and 28 mice were part of the immunolabeling experiments. Mice were single housed on a 12-12 hr day-night cycle (7:00-19:00 lights on) at a constant temperature of 22 °C. Animals received food and water ad libitum. Single caging was used to avoid potential negative effects of social interaction such as stress, fighting and hierarchical power. All experimental procedures were in compliance with the European Union Directive 2010/63/EU and approved by the Institutional Animal Care and Use Committee of Radboud University, Nijmegen, The Netherlands.

4.2 Object-in-Context task

To investigate memory accuracy in a hippocampal-dependent episodic-like memory, the object-in-context (OiC) task was used. In this task, the animal is trained to learn to associate an object (what) with the specific training context (where). Due to their intrinsic curiosity, mice spend more time exploring a new object, a new location of an object, or, as in our case, an object that is not expected to be in this specific context. Prior to training, mice were handled for 1-2 min. on four consecutive days, followed by three days of habituation in the training context. The animals were habituated in the same two contexts as those used for training (two round boxes of 40 cm. diameter and 40 cm. height with different modifications, contexts A and B) for 5 minutes in each for 3 days (Figure 2). One of the two boxes was gray with sawdust bedding and the other one had white stripes and dots modifying the walls and had corncob bedding to ensure that the environments would be noticeably different for the animals.

Following the habituation days, the animals were trained in the OiC task. For training, mice were placed in the first-round box (either context A or B) and could explore one set of two identical objects, either two glass jars or two light bulbs, for 5 minutes. Immediately afterwards, the animal was placed in the second-round box and could explore the other set of two identical objects for 5 minutes. The sequence of the two contexts and the object-context combinations were counterbalanced to avoid order effects. For retention testing, 24hr after training, the animals were placed in one of the

two training contexts (either A or B) with one exemplar of both training objects (one glass jar and one light bulb) for 5 minutes. The context used on the retention test was counterbalanced with half the animals being tested in context A and the other half in context B.

Both the training and testing trials were videotaped and analyzed after the end of the experiment. Total exploration time of each object in the two training contexts during training (to ensure that the animals did not develop a preference for one of the objects or a specific position of the objects) as well as during the retention test were measured. The experimenter scoring the behavior was fully blind to the experimental manipulations. Object exploration time was measured by the moments when the animal was actively investigating the object. Active exploration includes nose poking or scratching the object but not the time spent digging around or sitting on top of the object. To calculate a measure of memory, the Discrimination Index (DI%) was used. The DI% is defined as the time spent exploring the novel object-context combination minus the time spent exploring the familiar object-context combination, divided by the total exploration time of both objects, and all multiplied by one hundred [DI% = (Novel - Familiar)/(Novel + Familiar)*100].

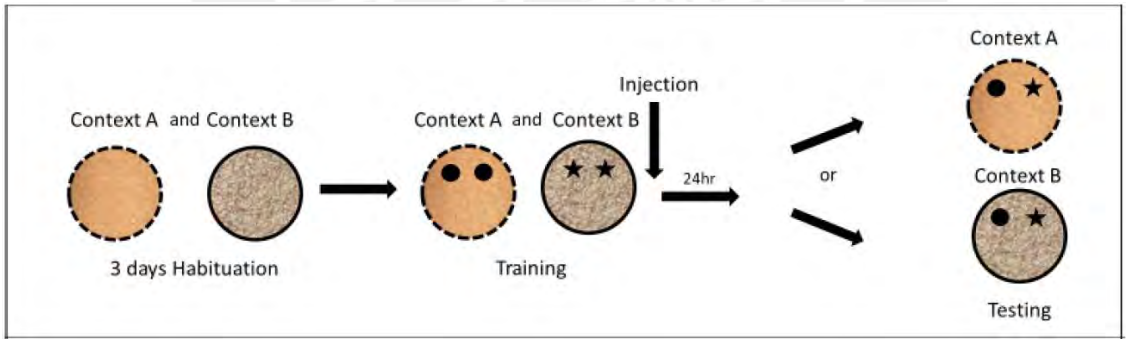

Figure 2: Experimental procedure. Animals are habituated in the same as the training and the testing contexts.

4.3 Systemic stress hormone Injection

The noradrenergic stimulant yohimbine or glucocorticoid corticosterone was injected immediately after the training session. Yohimbine is an alpha2-adrenoceptor antagonist that blocks the negative feedback of these receptors which increases NE-levels in the brain (Rang et al., 2014). For yohimbine, the doses used were 0.3, 1 and 3 mg/kg. Yohimbine was dissolved in saline. The control group was injected intraperitoneally with saline only. For corticosterone, the doses used were 1, 3 and 10

mg/kg. Corticosterone was first dissolved in 100% ethanol and subsequently diluted in saline to get a 5% ethanol solution. The control group for the corticosterone experiment was injected with 5% ethanol in saline only. The injection volume for all groups was 0.1ml/10g of body weight. Doses of yohimbine and corticosterone were chosen based on previous studies of our laboratory (Atsak et al., 2015; Roozendaal et al., 2006). This resulted in 4 groups of animals for the yohimbine treatments (3 doses and saline) and 4 groups of animals for the corticosterone treatments (3 doses and vehicle) (see Table 1). The behavioral results indicated that the concentration of 1 mg/kg for yohimbine and 3 mg/kg for corticosterone were most effective, based on the inverted U-shape model for drugs explained more in the results section. For the immunohistochemical experiments, these doses of yohimbine and corticosterone alone were used. For these latter experiments, both drugs were dissolved in 5% ethanol in saline. The vehicle control contained 5% ethanol in saline only.

Table 1
Injection drugs and Doses

Treatments	Yohimbine	Corticosterone
Doses	Saline	Vehicle
	0.3mg/kg	1mg/kg
	1mg/kg	3mg/kg
	3mg/kg	10mg/kg

4.4 Animal Sacrificing

In order to investigate the effect of the stress hormones on neural activity during the consolidation period, 29 animals were sacrificed 1hr after training and systemic drug treatment. The specific time point was chosen due to the neuronal assessment using c-Fos protein as a neuronal activity marker. The animals were anesthetized with an overdose of sodium pentobarbital (40-50mg/kg), followed by transcardial perfusion with 10 ml of phosphate-buffered saline (PBS) and 10 ml of 4% paraformaldehyde (PFA). The brains were extracted and further fixated for 24hr in 4% PFA in 0.1 M PBS (pH 7.4). For cryoprotection, the brains were immersed in a hypertonic solution of 30% sucrose in PBS (pH 7.4) for 4 days. Subsequently, the brains were stored at -80°C until slicing.

4.5 Tissue slicing

The whole brain was sliced in 30-μm-thick coronal slices using a cryostat (-20°C object temperature and -18°C environment temperature). Brain slices were placed in 1% PBS + 0.01% sodium azide (NaN3). Although the mPFC, amygdala and hippocampus are all areas of interest, this study mainly focused on the mPFC (Bregma 1.98 to 1.54 mm) (Figure 3) (Paxinos & Franklin, 2008).

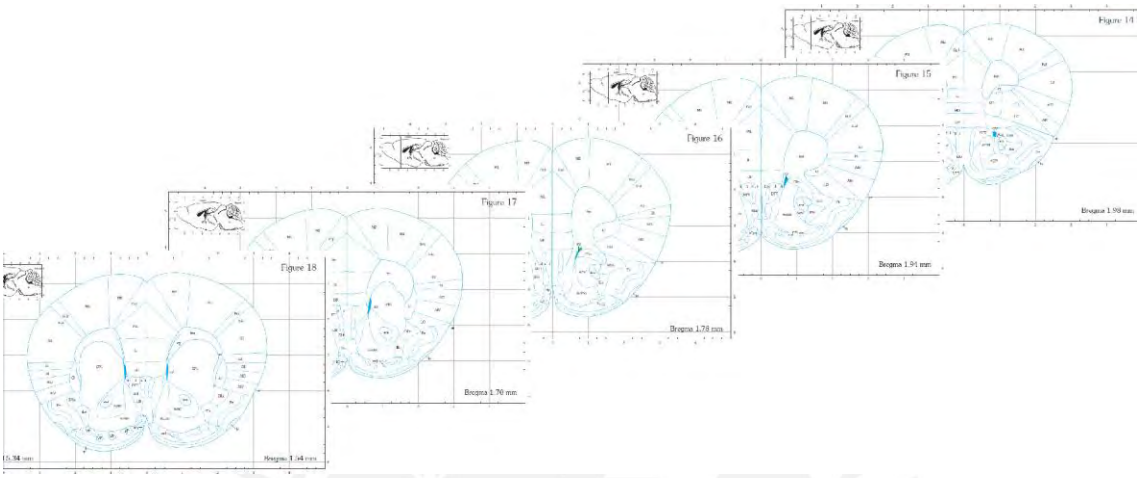

Figure 3: Anatomical representations of the ROI based on Paxinos and Franklin atlas (Paxinos & Franklin, 2008).

4.6 Immunohistochemistry

Immunohistochemistry was used to examine the area of interest. The selected antibodies in this study were c-Fos and glutamic acid decarboxylase, kDa 67 (GAD67) (Table 2), the brain slices were also stained with 4′,6-diamidino-2-phenylindole (DAPI). c-Fos as previously mentioned, is a protein that belongs to the immediate early expressed genes. This protein is found in a low baseline level but it elevated rapidly one hour after the activation of neurons (Hoffman et al., 1993), thus delivering a clear image of the which neurons were activated during the procedure of interest. GAD67 antibody is staining the GAD enzyme that is responsible for catalyzing the decarboxylation of glutamate to GABA and CO2 (Soghomonian & Martin, 1998), allowing identification of inhibitory cells. Lastly, DAPI is mostly used in histological studies due to its ability to stain DNA in the nuclei, thus providing a clear image of all cells (Kapuscinski, 1995).

The widely used procedure of fluorescent immunohistochemistry in free-floating sections was employed in this study. Pre-frontal cortical slices were washed three times for 10 minutes in 1xPBS in 12-well plates (2.5 ml) followed by incubation for 30

minutes in 0.5%Triton. Afterwards, the slices were cleaned up again three times for 10 minutes in 1xPBS. For pre-incubation, the slices were transferred to 24-well plates (500 µl) containing 5% Normal Donkey Serum (NDS), 1% Bovine Serum Albumin (BSA) in 1xPBS for 1hr at room temperature (RT). Next, slices were incubated in primary guinea pig antibodies anti-c-Fos (1:750) and mouse anti-GAD67 (1:500) in 0.1%BSA-c previously diluted in 1xPBS and 2% NDS overnight at RT. Later, the slices were transferred back to 12-well plates and washed three times for 10 minutes in 1xPBS. Then, incubated with the secondary antibodies Alexa Fluor 647 Donkey anti-guinea pig (1:750) and Alexa Fluor 488 anti-mouse (1:500) in 0.1% BSA-c and 2%NDS in 1xPBS for 3hrs in the dark at RT (Table 2). Subsequently, the slices were incubated in DAPI (1:5000) with 0.1% BSA-c in 1xPBS for 15 minutes and cleansed again three times for 10 minutes each in 1xPBS. The slices were mounted on gelatin-coated slides, left to dry, and coverslipped with Fluorsave. The slices were stored at 4°C.

