STRESS HORMONE EFFECTS ON THE HIPPOCAMPUS IN REGULATING EPISODIC-LIKE MEMORY CHUNAN GUO

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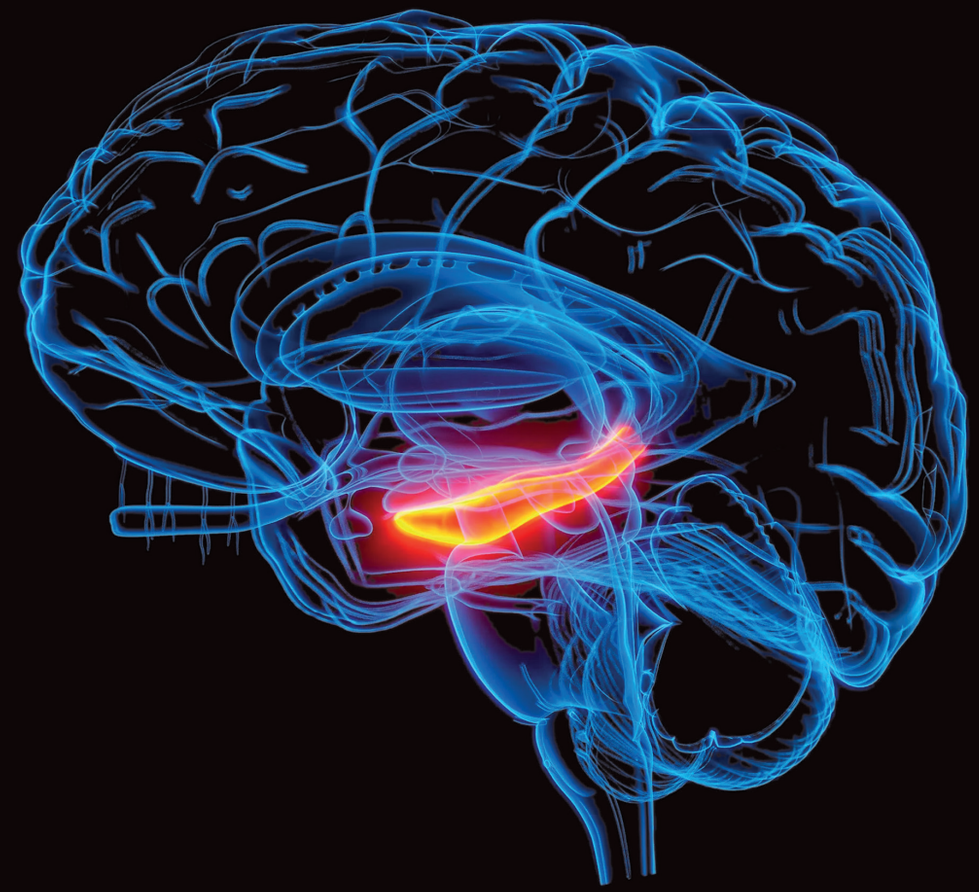
Stress hormone effects on the hippocampus in regulating episodic-like memory

Involvement of separation and linking of memory for multiple training events



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Chunan Guo

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Stress hormone effects on the hippocampus in regulating episodic-like memory: involvement of separation and linking of memory for multiple training events

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CHAPTER 1

General introduction

Emotionally arousing or stressful experiences typically induce strong and lasting memories (McGaugh, 2013). A large body of literature has shown that this strengthening of memories involves synergistic actions of both norepinephrine and glucocorticoid hormones (corticosterone in rodents, cortisol in humans) (Joëls et al., 2011; Roozendaal & McGaugh, 2011; de Quervain et al., 2017; Schwabe et al., 2022). The capacity to store and recall memories of biologically significant experiences is highly adaptive and crucial for our daily existence and survival (McGaugh, 2003). However, memories can be subject to multiple types of modifications beyond mere strengthening (Schacter, 1999). It is still debated to what extent the emotional impact of an experience additionally influences memory accuracy, fidelity, and susceptibility to incorporation of misinformation (Morgan et al., 2004a; Porter et al., 2008; Hoscheidt et al., 2014). Only recently, this topic has begun to attract attention in animal research, but a larger literature from human studies shows contradictory results: Some studies reported that arousal improves the accuracy of memories, resulting in vivid recall of emotionally arousing experiences (Ochsner, 2000; Steidl et al., 2006; Kensinger et al., 2007a; Segal et al., 2012), while other studies proposed that emotional memories are remembered in a more generalized manner, potentially leading to less accurate recollection of specific details (Morgan et al., 2004a; Richards & Gross, 2006; Levine & Edelstein, 2009). As aberrant memory processing of emotional information lies at the core of several stress-related disorders, including posttraumatic stress disorder and phobias (Lissek et al., 2014; Lis et al., 2020), an understanding of the mnemonic modifications produced by stress and emotional arousal might also bear significant clinical relevance. In this thesis, I will present a series of experiments in mice aimed at investigating the effects of the two major stress hormones norepinephrine and corticosterone on episodic-like quality of memory.

In the upcoming sections, I will first give an overview of prior animal research into the role of norepinephrine and glucocorticoids in modulating the consolidation process of memory as well as the primary brain mechanisms that have been identified as mediating these stress hormone effects. Then, I will describe literature that demonstrated that stress and stress hormones dynamically regulate many other aspects of memory as well, with a special focus on how they affect several quality aspects of memory, including accuracy and generalization. Most of these studies investigating memory quality involved human work, but in recent years also some interesting studies in animals became available. Then, I will summarize the findings of some recent experiments in rats showing that norepinephrine and corticosterone have an opposite influence on episodic-like quality of memory that requires the separation of memory representations for multiple training events. Lastly, I will present the scope of my thesis that aims at further investigating the effects of norepinephrine and corticosterone on this specific memory function and the underlying neurobiological mechanism in mice, followed by a brief explanation of the research question and general design for each of my experimental chapters.

1. Stress hormone signaling and its effects on memory consolidation

The concept of stress and its bodily effects has undergone continuous evolution in scientific understanding. Building upon Claude Bernard's theory of the internal milieu, Walter Cannon introduced the concept of homeostasis to explain the physiological "fight-or-flight" response exhibited by organisms when confronted with a threatening situation (Cannon, 1932). In a biological context, the term "stress" refers to the non-specific physiological response of the body to various homeostatic demands (Selye, 1936). The current knowledge is that the physiological function of the stress response includes coordinated autonomic, neuroendocrine, metabolic and immune responses to deal with the potential threats to homeostasis (Koolhaas et al., 2011; McEwen & Akil, 2020). A developing concept in stress neurobiology proposes that a primary purpose of the stress response is to mobilize energy in order to facilitate context-specific survival, rather than solely maintaining homeostatic systems at pre-challenge levels (Dallman et al., 2006; Nederhof & Schmidt, 2012). An adequate stress response not only allows individuals to respond acutely to dangerous or threatening situations, but also prepares them for future exposures by inducing long-term behavioral changes, including effects on learning and memory (McGaugh, 2013).

Stressful and emotionally stimulating events activate two major stress-response systems: The sympathetic nervous system and the hypothalamus-pituitary-adrenocortical (HPA) axis. Initially, the sympathetic nervous system is activated, which rapidly triggers the release of catecholamines such as epinephrine and norepinephrine from the adrenal medulla and sympathetic nerve endings (Mason, 1968; Jänig, 2014). Peripherally released catecholamines cannot directly enter the brain (Weil-Malherbe et al., 1959), but they can bind to adrenoceptors on the vagus nerve which then affects brain function by activating noradrenergic cell groups within the nucleus of the solitary tract (NTS) and locus coeruleus (LC). The activation of these noradrenergic cell groups will, in turn, elevate norepinephrine levels in the brain (McGaugh & Roozendaal, 2002). Norepinephrine is also directly released in the brain upon stress exposure by the activation of these noradrenergic cells in the NTS and LC (Aston-Jones & Cohen, 2005; Valentino & Van Bockstaele, 2008; Sara, 2009). Norepinephrine affects brain function by binding to locally expressed a- and β-adrenergic G-protein-coupled receptors, residing on the cell membrane, and which are ubiquitously present throughout the brain. Whereas activation of most adrenoceptor subtypes exerts excitatory effects, one specific subtype, i.e., the a2-adrenoceptor, is located predominantly presynaptically and provides negative feedback to the neuron, leading to a decrease in the amount of subsequent norepinephrine release (Rang et al., 2014).

In a more delayed fashion, stress also induces an activation of the HPA axis, which initiates a series of events that ultimately results in the release of glucocorticoid hormones from the adrenal cortex. During this process, the parvocellular cells of the paraventricular nucleus of the hypothalamus (PVN) release corticotropin-releasing hormone (CRH) and vasopressin (AVP) into the portal system. CRH (and AVP) stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which in turn triggers the synthesis and release of glucocorticoids from the adrenal cortex into the bloodstream (Axelrod & Reisine, 1984; Ulrich-Lai & Herman, 2009). Peripherally, glucocorticoids cause, among others, immunosuppressive effects and elevated blood glucose levels, impacting various metabolic processes (Wajchenberg et al., 1984; Sapolsky et al., 2000; Kuo et al., 2015). Due to their high lipophilicity, glucocorticoids easily penetrate the blood-brain barrier and bind to both mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) in the brain (McEwen et al., 1968; Reul et al., 1985; Spencer et al., 1990). MRs have a high affinity for glucocorticoids, whereas GRs have a lower affinity. During stress and circadian-induced increases in the frequency and intensity of glucocorticoid secretory bursts, GRs gradually become activated (Reul et al., 1985; de Kloet, 1991; De Kloet et al., 1998). Both MRs and GRs are initially located in the cytoplasm, but upon binding with their ligand, translocate to the nucleus (Koning et al., 2019). In the nucleus, they directly influence gene transcription by binding to glucocorticoid responsive elements on the DNA as either homodimers or heterodimers, recruiting co-repressors or coactivators (Datson et al., 2001). Additionally, they indirectly regulate gene expression by interacting with other stress-induced transcription factors to modulate their activity (De Bosscher et al., 2003; John et al., 2011; Koning et al., 2019; Provençal et al., 2020). In addition to their genomic actions, glucocorticoids can also exert rapid, non-genomic effects on neuroplasticity and memory (Finsterwald & Alberini, 2014; Gray et al., 2017). These effects are believed to occur through an interaction with a membrane-associated variant or variants of the steroid receptor (Johnson et al., 2005; Barsegyan et al., 2010; Karst et al., 2010; Riedemann et al., 2010; Roozendaal et al., 2010; Lee et al., 2011). The membrane-bound MR exhibits an approximately 10-fold lower affinity for its ligand than the intracellular MR (Karst & Joëls, 2005b), allowing it to play a prominent role in the behavioral stress response (Le Menuet & Lombès, 2014). Stimulation of this receptor rapidly and reversibly increases the frequency of spontaneous release of glutamate vesicles, raising neuronal excitability (Karst & Joëls, 2005b). Additionally, a membrane-bound GR has recently been identified which also rapidly alters neuronal function and this effect might involve the stimulation of endocannabinoid release (Di et al., 2003; Atsak et al., 2015). Through these mechanisms, glucocorticoids have the ability to modulate neuronal processing in a time-dependent manner, allowing for the generation of the most adaptive, dynamic response to stress (Joëls et al., 2011).

1.1 Adrenergic effects on memory consolidation

Extensive evidence indicates that epinephrine and norepinephrine enhance the consolidation of memory (McGaugh & Roozendaal, 2002). Early work has shown that the stimulant amphetamine, which increases peripheral epinephrine levels (Martinez Jr et al., 1980), enhances memory retention when administered to rats or mice immediately following training (Roozendaal et al., 1996a). Additionally, systemic administration of epinephrine given to rats immediately after inhibitory avoidance training was found to enhance later retention of that training experience (Gold & Van Buskirk, 1975), whereas epinephrine given two hours after the training event did not affect memory retention. These findings suggested that stress hormones released during emotional training may act as endogenous modulators of memory consolidation (McGaugh & Gold, 1989) within a critical time window (McGaugh & Gold, 1989; McGaugh, 2000). Many subsequent studies have shown that epinephrine administration induces both time- and dose-dependent enhancement of memory consolidation of different training experiences in rats and mice (Sternberg et al., 1985; Liang et al., 1986).

It is now well established that the memory-enhancing effect of peripheral epinephrine depends on increased norepinephrine levels in the brain. *In vivo* microanalysis work has demonstrated that systemic epinephrine administration elevates norepinephrine levels within the amygdala (Williams et al., 1998). Moreover, the administration of a β-adrenoceptor antagonist into the amygdala prevents the enhancing effect of systemic epinephrine on memory (Liang et al., 1986; Williams et al., 1998). These findings underscore the intricate interplay between peripheral epinephrine and centrally released norepinephrine in modulating memory consolidation. Even in the absence of stressful training conditions and associated increase in peripheral catecholamine levels, lower levels of emotional arousal are sufficient to induce the release of norepinephrine throughout the brain by a more specific activation of catecholamine-containing cells within the LC (Berridge, 2008; Atzori et al., 2016). Studies have revealed that both highly arousing training experiences, such as inhibitory avoidance training, and less arousing object recognition training trigger such release of norepinephrine (Hatfield & McGaugh, 1999; Quirarte et al., 1997; McIntyre et al., 2002; (Roozendaal et al., 2006; Nirogi et al., 2012). Extensive evidence supports a role for central norepinephrine in modulating memory consolidation for both aversive and more mildly arousing experiences (see also section 1.4) (Ferry et al., 1999; Barsegyan et al., 2014).

1.2 Glucocorticoid effects on memory consolidation

Numerous studies have demonstrated that glucocorticoids also facilitate the consolidation of memory for emotionally arousing experiences (Roozendaal & McGaugh, 1996c; De Kloet et al., 1998; McGaugh & Roozendaal, 2002; Okuda et al., 2004; Sandi & Pinelo-Nava, 2007; Roozendaal & McGaugh, 2011; de Quervain et al., 2017). Systemic

administration of corticosterone or the synthetic glucocorticoid dexamethasone immediately after inhibitory avoidance training was found to enhance later retention for the training experience (Roozendaal & McGaugh, 1996b; Roozendaal & McGaugh, 1996e; Roozendaal et al., 1999b). In contrast, eliminating endogenous corticosterone through either adrenalectomy or the administration of the glucocorticoid-synthesis inhibitor metyrapone has been shown to impair retention (Oitzl & De Kloet, 1992; Roozendaal et al., 1996e). Findings that GR agonists also enhance memory consolidation (Roozendaal & McGaugh, 1997b; Miranda et al., 2008), and that their blockade, but not that of MRs, shortly before or immediately after training impairs the formation of long-term memory, suggest a critical role for the GR in mediating these glucocorticoid effects on memory consolidation (Roozendaal et al., 1996d; Lupien et al., 2002; Roozendaal & McGaugh, 1997a; Roozendaal & McGaugh, 1997b). Similar to the effects of epinephrine and norepinephrine, glucocorticoids induce both time- and dose-dependent enhancement of memory consolidation (Roozendaal et al., 2006). It is important to note that, in accordance with an inverted-U shaped relationship of glucocorticoids on memory consolidation, the memory-modulatory effect of a specific glucocorticoid dosage depends also on other factors such as the task's intrinsic aversiveness. For instance, moderate doses of dexamethasone administered after training on a highly aversive water-maze spatial task can lead to memory impairment. This task is considerably more stressful for rats than inhibitory avoidance training, and therefore induces high levels of endogenous glucocorticoids (Roozendaal et al., 1996d). Notably, when the training conditions are modified to reduce stress, such as by elevating the water temperature in the maze, posttraining glucocorticoid injections have the potential to enhance consolidation processes (Sandi et al., 1997). Further, consistent with the effects of glucocorticoids influencing the memory consolidation process, other studies have shown that glucocorticoid administration is only effective when given within a critical time window after training. Research conducted by Sandi & Rose (1994) demonstrated that corticosterone injections to one day-old chicks effectively improved the consolidation processes for avoidance learning when administered within 60 minutes after the training, whereas later administration was ineffective. In another study by Flood et al. (1978), dexamethasone was found to enhance memory for avoidance learning in mice when injected even as late as 150 minutes after the training session.

1.3 Glucocorticoid-adrenergic interactions on memory consolidation

Numerous studies have shown that glucocorticoids and norepinephrine intimately interact in modulating memory consolidation. For instance, glucocorticoid administration after footshock training in the inhibitory avoidance task rapidly increased norepinephrine levels within the basolateral amygdala (BLA) (McReynolds et al., 2010).

Conversely, a β-adrenoceptor antagonist administered into the BLA prevented the effect of posttraining glucocorticoid administration on memory consolidation (Quirarte et al., 1997; Roozendaal et al., 1999a; Roozendaal et al., 2002). Research into the molecular mechanisms underlying this interaction suggests that glucocorticoids facilitate memory consolidation by permissively enhancing the norepinephrine-stimulated intracellular cAMP-protein kinase A (PKA) signaling cascade (Roozendaal et al., 2002). Other findings indicated that these glucocorticoid effects on increasing norepinephrine signaling occur too quickly to be mediated via transcriptional regulation in the nucleus (i.e., genomic effects) and likely involve rapid, nongenomic mechanisms (Roozendaal et al., 2002; Roozendaal et al., 2010; Chen et al., 2012; Zhou et al., 2012; Karst & Joëls, 2016).

The interaction between the glucocorticoid and noradrenergic systems could explain why glucocorticoids selectively enhance memory consolidation for emotionally arousing information or situations, which are associated with increased endogenous release of norepinephrine (Roozendaal et al., 2006). Corticosterone administration after object recognition training was found to enhance 24-hour memory of emotionally aroused rats that had no previous exposure to the training context. However, corticosterone did not enhance object memory in rats that had been extensively habituated to the training context, reducing emotional arousal caused by the novelty of the task (Okuda et al., 2004). As shown in Figure 1, systemic administration of the β-adrenoceptor antagonist propranolol immediately after object recognition training in non-habituated rats blocked the corticosterone-induced memory enhancement (Roozendaal et al., 2006), whereas a low dose of the noradrenergic stimulant yohimbine administered to well-habituated (i.e., low-aroused) rats immediately after object recognition training enabled the corticosterone-induced memory enhancement in a dose-dependent manner. Yet, posttraining injections of corticosterone and yohimbine separated by a 4-hour delay did not enhance memory consolidation (Roozendaal et al., 2006), which is consistent with findings of other studies indicating that the release of norepinephrine and glucocorticoids must occur in a precisely timed and synergistic manner (de Kloet et al., 2008; Joëls, 2008; Joëls et al., 2011; de Quervain et al., 2017).

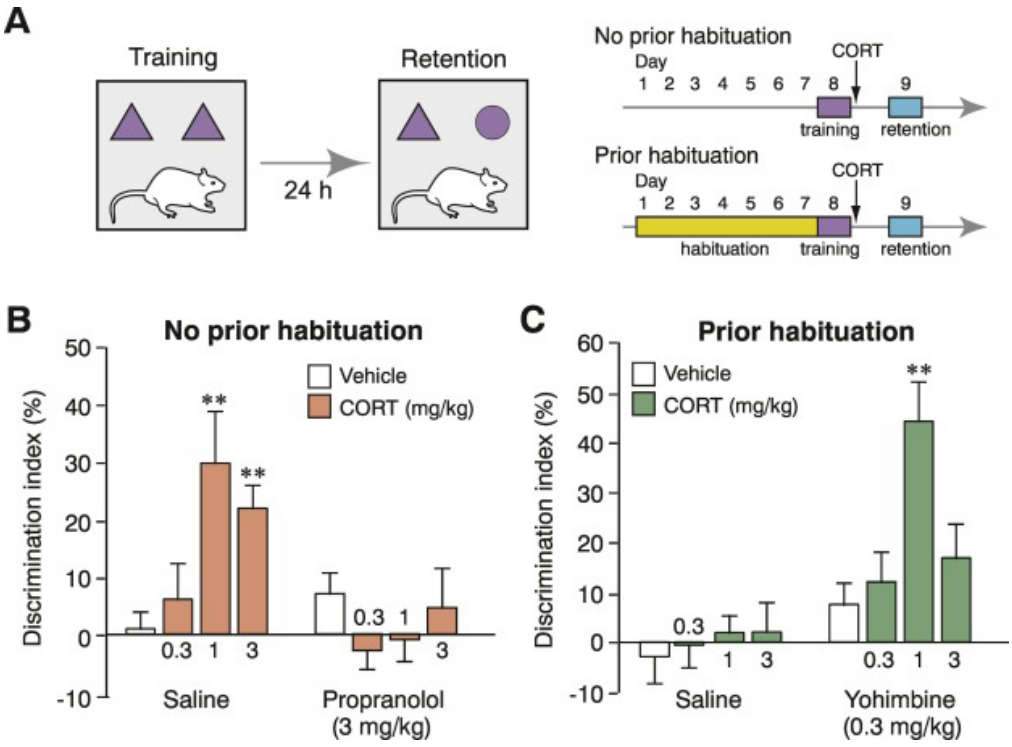

Figure 1. Glucocorticoid effects on memory consolidation for object recognition training require noradrenergic activation.

A, Rats were either habituated to the training context for 7 days (prior habituation) or not habituated (no prior habituation). On Day 8, they were given a 3-minute training trial during which they could freely explore two identical objects, followed by systemic drug administration. Retention was tested 24 hours later by placing the rats back into the apparatus for 3 minutes. On the retention trial, one object was similar to the training objects whereas the other was novel. **B**, Immediate posttraining administration of the β-adrenoceptor antagonist propranolol (3.0 mg/kg, s.c.) blocked the corticosterone-induced enhancement of object recognition memory in non-habituated rats. **C**, The noradrenergic stimulant yohimbine (0.3 mg/kg, s.c.) enabled the corticosterone effect on object recognition memory in habituated rats. Data represent the discrimination index (%) on the 24-hour retention trial, expressed as mean ± SEM. The discrimination index was calculated as the difference in time spent exploring the novel object, expressed as the ratio of the total time spent exploring both objects x100%. $^{**}p < 0.01$ as compared to the corresponding vehicle group (Roozendaal et al., 2006; Roozendaal et al., 2009a).

1.4 Stress hormone effects on memory consolidation involve different brain regions

Many studies have examined the effect of norepinephrine or glucocorticoid administration into specific brain regions on memory consolidation. Most studies have examined these effects in the BLA, where the direct administration of norepinephrine or glucocorticoids dose-dependently enhances the consolidation of memory of many different training

experiences (Roozendaal et al., 1996a; Roozendaal & McGaugh, 1997a; LaLumiere et al., 2003). Other studies have shown critical interactions between norepinephrine and glucocorticoids within the BLA. Intra-BLA infusions of a ß-adrenoceptor antagonist was found to block the memory-enhancing effects of systemic injections of dexamethasone or corticosterone as well as the effects of a locally infused selective GR agonist (Quirarte et al., 1997; Roozendaal et al., 2002; Roozendaal et al., 2006). Conversely, a GR antagonist administered into the BLA increased the dose of norepinephrine necessary to enhance memory, supporting the view that glucocorticoids facilitate intracellular noradrenergic signaling mechanisms (Roozendaal et al., 2002).

Consistent with the widely distributed expression of receptors for both norepinephrine and glucocorticoids throughout the brain, direct administration of norepinephrine or glucocorticoids into several other brain regions, including the hippocampus, dorsal striatum, anterior insular cortex and prefrontal cortex, following training has also been shown to enhance the consolidation of long-term memory (Liang et al., 1986; Liang et al., 1990; Ferry et al., 1999; Roozendaal & McGaugh, 2011). Whereas stress hormone infusions into the BLA have been shown to almost indiscriminately enhance memory for many different types of emotionally arousing training experiences, the effect of stress hormone administration into other brain regions appears to depend on the specific information acquired during the training experience (Nathan et al., 2004). For example, glucocorticoid administration into the hippocampus was found to enhance memory of spatial training in a water maze, whereas it did not affect memory of cued training in a water maze (Quirarte et al., 1997). Conversely, the same glucocorticoid administration into the dorsal striatum selectively enhanced memory of the cued training. Norepinephrine or glucocorticoid administration into the anterior insular cortex enhanced the consolidation of different forms of recognition memory, whereas such administration into the hippocampus did not affect this memory (Miranda et al., 2008; Roozendaal et al., 2010; Chen et al., 2022).

The BLA plays a crucial role in coordinating these different stress hormone effects on memory consolidation, by influencing neural plasticity and information storage processes in other brain regions (Pitkänen et al., 2000; Petrovich et al., 2001; Price, 2003; Sah et al., 2003; Roozendaal & McGaugh, 1997a; Roozendaal & McGaugh, 2011). For example, norepinephrine and corticosterone were both found to influence the effects of BLA electrical stimulation on dentate gyrus long-term potentiation (Akirav & Richter-Levin, 2002). Furthermore, noradrenergic activation of the BLA after inhibitory avoidance was found to increase experience-dependent molecular markers of neuroplasticity within the hippocampus (McIntyre et al., 2005), whereas an inhibition of noradrenergic activity within the BLA prevented the memory-enhancing effect induced by norepinephrine or glucocorticoid administration (as well as that of many other neuroactive agents) into other brain regions, including the hippocampus, anterior insular cortex and prefrontal

cortex (Roozendaal et al., 1999a; Roozendaal et al., 2009b; Chen et al., 2018; Barsegyan et al., 2019). For an extensive review on BLA interactions with other brain regions in regulating neuroplasticity and memory consolidation, see & McGaugh (2011).

2 Stress hormone effects on quality aspects of memory

Stress and stress hormones not merely strengthen memories, but they affect many other aspects of memory processing as well. This topic has been particularly investigated in humans, and has received considerably less attention in animal studies. These studies revealed that emotional arousal and stress trigger time-dependent alterations in memory, bolstering some memory processes while impairing others (Schwabe et al., 2022) (Figure 2). These time-dependent shifts in memory are closely linked to the temporal patterns of action exhibited by norepinephrine and glucocorticoids. Notably, memory for crucial aspects of the stressful event itself is generally increased and more vivid; a phenomenon often observed in the wake of acute stress (Dandolo & Schwabe, 2018; Moscovitch & Gilboa, 2021). Simultaneously, stress can hinder the creation of information unrelated to the stressor itself (de Quervain et al., 1998; Maroun & Akirav, 2008; Roozendaal & McGaugh, 2011; Schwabe et al., 2012; de Quervain et al., 2017). Moreover, stress has been found to promote a shift from 'cognitive' hippocampus-dependent forms of learning and memory toward striatal-dependent habitual forms of learning and memory (Balleine & O'Doherty, 2010; Schwabe et al., 2012). This can manifest as reduced memory flexibility, resulting in challenges with goal-directed learning, compromised memory updating, and difficulties in transferring memories to novel situations (Shohamy & Adcock, 2010; Quaedflieg et al., 2020). Both the augmenting and diminishing effects of stress are primarily attributed to the swift actions of norepinephrine and glucocorticoids. In contrast, the delayed genomic actions of glucocorticoids may raise the threshold for the encoding of new information in the aftermath of stress exposure (Henckens et al., 2012; Quaedflieg et al., 2020; Zerbes et al., 2022), which could serve as a protective mechanism, guarding the consolidation of memories related to the stressful event from interference (Roozendaal et al., 2002; Schwabe et al., 2022).

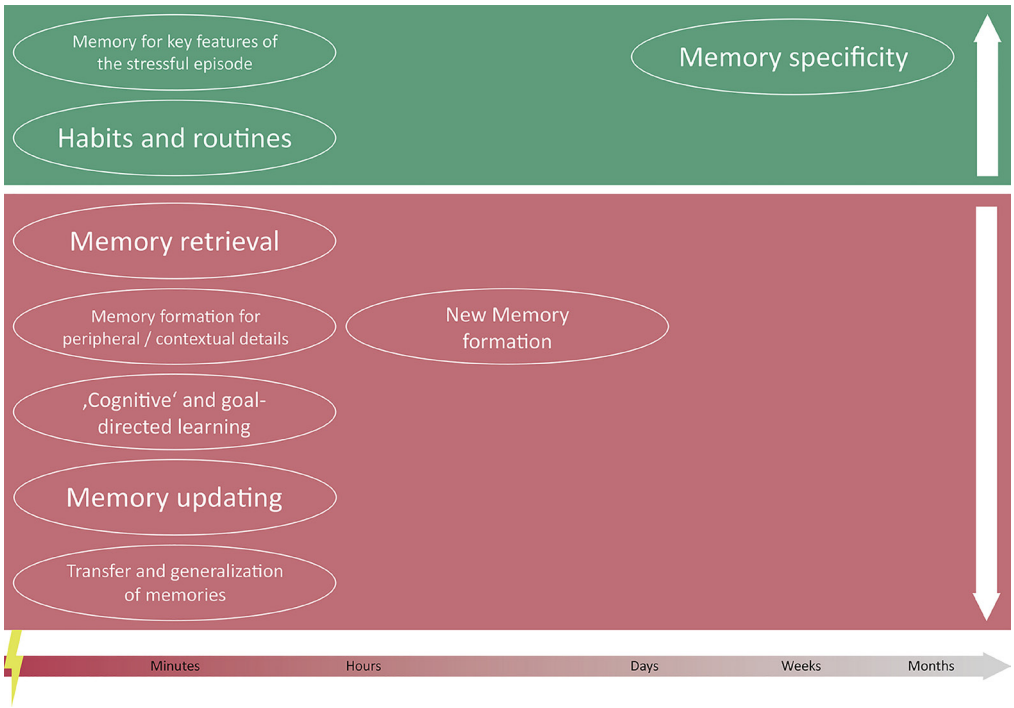

Figure 2. Acute stress induces time-dependent changes in memory, enhancing some processes (green) while impairing others (red).