Table 2
Immunohistochemistry materials

Name	Raised in	React with	Manufacturer	Catalogue no.
Anti-CFOS	Guinea Pig	CFOS	Synaptic Systems	226 004
Anti-Gad67	Mouse	GAD67	Sigma-Aldrich	MAB5406-25ug
Alexa Fluor 647-conjugated AffiniPure Donkey Anti-Guinea Pig IgG	Donkey	Guinea Pig	Jackson ImmunoResearch	706-605-148
Alexa Fluor 488 donkey anti-mouse IgG	Donkey	Mouse	Invitrogen	A21202
DAPI		Chromatin	Thermo Scientific	62248

4.7 Cell Counting

Images were acquired on an Automated High-Content Fluorescence Microscope (Leica, DMI 6000B, Radboudumc, Nijmegen) with a 20x magnification. The

excitations were: 30 ms with 1 ms gain for DAPI, 700 ms with 2 ms gain for c-Fos and 150 ms with 1 ms gain for GAD67. ImageJ software was used to calculate cells and estimate area sizes (Rueden et al., 2017). The mPFC was first divided into three regions: Cg1, Prl and IL. Then each region was subdivided into five sections representing cortical layers (L), because one objective was to explore if there were also differences between the layers' activity due to drug treatment. In coronal sections, the borders between L1, L2/L3 and L4, L5, and L6 were identified based on GAD67 staining. The borders of cortical layers were defined using the inflection points between the curves from the pial surface to the white matter (Almási et al., 2019). The number of cells expressing c-Fos, GAD67, and those expressing both markers were counted. Due to the scarce number of cells in LI, this cortical layer was not taken into consideration for the cell counting. Cortical regions were identified with respect to a stereotactic mouse brain atlas (Figure 4) (Paxinos & Franklin, 2008) and the online version of the Allen Mouse Brain Atlas (2008) was used as guideline for identifying cortical layers (Figure 5). All areas were first counted in cells/pixel, then transformed into cells/μm, and then cells/mm based on the ratio between pixels and μm in our images. The absolute non-GABAergic activity defined as all c-Fos-expressing cells minus the cells that co-expressed c-Fos and GAD67 [c-Fos-(c-Fos+GAD67)] is main topic for this research. The absolute GABAergic activity was defined as the number of cells that co-expressed c-Fos and GAD67. Finally, the total number of GABAergic neurons was defined as the number of cells that expressed GAD67 minus the cells that co-expressed c-Fos and GAD67 [GAD67-(c-Fos+GAD67)].

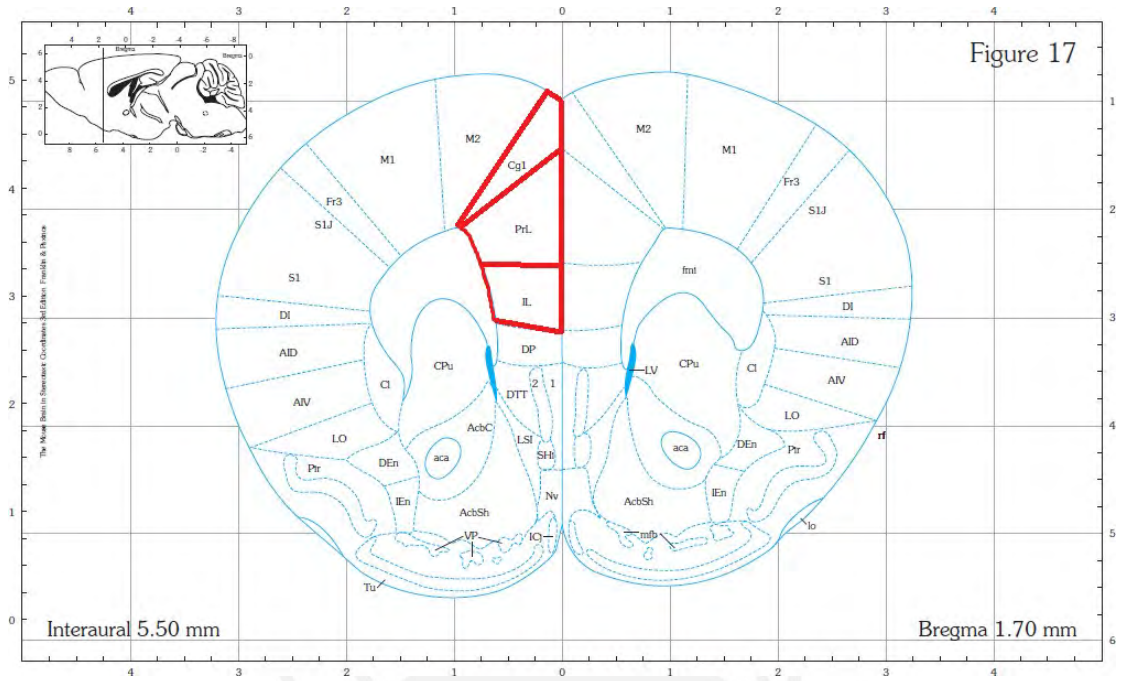

Figure 4: Areas measured, boarders between Cg1, Prl and IL.

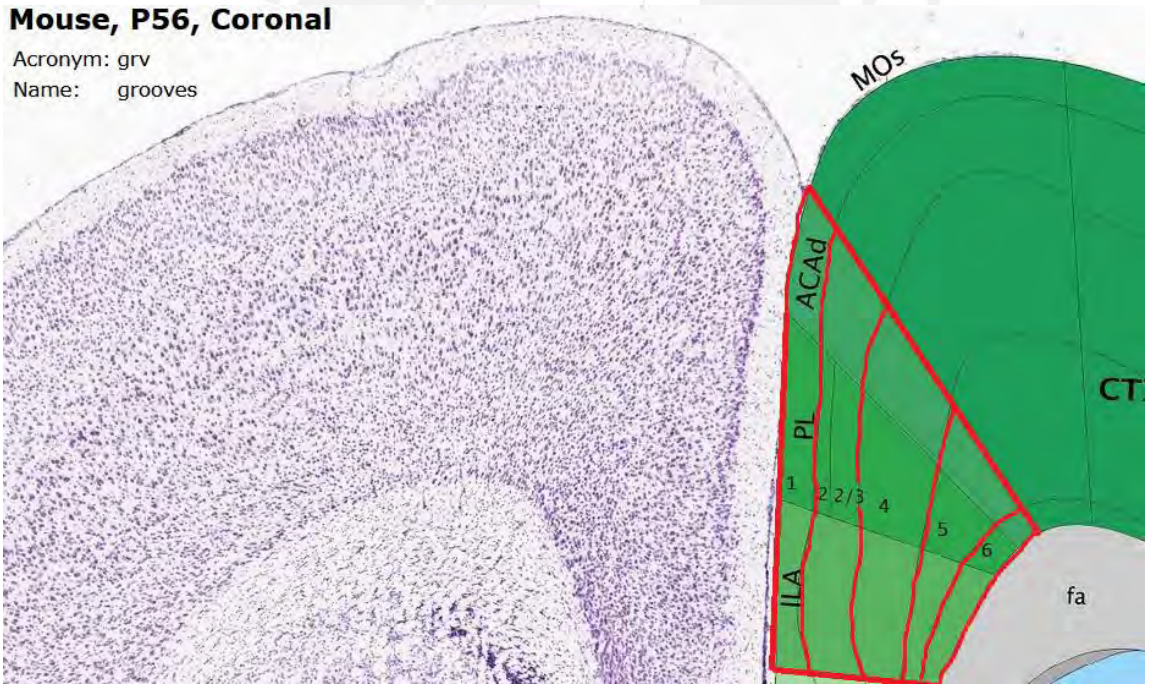

Figure 5: Areas measured, boarders between L1, L2/L3 and L4, L5, and L6 from the pial surface to the white matter inside Cg1, PrL and IL regions.

Note: PL stands for prelimbic cortex in Allen Mouse Brain Atlas (2008).

[a] Numbers in PL represents cortical layers in PL and throughout the other mPFC regions.

4.8 Statistics

All statistical analyses were performed using IBM SPSS Statistics 23. In order to compare the discrimination index and total exploration time of the animals during both training and testing, one-way ANOVAs with drug treatment as between-subject variable and LSD post-hoc tests were used. To contrast the differences of the activation in the brain regions, separated analyses for each of the three medial pre-frontal cortex regions were employed. For each region, a mixed-design repeated measures ANOVAs with drug treatment as between-subject variable and cortical layers as within-subject variable and Tukey's post-hoc analysis were used. For all comparisons, a p-value less than 0.05 was considered as statistically significant. The calculations were made based on mean ± SE.

5. Results

5.1. Behavioral Results

5.1.1 Effect of yohimbine and corticosterone on object-in-context memory: Role of habituation in the training context.

Mice were trained and tested on the OiC task as described in the method section. Initially, the animals were habituated in the same context as the one used for training and testing on the OiC task. The drugs were injected immediately after training and the retention test followed 24 hr later. The experiments came to uncover a much-unpredicted result. Based on previous findings (Roozendaal & Mirone, 2020), it would be expected that yohimbine would enhance memory accuracy, but that corticosterone-treated animals show an impaired memory accuracy, as previously shown for the inhibitory avoidance discrimination task. Contrary to this hypothesis, the findings indicate that both yohimbine and corticosterone administration enhanced memory accuracy (Figure 6). One-way ANOVAs for discrimination index revealed a significant yohimbine effect ($F(3,52)=4.80$, $p=0.005$, $\eta p2=0.39$) as well as corticosterone effect ($F(3,53)=4.85$, $p=0.005$, $\eta p2=0.53$). Further, post hoc analyses showed that the discrimination index of the 1 mg/kg yohimbine group ($n=15$, $M=26.9$, $SD=20.6$) was significantly different from that of the vehicle group ($n=14$, $M=6.1$, $SD=18.6$; $p=0.003$). It was also significantly distinct from the 0.3mg/kg dose group ($n=12$, $M=11.76$, $SD=22$; $p=0.036$) and the 3 mg/kg dose group ($n=15$, $M=12.34$, $SD=19.9$; $p=0.001$). Importantly, the low and the high dose of yohimbine did not show any difference from the saline group.

For corticosterone, the 3 mg/kg group ($n=15$, $M=15.66$, $SD=14.77$) had a significantly greater discrimination index compared to the vehicle group ($n=15$, $M=4.72$, $SD=10.42$; $p=0.024$). The discrimination index of the 3 mg/kg group was also significantly different from that of the 1 mg/kg dose group ($n=13$, $M=-1.2$, $SD=11.13$; $p=0.001$) and 10 mg/kg dose group ($n=14$, $M=1.04$, $SD=12.57$; $p=0.004$). Moreover, the discrimination index of the lower or higher dose groups did not differ significantly from that of the vehicle group. Training data are reported as the total exploration time of the objects, left and right and in the two boxes. The findings indicate that the animals do not have any preference to explore the object placed on either the right or left side. Neither do they explore the objects differently in the two boxes. Likewise, all possible

combinations of pairings of objects and contexts and left and right side were done in a counterbalanced order to exclude all kinds of biases.