These time-dependent changes in memory are thought to be directly linked to the temporal profiles of action of major stress mediators (Schwabe et al., 2022).

Other studies have examined the impact of stress and emotional arousal on quality aspects of memory. Some research suggests that emotional arousal can improve memory accuracy, resulting in vivid and detailed recall of emotionally charged experiences. These studies point to the idea that emotions can enhance memory formation of specific details in memory (Ochsner, 2000; Steidl et al., 2006; Kensinger et al., 2007a; Segal et al., 2012). However, a study by Rimmele and colleagues revealed that sometimes there is a disconnect between the subjective feeling of remembering and the objective accuracy of memory when it comes to negative and neutral scenes (Rimmele et al., 2011). When participants were asked to make "remember" judgments, they were more likely to do so for negative scenes compared to neutral ones. However, when it came to memory for contextual details and associations, participants showed actually poorer performance in terms of objective memory accuracy for negative scenes. In essence, the subjective sense of remembering was influenced by the emotional valence of the scenes, but this did not translate into more accurate memory for the finer details or associations in the negative scenes (Rimmele et al., 2011). Other studies are consistent with the view that memories

for emotional information or when they are encoded during stressful or arousing situations may be remembered in a more generalized manner (Morgan et al., 2004a; Richards & Gross, 2006; Levine & Edelstein, 2010). This perspective suggests that stress exposure or emotional arousal might lead to less accurate memory for specific details, potentially promoting more gist-based or generalized memory processing. Other studies have investigated the effects of stress on the contextualization (context-dependency) of episodic memories in humans. Some studies found that cortisol had contrasting effects on emotional memory contextualization over time: Its rapid effects impaired this process, while its slower, presumably genomic, effects enhanced contextualization (van Ast et al., 2013; Sazma et al., 2019). However, the contextualization of neutral memory remained unchanged by cortisol, regardless of the timing of the administration (van Ast et al., 2013).

Thus, whereas some of these studies indicated that stress and emotional arousal enhanced memory accuracy, other studies found the exact opposite effect and indicated that stress and emotional arousal might enhance the generalization of memory. It should be noted that these human studies have employed many different types of memory tasks and different readout measures. Further, the usage and definitions of the terms 'accuracy' and 'generalization' varied across these studies, which complicates direct comparison of findings. The experimental procedures also drastically varied in terms of their stressfulness or emotionality: Some studies investigated differences in accuracy or generalization for emotionally arousing vs neutral encoded information, whereas other studies examined memory quality after exposing participants to an actual stress challenge. It is likely that these different experimental procedures were associated with differences in endogenous stress hormone release. This might not only be true for the magnitude of stress hormone release, but also for the type of stress hormone (i.e., norepinephrine vs cortisol) that was released. Unfortunately, most studies did not actually measure stress hormone levels in their experiments. Thus, with some exceptions, the findings of these experiments do not allow any conclusion with respect to the role of norepinephrine and glucocorticoids in influencing these quality aspects of memory.

More recently, animal studies started to examine the effect of stress and stress hormone manipulation on memory accuracy and generalization. A number of studies indicated that more aversive training protocols (i.e., higher shock intensity) during fear conditioning were associated with a transition from accurate to generalized (i.e., less accurate) fear expression (Ghosh & Chattarji, 2015; dos Santos Corrêa et al., 2019). Some studies have implicated an important role for corticosterone in this effect. For example, higher training-induced corticosterone levels or posttraining corticosterone administration induced generalization of contextual fear conditioning (Kaouane et al., 2012; dos Santos Corrêa et al., 2019; Lesuis et al., 2021). Dos Santos Corrêa et al. (2019) and Lesuis et al., (2021) found that this generalization effect after contextual fear conditioning was associated

with more pronounced freezing behavior to a novel context. Kaouane et al. (2012) found that corticosterone administration into the hippocampus impaired the ability to correctly associate the context as the predictor of the threat, instead it increased freezing to an innocuous cue that does not signal threat. It should be noted that most of these studies examined the role of corticosterone in memory generalization, and thus it is not known whether norepinephrine administration might induce similar effects. A recent study by Song et al. (unpublished findings) indicated that the noradrenergic stimulant yohimbine administered immediately after training on an object recognition task increased the detailedness of object memory, suggesting that norepinephrine might have a different influence on quality aspects of memory. However, these studies examined different aspects of memory quality. There are no studies that directly compared the effect of norepinephrine and glucocorticoid administration on such different quality aspects of memory.

2.2 Norepinephrine and glucocorticoid effects on episodic-like accuracy of memory

In recent years, our laboratory has performed a series of experiments in rats to directly compare the effect of norepinephrine and corticosterone administration on memory accuracy using a dual-event inhibitory avoidance task. In this task, originally termed the inhibitory avoidance discrimination task (Atucha & Roozendaal, 2015), rats are trained sequentially in two different inhibitory avoidance apparatuses with a brief delay, but receive an electric shock only in the latter context. After 48 hours, retention latencies are tested in both training contexts as well as in a novel context to test whether the rats associated the shock experience with the correct training context (as evidenced by long latencies in the shock context, but not the other contexts). Vehicle-treated control rats that were trained with an interval of 1 minute between the two training events displayed similar retention latencies in the shock box and non-shock box, which were both longer than those in the novel box (Roozendaal & Mirone, 2020)(Figure 3). These findings thus indicate that these rats had not accurately associated the shock experience with the correct training event. In contrast, control rats trained with a longer interval of 2 minutes between the two training events, i.e., an easier version of the task, were able to accurate associated the shock experience with the correct training event as indicated by longer retention latencies in the shock box compared to both the non-shock box and novel box.

Systemic administration of the noradrenergic stimulant yohimbine given immediately after the training session was found to enhance both the accuracy and strength of memory, as evidenced by longer retention latencies in the shock box, but shorter retention latencies in the safe non-shock box (Roozendaal & Mirone, 2020). Conversely, corticosterone administration after the training session enhanced retention latencies in both the shock and non-shock training contexts. Corticosterone treatment did not

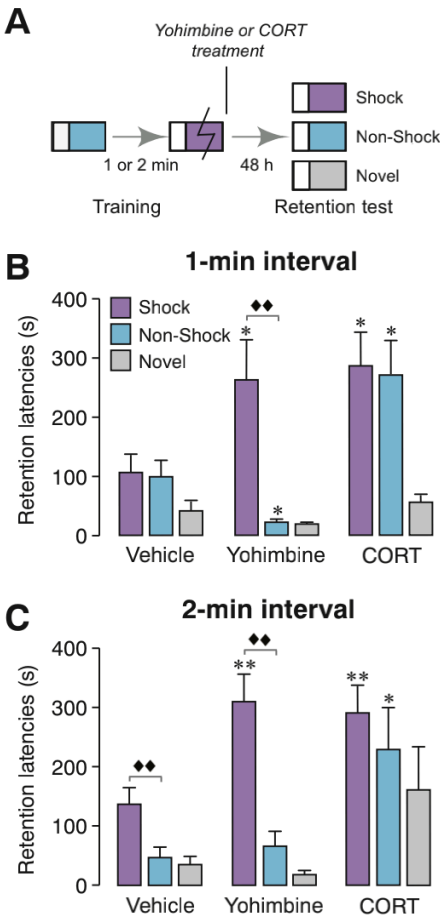

Figure 3. Opposite effects of norepinephrine and corticosterone (CORT) on accuracy of an episodic-like memory.

A, Experimental design of the dual-event inhibitory avoidance task. During training, rats visit two distinct inhibitory avoidance apparatuses with either a 1- or 2-minute interval, but only receive a footshock upon entering the dark compartment of the latter context, i.e., the shock box. The noradrenergic stimulant yohimbine (1 mg/kg, s.c.) or corticosterone (CORT, 3 mg/kg, s.c.) was administered immediately after the training. Forty-eight hours later, retention latencies to enter the dark compartment of these two previously encountered training contexts (shock box and non-shock box) as well as of a novel box were tested in a counterbalanced order. Each apparatus has the same geometry, but the non-shock box and novel box have distinct visual contextual features (i.e., stripes and circles taped to the wall) as well as distinct tactile features by tape placed on the floor. **B**, Step through latencies (mean + SEM) in seconds on the 48-hour retention test of rats trained on the inhibitory avoidance discrimination task with a 1-minute interval ('difficult version') between the two training events. Vehicle-treated rats did not show an accurate shock-context association as indicated by similar retention latencies in the shock box and non-shock box. Yohimbine enhanced both the accuracy and strength of memory as shown by specific long latencies in the shock box. By contrast, corticosterone enhanced latencies in both the shock box and non-shock box. **C**, Step-through latencies (mean + SEM) in seconds on the 48-hour retention test of rats trained on the dual-event inhibitory avoidance task with a 2-minute interval ('easy version') between the two training episodes. Vehicle-treated rats showed accurate memory of the shock context association. Yohimbine strengthened memory while maintaining memory accuracy. In contrast, corticosterone again enhanced latencies in both the shock box and non-shock box. $*p < 0.05$, $**p < 0.01$ vs vehicle; $\blacklozenge\blacklozenge p < 0.01$ shock box vs non-shock box (Roozendaal & Mirone, 2020).

significantly affect retention latencies in the novel context. These findings thus indicate that the noradrenergic and glucocorticoid systems, while both strengthening memory of the shock experience *per se*, have seemingly opposite effects on the accuracy of this episodic-like memory (Figure 3). These findings could, at least in part, explain the opposite effects of stress and emotional arousal on accuracy vs generalization of memory in the human literature.

2.3 Norepinephrine enhances the consolidation of pattern-separated memories

To examine the neural mechanism of how noradrenergic activation enhances memory accuracy on this task, a series of additional experiments were performed. Norepinephrine administration into the BLA after training on the dual-event inhibitory avoidance task was found to enhance episodic-like memory by promoting the separation of memory for the two training events into distinct memory representations (Atucha et al., under revision). This pattern separation process is closely linked to the operation of the dorsal blade of the dentate gyrus (dDG) within the hippocampus (Marr, 1971; Leutgeb et al., 2007; Yassa & Stark, 2011; Bekinschtein et al., 2013; Rolls, 2016). Norepinephrine administration into the BLA was found to enhance the consolidation of pattern-separated memories within the dDG via post-transcriptional regulation of gene expression by a down-regulation of miR-134 (Atucha et al., under revision). MicroRNAs (miRs) are small, non-coding RNAs that are capable of RNA silencing by binding to complementary sequences on the 3' untranslated regions (3'UTR) of their target mRNA (He & Hannon, 2004). MiR-134 serves as a critical regulator of the cAMP response element-binding (CREB) and brain-derived neurotrophic factor (BDNF) pathways (Schratt et al., 2006; Gao et al., 2010). Importantly, this norepinephrine-induced down-regulation of miR-134 within the dDG was causally linked with an up-regulation of both CREB and BDNF mRNA levels. CREB and BDNF are canonical memory mechanisms that play critical roles in memory consolidation (Silva et al., 1998; Bramham & Messaoudi, 2005). Moreover, BDNF is an important modulator of hippocampal neurogenesis (i.e., the generation of new neurons from neural stem cells) by its contribution to cell proliferation and differentiation (Waterhouse et al., 2012), and an interaction of BDNF with newborn cells within the dDG has been implicated in the consolidation of overlapping memories (Bekinschtein et al., 2014).

Levels of miR-134 can be experimentally manipulated by the administration of either an antagomir (i.e., the exact complementary sequence of miR-134) or mimic (i.e., exact copy of miR-134). Selective down-regulation of miR-134 in the hippocampus after training on the dual-event inhibitory avoidance task was found to enhance memory accuracy. Down-regulation of miR-134 in the hippocampus did not affect the strength of the memory, indicating again that this hippocampal mechanism is selectively involved in regulating norepinephrine effects on memory accuracy. This was further supported by

the finding that an up-regulation of miR-134 levels in the hippocampus after the training selectively blocked the effect of norepinephrine administration into the BLA on inducing memory accuracy, but did not alter the norepinephrine effect on strengthening the memory. Norepinephrine administration into the BLA now increased retention latencies in both the shock box and non-shock box. Importantly, retention latencies in the novel box were not affected, indicating that the effect could not be explained by a complete generalization of memory across contexts.

Based on these findings, it was hypothesized that this norepinephrine-induced down-regulation of miR-134 within the BLA-dDG circuit might play a selective role in separating the memory of the two training events into distinct memories. To test this hypothesis, it was investigated whether norepinephrine induced a down-regulation of miR-134 in the dDG under training conditions that do not require the separation of memory of different training events. When animals were trained on a single-event inhibitory avoidance task or exposed twice to the same shock context, norepinephrine administration into the BLA did not down-regulate miR-134 levels in the dDG. These findings thus indicate that noradrenergic activation of the BLA selectively engages this miR-134 mechanism in the dDG after training on an episodic-like task when overlapping information of multiple events is present. Consistent with these findings, Segal et al., 2012 found previously that noradrenergic activation (tested using salivary alpha-amylase) after exposure to fearful stimuli was correlated with enhanced subsequent pattern separation performance in humans. Other human studies are consistent with the idea that emotional arousal might support the segmentation of continuous experience into distinct and memorable episodes. It was found that changes in context, or event boundaries, elicit a burst of (noradrenergic) arousal which guides the segmentation of adjacent episodes in later memory (Clewett et al., 2020). It has further been proposed that competing memories via this mechanism are adaptively segmented to protect emotional memories from immediate sources of interference (Dunsmoor et al., 2018). It should be noted, however, that these latter human studies focused on the role of arousal on event segmentation during the actual encoding of information, and not during the posttraining consolidation phase.

Opposite to the effect of norepinephrine, corticosterone was found to reduce episodic-like accuracy of memory on the dual-event inhibitory avoidance task (Roozendaal & Mirone, 2020). As mentioned above, corticosterone increased retention latencies in both the shock box and non-shock box, but importantly left retention latencies in the novel box unaltered. Thus, this finding stands in contrasts to those of other studies indicating that elevated corticosterone levels after contextual fear conditioning increased freezing responses in a novel context (dos Santos Corrêa et al., 2019; Lesuis et al., 2021). Rather, these findings support the view that corticosterone administration after training on

the dual-event inhibitory avoidance task might induce a linking of memory of the two training events. Such a linking of memory of training events is then possibly caused by an opposite regulation of this hippocampal pattern separation mechanism within the dDG. Interestingly, corticosterone has previously been shown to increase overall activity in the DG (and thus disrupt the typical sparse regional encoding) (Lesuis et al., 2021), and to reduce hippocampal BDNF expression (Smith et al., 1995; Schaaf et al., 1998). DG hyperactivity was associated with an impaired memory for the contextual aspects of the training event, indicating a potential impairment in hippocampal function. Such an interpretation would be consistent with findings of electrophysiological studies indicating that glucocorticoids typically impair hippocampal long-term potentiation (LTP), a fundamental form of synaptic neuroplasticity (Foy et al., 1987; Shors et al., 1990; Diamond et al., 1992). However, as mentioned previously, glucocorticoids do not always impair memory on hippocampus-dependent tasks. Some studies showed that glucocorticoid administration enhances hippocampus-dependent memory in paradigms such as contextual fear conditioning, inhibitory avoidance and water-maze spatial tasks (Roozendaal & McGaugh, 1996a). This duality underscores the complexity of the relationship between glucocorticoids and hippocampal-dependent memory processes.

These findings show that the effects of norepinephrine and corticosterone critically depend on the type of memory process tested. Understanding how different stress hormones might have distinct effects on the separation of memory for multiple events can provide insight into how the brain organizes and differentiates between overlapping memories under stress. Moreover, gaining a deeper understanding of the intricate relationship between these mechanisms and their functional consequences is crucial for comprehending the mnemonic modifications found in stress-related disorders.

3. Scope and outline of this thesis

In this thesis, I investigated the hypothesis that norepinephrine and corticosterone induce opposite effects on memory accuracy after training on a task that requires the separation of memory representations of multiple training events, but that norepinephrine and corticosterone induce similar effects on hippocampus-dependent memory under training conditions that do not require the separation of memory of different training events.

In **Chapter 2,** I examined the effect of norepinephrine and corticosterone on object-in-context memory, a hippocampus-dependent memory task in which two object presentation events during the training session are distinguished by the contexts in which they appear (Dix & Aggleton, 1999; Eacott & Norman, 2004; Barsegyan et al., 2014;

Balderas et al., 2015). Thus, similar to the dual-event inhibitory avoidance task, accurate performance on this task requires the separation of memory for the two learning events. Yet, the task has as benefit that both training episodes are of equal valence, excluding alternative interpretations related to the higher saliency of the training episode in the shock box. Different doses of the noradrenergic stimulant yohimbine or corticosterone were administered systemically immediately after training on the object-in-context task. A retention test 1 day later assessed whether the mice had correctly associated the object presentations with the corresponding training contexts. To experimentally manipulate the necessity to separate memory of the two training events, animals received three habituation sessions to either the two training contexts or two different contexts prior to the training session. I hypothesized that yohimbine and corticosterone administration would induce opposite effects on object-in-context memory of mice that had no prior habituation to the training contexts by regulating a hippocampal mechanism that facilitates either a separation or linking of memory of the two training events, respectively. However, both yohimbine and corticosterone should enhance object-in-context memory of mice that were previously familiarized with the training contexts and thus already had formed memories of the two training contexts. To examine whether prior context habituation alters the contribution of brain regions involved in episodic/contextual (i.e., the hippocampus) and object (i.e., anterior insular and perirhinal cortices) memory to the yohimbine and corticosterone effect on object-in-context memory, I also assessed posttraining neuronal activity in these regions. To examine neuronal activity, I analyzed local c-Fos expression, a well-established molecular marker for activated cells (Minatohara et al., 2016) 1 hour after the training. I additionally assessed the co-expression of c-Fos with GAD67, a GABAergic marker, to dissociate excitatory vs inhibitory neuronal activity in the hippocampus. Both the effects on neuronal activity in the hippocampal subregions *per se* as well as the correlations in activity between subregions were assessed as a proxy for hippocampal function.

In **Chapter 3**, I aimed to provide causal evidence for the hypothesis that the effect of norepinephrine on enhancing object-in-context memory in non-habituated mice does require the hippocampus, but that prior habituation to the training contexts renders this norepinephrine effect hippocampus independent. Therefore, I combined posttraining systemic administration of yohimbine with Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetics to selectively silence the hippocampus during both the training on the object-in-context task and the posttraining consolidation period. Mice received bilateral intracranial injections into the hippocampus of an adeno-

associated virus that induces the expression of an excitatory DREADD receptor selectively in inhibitory GABAergic neurons, or a control virus. Following viral transfection, mice received three habituation sessions to either the two training contexts or two different contexts. On the training day, the hippocampus was chemogenetically inactivated prior to the training session, and yohimbine was administered immediately posttraining. The effect of DREADD-mediated inhibition of hippocampal activity on the effects of yohimbine administration on object-in-context memory in the two habituation conditions was tested in a retention test 1 day later. Specifically, I aimed to test the hypothesis that hippocampal inactivation would impair object-in-context memory of yohimbine-treated mice that had been habituated to two different contexts prior to training, but that hippocampal inactivation would have no effect in mice that had been habituated to the two training contexts.

To further examine whether norepinephrine and corticosterone solely induce opposite effects on hippocampus-dependent memory after training on a task that requires the separation of overlapping memory representations for multiple training events, in **Chapter 4**, yohimbine and corticosterone were administered after training on an object location task. In this task, spatial memory is formed by associating an object with a specific location within the training context, which also critically depends on the hippocampus (Balderas et al., 2008; Roozendaal et al., 2010; Barsegyan et al., 2019). However, the training experience comprises a single event, and thus the animals do not have to separate overlapping memory representations. Different doses of the noradrenergic stimulant yohimbine or corticosterone were administered systemically immediately after the training session. At a 1-day retention test, the mice were re-exposed to the same context with the same two objects, but one of the objects had been moved to a novel location. To investigate whether prior context habituation influenced the effect of yohimbine and corticosterone administration on object location memory, and hippocampal involvement herein, animals received three habituation sessions to either the training context or a different context prior to the training session. I examined the effects of stress hormone manipulation and context habituation on posttraining neuronal activity in the hippocampus by assessing c-Fos and GAD67 expression similarly to the approach in Chapter 2.

Lastly, in **Chapter 5**, I summarize the main findings of this thesis and provide general conclusions and future prospects.

CHAPTER 2

Opposite effects of norepinephrine and glucocorticoids on episodic-like memory in an object-in-context task: role of context habituation

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Abstract

Extensive evidence indicates that the stress hormones norepinephrine and glucocorticoids create strong and lasting memories. However, considerably less is known of how these two hormones might affect the quality of these strengthened memories. In the present study, we examined whether systemic administration of the noradrenergic stimulant yohimbine and corticosterone induce opposite effects specifically on episodic-like memory in a task that requires the separation of memory for multiple training events. For this, mice were trained on an object-in-context task, a hippocampus-dependent task in which two object presentation events during the training session are distinguished by the contexts in which they appear. To manipulate the need for separating memory of the two training events, animals underwent different habituation sessions to either the two training contexts or two distinct contexts before the training session. We found that yohimbine administered immediately after the training session dose-dependently enhanced object-in-context memory assessed 24 h later, whereas corticosterone impaired this memory in mice that were not habituated to the training contexts. However, both yohimbine and corticosterone enhanced object-in-context memory when mice were previously familiarized with these contexts. To explore whether prior context habituation might alter the contribution of the hippocampus in regulating the yohimbine and corticosterone effect on object-in-context memory, we assessed posttraining neuronal activity within different hippocampal regions as well as in other brain regions that are relevant to some aspects of object recognition memory. These findings suggest that yohimbine and corticosterone administration induced opposite effects on object-in-context memory in non-habituated animals by regulating a hippocampal mechanism that facilitates either a separation or linking of memory of the two training events, respectively. Yet, habituation to the training contexts might already have generated memories for the two training contexts, in which the object information merely needed to be added. Thereby, the enhancing effects of both yohimbine and corticosterone in this condition might be mediated by a strengthening of memory for the objects *per se* by the involvement of other brain regions.

Keywords: norepinephrine; glucocorticoids; object-in-context memory; memory separation; memory linking; pattern separation.

Introduction

Extensive evidence indicates that stressful and emotionally arousing experiences induce strong and lasting memories by the activation of different hormonal systems (McGaugh, 2000; Sara, 2009; Joëls et al., 2011; Roozendaal & McGaugh, 2011; Takeuchi et al., 2016; de Quervain et al., 2017; Bahtiyar et al., 2020; Schwabe et al., 2022). First, stress rapidly activates the sympathetic nervous system, which induces the release of catecholamines, such as epinephrine and norepinephrine, from the adrenal medulla and sympathetic nerve endings as well as from noradrenergic cell groups within the brain (Mason, 1968). In a delayed fashion, stress also induces the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which culminates in the release of glucocorticoid hormones (corticosterone in rodents, cortisol in humans) (Ulrich-Lai & Herman, 2009). An impressive body of literature indicates that both norepinephrine and glucocorticoids, in a predominantly synergistic fashion, enhance the consolidation of memory of emotionally arousing experiences (Roozendaal, Okuda, de Quervain, et al., 2006).

Considerably less is known of how these two hormonal systems might also affect the quality of these strengthened memories. Human behavioral studies have reported conflicting findings (Morgan et al., 2004a; Porter et al., 2008; Hoscheidt et al., 2014). Whereas some studies indicated that emotional arousal also increases the accuracy of memory, such that participants could remember more details of an experience (Kensinger et al., 2007a; Segal et al., 2012), other studies reported that emotional memories are strengthened in a generalized fashion, making that particularly the gist of an experience is recalled (Payne et al., 2002; Talarico & Rubin, 2003; Morgan et al., 2004a; Sharot et al., 2004; Schwabe & Wolf, 2009; Rimmele et al., 2011). Recent findings from animals studies investigating this topic have suggested that norepinephrine and glucocorticoids induce opposite effects on the accuracy of memory which might, in part, explain the conflicting findings from stress exposure in human experiments. In our laboratory, we have previously investigated noradrenergic and glucocorticoid effects on episodic-like memory in a dual-event inhibitory avoidance task. In this task, rats were subsequently trained in two different inhibitory avoidance apparatuses with a brief interval, but received footshock in only one of these two contexts (Atucha & Roozendaal, 2015). It was found that the noradrenergic stimulant yohimbine administered systemically immediately after the training session not only enhanced memory of the shock experience *per se*, but also increased the rats' ability on a later retention test to successfully discriminate in which of these two contexts they had received footshock. In contrast, posttraining systemic corticosterone administration induced a generalized strengthening of memory of the training with rats displaying increased retention latencies in both training contexts (Atucha & Roozendaal, 2015; Roozendaal & Mirone, 2020). Further findings provided support for the view that noradrenergic activation enhances episodic-like memory on

this task by facilitating the separation of overlapping memory representations to enable the selective strengthening of correct associations into long-term memory (Atucha et al., under revision). Based on these findings, it could be hypothesized that corticosterone impairs episodic-like memory on this task by supporting a linking of memory of the two training events, but this has not been investigated.

Episodic-like and contextual memories depend critically on the hippocampus (Tulving & Markowitsch, 1998; Eichenbaum, 2017b), and several studies have shown that norepinephrine and corticosterone regulate hippocampal function to induce their hormone-specific effect on episodic-like or contextual memories (Kaouane et al., 2012; Raybuck & Lattal, 2014; Atucha et al., 2017; dos Santos Corrêa et al., 2019; Lesuis et al., 2020). Pattern separation is closely linked to the operation of the dorsal blade of the dentate gyrus (dDG) within the hippocampus (Marr, 1971; Leutgeb et al., 2007; Yassa & Stark, 2011; Bekinschtein et al., 2013; Rolls, 2016). Interestingly, some studies have shown that prior knowledge of the training context could change the contribution of the hippocampus to the formation of contextual memories. For example, rats with lesions of their hippocampus were able to successfully acquire a contextual fear conditioning task if they had previously been exposed to the training context (Young et al., 1994). Similarly, it has been shown that the degree of contextual familiarity influences the effect of hippocampal inactivation on object recognition memory (Oliveira et al., 2010). Whereas hippocampal inactivation after a short habituation period (creating limited context familiarity) enhanced long-term object recognition memory, hippocampal inactivation did not affect long-term recognition memory after a longer contextual habituation period. Thus, these findings suggest that prior knowledge of the training context might reduce the involvement of the hippocampus in acquiring these learning tasks. However, we do not know if prior context habituation might also alter hippocampal involvement in regulating the effect of norepinephrine and corticosterone on episodic-like memory and the separation of memory for multiple training events.

In the present study, we examined whether norepinephrine and corticosterone induce opposite effects on episodic-like memory in a task that requires the separation of multiple memory representations. For this, mice were trained on an object-in-context task, a hippocampus-dependent memory task in which two object presentation events, of similar neutral valence, during the training session are distinguished by the contexts in which they appear (Dix & Aggleton, 1999; Eacott & Norman, 2004; Barsegyan et al., 2014; Balderas et al., 2015). Thus, similar to the dual-event inhibitory avoidance task, accurate performance on this task requires the separation of memory for the two learning events. To investigate whether prior context habituation alters the effects of norepinephrine and corticosterone, animals received three habituation sessions to either

the two training contexts or two different contexts prior to the training session. Different doses of the noradrenergic stimulant yohimbine or corticosterone were administered systemically immediately after the training session. A retention test 24 h later assessed whether the mice had correctly associated the object presentations with the specific training contexts. We also examined how prior context habituation might alter the effect of posttraining yohimbine and corticosterone administration on hippocampal activity during the post-learning consolidation period. For this. we examined c-Fos expression, a well-established molecular marker for activated cells (Minatohara et al., 2016), 1 h after training in different hippocampal subregions as well as in other brain regions that are relevant to some aspects of recognition memory, i.e., perirhinal cortex (PRh), anterior insular cortex (aIC) and basolateral amygdala (BLA) (Roozendaal et al., 2008; Roozendaal et al., 2010). We assessed the effects of prior context habituation and hormone administration on neuronal activity within these brain regions *per se*, as well as across-animal correlations in activity to investigate effects on neuronal connectivity.