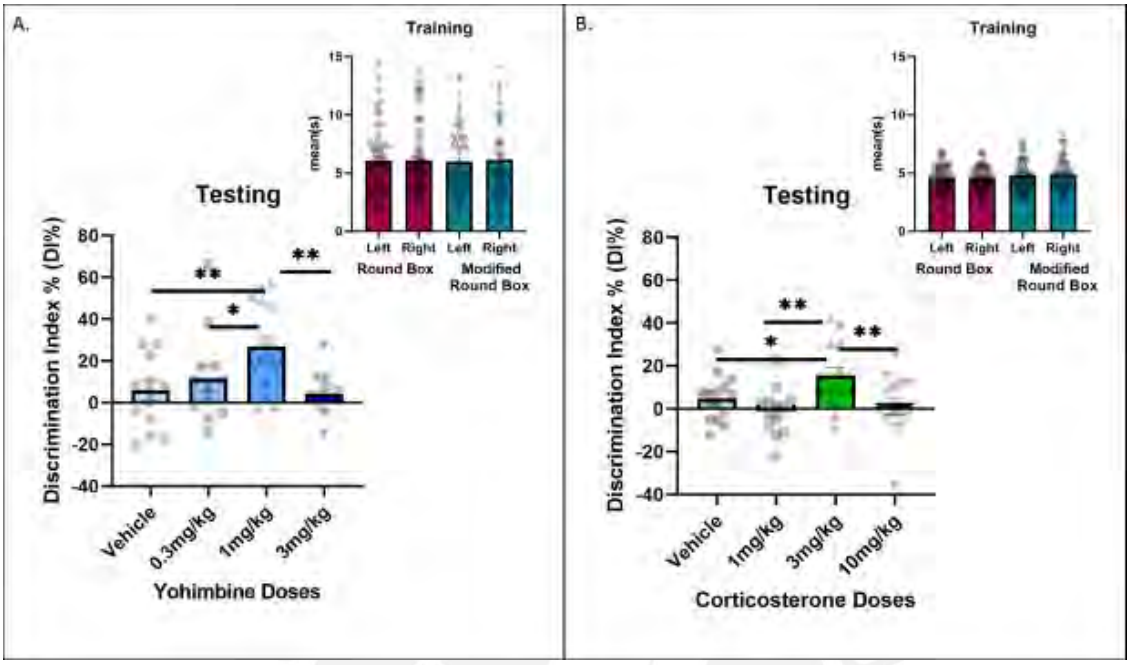

*Figure 6: The effect of the stress hormones as the discrimination index in memory accuracy. Animals were habituated in the same two contexts as the training and testing box. The results are reported by the discrimination index. Data are expressed as mean ± SEM. Dots, squares and triangles in the different graphs represent individual data points. *P < 0.05, **P < 0.01, n=~15 mice per group. A) The dose-dependent effect of yohimbine in memory accuracy. B) The dose-dependent effect of Corticosterone in memory accuracy. Next to the testing, the training results are reported as the total exploration time of the objects and are presented showing the absence of biases based on the position of the object and the context (n~15 mice per group).*

5.2 Immunohistological results

To examine how the two stress hormones affect the mPFC activity during the post-learning consolidation phase, immunohistochemical staining on the brains of mice was performed. Specifically, the aim was to study the effect of the two stress hormones on neuronal activity within the cortical layers of subareas Cg1, PrL and IL. For this experiment, animals were only treated with the most effective dose of yohimbine (1.0 mg/kg) or corticosterone (3 mg/kg). The training conditions were the same as the ones described in the previous section and no biases based on the context, the object or the position of the object were observed. Drug treatment was given immediately after the training and the animals were sacrificed 1hr after training to be able to observe neuronal

activity during consolidation. For immunostaining, two antibodies (c-Fos and GAD67) that stain for active cells and GABAergic inhibitory neurons respectively and DAPI for nuclei (Figure 7A) were used. For this anatomical investigation, the measured areas were Cg1, PrL and IL (Figure 7C). The co-localization of the c-Fos and GAD67, which give us information about the active GABAergic cells (Figure 7B) was also a research objective. The hypothesis was that yohimbine and corticosterone administration might have opposite effects on mPFC activity.

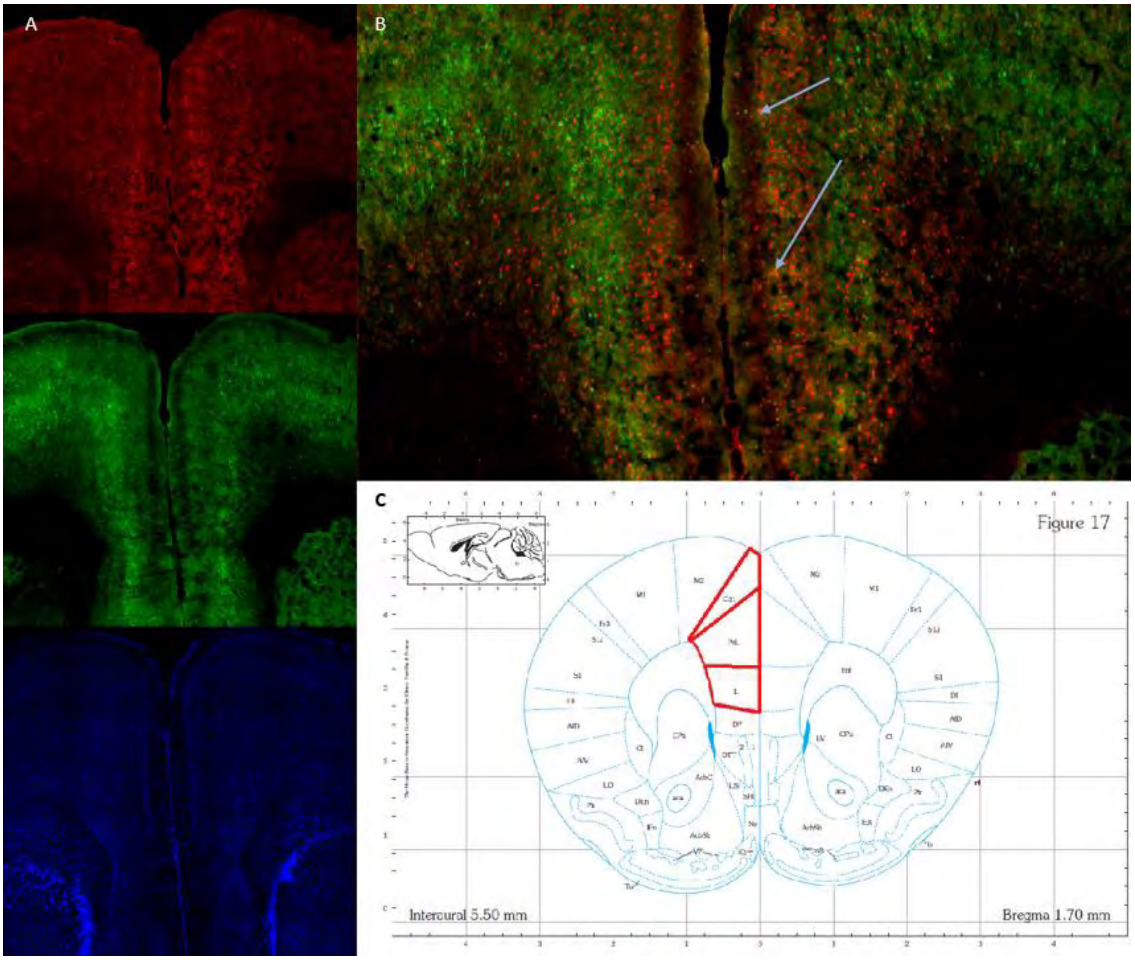

Figure 7: A) Staining example mPFC, top c-Fos middle GAD-67 and bottom DAPI staining. B) Colocalization of c-Fos and GAD67. C) Example of areas measured pointed on a picture taken by Paxinos and Franklin atlas (Paxinos and Franklin, 2008).

5.2.1 The effects of yohimbine and corticosterone on non-GABAergic activity after OiC training.

In this part of the experiment, the aim was to examine the mPFC activity under the effect of the two stress hormones. The explored activity here is the non-GABAergic one. When referring to the non-GABAergic activity, it indicates active neurons that express c-Fos but not expressing GAD67 (c-Fos-GAD67).

5.2.1 a Systemic yohimbine injection increased, but corticosterone did not affect, activity of non-GABAergic cells in Cg1 and PrL. No effect of drug treatment was observed in IL region.

It was studied how the excitatory cells are activated in different areas of the mPFC under the effect of post training administration of yohimbine or corticosterone. Our analysis came to support only in part the findings of the behavioral study. For the c-Fos expressing cells normalized per area, cells that express only c-Fos but not GAD67 per area reported on mm2 were counted. Mixed-design repeated measure ANOVAs for each mPFC region was used to explore if there were interactions between cortical layers and drug treatment. However, the results revealed no significant interaction between Layers and Drug Treatment in Cg1 (Wilk's Lambda=0.59, $F(6,46)=2.25$, $p=0.055$), in PrL (Wilk's Lambda=0.62, $F(6,46)=2.08$, $p=0.073$) or in IL region (Wilk's Lambda=0.70, $F(6,46)=2.25$, $p=0.199$). Nonetheless, animals treated with yohimbine showed significantly more c-Fos expressing non-GABAergic cells in Cg1 (Figure 8) and PrL (Figure 9) regions. Specifically, for the number of c-Fos-expressing non-GABAergic cells in Cg1 a significant main effect of drug was observed ($F(2,25)=7.38$, $p=0.003$, $\eta p2=0.37$). Further post-hoc analysis indicated that yohimbine-treated mice ($n=10$, $M=1363.28$) had significantly more c-Fos expressing non-GABAergic cells than those administered vehicle ($n=9$, $M=883.34$), $t(12.94)=-2.84$, $p=0.014$. and those administered corticosterone ($n=9$, $M=791.79$), $t(17)=-3.13$, $p=0.006$.

Likewise, for the number of c-Fos-expressing non-GABAergic cells in PrL a significant main effect of drug was revealed ($F(2,25)=6.05$, $p=0.007$, $\eta p2=0.33$). Further post-hoc analysis indicated that yohimbine-treated mice ($n=10$, $M=1344.58$) had significantly more c-Fos expressing non-GABAergic cells than those administered vehicle ($n=9$, $M=933.08$), $t(14.38)=-2.28$, $p=0.039$ and those administered corticosterone ($n=9$, $M=811.82$), $t(11.99)=-3.16$, $p=0.008$. On the other hand, no main effect of drug for the number of c-Fos-expressing non-GABAergic cells was observed in IL region ($F(2,25)=2.20$, $p=0.131$) (Figure 10).