Material and methods

Animals

Four-hundred-and-twenty male CB57BL/6J mice (10-14 weeks old at time of behavioral experiments) from Charles River Breeding Laboratories (Kisslegg, Germany) were kept in a temperature-controlled (22 °C) vivarium room and maintained on a 12:12-h light:dark regimen (7:00 – 19:00 h lights on). The vivarium room had a light intensity of 47 lux and humidity of 72%. Mice had *ad libitum* access to food and water. Mice were single housed 7 days prior to the start of the experiment and remained single housed throughout to avoid potential stress induced by hierarchical status or fighting and prevent testing order effects. Training and testing was performed during the light phase of the cycle, between 10:00 and 16:00 h, at the nadir of the diurnal cycle of corticosterone. All experimental procedures were in compliance with European Union Directive 2010/63/EU and approved by the Central Authority for Scientific Procedures on Animals (CCD), The Hague, The Netherlands. All efforts were made to minimize animal suffering and to reduce the number of animals.

Object-in-context task

The animals were trained and tested in two gray round plastic boxes (40 cm diameter, 40 cm height) placed next to one another in a dimly illuminated experimental room. One box had gray inner walls and the floor was covered with sawdust. The other box had white stripes and dots modifying the walls and contained corncob bedding to make this a distinctly different contextual environment from the other box. The objects to be discriminated were white glass light bulbs (6 cm diameter, 11 cm length) and transparent

glass jars (5.5 cm diameter, 5 cm height), secured to the floor of the boxes with Velcro tape.

Prior to training, mice were first handled for 2 min each on 4 consecutive days to become accustomed to the experimenter. Subsequently, the animals received three days of habituation to reduce novelty stress which is required to guarantee sufficient exploration of the objects on the training session (Stefanko et al., 2009). Some experimental groups were habituated to the same two contexts as those used for training (two round boxes with different modifications, contexts A and B) for 3 consecutive days. Other experimental groups were habituated to two different contexts (two square boxes (40 cm width, 40 cm length, 40 cm height) with different modifications, contexts X and Y) for 3 days (Figure 1A). In each condition (either the round or square boxes), one box was gray with sawdust bedding and the other one had white stripes and dots on the walls and had corncob bedding. During the habituation, the animals could explore each context without any objects. The duration of habituation to each context was dependent on the experimental group and was always identical to the duration of the training session in each context.

On the training session, the mice were placed in the first box (context A or B), and were able to explore one set of two identical objects (either two glass jars or two light bulbs), placed 5 cm away from the edge of the box. Immediately after the first context exposure, mice were placed in the second box (context B or A), containing the other set of two identical objects. The sequence of the two contexts and the object-context combinations were counterbalanced across animals. To assess drug-induced memory enhancement, animals were trained in each context for 5 min, whereas for the assessment of drug-induced memory impairment, animals were trained in each context for 7 min. To determine the effect of training duration on memory performance, other experimental groups were trained for 7, 8, 9 or 10 min in each context. To avoid the presence of olfactory trails, feces were removed, bedding was stirred, and the objects were thoroughly cleaned with 70% ethanol in between animals. Immediately after the training session, the animals received a systemic drug injection and were placed back in their home cage. Some mice were sacrificed at 1 h after training and drug treatment for immunohistochemical assessment of neuronal activity. Other mice were left undisturbed until the retention test 24 h later. For retention testing, the animals were placed in one of the two training contexts with one exemplar of both training objects for 5 min, regardless of the duration of the training session. Both the context used on the retention test and the positioning of the novel object-in-context association within that context, i.e., left or right, were counterbalanced across animals.

Mice' behavior during training and retention test was recorded with a video camera mounted above the experimental apparatus. Videos were analyzed offline by a trained

observer blind to the treatment condition, and the time spent exploring each object was scored. Object exploration was defined as actual active interaction with an object, i.e., pointing the nose to the object at a distance of <1 cm and/or touching it with the nose (Okuda et al., 2004; Leger et al., 2013; Song et al., 2020). Turning around, climbing or sitting on an object *per se* was not included in exploration time as the animals then often do not actively engage in exploring the object but rather exhibit grooming behavior or are using the object as platform to scan the environment (Roozendaal et al., 2006). In order to analyze memory performance, a discrimination index (DI%) was calculated as the difference in time exploring the novel and familiar object-in-context combination, expressed as the ratio of the total time spent exploring both objects (i.e., [Time Novel - Time Familiar] / [Time Novel + Time Familiar] x 100%). Two mice showing a total exploration time of <2 s during training and/or testing were removed from further analyses (Okuda et al., 2004).

Systemic drug administration

For the behavioral experiments, the noradrenergic stimulant yohimbine (0.3, 1 or 3 mg/kg; 17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride; Sigma-Aldrich), an α_2-adrenoceptor antagonist that increases norepinephrine levels in the periphery and brain (Szemeredi et al., 1991), was dissolved in saline, whereas the control group received saline only. Corticosterone (1, 3 or 10 mg/kg, Sigma-Aldrich) was first dissolved in 100% ethanol and subsequently diluted in saline to get a 5% ethanol solution, and the control group was injected with a vehicle containing 5% ethanol in saline. Drugs were administered intraperitoneally in a volume of 0.01 mL/g of body weight, immediately after the training session. Doses of yohimbine and corticosterone were selected based on previous studies (Cai et al., 2006; Song et al., 2021) For the immunohistochemical experiments, both yohimbine (1 mg/kg) and corticosterone (3 mg/kg) were dissolved in a vehicle containing 5% ethanol in saline. Drug solutions were prepared freshly before each experiment.

Immunohistochemistry

Mice were anesthetized with an overdose of sodium pentobarbital (40-50 mg/kg) 1 h after training and drug treatment, followed by transcardial perfusion with 10 mL of ice-cold phosphate-buffered saline (PBS) and 10 mL of ice-cold 4% paraformaldehyde (PFA) (pH 7.4). Brains were extracted, post-fixed in 4% PFA in 0.1 M PBS (pH 7.4) for 24 h, and then transferred to a 30% sucrose solution in 0.1 M PBS at 4 °C for 4 days . Coronal slices of 30 µm thickness were cut on a cryostat, collected in 0.1 M PBS with 0.01% sodium azide, and stored at 4 °C. For immunohistochemistry procedures, three to four sections of each of the brain regions investigated were selected according to the Franklin and Paxinos mouse brain atlas (Franklin & Paxinos, 2007): hippocampus (anteroposterior (AP), -1.58 to -2.06 mm), PRh (AP, -1.58 to -2.06 mm), aIC (AP, +1.42 to +1.18 mm) and BLA

(AP, -1.59 to -2.78 mm). Sections were rinsed in 0.5% Triton in PBS for 30 min at room temperature (RT), washed three times in PBS for 10 min per wash, and then blocked in 5% Normal Donkey Serum (NDS, Jackson ImmunoResearch Laboratories) and 1% Bovine Serum Albumin (BSA, Thermo Fisher) in PBS for 1 h at RT. Next, sections were incubated with primary antibodies (c-Fos; guinea pig anti-c-Fos, 1:750, 226 004 Synaptic Systems, glutamic acid decarboxylase 67 (GAD67); mouse anti-GAD67; 1:500, MAB5406-25ug Sigma-Aldrich) in PBS containing 2% NDS and 0.1% acetylated BSA (BSA-c, Aurion) overnight at RT. Afterwards, sections were washed three times in PBS for 10 min per wash, followed by incubation with fluorophore-conjugated secondary antibodies Donkey anti-guinea pig Alexa Fluor 647 (1:750, Jackson ImmunoResearch) and Donkey anti-mouse Alexa Fluor 488 (1:500, Invitrogen) in PBS with 2% NDS and 0.1% BSA-c for 3 h at RT. All procedures starting from the secondary antibody incubation onwards were performed in the dark. Subsequently, sections were incubated with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI, 1:5,000) in PBS with 0.1% BSA-c for 15 min, then washed three times in PBS for 10 min per wash, mounted on gelatin-coated slides, left to dry, and coverslipped with Fluorsave mounting medium (Sigma-Aldrich). The slides were stored at 4 °C.

Imaging and quantification

Images were acquired on an Automated High-Content Fluorescence Microscope (Leica, DMI 6000B, Germany) with a 20x magnification. ImageJ software was used to count labeled cells and measure area sizes (Rueden et al., 2017). The regions of interest were identified with a stereotactic mouse brain atlas (Franklin & Paxinos, 2007). For the hippocampus and BLA, the regions were manually drawn according to the Allen Mouse Brain Atlas (http://portal.brain-map.org/). For the analysis of c-Fos-positive neurons, the hippocampus was divided into its four main subregions: granule cell layer of the dDG, granule cell layer of the dentate gyrus ventral blade (vDG), pyramidal cell layer of the cornu ammonis 3 (CA3) and cornu ammonis 1 (CA1). For the analysis of c-Fos and GAD67 double-positive neurons, we looked additionally in the *striatum radiatum* of the CA3 (CA3sr) and CA1 (CA1sr). The aIC was divided into the agranular aIC dorsal part (AID), agranular aIC ventral part (AIV), dysgranular aIC (DI), and granular aIC (GI), in which two squared areas (80 x 80 µm) were selected to cover layers II/III and layers V/VI, respectively. For the PRh, two squared areas (200 x 200 µm) covering layers II/III and layers V/VI were used. For each region of interest, the number of c-Fos-positive and GAD67-positive cells and double-positive neurons was counted manually by a researcher blind to the treatment condition, and then converted to number of cells per mm^2. Relative GABAergic activity was calculated as the number of neurons showing co-localization of c-Fos and GAD67, expressed as the percentage of the total number of GAD67-positive neurons.

Statistics

Data are expressed as mean ± SEM. Statistical analyses were performed using IBM SPSS statistics version 25. Total object exploration time in each of the two contexts during the training session was analyzed using linear mixed models with drug treatment group (saline, yohimbine 0.3, 1, or 3 mg/kg, and vehicle, corticosterone 1, 3, or 10 mg/kg, respectively) (if appropriate), and habituation condition (different or same) as between-subject parameters, and object exploration time in the first and second box as within-subject parameter. Moreover, as within-subject parameters, the training session number (first or second episode), the object explored (bulb vs. jar) and training context (A vs. B) were included. Noteworthy, all within-subject variables were counterbalanced across animals. The DI% and total object exploration time at the 5-min retention test were analyzed with two-way ANOVAs with drug treatment (3 drug doses and respective vehicle) and habituation condition (different or same) both as between-subject parameters. The DI% and total object exploration time for the 7-min training group (in the different habituation condition only) was analyzed with a one-way ANOVA with drug treatment group (yohimbine 1 mg/kg, corticosterone 3 mg/kg or vehicle) as between-subject parameter, the training session number (first or second episode), the object explored (bulb vs. jar) and training context (A vs. B) were added as within-subject parameters. When appropriate, Tuckey *post-hoc* tests were used to determine the source of the significance. One-sample *t*-tests were used to determine whether the DI% was different from zero (i.e., chance level) and thus whether learning had occurred.

Immunohistochemistry data for the hippocampal subregions as well as the BLA was analyzed by two-way ANOVAs, with drug treatment (yohimbine 1 mg/kg, corticosterone 3 mg/kg or vehicle) and habituation condition (different or same) as between-subject variables. Neuronal activity in the PRh and aIC was analyzed using a linear mixed model with drug treatment and habituation condition as between-subject variables and cortical layers and/or subregion as within-subject variables. Significant effects of drug treatment were followed up by tests for yohimbine and corticosterone treatment separately. *Post hoc* independent-sample *t*-tests between appropriate groups were conducted to determine the source of significance. Finally, Pearson correlations were calculated to determine correlations between c-Fos-expression data in different brain regions. Correlations were statistically compared by running a Fisher r-to-z transformation and one-tailed tests for significance based on our hypotheses. For all statistical tests, $p < 0.05$ was accepted for statistical significance, except for the Pearson correlations where we kept a more stringent threshold of $p < 0.01$. The figures only display significant *post hoc* comparisons unless stated otherwise. The number of mice per group is indicated in the figure legends.

Results

Posttraining noradrenergic stimulation dose-dependently enhances object-in-context memory independent of the habituation condition

In the first experiment, we examined whether posttraining systemic administration of the noradrenergic stimulant yohimbine (0.3, 1 or 3 mg/kg) enhances object-in-context memory and whether the effect depends on the habituation condition. Mice were habituated for three sessions to either the different or same contexts, trained the next day for 5 min on the object-in-context task followed by yohimbine treatment, and retention was tested 24 h later (Figure 1A). Total object exploration time during training was different for the two habituation conditions ($F_{(1,171)}$ = 11.84, p = 0.001), with animals showing more object exploration in the same habituation condition. Critically, total object exploration time during training did not differ between drug treatment groups ($F_{(3,171)}$ = 1.14, p = 0.33, Table I). Further, total object exploration time during training was not dependent on training context (A vs. B, $F_{(1,171)}$ = 0.58, p = 0.44), training session (first vs. second, $F_{(1,171)}$ = 0.31, p = 0.58) or type of object (jars vs bulbs, $F_{(1,171)}$ = 0.22, p = 0.64). Total object exploration time during the retention test was also different for the two habituation conditions ($F_{(1,60)}$ = 33.68, p < 0.001), with animals in the same habituation condition showing again more total object exploration. However, total object exploration time during the retention test did not differ between drug treatment groups ($F_{(3,60)}$ = 0.65, p = 0.59) or drug treatment X habituation condition interaction ($F_{(3,60)}$ = 0.97, p = 0.41).