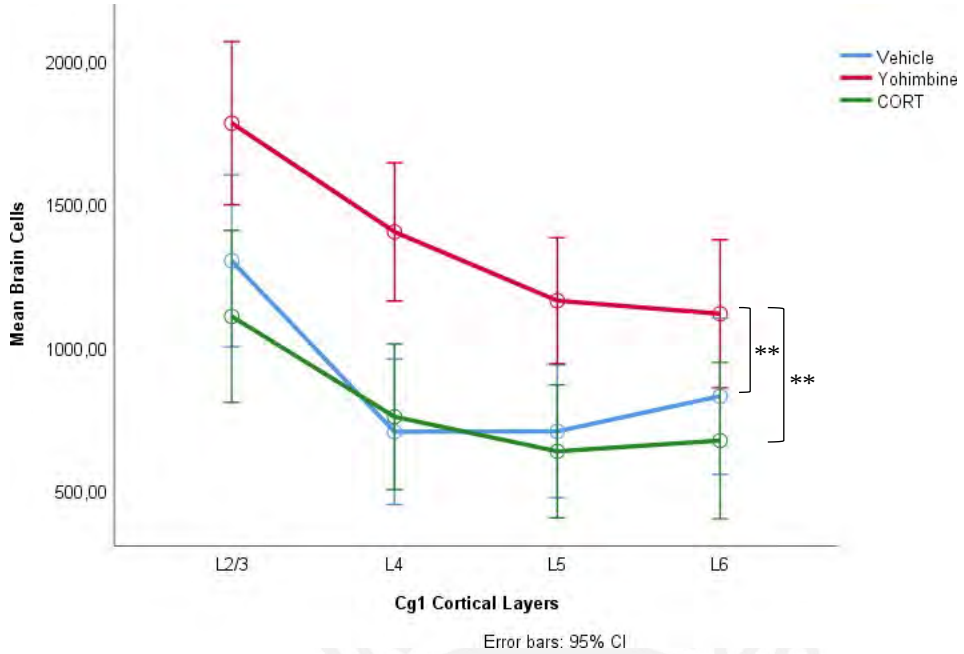

Figure 8: Effect of drug treatment in Cg1 brain activity by cortical layer.
Overall yohimbine increased Cg1 brain activity in all cortical layers and not in a layer specific manner.
***p < 0.01, n=~11.*

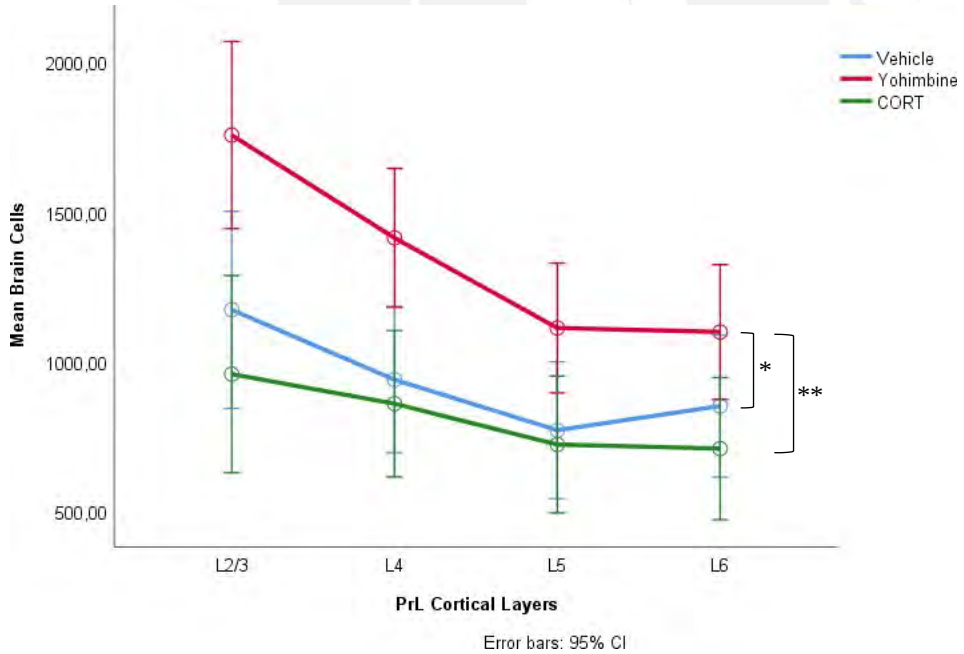

Figure 9: Effect of drug treatment in PrL brain activity by cortical layer.
Overall yohimbine increased PrL brain activity in all cortical layers and not in a layer specific manner.
***p < 0.01, n=~11.*

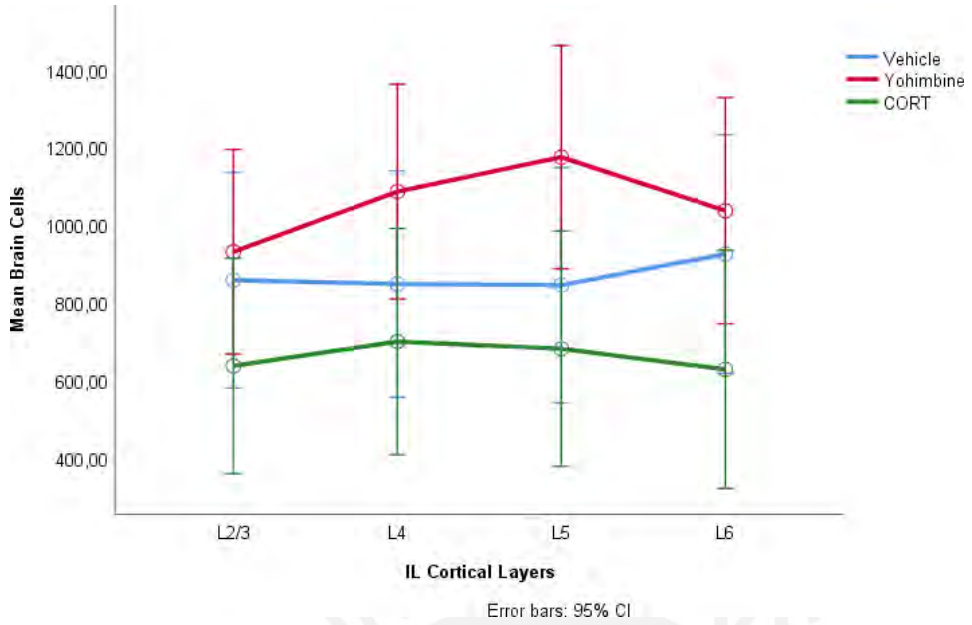

Figure 10: Effect of drug treatment in IL brain activity by cortical layer. No effect of drug was observed for the number of c-Fos-expressing non-GABAergic cells in IL region, p=0.131, n=~11.

5.2.2 No effect of drugs in GABAergic activity or in the total number of GABAergic neurons.

The objective here was to examine if the excitatory activity is the only one that changes due to different manipulations in the brain. By investigating GAD67 and c-Fos activation in GABAergic neurons, a clearer view on the stimulation of the mPFC under the effect of the stress hormones could be obtained. When referring to GABAergic neurons' activity measured by c-Fos expression, it indicates the number of cells that co-expressed c-Fos and GAD67. On the other hand, when total number of GABAergic neurons is mentioned, it indicates the number of cells that expressed GAD67. For GABAergic activity mixed-design repeated measure ANOVAs revealed no significant interaction between Layers and Drug Treatment in Cg1 (Wilk's Lambda=0.85, $F(6,46)=0.68$, $p=0.670$), Prl (Wilk's Lambda=0.80, $F(6,46)=0.89$, $p=0.511$) or IL region (Wilk's Lambda=0.87, $F(6,46)=0.54$, $p=0.776$). In addition, no main effect of drug effect was observed for GABAergic activity in Cg1 ($F(2,25)=0.01$, $p=0.987$), Prl ($F(2,25)=0.12$, $p=0.892$) or IL region ($F(2,25)=0.13$, $p=0.879$). Similarly, for total number of GABAergic neurons mixed-design repeated measure ANOVAs revealed no significant interaction between Layers and Drug Treatment in Cg1 (Wilk's

Lambda=0.77, $F(6,46)=1.09$, $p=0.384$), Prl (Wilk's Lambda=0.64, $F(6,46)=1.92$, $p=0.097$) or IL region (Wilk's Lambda=0.83, $F(6,46)=0.75$, $p=0.611$). In addition, no main effect of drug effect was observed for GABAergic activity in Cg1 ($F(2,25)=1.05$, $p=0.364$), Prl ($F(2,25)=1.98$, $p=0.159$) or IL region ($F(2,25)=2.65$, $p=0.090$).

6. Discussion

In this study, the aim was to investigate the effect of noradrenaline and glucocorticoids on memory accuracy in the OiC task, and the changes in mPFC brain activity related to the stress hormones induction by drug treatment. Our behavioral results indicate that both hormones enhance memory accuracy when the animals had prior knowledge of the training contexts. Nonetheless, our histological analysis further revealed that only yohimbine increased the number of c-Fos-expressing non-GABAergic cells in the Cg1 and PrL regions of the mPFC. No other effect of drug was observed for others brain cells type and mPFC regions.

6.1 Stress hormone effects on the OiC task under the influence of systems consolidation caused by different habituation conditions.

On the OiC task, it was expected that yohimbine would improve memory accuracy and corticosterone would induce memory generalization based on prior findings (Roozendaal & Mirone, 2020). In the current study, this prediction was not confirmed by the results. Yohimbine, given immediately after the training session, improved memory accuracy on a 24-hour retention test in a dose-dependent manner. Yohimbine at a dose of 1 mg/kg evidenced to be the most effective. The doses of 0.3 mg/kg and 3 mg/kg had no effect on memory accuracy, showing an inverted U-shape pattern for this drug dose dependency. Contrary to expectations, corticosterone treatment also improved memory accuracy in a dose-dependent manner. The most effective dose was 3 mg/kg, and neither the lower (1 mg/kg) nor the higher (10 mg/kg) doses affected memory accuracy, following also an inverted U-shaped pattern for the effectiveness of this drug.

These initial findings were intriguing. Thus, differences in the experimental design between the inhibitory avoidance discrimination task (previously used in other studies) and the OiC task were analyzed in an attempt to establish methodological explanations. A remarkable difference is the degree of emotional arousal triggered by each of the task situations. In that respect, for the inhibitory avoidance discrimination task the animals are trained in two distinct inhibitory avoidance apparatuses, but they only get an electric footshock when entering into the second training apparatus dark compartment. The animals ability to associate the shock experience with the actual shock context is subsequently tested (Atucha et al., 2017). Therefore, the shock

experience in interaction with the drug treatment could be a factor for the differences in memory accuracy observed between the two tasks. However, the intensity of the shock is typically very low (0.18 to 0.38 mA for 1 sec) and as a result likely only mildly stressful (Roozendaal & Mirone, 2020; Schutsky et al., 2011).

The habituation process is another significant distinction between the two tasks. The inhibitory avoidance discrimination task has previously been performed in rats without prior habituation to the training environments. Mice must become acclimated to the surroundings in which they will be trained later. Otherwise, mice may spend more time examining the environment safety or hiding in a corner rather than completing the task (Buccafusco, 2000). Consequently, mice were habituated to each of the two training contexts for three days previous to the training day. Three days of acclimatization proved to be enough to alleviate the stress elicited by the new surroundings (for details see protocol in method section). Nonetheless, prior exposure to the training environment may lead to system consolidation, making context memory less dependent on the hippocampus and more dependent on the mPFC (Winocur & Moscovitch, 2011). Thereby, when the animals were exposed to the training phase, it is possible they recognized the contexts, and this might have influenced the memory enhancing effects observed in the two drug conditions. In that regard, recently Timplalexi (2020) has shown that when mice are habituated in different contexts from the ones used later for training and testing in the OiC task, stress hormones influence memory in a similar way as in the inhibitory avoidance discrimination task. That is, yohimbine improved memory accuracy in a dose-dependent manner and corticosterone, on the other hand, had no effect on memory. However, it should be noted that vehicle control animals showed no memory of the training session, so corticosterone administration could not further impair memory on this task (Timplalexi, 2020). Hence, the findings of the current study are novel because they reveal that the two stress hormones can have a similar effect on memory accuracy, but that might be dependent on the familiarity with the training context.

6.2 Yohimbine enhances non-GABAergic activity in all cortical layers of Cg1 and PrL regions. No effect of drug was observed in IL activity.

Our cellular analysis supports only in part the behavioral outcomes. It was found that yohimbine influenced non-GABAergic c-Fos activity in Cg1 and PrL. Hence, the

memory-enhancing dose of yohimbine also enhanced c-Fos activity in excitatory cells of mPFC regions. This is consistent with the idea that these cortical regions play important roles in different aspects of recognition memory in rodents (Morici et al., 2015; Tanimizu et al., 2018; Warburton & Brown, 2015). Specifically, robust changes in neural activity of the anterior cingulate cortex (ACC) of mice, which includes Cg1 region, have been associated with the exploration of objects, former object locations and the introduction of novel objects into the environment (Weible et al., 2009). Moreover, a study observed that micro-infusion of the GABAa receptor agonist, muscimol, into the ACC to reversibly inactivate the area impaired novel object recognition memory after a 24h delay (Pezze et al., 2017). Likewise, animals with lesions in the PrL were unable to discriminate between objects that had been sampled at different time points in a Recency Task (Nelson et al., 2011). Additionally, infusion of SKF81297, a dopamine D1 receptor agonist, into the PrL impaired the recognition of a novel object after a 10 min delay without affecting activity or exploration, suggesting an involvement of PrL D1 receptors in the formation and retrieval/expression of novel object recognition (Pezze et al., 2015).

On the other hand, it was not confirmed that yohimbine influenced non-GABAergic c-Fos activity in IL region. This finding differs from other studies which have observed that the IL is involved in memory recognition in rodents (Barker & Warburton, 2020; DeVito & Eichenbaum, 2010; Nelson et al., 2011; Tanimizu et al., 2018). However, some of these studies did not only target the IL, they applied lesions to different regions of the mPFC along with the IL (Barker & Warburton, 2020; DeVito & Eichenbaum, 2010). Thus, it is difficult to assess the actual relevance of the IL in the recognition memory tasks of those studies. Although Nelson et al. (2011) reported that targeted lesions specifically into the IL disrupted performance in an object location task, those lesions were not as anatomically selective as expected. Quantification of the selectivity of those lesions indicated a robust depletion of IL (~74%) but also a ~49% depletion of the PrL suggesting some spread of the toxin dorsally. Importantly, permanent or *reversible* lesions are susceptible to unintentional off-target effects that might inactivate other brain regions besides the targeted one (Bell & Bultitude, 2018). Thus, it is hard to determine to what extent PrL depletion was also involved in the impairment of object location recognition in Nelson et al. study.

Nonetheless, Tanimizu et al. (2018) measured mPFC c-fos activity of mice after training and observed higher c-fos-positive cells in PrL and IL of mice that were previously habituated to a Novel Object Recognition task (NOR) in contrast to those that were not habituated to the task. Therefore, those findings differ from the ones observed in this study. In that regard, some important distinctions need to be considered between Tanimizu et al. (2018) study from this research. First, mPFC activity between habituated animals vs. non-habituated animals was not compared. Further investigations concerning the OiC task could implement a non-habituated condition in the study design to assess the effects of lack of habituation in mPFC activity. Secondly, differences in mPFC activity were explored due to different pharmacological treatments, while Tanimizu et al. (2018) studied mPFC neural differences according to previous habituation or non-habituation conditions without drug treatment. Thirdly, the NOR task used by Tanimizu et al. (2018) placed two different objects for exploration in only one training context whereas two sets with two identical objects in two training contexts were placed in this experiment. Consequently, in each study the animals performed different kind of context-object associations which could have been reflected in differences in IL activity.

Finally, in this study no differences in brain activity were observed between the cortical layers of any of the mPFC regions due to drug treatment. In turn, it was found an overall increase of non-GABAergic c-Fos activity in both Cg1 and PrL of mice treated with yohimbine after training compared to those that received a vehicle or corticosterone. Yohimbine is a noradrenergic stimulant. In that respect, it has been evidence of increased noradrenaline levels in the mPFC when rodents perform correctly in a spatial working memory task (Rossetti & Carboni, 2005). Moreover, injection of a β adrenergic receptor antagonist into the PrL 2hr post training produced amnesia in rodents trained in an associative learning odor-discrimination task, suggesting a delayed role of noradrenaline in a late phase of long-term memory consolidation (Tronel et al., 2004).

To our knowledge, it has not been reported previously any specific distinction among mPFC cortical layers activity during the consolidation memory stage of the OiC task. Thus, these results suggest that yohimbine generally increases activity over all cortical layers in Cg1 and PrL regions and not in a layer specific manner during the

consolidation of new memories in the OiC task. Taking all these elements into consideration, it can be inferred that noradrenergic activation is related to a general increment of excitatory cells in all cortical layers of Cg1 and PrL and that strengthens consolidation process and memory accuracy in the OiC task. This outcome is coherent with the fact that locus coeruleus (LC) noradrenergic projections into the mPFC have modulatory effects on working memory and attention (Caetano et al., 2013; Mather et al., 2015; Ramos & Arnsten, 2007). It has been argued that these operations are likely to be important for the mPFC capacity to assess the contextual relevance and emotional valence of potentially threatening stimuli for mounting adaptive responses (Radley et al., 2008).

6.3 Corticosterone did not alter non-GABAergic activity, GABAergic activity or the total number of GABAergic neurons in the mPFC

Because corticosterone treated animals showed enhanced memory in the OiC task, it was expected to find differences in mPFC activation between corticosterone and vehicle for any of the cells' types addressed in this study, and/or between corticosterone and yohimbine for GABAergic activity or the total number of GABAergic neurons. However, no difference in mPFC activity between corticosterone and the other drug conditions was found. These findings suggest that corticosterone memory enhancement effect in the OiC task was neither due to an inhibitory regulation process nor a stimulation of excitatory cells within mPFC. In that respect, even though the regulatory role of GABAergic activity in memory processes have been documented in different studies (Gasbarri & Pompili, 2014; Kalueff & Nutt, 1996; Tabassum et al., 2017), it has not been assessed if glucocorticoids memory effects is related to changes in GABAergic activity within the mPFC of rodents.

Importantly, there is evidence that glucocorticoid-induced enhancement of memory consolidation involves a neural circuitry that include the BLA and its projections to efferent brain regions (McGaugh, 2002; McGaugh, 2004; Roozendaal, McEwen, et al., 2009), whereas glucocorticoid impairment of working memory depends primarily on influences within the mPFC (Roozendaal et al., 2004). Therefore, stress-level glucocorticoid effects on both memory consolidation and working memory depend critically on interactions between the BLA and mPFC (Roozendaal et al., 2004; Roozendaal et al., 2009). Furthermore, it has been argued that stress exposure or

glucocorticoid administration alters mPFC activity in such a way that it impairs working memory and concurrently enhances memory consolidation (Barsegyan et al., 2010). A temporary decrement of working memory capacity might inhibit interference, thus protecting newly encoded information from distraction and thereby, enable a memory enhancement of emotionally significant experiences (Bunge et al., 2001; Kapur et al., 1995).

In that regard, Barsegyan et al. (2010) found that a glucocorticoid receptor (GR) agonist administered into mPFC of rats both enhanced memory consolidation and impaired working memory. These memory effects were mediated by activation of a membrane-bound steroid receptor and depended on noradrenergic activity within the mPFC to increase levels of cAMP dependent protein kinase1. Moreover, in another study they observed that GR agonist administration into the PrL enhanced object recognition memory via functional interactions with the anterior insular cortex (aIC) and dorsal hippocampus (dHPC). Specifically, GR agonist induction into the PrL reduced c-fos activity in the aIC and increased c-fos activity in the dHPC, but no changes in c-fos activity in the PrL was reported. In addition, propranolol into the BLA completely blocked this effect, indicating that BLA noradrenergic activity is essential for enabling this neural process during the post-learning consolidation period (Barsegyan et al., 2019). Accordingly, the memory consolidation enhancement of corticosterone treated mice observed in this study most likely followed these neural pathways. However, we did not analyzed activity within other brain regions which could give us a broad understanding of the results from the behavioral task. Nevertheless, in line with Barsegyan et al. (2010) findings, it was observed that mPFC activity related to memory enhancement was influenced by noradrenergic stimulation due to yohimbine treatment, which was discussed in the previous section.

6.4 Criticism

1 Protein kinase A (PKA) is a family of enzymes whose activity is dependent on cellular levels of cyclic AMP (cAMP). PKA is also known as cAMP-dependent protein kinase. PKA has several functions in the cell, including regulation of glycogen, sugar, and lipid metabolism (Turnham & Scott, 2016). Evidence indicates that an activation of PKA within the mPFC impairs working memory (Taylor, Birnbaum, Ubriani, & Arnsten, 1999), whereas increased cAMP/PKA activity within the BLA and hippocampus seems necessary for influencing long-term neuroplasticity and memory formation (Schafe & LeDoux, 2000).

The current study was the first to investigate the effects of both noradrenaline and glucocorticoids in mPFC activity in an episodic-like low-arousing memory task in mice. It is important to consider that the results presented here might be influenced by the habituation condition prior to the training and testing phase. In that regard, Timplalexi (2020) has shown that different habituation conditions elicited different effects on memory due to corticosterone or yohimbine treatment, as well as differences in hippocampal activation. Here, brains of animals conditioned in the same context for training and testing were analyzed. Thus, the effects of both stress hormones in mPFC activity under a no-habituation condition or habituation in a different context from the ones used in training and testing remain unknown. Besides, there are studies showing that habituation can be divergent depending on the mice strain. Hence, if another strain would be used that might lead to different habituation outcomes. Furthermore, HPA-axis response to habituation conditions can also differs between mice strains (Ryabinin et al., 1999).