At the 24-h retention test, a two-way ANOVA for the DI% indicated a significant main effect of drug treatment ($F_{(3,60)}$ = 6.47, p = 0.001), but no effect of habituation condition ($F_{(1,60)}$ = 0.13, p = 0.72) or drug treatment X habituation condition interaction ($F_{(3,60)}$ = 1.20, p = 0.32, Figure 1B). Object-context primacy, i.e., whether the novel object-in-context association was encountered during the first or second training episode, did not influence the DI%, nor did it interact with any other factors (all p's > 0.06). Tukey's *post-hoc* analyses revealed that the 1 mg/kg yohimbine group in both habituation conditions had a significantly greater DI% than the respective saline group (different: $t_{(29)}$ = 3.59, p = 0.001, same: $t_{(27)}$ = 3.11, p = 0.004). Further, one-sample t-tests indicated that the 1 mg/kg yohimbine group in both habituation conditions showed successful memory performance, with the DI% being significantly greater than zero (different: $t_{(15)}$ = 6.47, p < 0.001, same: $t_{(14)}$ = 4.57, p < 0.001), whereas the two saline groups did not display any memory (different: $t_{(14)}$ = 0.08, p = 0.94, same: $t_{(13)}$ = 0.81, p = 0.44). Mice of the different habituation condition treated with the other two doses of yohimbine also showed no memory for the object-context association (0.3 mg/kg: $t_{(13)}$ = 0.48, p = 0.64, 3 mg/kg: $t_{(14)}$ = 1.48, p = 0.16). Mice of the same habituation condition treated with the lower dose of 0.3 mg/kg yohimbine showed memory ($t_{(13)}$ = 2.31, p = 0.04), whereas mice treated the higher dose of 3 mg/kg yohimbine did not ($t_{(13)}$ = 0.08, p = 0.94). These findings indicate that

yohimbine dose-dependently enhanced memory for the association of the object and the context in both habituation conditions.

Posttraining corticosterone administration dose-dependently enhances object-in-context memory only when previously habituated to the same context

In the second experiment, we examined whether posttraining systemic corticosterone (1, 3, or 10 mg/kg) administration enhances object-in-context memory and whether the effect is dependent on the habituation condition. Similar to the yohimbine experiment, mice were first habituated for three sessions to either the different or same contexts, trained for 5 min on the object-in-context task followed by corticosterone treatment, and retention was tested 24 h later. Similar to the first experiment, total object exploration time during training was different for the two habituation conditions ($F_{(1,86)}$ = 10.97, p = 0.001), with animals showing more object exploration in the same habituation condition. Again, total object exploration time during training did not differ between drug treatment groups ($F_{(3,156)}$ = 0.49, p = 0.68, Table I), and was not dependent on training context (A vs. B: $F_{(1,156)}$ = 3.61, p = 0.06), training session (first vs. second, $F_{(1,156)}$ = 0.14, p = 0.71) or type of object (jars vs bulbs: $F_{(1,156)}$ = 1.22, p = 0.27). Total object exploration time during the retention test was not influenced by drug treatment ($F_{(3,56)}$ = 0.46, p = 0.71), habituation condition ($F_{(1,56)}$ = 1.04, p = 0.31) or drug treatment X habituation condition interaction effects ($F_{(3,56)}$ = 1.32, p = 0.28).

At the 24-h retention test, a two-way ANOVA for the DI% revealed no main effect of drug treatment ($F_{(3,56)}$ = 0.60, p = 0.62), but indicated a significant effect of habituation condition ($F_{(1,56)}$ = 7.30, p = 0.009) as well as drug treatment X habituation condition interaction effect ($F_{(3,56)}$ = 3.01, p = 0.04, Figure 1C). Again, object-context primacy did not influence the DI%, nor did it interact with any of the other factors (all p's > 0.08). Tukey's *post-hoc* analyses revealed that the 3 mg/kg corticosterone group in the same habituation condition had a significantly greater DI% than the vehicle group ($t_{(28)}$ = 2.84, p = 0.008), whereas in the different habituation condition these two groups did not differ from each other ($t_{(25)}$ = -0.35, p = 0.73). Moreover, the DI% of the 3 mg/kg corticosterone group in the same habituation condition was significantly greater than that of the 3 mg/kg corticosterone group in the different habituation condition ($t_{(28)}$ = 2.80, p = 0.009). One-sample t-tests indicated that the 3 mg/kg corticosterone group in the same habituation condition showed successful memory, with the DI% being significantly greater than zero ($t_{(28)}$ = 2.84, p < 0.01), whereas mice treated with vehicle ($t_{(14)}$ = 1.22, p = 0.24) or the other doses of corticosterone did not (1 mg/kg: $t_{(12)}$ = 0.39, p = 0.71; 10 mg/kg: $t_{(13)}$ = 0.27, p = 0.80). In contrast, none of the drug treatment groups in the different habituation condition showed evidence of memory (vehicle: $t_{(12)}$ = 0.53, p = 0.61; 1 mg/kg: $t_{(12)}$ = 0.78, p = 0.46; 3 mg/kg: $t_{(13)}$ = -0.005, p = 0.99; 10 mg/kg: $t_{(12)}$ = 0.58, p = 0.57).

These findings indicate that corticosterone dose-dependently enhanced memory for the association of the object and the context when mice were previously habituated to the training contexts, but not when habituated to the different contexts.

Table I. Object exploration time during training and retention test

Treatment group	Habituation condition	First box training (s)	Second box training (s)	Retention test (s)
VEH (*n* = 15)	Different	7.8 ± 1.5	7.3 ± 1.8	7.5 ± 2.0
YOH 0.3 mg/kg (*n* = 14)	Different	8.2 ± 1.2	7.8 ± 1.5	6.7 ± 1.3
YOH 1 mg/kg (*n* = 16)	Different	8.4 ± 2.2	7.9 ± 1.6	7.5 ± 1.8
YOH 3 mg/kg (*n* = 15)	Different	8.8 ± 2.3	8.7 ± 1.7	7.0 ± 1.4
VEH (*n* = 14)	Same	8.5 ± 2.3	9.3 ± 3.8	10.5 ± 2.7
YOH 0.3 mg/kg (*n* = 15)	Same	10.7 ± 3.8	10.3 ± 3.5	9.4 ± 3.3
YOH 1 mg/kg (*n* = 15)	Same	9.1 ± 2.9	9.9 ± 2.8	10.0 ± 2.9
YOH 3 mg/kg (*n* = 14)	Same	10.5 ± 3.7	9.5 ± 3.3	11.0 ± 4.0
VEH (*n* = 13)	Different	7.9 ± 1.5	8.7 ± 2.2	7.0 ± 2.4
CORT 1 mg/kg (*n* = 13)	Different	7.9 ± 1.8	8.6 ± 1.6	7.7 ± 2.2
CORT 3 mg/kg (*n* = 14)	Different	8.3 ± 2.8	9.0 ± 1.5	7.2 ± 2.0
CORT 10 mg/kg (*n* = 13)	Different	8.6 ± 2.1	8.5 ± 1.7	8.2 ± 1.8
VEH (*n* = 15)	Same	9.2 ± 2.3	8.6 ± 1.5	7.0 ± 1.4
CORT 1 mg/kg (*n* = 13)	Same	10.1 ± 2.4	9.1 ± 1.7	7.4 ± 1.4
CORT 3 mg/kg (*n* = 15)	Same	9.7 ± 2.6	9.7 ± 2.9	7.2 ± 2.0
CORT 10 mg/kg (*n* = 14)	Same	9.5 ± 3.1	8.7 ± 1.7	7.0 ± 1.8

Data are shown as mean ± SEM

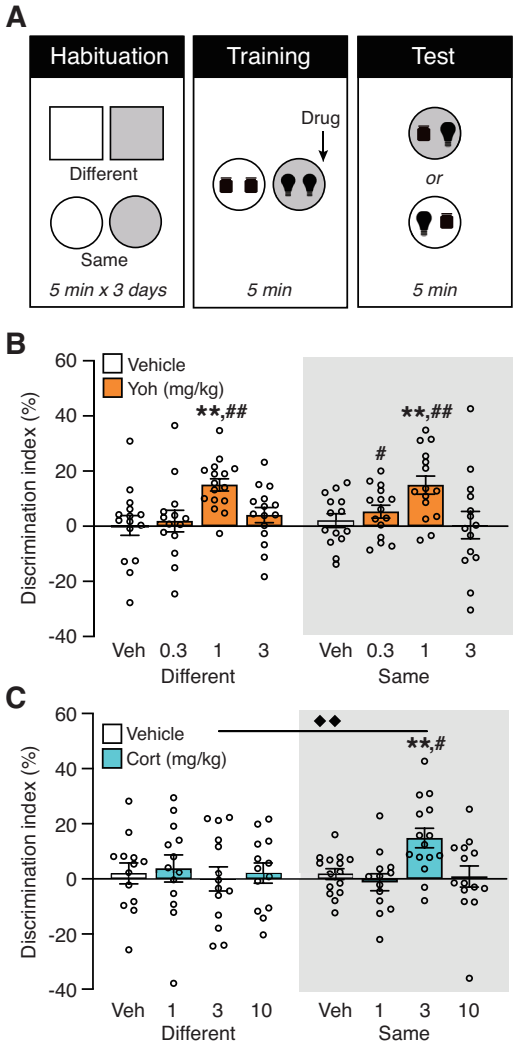

Figure 1. Effect of posttraining yohimbine and corticosterone administration on object-in-context memory under the different and same habituation conditions.

A. Experimental design of the object-in-context task. Mice were initially habituated for 5 min to either the two training contexts (same) or two different contexts (different) on three consecutive days. Afterwards, they were trained on the object-in-context task during which they could explore a set of two identical objects in the first training context for 5 min and then a different set of two identical objects in the second training context, followed immediately by an intraperitoneal injection of yohimbine (YOH; 0.3, 1, or 3 mg/kg), corticosterone (CORT; 1, 3, or 10 mg/kg) or their respective vehicle solutions. Retention was tested 24 h later during which the mice could explore one exemplar of each of the two training objects in one of the two training contexts for 5 min. **B.** Posttraining yohimbine administration dose-dependently enhanced object-in-context memory in both habituation conditions. Different habituation condition, VEH: $n = 15$, YOH 0.3 mg/kg: $n = 15$, YOH 1 mg/kg: $n = 16$, YOH 3 mg/kg: $n = 15$; same habituation condition, VEH: $n = 14$, YOH 0.3 mg/kg: $n = 15$, YOH 1 mg/kg: $n = 15$, YOH 3 mg/kg: $n = 14$. **C.** Posttraining corticosterone administration dose-dependently enhanced object-in-context memory in the same habituation condition, but had no effect in the different habituation condition. Different habituation condition, VEH: $n = 13$, CORT 1 mg/kg: $n = 13$, CORT 3 mg/kg: $n = 14$, CORT 10 mg/kg: $n = 13$; same habituation condition, VEH: $n = 15$, CORT 1 mg/kg: $n = 13$, CORT 3 mg/kg: $n = 15$, CORT 10 mg/kg: $n = 14$. Data are shown as mean ± SEM, dots represent individual data points. $^{**}p < 0.01$ vs. VEH; $^{◆◆}p < 0.01$ different vs. same habituation condition, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. chance level.

Posttraining corticosterone administration impairs object-in-context memory of animals trained for 7 min when previously habituated to a different context

Training settings in the experiments above did not allow for the assessment of potential impairment of object-in-context memory as vehicle/saline-treated mice did not show successful memory performance to start with. Therefore, we next aimed at developing training settings that would generate a weak memory, exploring training durations of 7, 8, 9 and 10 min, with the intention to create training settings that would allow for both memory enhancement and impairment to be detected (Figure 2A). In this experiment, all animals were habituated for three sessions to the different context condition. Total object exploration time during training depended on the duration of the training session ($F_{(3,46)} = 6.24$, $p = 0.01$). Further tests for the different training settings separately revealed that total object exploration time during training did not depend on training context (A vs. B, all p's > 0.33), training session (first vs. second, all p's > 0.27) or type of object (jars vs. bulbs, all p's > 0.18; Table II). Total object exploration time during the retention test was dependent on the duration of the training session ($F_{(3,50)} = 3.22$, $p = 0.03$) with mice trained for 10 min showing more total object exploration time on the retention test than animals trained for 8 min. All other comparisons were not significant. Total object exploration time during the retention test was not influenced by object-context primacy, nor did object-context primacy interact with any of the other factors (all p's > 0.12). One-sample t-tests for the DI% indicated that mice trained for 9 or 10 min showed significant 24-h object-in-context memory (9 min: $t_{(13)} = 4.53$, $p < 0.01$, 10 min: $t_{(13)} = 4.16$, $p < 0.01$), whereas mice trained for 7 or 8 min did not (7 min: $t_{(13)} = 1.46$, $p = 0.17$, 8 min: $t_{(9)} = 2.00$, $p = 0.08$, Figure 2B). Yet, we aimed for a weak memory for this experiment, as a relatively low DI% might enable the detection of bidirectional modulation of memory by the two stress hormones (i.e., both enhancing and impairing effects). Therefore, we implemented a 7-min training duration for the next experiment.

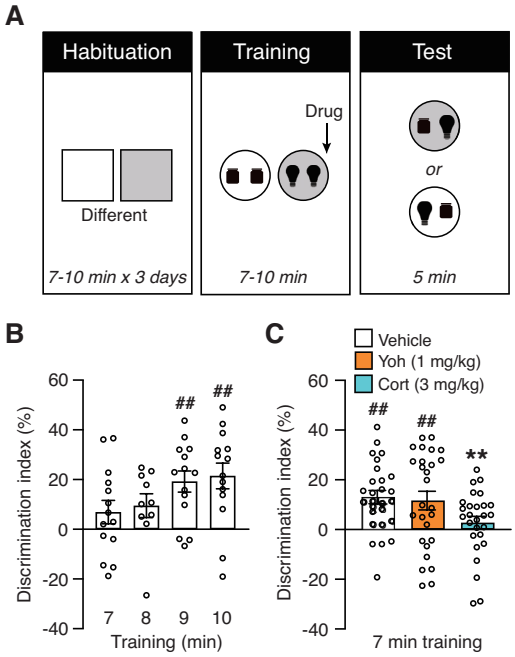

Figure 2. Effect of posttraining yohimbine and corticosterone administration on object-in-context memory of animals trained for 7 min under the different habituation condition.

A. Experimental design of the object-in-context task. Mice were initially habituated for 7-10 min to two different contexts on three consecutive days. Afterwards, they were trained on the object-in-context task during which they could explore a set of two identical objects in the first training context for 7-10 min and then a different set of two identical objects in the second training context for 7-10 min. Posttraining intraperitoneal injection of yohimbine (YOH 1 mg/kg), corticosterone (CORT 3 mg/kg) or vehicle was given to mice that were trained for 7 min in each context. Retention was tested 24 h later during which the mice could explore one exemplar of each of the two training objects in one of the two training contexts for 5 min. **B.** Effect of training duration on 24-h object-in-context memory. 7 min: $n = 14$, 8 min: $n = 10$, 9 min: $n = 14$, 10 min: $n = 14$. **C.** Posttraining administration of corticosterone after a 7-min training session impaired object-in-context memory in the different habituation condition, whereas yohimbine administration did not have an effect . VEH: $n = 27$, YOH 1 mg/kg: n = 27, CORT 3 mg/kg: $n = 27$. Data are shown as mean ± SEM, dots represent individual data points. $^{**}p < 0.01$ vs. VEH, $^{\#\#}p < 0.01$ vs. chance level.