In addition, only the mPFC was explored in this study. Thus, there can only be speculations about the involvement of other brain areas, like the BLA, which is known to play a key role in memory consolidation dynamics. Secondly, immunolabeling was the only technique used to examine brain activity. Even though this is a useful tool for neuronal study, other techniques like iDISCO may offer a more comprehensive view of neural activity. iDISCO allows to obtain 3D images of specific molecules labelled throughout a whole tissue. Although, these findings suggest a relation among habituation, drug effect and brain activity, additional research is needed to determine the causal connections between those factors.

6.5 Future directions

In the future, the findings presented here could be useful to investigate in depth the habituation effects in connection with stress hormones activity in the brain. This study only used animals habituated to the same context as the training context. However, as mentioned above, recent data from Timplalexi (2020) compared this same habituation condition against a group of mice habituated to a different context from the training context. At a behavioral level, they observed that mice habituated to a different context from the training context only showed memory enhancement under the effect of yohimbine, but not corticosterone. For animals habituated in the same context as the

training context, the behavioral results are the same. However, they only observed differences in hippocampal activity in mice that were habituated in a different context from the training context. Thus, their results suggest that habituation to the training context appeared to reduce the effect of the two stress hormones on altering hippocampal activity during the consolidation period. Interestingly, it was observed that in those animals yohimbine increased activation of the mPFC, but corticosterone did not affect mPFC activity in this study. Taken together, Timplalexi (2020) findings and the present results indicate that corticosterone memory enhancement effect might not be directly related to hippocampal or mPFC activity when mice have previous knowledge of the training context. Certainly, this is very interesting and raises the important question of which brain region(s) is/are then involved. Future studies could address this question by examining other brain regions like the BLA and cortical areas surrounding the hippocampus after corticosterone treatment in this task. Moreover, since the mPFC of the animals not habituated on the same context as the training context has not been analyzed in either of the two studies, it remains unknow how the mPFC is working during memory consolidation process when mice have not prior knowledge of the training context. Naturally, this would offer a broader understanding of the circumstantial role of the stress hormones in the mPFC. Future studies should address this issue.

Furthermore, study the possible effects of habituation in the inhibitory avoidance task would be interesting for future research. Considering that changes in mPFC activity related to stress hormones administration were observed in this study but these results are only correlational, investigate the causal dynamics between stress hormones and mPFC on memory accuracy is still an important question to explore. Moreover, it would be interesting to label the types of excitatory and inhibitory cells to see if the excitatory neurons are glutamatergic as expected and the GABAergic neurons are parvalbumin or somatostatin cells. This would lead to a greater understanding of the physiology and function of the cells under investigation.

7. Conclusion

The current study suggests that both yohimbine and corticosterone enhance memory of mice that have been trained in an episodic-like low-arousing memory task. Changes in the activity of non-GABAergic neurons in the mPFC reflect this on a

cellular level. Yohimbine appears to improve memory accuracy by boosting noradrenaline in the synaptic cleft and increasing brain activity in the mPFC. Corticosterone improved memory accuracy, but this was not reflected by changes in non-GABAergic or GABAergic activity in the mPFC. In general, this could mean that glucocorticoids might be enhancing memory by activation of other brain regions or through neural pathways that have not been addressed in this study.

8. References

Akirav, I., & Maroun, M. (2006). Ventromedial prefrontal cortex is obligatory for consolidation and reconsolidation of object recognition memory. *Cerebral cortex, 16*(12), 1759-1765.

Almási, Z., Dávid, C., Witte, M., & Staiger, J. F. (2019). Distribution Patterns of Three Molecularly Defined Classes of GABAergic Neurons Across Columnar Compartments in Mouse Barrel Cortex. *Frontiers in Neuroanatomy, 13*(45). doi: 10.3389/fnana.2019.00045

Ameen-Ali, K., Easton, A., & Eacott, M. (2015). Moving beyond standard procedures to assess spontaneous recognition memory. *Neuroscience & Biobehavioral Reviews, 53*, 37-51.

Atsak, P., Hauer, D., Campolongo, P., Schelling, G., Fornari, R. V., & Roozendaal, B. (2015). Endocannabinoid signaling within the basolateral amygdala integrates multiple stress hormone effects on memory consolidation. *Neuropsychopharmacology, 40*(6), 1485-1494.

Atucha, E., Vukojevic, V., Fornari, R. V., Ronzoni, G., Demougin, P., Peter, F., . . . Roozendaal, B. (2017). Noradrenergic activation of the basolateral amygdala maintains hippocampus-dependent accuracy of remote memory. *Proceedings of the National Academy of Sciences, 114*(34), 9176-9181. doi: 10.1073/pnas.1710819114

Barker, G., & Warburton, E. (2020). Putting objects in context: A prefrontal–hippocampal–perirhinal cortex network. *Brain and Neuroscience Advances, 4*, 2398212820937621.

Barker, G. R., Bird, F., Alexander, V., & Warburton, E. C. (2007). Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *Journal of Neuroscience, 27*(11), 2948-2957.

Barker, G. R. I., & Warburton, E. C. (2015). Object-in-place associative recognition memory depends on glutamate receptor neurotransmission within two defined hippocampal-cortical circuits: a critical role for AMPA and NMDA receptors in the hippocampus, perirhinal, and prefrontal cortices. *Cerebral cortex, 25*(2), 472-481.

Barsegyan, A., Mackenzie, S. M., Kurose, B. D., McGaugh, J. L., & Roozendaal, B. (2010). Glucocorticoids in the prefrontal cortex enhance memory consolidation and impair working memory by a common neural mechanism. *Proceedings of the National Academy of Sciences, 107*(38), 16655-16660. doi: 10.1073/pnas.1011975107

Barsegyan, A., McGaugh, J. L., & Roozendaal, B. (2014). Noradrenergic activation of the basolateral amygdala modulates the consolidation of object-in-context recognition memory. *Frontiers in behavioral neuroscience, 8*, 160.

Barsegyan, A., Mirone, G., Ronzoni, G., Guo, C., Song, Q., van Kuppeveld, D., . . . Roozendaal, B. (2019). Glucocorticoid enhancement of recognition memory via basolateral amygdala-driven facilitation of prelimbic cortex interactions. *Proceedings of the National Academy of Sciences, 116*(14), 7077-7082. doi: 10.1073/pnas.1901513116

Bell, A. H., & Bultitude, J. H. (2018). Methods matter: A primer on permanent and reversible interference techniques in animals for investigators of human

neuropsychology. *Neuropsychologia, 115*, 211-219. doi: 10.1016/j.neuropsychologia.2017.09.019
Buccafusco, J. J. (2000). *Methods of behavior analysis in neuroscience*: Crc Press.
Bunge, S. A., Ochsner, K. N., Desmond, J. E., Glover, G. H., & Gabrieli, J. D. (2001). Prefrontal regions involved in keeping information in and out of mind. *Brain, 124*(10), 2074-2086.
Caetano, M., Jin, L., Harenberg, L., Stachenfeld, K., Arnsten, A., & Laubach, M. (2013). Noradrenergic control of error perseveration in medial prefrontal cortex. *Frontiers in Integrative Neuroscience, 6*(125). doi: 10.3389/fnint.2012.00125
Cahill, L., Babinsky, R., Markowitsch, H. J., & McGaugh, J. L. (1995). The amygdala and emotional memory. *Nature, 377*(6547).
Cahill, L., & McGaugh, J. L. (1998). Mechanisms of emotional arousal and lasting declarative memory. *Trends in neurosciences, 21*(7), 294-299.
Cahill, L., Prins, B., Weber, M., & McGaugh, J. L. (1994). β-Adrenergic activation and memory for emotional events. *Nature, 371*(6499), 702-704.
Chandler, V. L., Maler, B. A., & Yamamoto, K. R. (1983). DNA sequences bound specifically by glucocorticoid receptor in vitro render a heterologous promoter hormone responsive in vivo. *Cell, 33*(2), 489-499.
Davis, S., Poirier, R., Renaudineau, S., Poucet, B., Save, E., & Laroche, S. (2010). The formation and stability of recognition memory: what happens upon recall? *Frontiers in behavioral neuroscience, 4*, 177.
De Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience, 6*(6), 463-475. doi: 10.1038/nrn1683
De Kloet, E. R., Karst, H., & Joëls, M. (2008). Corticosteroid hormones in the central stress response: Quick-and-slow. *Frontiers in neuroendocrinology, 29*(2), 268-272. doi: https://doi.org/10.1016/j.yfrne.2007.10.002
de Quervain, D., Schwabe, L., & Roozendaal, B. (2017). Stress, glucocorticoids and memory: implications for treating fear-related disorders. *Nature Reviews Neuroscience, 18*(1), 7-19. doi: 10.1038/nrn.2016.155
de Quervain, D. J. F., Aerni, A., Schelling, G., & Roozendaal, B. (2009). Glucocorticoids and the regulation of memory in health and disease. *Frontiers in neuroendocrinology, 30*(3), 358-370. doi: https://doi.org/10.1016/j.yfrne.2009.03.002
Dere, E., Huston, J. P., & Silva, M. A. D. S. (2005a). Episodic-like memory in mice: simultaneous assessment of object, place and temporal order memory. *Brain research protocols, 16*(1-3), 10-19.
Dere, E., Huston, J. P., & Silva, M. A. D. S. (2005b). Integrated memory for objects, places, and temporal order: evidence for episodic-like memory in mice. *Neurobiol Learn Mem, 84*(3), 214-221.
Dere, E., Huston, J. P., & Silva, M. A. D. S. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience & Biobehavioral Reviews, 31*(5), 673-704.
Dere, E., Kart-Teke, E., Huston, J., & Silva, M. D. S. (2006). The case for episodic memory in animals. *Neuroscience & Biobehavioral Reviews, 30*(8), 1206-1224.
DeVito, L. M., & Eichenbaum, H. (2010). Distinct contributions of the hippocampus and medial prefrontal cortex to the "what-where-when" components of episodic-like memory in mice. *Behav Brain Res, 215*(2), 318-325. doi: 10.1016/j.bbr.2009.09.014

DeVito, L. M., & Eichenbaum, H. (2011). Memory for the order of events in specific sequences: contributions of the hippocampus and medial prefrontal cortex. *Journal of Neuroscience, 31*(9), 3169-3175.

Diorio, D., Viau, V., & Meaney, M. J. (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *Journal of Neuroscience, 13*(9), 3839-3847.

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav Brain Res, 99*(2), 191-200.

Eacott, M. J., & Norman, G. (2004). Integrated memory for object, place, and context in rats: a possible model of episodic-like memory? *Journal of Neuroscience, 24*(8), 1948-1953.

Eichenbaum, H. (2017). Prefrontal–hippocampal interactions in episodic memory. *Nature Reviews Neuroscience, 18*(9), 547-558. doi: 10.1038/nrn.2017.74

Euston, David R., Gruber, Aaron J., & McNaughton, Bruce L. (2012). The Role of Medial Prefrontal Cortex in Memory and Decision Making. *Neuron, 76*(6), 1057-1070. doi: https://doi.org/10.1016/j.neuron.2012.12.002

Farovik, A., Dupont, L. M., Arce, M., & Eichenbaum, H. (2008). Medial prefrontal cortex supports recollection, but not familiarity, in the rat. *Journal of Neuroscience, 28*(50), 13428-13434.

Galvez, R., Mesches, M. H., & Mcgaugh, J. L. (1996). Norepinephrine release in the amygdala in response to footshock stimulation. *Neurobiol Learn Mem, 66*(3), 253-257.

Gasbarri, A., & Pompili, A. (2014). The role of GABA in memory processes *Identification of neural markers accompanying memory* (pp. 47-62): Elsevier.

Goddard, G. V. (1964). Amygdaloid stimulation and learning in the rat. *Journal of comparative and physiological psychology, 58*(1), 23.

Gold, P. E., & van Buskirk, R. (1978). Posttraining brain norepinephrine concentrations: correlation with retention performance of avoidance training and with peripheral epinephrine modulation of memory processing. *Behavioral biology, 23*(4), 509-520.

Gold, P. E., & Van Buskirk, R. B. (1975). Facilitation of time-dependent memory processes with posttrial epinephrine injections. *Behavioral biology, 13*(2), 145-153.

Gonzalez, C., Kramar, C., Garagoli, F., Rossato, J. I., Weisstaub, N., Cammarota, M., & Medina, J. H. (2013). Medial prefrontal cortex is a crucial node of a rapid learning system that retrieves recent and remote memories. *Neurobiol Learn Mem, 103*, 19-25.

Gorelick, D., Bozewicz, T., & Bridger, W. (1975). The role of catecholamines in animal learning and memory. *Catecholamines and behavior, 2*, 1-30.

Granon, S., & Poucet, B. (2000). Involvement of the rat prefrontal cortex in cognitive functions: a central role for the prelimbic area. *Psychobiology, 28*(2), 229-237.

Hannesson, D., Vacca, G., Howland, J., & Phillips, A. (2004). Medial prefrontal cortex is involved in spatial temporal order memory but not spatial recognition memory in tests relying on spontaneous exploration in rats. *Behav Brain Res, 153*(1), 273-285.

Hardebo, J. E., & Owman, C. (1980). Barrier mechanisms for neurotransmitter monoamines and their precursors at the blood- brain interface. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, 8*(1), 1-11.

Hatfield, T., & McGaugh, J. L. (1999). Norepinephrine infused into the basolateral amygdala posttraining enhances retention in a spatial water maze task. *Neurobiol Learn Mem, 71*(2), 232-239.
Haycock, J. W., Van Buskirk, R., Ryan, J. R., & McGaugh, J. L. (1977). Enhancement of retention with centrally administered catecholamines. *Experimental neurology, 54*(2), 199-208.
Hoffman, G. E., Smith, M. S., & Verbalis, J. G. (1993). c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Frontiers in neuroendocrinology, 14*(3), 173-213.
Hoscheidt, S. M., LaBar, K. S., Ryan, L., Jacobs, W. J., & Nadel, L. (2014). Encoding negative events under stress: High subjective arousal is related to accurate emotional memory despite misinformation exposure. *Neurobiol Learn Mem, 112*, 237-247.
Jin, J., & Maren, S. (2015). Prefrontal-Hippocampal Interactions in Memory and Emotion. *Frontiers in Systems Neuroscience, 9*(170). doi: 10.3389/fnsys.2015.00170
Jobson, D. D., Hase, Y., Clarkson, A. N., & Kalaria, R. N. (2021). The role of the medial prefrontal cortex in cognition, ageing and dementia. *Brain communications, 3*(3), fcab125.
Kalueff, A., & Nutt, D. J. (1996). Role of GABA in memory and anxiety. *Depress Anxiety, 4*(3), 100-110. doi: 10.1002/(sici)1520-6394(1996)4:3<100::aid-da2>3.0.co;2-k
Kapur, S., Craik, F., Jones, C., Brown, G., Houle, S., & Tulving, E. (1995). Functional role of the prefrontal cortex in retrieval of memories: A PET study. Neuroreport: An International Journal for the Rapid Communication of Research in Neuroscience, 6 (14), 1880–1884.
Kapuscinski, J. (1995). DAPI: a DNA-specific fluorescent probe. *Biotechnic & Histochemistry, 70*(5), 220-233.
Kesner, R. P., & Churchwell, J. C. (2011). An analysis of rat prefrontal cortex in mediating executive function. *Neurobiol Learn Mem, 96*(3), 417-431.
Kolb, B. (1984). Functions of the frontal cortex of the rat: a comparative review. *Brain Research Reviews, 8*(1), 65-98.
Kolb, B. E., & Tees, R. C. (1990). *The cerebral cortex of the rat*: The MIT Press.
Kovács, G. L., Telegdy, G., & Lissák, K. (1977). Dose-dependent action of corticosteroids on brain serotonin content and passive avoidance behavior. *Hormones and Behavior, 8*(2), 155-165.
Laubach, M., Amarante, L. M., Swanson, K., & White, S. R. (2018). What, if anything, is rodent prefrontal cortex?. *Eneuro, 5*(5).
Levine, L. J., Lench, H. C., Stark, C. E., Carlson, S. J., Carpenter, Z. K., Perez, K. A., . . . Frithsen, A. (2020). Predicted and remembered emotion: tomorrow's vividness trumps yesterday's accuracy. *Memory, 28*(1), 128-140.
Liang, K. C. (2001). Epinephrine modulation of memory: Amygdala activation and regulation of long-term memory storage.
Liang, K. C., Juler, R. G., & McGaugh, J. L. (1986). Modulating effects of posttraining epinephrine on memory: involvement of the amygdala noradrenergic system. *Brain Res, 368*(1), 125-133. doi: 10.1016/0006-8993(86)91049-8
Loizou, L. (1970). Uptake of monoamines into central neurones and the blood-brain barrier in the infant rat. *British journal of pharmacology, 40*(4), 800.
Lonergan, M. H., Olivera-Figueroa, L. A., Pitman, R. K., & Brunet, A. (2013). Propranolol's effects on the consolidation and reconsolidation of long-term

emotional memory in healthy participants: a meta-analysis. *Journal of psychiatry & neuroscience : JPN, 38*(4), 222-231. doi: 10.1503/jpn.120111
Makara, G. B., & Haller, J. (2001). Non-genomic effects of glucocorticoids in the neural system: evidence, mechanisms and implications. *Progress in neurobiology, 65*(4), 367-390.
Mather, M., Clewett, D., Sakaki, M., & Harley, C. W. (2015). Norepinephrine ignites local hotspots of neuronal excitation: How arousal amplifies selectivity in perception and memory. *Behavioral and Brain Sciences, 39*, e200. doi: 10.1017/S0140525X15000667
McDonald, R. J., & White, N. M. (2013). A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum.
McEwen, B. S., De Kloet, E. R., & Rostene, W. (1986). Adrenal steroid receptors and actions in the nervous system. *Physiol Rev, 66*(4), 1121-1188. doi: 10.1152/physrev.1986.66.4.1121
McGaugh, J. L. (2000). Memory--a century of consolidation. *Science, 287*(5451), 248-251.
McGaugh, J. L. (2002). Memory consolidation and the amygdala: a systems perspective. *Trends in neurosciences, 25*(9), 456-461. doi: https://doi.org/10.1016/S0166-2236(02)02211-7
McGaugh, J. L. (2003). *Memory and emotion: The making of lasting memories*: Columbia University Press.
McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu. Rev. Neurosci., 27*, 1-28.
McGaugh, J. L., & Roozendaal, B. (2002). Role of adrenal stress hormones in forming lasting memories in the brain. *Current opinion in neurobiology, 12*(2), 205-210.
McIntyre, C. K., Hatfield, T., & McGaugh, J. L. (2002). Amygdala norepinephrine levels after training predict inhibitory avoidance retention performance in rats. *European Journal of Neuroscience, 16*(7), 1223-1226.
McIntyre, C. K., Miyashita, T., Setlow, B., Marjon, K. D., Steward, O., Guzowski, J. F., & McGaugh, J. L. (2005). Memory-influencing intra-basolateral amygdala drug infusions modulate expression of Arc protein in the hippocampus. *Proceedings of the National Academy of Sciences, 102*(30), 10718-10723.
Mitchell, J. B., & Laiacona, J. (1998). The medial frontal cortex and temporal memory: tests using spontaneous exploratory behaviour in the rat. *Behav Brain Res, 97*(1-2), 107-113.
Morgan, C. A., 3rd, Hazlett, G., Doran, A., Garrett, S., Hoyt, G., Thomas, P., . . . Southwick, S. M. (2004). Accuracy of eyewitness memory for persons encountered during exposure to highly intense stress. *Int J Law Psychiatry, 27*(3), 265-279. doi: 10.1016/j.ijlp.2004.03.004
Morici, J. F., Bekinschtein, P., & Weisstaub, N. V. (2015). Medial prefrontal cortex role in recognition memory in rodents. *Behav Brain Res, 292*, 241-251.
Nelson, A. J., Cooper, M. T., Thur, K. E., Marsden, C. A., & Cassaday, H. J. (2011). The effect of catecholaminergic depletion within the prelimbic and infralimbic medial prefrontal cortex on recognition memory for recency, location, and objects. *Behav Neurosci, 125*(3), 396-403. doi: 10.1037/a0023337
Oitzl, M. S., & De Kloet, E. R. (1992). Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. *Behavioral neuroscience, 106*(1), 62.

Okuda, S., Roozendaal, B., & McGaugh, J. L. (2004). Glucocorticoid effects on object recognition memory require training-associated emotional arousal. *Proceedings of the National Academy of Sciences, 101*(3), 853-858.

Öngür, D., & Price, J. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral cortex, 10*(3), 206-219.

Parsons, R. G., & Ressler, K. J. (2013). Implications of memory modulation for post-traumatic stress and fear disorders. *Nature neuroscience, 16*(2), 146-153.

Paxinos, G., & Franklin, K. (2008). The Mouse Brain in Stereotaxic Coordinates, Compact| 978-0-12-374244-5| Elsevier. The Mouse Brain in Stereotaxic Coordinates.

Peters, G. J., David, C. N., Marcus, M. D., & Smith, D. M. (2013). The medial prefrontal cortex is critical for memory retrieval and resolving interference. *Learning & memory (Cold Spring Harbor, N.Y.), 20*(4), 201-209. doi: 10.1101/lm.029249.112

Pezze, M. A., Marshall, H. J., Fone, K. C., & Cassaday, H. J. (2015). Dopamine D1 receptor stimulation modulates the formation and retrieval of novel object recognition memory: Role of the prelimbic cortex. *Eur Neuropsychopharmacol, 25*(11), 2145-2156. doi: 10.1016/j.euroneuro.2015.07.018

Pezze, M. A., Marshall, H. J., Fone, K. C., & Cassaday, H. J. (2017). Role of the anterior cingulate cortex in the retrieval of novel object recognition memory after a long delay. *Learning & memory (Cold Spring Harbor, N.Y.), 24*(7), 310-317. doi: 10.1101/lm.044784.116

Preuss, T. M. (1995). Do rats have prefrontal cortex? The Rose-Woolsey-Akert program reconsidered. *Journal of cognitive neuroscience, 7*(1), 1-24.