Table II. Effect of training duration on object exploration time during training and retention test

Training duration	Habituation condition	First box training (s)	Second box training (s)	Retention test (s)
7 min ($n = 14$)	Different	14.7 ± 2.8	14.7 ± 2.4	11.9 ± 6.3
8 min ($n = 10$)	Different	14.8 ± 1.5	15.4 ± 2.1	10.0 ± 2.5
9 min ($n = 14$)	Different	17.5 ± 3.3	16.2 ± 3.5	10.5 ± 7.8
10 min ($n = 14$)	Different	16.9 ± 2.7[1,2]	18.0 ± 2.6[1,2]	14.4 ± 4.8[2]

Data are shown as mean ± SEM

[1]$p < 0.05$ vs 7 min group; [2]$p < 0.05$ vs 8 min group

Thus, mice were habituated to the different context condition and then trained for 7 min in each of the two training contexts and administered effective dosages of yohimbine (1 mg/kg), corticosterone (3 mg/kg) or vehicle. Total object exploration time during training did not differ between drug treatment groups ($F_{(2,71)} = 0.05$, $p = 0.83$, Table IIII), nor was it dependent on training context (A vs. B, $F_{(1,71)} = 1.51$, $p = 0.22$), training session (first vs. second, $F_{(1,71)} = 0.05$, $p = 0.82$) or type of object (jars vs. bulbs, $F_{(1, 71)} = 0.11$, $p = 0.75$). Total object exploration time during the retention test was also not affected by drug treatment ($F_{(2,57)} = 1.49$, $p = 0.23$), testing session (first vs. second, $F_{(1,57)} = 0.36$, $p = 0.55$), type of object (jars vs. bulbs, $F_{(1, 57)} = 0.30$, $p = 0.58$) or any interaction between these factors (all p's > 0.23, Table III).

At the 24-h retention test, a one-way ANOVA for the DI% revealed a main effect of drug treatment ($F_{(2,75)} = 3.36$, $p = 0.04$, Figure 2C). Object-context primacy again did not influence the DI%, nor did it interact with any of the other factors (all p's > 0.33). One-sample t-tests indicated that both the vehicle ($t_{(26)} = 4.93$, $p < 0.001$) and yohimbine groups ($t_{(26)} = 3.11$, $p = 0.004$) showed successful memory, whereas the corticosterone group showed no evidence of memory (i.e., the DI% did not differ from zero: $t_{(26)} = 1.05$, $p = 0.30$). Moreover, Tukey's *post-hoc* analyses revealed that the corticosterone group had a significantly smaller DI% than the vehicle group ($t_{(52)} = -2.81$, $p < 0.01$), whereas the difference with the yohimbine group just failed to reach significance ($t_{(52)} = -1.97$, $p = 0.054$). Yohimbine and vehicle-treated mice did not significantly differ in memory performance ($t_{(52)} = -0.31$, $p = 0.76$). These findings indicate that with more extensive training corticosterone impairs object-in-context memory when mice are habituated to a different context prior to training, whereas yohimbine does not.

Table III. Object exploration time during training and retention test

Treatment group	Habituation condition	First box training (s)	Second box training (s)	Retention test (s)
VEH ($n = 27$)	Different	14.7 ± 2.5	15.2 ± 3.0	8.9 ± 2.1
YOH 1 mg/kg ($n = 27$)	Different	15.4 ± 3.4	15.2 ± 3.5	9.7 ± 2.4
CORT 3 mg/kg ($n = 27$)	Different	15.3 ± 3.4	15.0 ± 3.3	8.5 ± 2.2

Data are shown as mean ± SEM

Posttraining yohimbine and corticosterone administration have distinct effects on post-learning neuronal activity, which further depend on the habituation condition

Our behavioral findings show that yohimbine enhanced object-in-context memory in both habituation conditions. In contrast, the corticosterone effect on object-in-context memory was different for the two habituation conditions: Corticosterone enhanced memory when mice were habituated to the two training contexts, but it impaired memory when they were habituated to two different contexts. These findings suggest that the two stress hormones might also induce distinct effects on the underlying neural circuitry that would further depend on the habituation condition. To examine the effect of posttraining yohimbine and corticosterone administration on neuronal activity, we assessed expression of the immediate-early gene c-Fos within the hippocampus and other brain regions of interest (i.e., PRh, aIC and BLA) during the post-learning consolidation period. For this experiment, yohimbine and corticosterone dosages were restricted to the behaviorally effective doses (1 mg/kg yohimbine and 3 mg/kg corticosterone). Mice were first habituated for three sessions to either the different or same contexts, and then trained for 5 min on the object-in-context task. Drug administration was given immediately after the training, and mice were sacrificed 1 h later for tissue collection.

Total object exploration time during the training session again differed between the two habituation conditions ($F_{(1,70)} = 5.32$, $p = 0.02$), with animals of the same habituation condition showing more object exploration behavior. However, total object exploration time did not differ between drug treatment groups ($F_{(2,70)} = 2.19$, $p = 0.12$), training context (A vs. B, $F_{(1,70)} = 2.87$, $p = 0.95$), training session (first vs. second, $F_{(1,70)} = 0.04$, $p = 0.85$) or type of object (jars vs. bulbs, $F_{(1,70)} = 1.27$, $p = 0.26$), nor did any of the interactions between these factors influence total object exploration time during training (all p's > 0.05, Table IV).

Table IV. Object exploration time during training of the experimental groups used for immunohistochemistry

Treatment group	Habituation condition	First box training (s)	Second box training (s)
VEH ($n = 10$)	Different	8.3 ± 1.0	8.0 ± 1.5
YOH 1 mg/kg ($n = 10$)	Different	8.0 ± 1.4	7.8 ± 1.2
CORT 3 mg/kg ($n = 10$)	Different	7.4 ± 1.0	8.0 ± 1.3
VEH ($n = 10$)	Same	9.1 ± 2.1	9.9 ± 1.0
YOH 1 mg/kg ($n = 10$)	Same	8.6 ± 1.4	8.1 ± 1.3
CORT 3 mg/kg ($n = 9$)	Same	8.4 ± 2.0	8.3 ± 1.5

Data are shown as mean ± SEM

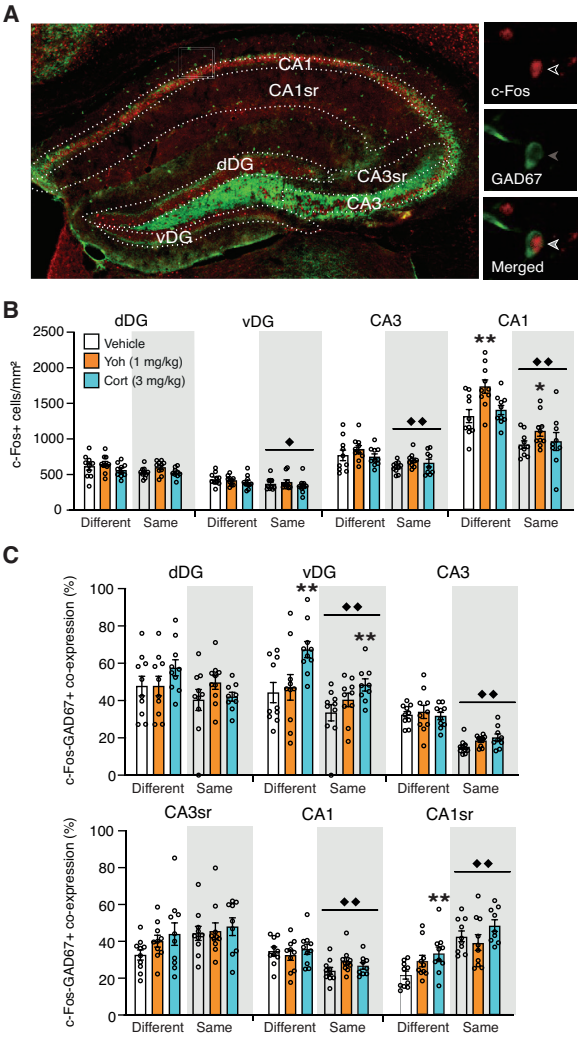

Figure 3. Effect of posttraining yohimbine and corticosterone administration on neuronal activity in the hippocampus in the two habituation conditions.

A. Diagram illustrating the different regions of interest: dorsal blade of the dentate gyrus granule cell layer (dDG), ventral blade of the dentate gyrus granule cell layer (vDG), CA3 pyramidal cell layer (CA3), CA3 *stratum radiatum* (CA3sr), CA1 pyramidal cell layer (CA1), CA1 *stratum radiatum* (CA1sr). The areas drawn show the exact regions in which the number of c-Fos+ cells and c-Fos+GAD67+ cells were counted. **B.** Posttraining administration of yohimbine increased the number of c-Fos-expressing cells in the CA1 in both habituation conditions, whereas corticosterone administration did not affect the number of c-Fos-expressing cells in the hippocampus. The number of c-Fos-expressing cells was overall lower in the same habituation condition compared to the different habituation condition in the vDG, CA3 and CA1. **C.** Posttraining administration of corticosterone increased relative c-Fos-GAD67 co-expression in the vDG and CA1sr. Posttraining administration of yohimbine did not affect relative c-Fos-GAD67 co-expression in the hippocampus. Relative c-Fos-GAD67 co-expression in the vDG and CA1 was higher in the different habituation condition, whereas it was higher in the CA3sr and CA1sr in the same habituation condition. Different habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 10$; same habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 9$. Data are shown as mean ± SEM, dots represent individual data points. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. VEH; $^{\blacklozenge}p < 0.05$, $^{\blacklozenge\blacklozenge}p < 0.01$ different vs. same habituation condition.

Hippocampus

We analyzed drug treatment and habituation condition effects on the total number of c-Fos-expressing neurons within the cell layer of the four main hippocampal subregions (dDG, vDG, CA3 and CA1) separately, based on their distinct roles in memory processing (Kesner and Rolls, 2015) (Figure 3A and B). Moreover, we examined the co-localization of c-Fos with the GABAergic marker GAD67, normalized to the total number of GAD67-expressing neurons (i.e., relative c-Fos-GAD67 co-expression), to provide us information about drug treatment and habituation condition effects on GABAergic activity (Figure 3A and C). For the dDG and vDG, relative c-Fos-GAD67 co-expression was determined within the granule cell layer, whereas for the CA1 and CA3, we assessed relative c-Fos-GAD67 co-expression separately within both the pyramidal cell layer and the *stratum radiatum*, which contains many GABAergic cells (Andersen et al., 1982) (Figure 3A). The total number of GAD67-expressing neurons within these subregions is reported in the supplemental results (Figure S1). All hippocampal subregions are known to express high levels of different types of adrenoceptors as well as glucocorticoid receptors (http://portal.brain-map.org/). Further, whereas adrenoceptors are predominantly found in glutamatergic cell types, glucocorticoid receptors are found in both glutamatergic and GABAergic cell types in the hippocampus (http://portal.brain-map.org/).

In the dDG, a two-way ANOVA for the number of c-Fos-expressing cells indicated no significant main effect of drug treatment ($F_{(2,53)} = 2.95$, $p = 0.06$) or habituation condition ($F_{(1,53)} = 3.97$, $p = 0.052$), and also not a significant drug treatment X habituation condition interaction effect ($F_{(2,53)} = 0.26$, $p = 0.77$, Figure 3B). In the vDG, we also found no drug treatment ($F_{(2,53)} = 1.06$, $p = 0.35$) or drug treatment X habituation condition interaction effect ($F_{(2,53)} = 0.32$, $p = 0.73$). However, we found a main effect of habituation condition ($F_{(1,53)} = 4.04$, $p = 0.047$), which was caused by fewer c-Fos-expressing cells in the same habituation condition. In the CA3, we also found no drug treatment ($F_{(2,53)} = 1.81$, $p = 0.17$) or drug treatment X habituation condition interaction effect ($F_{(2,53)} = 0.35$, $p = 0.71$). Again, we found a main effect of habituation condition ($F_{(1,53)} = 12.32$, $p = 0.001$) caused by fewer c-Fos-expressing cells in the same habituation condition. In the CA1, we observed a main effect of drug treatment ($F_{(2,53)} = 7.05$, $p = 0.002$), but no significant drug treatment X habituation condition interaction effect ($F_{(2,53)} = 0.99$, $p = 0.38$). Moreover, we found a main effect of habituation condition ($F_{(1,53)} = 49.53$, $p < 0.001$), caused by fewer c-Fos-expressing cells in the same habituation condition. Follow-up analysis indicated that the drug treatment effect was caused by yohimbine ($F_{(1,36)} = 14.38$, $p = 0.001$) and not corticosterone ($F_{(1,35)} = 0.56$, $p = 0.46$). Yohimbine significantly increased c-Fos expression in both the different ($t_{(18)} = 3.13$, $p = 0.006$) and same habituation condition ($t_{(18)} = 2.14$, $p = 0.046$).