Quirarte, G. L., Roozendaal, B., & McGaugh, J. L. (1997). Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences, 94*(25), 14048-14053.

Radley, J. J., Williams, B., & Sawchenko, P. E. (2008). Noradrenergic innervation of the dorsal medial prefrontal cortex modulates hypothalamo-pituitary-adrenal responses to acute emotional stress. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 28*(22), 5806-5816. doi: 10.1523/jneurosci.0552-08.2008

Ramos, B. P., & Arnsten, A. F. T. (2007). Adrenergic pharmacology and cognition: focus on the prefrontal cortex. *Pharmacology & therapeutics, 113*(3), 523-536. doi: 10.1016/j.pharmthera.2006.11.006

Rang, H. P., Ritter, J. M., Flower, R. J., & Henderson, G. (2014). *Rang & Dale's Pharmacology E-Book: with STUDENT CONSULT Online Access*: Elsevier Health Sciences.

Reis, F. M., Almada, R. C., Fogaça, M. V., & Brandão, M. L. (2016). Rapid Activation of Glucocorticoid Receptors in the Prefrontal Cortex Mediates the Expression of Contextual Conditioned Fear in Rats. *Cereb Cortex, 26*(6), 2639-2649. doi: 10.1093/cercor/bhv103

Reul, J., & Kloet, E. D. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology, 117*(6), 2505-2511.

Rimmele, U., Davachi, L., Petrov, R., Dougal, S., & Phelps, E. A. (2011). Emotion enhances the subjective feeling of remembering, despite lower accuracy for contextual details. *Emotion, 11*(3), 553.

Roozendaal, B. (2000). Glucocorticoids and the regulation of memory consolidation. *Psychoneuroendocrinology, 25*(3), 213-238.
Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews Neuroscience, 10*(6), 423-433.
Roozendaal, B., & McGaugh, J. L. (1997). Glucocorticoid receptor agonist and antagonist administration into the basolateral but not central amygdala modulates memory storage. *Neurobiol Learn Mem, 67*(2), 176-179.
Roozendaal, B., & McGaugh, J. L. (2011). Memory modulation. *Behavioral neuroscience, 125*(6), 797.
Roozendaal, B., McReynolds, J. R., & McGaugh, J. L. (2004). The basolateral amygdala interacts with the medial prefrontal cortex in regulating glucocorticoid effects on working memory impairment. *Journal of Neuroscience, 24*(6), 1385-1392.
Roozendaal, B., McReynolds, J. R., Van der Zee, E. A., Lee, S., McGaugh, J. L., & McIntyre, C. K. (2009). Glucocorticoid effects on memory consolidation depend on functional interactions between the medial prefrontal cortex and basolateral amygdala. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 29*(45), 14299-14308. doi: 10.1523/JNEUROSCI.3626-09.2009
Roozendaal, B., & Mirone, G. (2020). Opposite effects of noradrenergic and glucocorticoid activation on accuracy of an episodic-like memory. *Psychoneuroendocrinology, 114*, 104588. doi: 10.1016/j.psyneuen.2020.104588
Roozendaal, B., Okuda, S., Van der Zee, E. A., & McGaugh, J. L. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences, 103*(17), 6741-6746. doi: 10.1073/pnas.0601874103
Roozendaal, B., Portillo-Marquez, G., & McGaugh, J. L. (1996). Basolateral amygdala lesions block glucocorticoid-induced modulation of memory for spatial learning. *Behavioral neuroscience, 110*(5), 1074.
Roozendaall, B., Bohus, B., & McGaugh, J. L. (1996). Dose-dependent suppression of adrenocortical activity with metyrapone: effects on emotion and memory. *Psychoneuroendocrinology, 21*(8), 681-693.
Rossetti, Z. L., & Carboni, S. (2005). Noradrenaline and dopamine elevations in the rat prefrontal cortex in spatial working memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 25*(9), 2322-2329. doi: 10.1523/jneurosci.3038-04.2005
Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC bioinformatics, 18*(1), 529.
Runyan, J. D., & Dash, P. K. (2004). Intra-medial prefrontal administration of SCH-23390 attenuates ERK phosphorylation and long-term memory for trace fear conditioning in rats. *Neurobiol Learn Mem, 82*(2), 65-70.
Ryabinin, A. E., Wang, Y.-M., & Finn, D. A. (1999). Different levels of Fos immunoreactivity after repeated handling and injection stress in two inbred strains of mice. *Pharmacology Biochemistry and Behavior, 63*(1), 143-151.
Sanchez, E. R., Housley, P. R., & Pratt, W. B. (1986). The molybdate-stabilized glucocorticoid binding complex of L-cells contains a 98–100 kdalton steroid binding phosphoprotein and a 90 kdalton nonsteroid-binding phosphoprotein that is part of the murine heat-shock complex. *Journal of Steroid Biochemistry, 24*(1), 9-18.

Sapolsky, R. M., McEwen, B. S., & Rainbow, T. C. (1983). Quantitative autoradiography of [3H] corticosterone receptors in rat brain. *Brain research, 271*(2), 331-334.

Schafe, G. E., & LeDoux, J. E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *Journal of Neuroscience, 20*(18), RC96-RC96.

Schutsky, K., Ouyang, M., Castelino, C. B., Zhang, L., & Thomas, S. A. (2011). Stress and glucocorticoids impair memory retrieval via β2-adrenergic, Gi/o-coupled suppression of cAMP signaling. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 31*(40), 14172-14181. doi: 10.1523/jneurosci.2122-11.2011

Segal, S. K., & Cahill, L. (2009). Endogenous noradrenergic activation and memory for emotional material in men and women. *Psychoneuroendocrinology, 34*(9), 1263-1271.

Segal, S. K., Stark, S. M., Kattan, D., Stark, C. E., & Yassa, M. A. (2012). Norepinephrine-mediated emotional arousal facilitates subsequent pattern separation. *Neurobiol Learn Mem, 97*(4), 465-469.

Sekeres, M. J., Winocur, G., Moscovitch, M., Anderson, J. A., Pishdadian, S., Martin Wojtowicz, J., . . . Grady, C. L. (2018). Changes in patterns of neural activity underlie a time- dependent transformation of memory in rats and humans. *Hippocampus, 28*(10), 745-764.

Sharot, T., Delgado, M. R., & Phelps, E. A. (2004). How emotion enhances the feeling of remembering. *Nature neuroscience, 7*(12), 1376-1380.

Soghomonian, J.-J., & Martin, D. L. (1998). Two isoforms of glutamate decarboxylase: why? *Trends in pharmacological sciences, 19*(12), 500-505.

Soltani Zangbar, H., Ghadiri, T., Seyedi Vafaee, M., Ebrahimi Kalan, A., Fallahi, S., Ghorbani, M., & Shahabi, P. (2020). Theta Oscillations Through Hippocampal/Prefrontal Pathway: Importance in Cognitive Performances. *Brain Connectivity, 10*(4), 157-169.

Tabassum, S., Ahmad, S., Madiha, S., Khaliq, S., Shahzad, S., Batool, Z., & Haider, S. (2017). Impact of oral supplementation of Glutamate and GABA on memory performance and neurochemical profile in hippocampus of rats. *Pakistan Journal of Pharmaceutical Sciences, 30*.

Talarico, J. M., & Rubin, D. C. (2003). Confidence, not consistency, characterizes flashbulb memories. *Psychological science, 14*(5), 455-461.

Tanimizu, T., Kono, K., & Kida, S. (2018). Brain networks activated to form object recognition memory. *Brain research bulletin, 141*, 27-34.

Taylor, J. R., Birnbaum, S., Ubriani, R., & Arnsten, A. F. (1999). Activation of cAMP-dependent protein kinase A in prefrontal cortex impairs working memory performance. *Journal of Neuroscience, 19*(18), RC23-RC23.

Timplalexi, M. P. (2020). *Effect of yohimbine and corticosterone on object-in-context memory: Role of habituation in the training context* [Unpublished master's thesis]. Donders Graduate School for Cognitive Neuroscience.

Tronel, S., Feenstra, M. G., & Sara, S. J. (2004). Noradrenergic action in prefrontal cortex in the late stage of memory consolidation. *Learning & memory (Cold Spring Harbor, N.Y.), 11*(4), 453-458. doi: 10.1101/lm.74504

Trzaskowski, B., Latek, D., Yuan, S., Ghoshdastider, U., Debinski, A., & Filipek, S. (2012). Action of molecular switches in GPCRs-theoretical and experimental studies. *Current medicinal chemistry, 19*(8), 1090-1109.

Tulving, E. (1972). Episodic and semantic memory. *Organization of memory, 1*, 381-403.
Turnham, R. E., & Scott, J. D. (2016). Protein kinase A catalytic subunit isoform PRKACA; History, function and physiology. *Gene, 577*(2), 101-108. doi: 10.1016/j.gene.2015.11.052
Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience, 10*(6), 397-409.
Uylings, H. B., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex? *Behav Brain Res, 146*(1-2), 3-17.
Uylings, H. B., & van Eden, C. G. (1991). Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans *Progress in brain research* (Vol. 85, pp. 31-62): Elsevier.
van Stegeren, A. H., Wolf, O. T., Everaerd, W., Scheltens, P., Barkhof, F., & Rombouts, S. A. (2007). Endogenous cortisol level interacts with noradrenergic activation in the human amygdala. *Neurobiol Learn Mem, 87*(1), 57-66.
Villain, H., Benkahoul, A., Drougard, A., Lafragette, M., Muzotte, E., Pech, S., . . . Roullet, P. (2016). Effects of propranolol, a β-noradrenergic antagonist, on memory consolidation and reconsolidation in mice. *Frontiers in behavioral neuroscience, 10*, 49.
Walsh, T. J., & Palfai, T. (1979). Peripheral catecholamines and memory: Characteristics of syrosingopine-induced amnesia. *Pharmacology Biochemistry and Behavior, 11*(4), 449-452.
Warburton, E. C., & Brown, M. W. (2015). Neural circuitry for rat recognition memory. *Behav Brain Res, 285*, 131-139. doi: 10.1016/j.bbr.2014.09.050
Weible, A. P., Rowland, D. C., Monaghan, C. K., Wolfgang, N. T., & Kentros, C. G. (2012). Neural correlates of long-term object memory in the mouse anterior cingulate cortex. *Journal of Neuroscience, 32*(16), 5598-5608.
Weible, A. P., Rowland, D. C., Pang, R., & Kentros, C. (2009). Neural correlates of novel object and novel location recognition behavior in the mouse anterior cingulate cortex. *Journal of neurophysiology, 102*(4), 2055-2068.
Weil-Malherbe, H., Axelrod, J., & Tomchick, R. (1959). Blood-brain barrier for adrenaline. *Science, 129*(3357), 1226-1227.
Winocur, G., & Moscovitch, M. (2011). Memory transformation and systems consolidation. *Journal of the International Neuropsychological Society: JINS, 17*(5), 766.