Next, we examined relative c-Fos-GAD67 co-expression in the hippocampal subregions. In the dDG, we found no drug treatment ($F_{(2,53)} = 0.92$, $p = 0.41$), habituation condition ($F_{(1,53)} = 3.76$, $p = 0.06$), or drug treatment X habituation condition interaction effect ($F_{(2,53)} = 1.25$, $p = 0.30$, Figure 3C). In the vDG, however, we observed a main effect of drug treatment ($F_{(2,53)} = 5.89$, $p = 0.005$), together with a significant drug treatment X habituation condition interaction effect ($F_{(2,53)} = 3.61$, $p = 0.03$). Further, we found a main effect of habituation condition ($F_{(1,53)} = 177.26$, $p < 0.001$), which was caused by a lower relative c-Fos-GAD67 co-expression in the same habituation condition. Follow-up analysis indicated that this drug treatment effect was caused by corticosterone (main effect: $F_{(1,35)} = 13.52$, $p < 0.001$; corticosterone X habituation condition: $F_{(1,35)} = 7.54$, $p = 0.009$) and not yohimbine (main effect: $F_{(1,36)} = 0.25$, $p = 0.62$; yohimbine X habituation condition: $F_{(1,36)} = 0.02$, $p = 0.89$). Corticosterone treatment significantly increased relative c-Fos-GAD67 co-expression in both the different ($t_{(18)} = 3.34$, $p = 0.004$) and same habituation condition ($t_{(17)} = 2.98$, $p = 0.008$), but showed a larger absolute increase in the different habituation condition (22.7% point difference with vehicle) than in the same habituation condition (14.7% point difference with vehicle). In the CA3 pyramidal cell layer, we found no drug treatment ($F_{(2,53)} = 0.88$, $p = 0.42$) or drug treatment X habituation condition interaction ($F_{(2,53)} = 0.97$, $p = 0.39$). We did find a significant main effect of habituation condition ($F_{(2,53)} = 76.40$, $p < 0.001$), which was caused by a lower relative c-Fos-GAD67 co-expression in the same habituation condition. In the CA3 *stratum radiatum,* we found no drug treatment ($F_{(2,53)} = 1.49$, $p = 0.24$), habituation condition ($F_{(1,53)} = 3.95$, $p = 0.052$) or drug treatment X habituation condition interaction effect ($F_{(2,53)} = 0.45$, $p = 0.64$). In the CA1 pyramidal cell layer, we found no drug treatment ($F_{(2,53)} = 0.37$, $p = 0.69$) or drug X habituation condition interaction effect ($F_{(2,53)} = 1.40$, $p = 0.26$). However, we found a significant main effect of habituation condition ($F_{(1,53)} = 16.61$, $p < 0.001$), which was caused by a lower relative c-Fos-GAD67 co-expression in the same habituation condition. In the CA1 *stratum radiatum*, we found a main effect of drug treatment ($F_{(2,57)} = 4.31$, $p = 0.02$), in the absence of a drug treatment X habituation condition interaction ($F_{(2,57)} = 1.67$, $p = 0.19$). Further, we found a main effect of habituation condition ($F_{(1,57)} = 37.37$, $p < 0.001$), which was caused by a higher relative c-Fos-GAD67 co-expression in the same habituation. Follow-up analysis revealed that the drug treatment effect was caused by corticosterone ($F_{(1,35)} = 8.67$, $p = 0.006$) and not yohimbine ($F_{(1,36)} = 0.44$, $p = 0.51$). Corticosterone increased relative c-Fos-GAD67 co-expression in the different habituation condition ($t_{(18)} = 2.82$, $p = 0.01$) whereas the effect was not significant in the same habituation condition ($t_{(17)} = 1.37$, $p = 0.19$).

We next investigated whether drug treatment and habituation condition might also influence correlations in activity across the hippocampal subregions as a proxy for their functional connectivity. Therefore, we calculated Pearson correlations for c-Fos-expression data between each of the four hippocampal subregions for each of the three drug treatment groups and two habituation conditions (Figure 4A). In the different

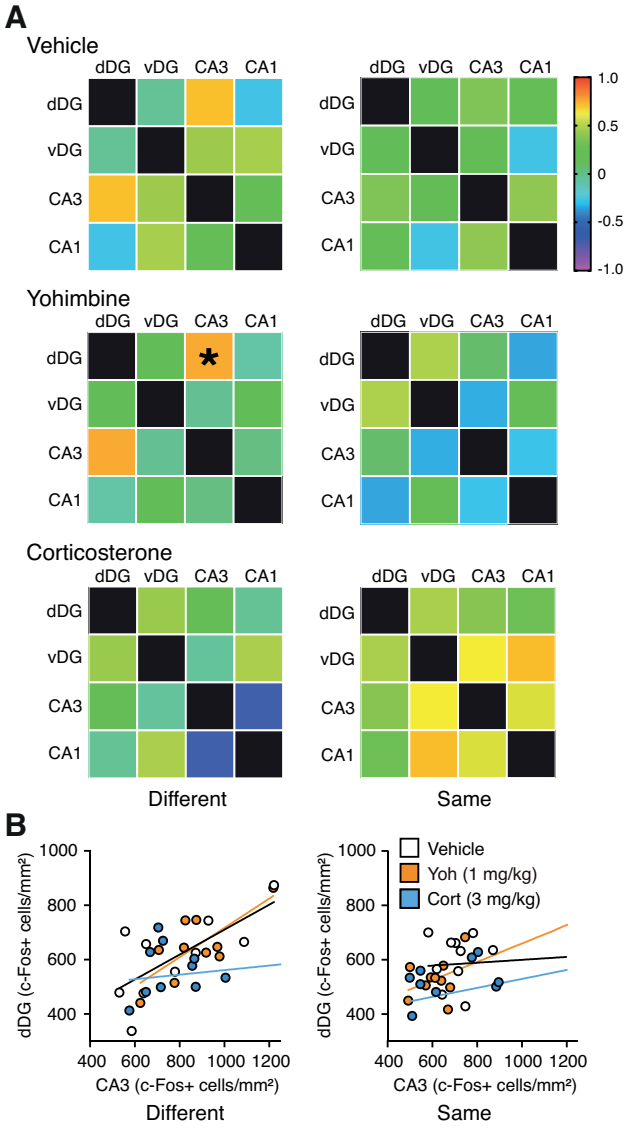

Figure 4. Across-animal correlations in the number of c-Fos-expressing cells in the cell layers of the hippocampal subregions.

In the different habituation condition, a significant positive correlation between dDG-CA3 was observed in the yohimbine group. No significant correlations were observed in the same habituation condition. Different habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 10$; same habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 9$. *$p < 0.01$

habituation condition, we observed a positive correlation between the number of c-Fos-expressing cells in the dDG and CA3 of mice treated with yohimbine ($r = 0.74$; $p = 0.01$), which was absent in mice treated with corticosterone ($r = 0.12$; $p = 0.75$; Figure 4B). Interestingly, a functional interaction between the dDG and CA3 has been associated with pattern separation and memory accuracy (Leutgeb et al., 2007; Lee et al., 2020). Further, direct comparison of these correlations by Fisher r-to-z transformation, implementing one-tailed tests for significance (based on our hypothesis that the opposite effects of yohimbine and corticosterone on object-in-context memory are reflected by also opposite effects on the dDG-CA3 pathway), indicated that the strength of this correlation in corticosterone-treated mice was significantly weaker as compared to that of mice treated with yohimbine ($Z = 1.71$, $p = 0.04$), but not as compared to that of mice treated with vehicle ($Z = 1.42$, $p = 0.08$). It should be noted that corticosterone effects on neuronal activity were assessed after a 5-min training session which impaired object-in-context memory relative to yohimbine but not to vehicle treatment. The functional interaction between the dDG-CA3 in yohimbine-treated mice observed in the different habituation condition was no longer present when mice were previously habituated to the training context (Figure 4A and B), resulting in a significant difference in dDG-CA3 crosstalk in yohimbine-treated mice between the two habituation conditions ($Z = 1.71$, $p = 0.04$).

Thus, our finding that yohimbine increased total c-Fos expression in the hippocampal CA1 cell layer (reflecting mainly glutamatergic activity), whereas corticosterone increased GABAergic activity in the vDG and CA1 *stratum radiatum* seems to fit with the opposite effect of yohimbine and corticosterone administration on object-in-context memory. Moreover, whereas mice treated with yohimbine displayed a significant correlation in neural activity between the dDG and CA3 subregions, this correlation was not present in mice treated with corticosterone. Yet, these findings only hold true for the different habituation condition. In the same habituation condition, we found that both yohimbine and corticosterone treatment enhanced object-in-context memory. Hippocampal activity of mice in the same habituation condition was generally lower compared to that of mice in the different habituation condition. Moreover, we observed no significant drug treatment effects on functional connectivity between hippocampal subregions in the same habituation condition, and a significant reduction in the crosstalk between the dDG and CA3 in yohimbine-treated mice compared to the different habituation condition. These findings thus suggest that prior context familiarization reduces hippocampal involvement in the object-in-context task. As such, the effects of yohimbine and corticosterone on enhancing object-in-context memory in the same habituation condition might involve other brain regions.

Perirhinal cortex

We next investigated drug treatment effects on neuronal activity in the PRh because of its critical involvement in object recognition memory (Ennaceur & Aggleton, 1997; Warburton et al., 2003; Massey et al., 2008). We assessed the number of c-Fos-positive cells in both the input (cortical layers II/III) and output (cortical layers V/VI) regions (Figure 5A). A linear mixed model, including drug treatment and habituation condition as between-subject factors and cortical layer as within-subject factor, indicated a main effect of drug treatment ($F_{(2,51)} = 8.77$, $p = 0.001$) as well as significant drug treatment X layer ($F_{(2,51)} = 4.02$, $p = 0.02$) and drug treatment X habituation condition X layer interaction effects ($F_{(2,51)} = 4.92$, $p = 0.01$; Figure 5B). We also found a significant main effect of habituation condition ($F_{(1,51)} = 8.01$, $p = 0.007$), which was caused by fewer c-Fos-expressing cells in the same habituation condition. All other main or interaction effects were not significant (all p's > 0.46).

Follow-up analysis indicated that yohimbine increased c-Fos expression in both cortical layers independent of habituation condition (main effect: $F_{(1,35)} = 15.99$, $p < 0.001$; yohimbine X habituation condition: $F_{(1,35)} = 0.60$, $p = 0.44$; yohimbine X layer: $F_{(1,35)} = 3.33$, $p = 0.08$; yohimbine X habituation condition X layer: $F_{(1,35)} = 0.17$, $p = 0.69$) (*post hoc* tests: different: layer II/III: $t_{(18)} = 3.06$, $p = 0.006$; layer V/VI: $t_{(18)} = 1.76$, $p = 0.09$; same: layer II/III: $t_{(17)} = 2.87$, $p = 0.01$; layer V/VI: $t_{(17)} = 2.87$, $p = 0.01$). In contrast, the corticosterone effect showed a significant interaction with both habituation condition and layer (main effect: $F_{(1,34)} = 8.89$, $p = 0.005$; corticosterone X habituation condition X layer: $F_{(1,34)} = 10.33$, $p = 0.003$). In the different habituation condition, corticosterone significantly increased c-Fos expression in layer II/III ($t_{(18)} = 2.27$, $p = 0.04$), but not in layer V/VI ($t_{(18)} = 1.69$, $p = 0.11$), whereas in the same habituation condition corticosterone increased c-Fos expression in layer V/VI ($t_{(16)} = 3.26$, $p = 0.005$), but not in layer II/III ($t_{(16)} = 0.82$, $p = 0.43$). (Figure 5B)

Next, we examined drug treatment and habituation condition effects on relative c-Fos-GAD67 co-expression in the PRh (see Figure S2 for the total number of GAD67-expressing neurons). We found significant main effects of drug treatment ($F_{(2,51)} = 3.35$, $p = 0.04$) and layer ($F_{(1,51)} = 9.82$, $p = 0.003$), as well as a significant habituation condition X layer interaction effect ($F_{(1,51)} = 5.51$, $p = 0.02$; Figure 5C). We found no main effect of habituation condition ($F_{(1,51)} = 1.34$, $p = 0.25$) or interactions with drug treatment (all p's > 0.10). Follow-up analysis indicated that yohimbine significantly increased relative c-Fos-GAD67 co-expression in the PRh ($F_{(1,35)} = 8.23$, $p = 0.007$). *Post hoc* tests indicated that yohimbine significantly increased relative c-Fos-GAD67 co-expression in layer II/III (different: $t_{(18)} = 2.53$, $p = 0.02$; same: $t_{(17)} = 2.64$, $p = 0.02$), but had no effect in layer V/VI (different: $t_{(18)} = 0.82$, $p = 0.43$; same: $t_{(17)} = 0.72$, $p = 0.48$). In contrast, corticosterone did not increase relative c-Fos-GAD67 co-expression ($F_{(1,34)} = 1.49$, $p = 0.23$). (Figure 5C)

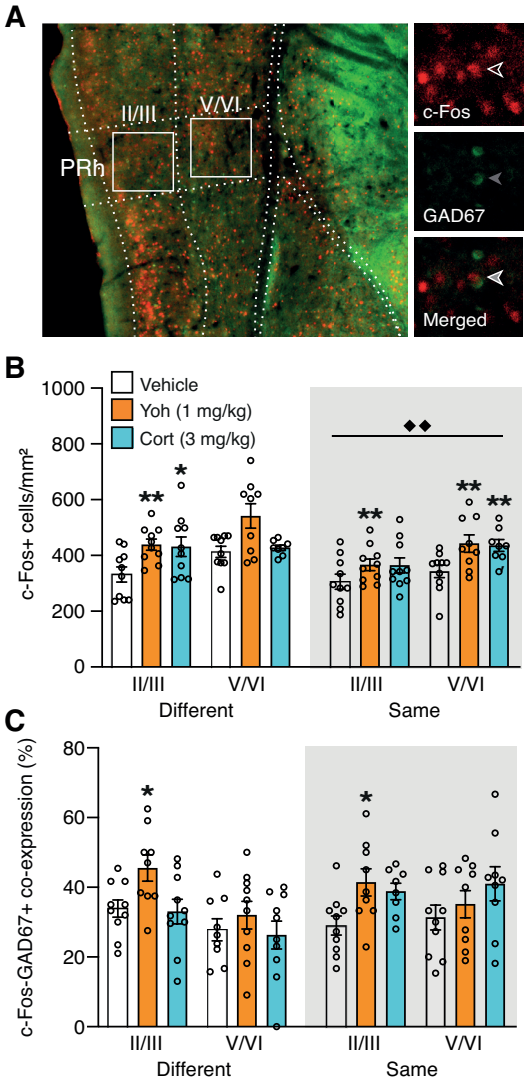

Figure 5. Effect of posttraining yohimbine and corticosterone administration on neuronal activity in the perirhinal cortex in the two habituation conditions.

A. Diagram illustrating the perirhinal cortex (PRh) with its different layers of interest: layers II/III and layers V/VI. The areas drawn show the exact regions in which the number of c-Fos-expressing cells and cFos-GAD67 co-expressing cells were counted. **B.** Posttraining yohimbine administration increased the number of c-Fos-expressing cells in the PRh independent of habituation condition and cortical layer, whereas corticosterone administration specifically increased the number of c-Fos-expressing cells in layer II/III in the different habituation condition, and in layer V/VI in the same habituation condition. Overall, the number of c-Fos-expressing cells was lower in the same compared to the different habituation condition. **C.** Posttraining yohimbine administration overall increased relative c-Fos-GAD67 co-expression in the PRh, whereas corticosterone did not have an effect. Different habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 10$; same habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 9$. Data are shown as mean ± SEM, dots represent individual data points. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. VEH; $^{\blacklozenge\blacklozenge}p < 0.01$ different vs. same habituation condition.

Thus, both yohimbine and corticosterone treatment after object-in-context training increased c-Fos expression within the PRh, whereas only yohimbine also enhanced relative c-Fos-GAD67 activity. The yohimbine effects were independent of habituation condition, whereas corticosterone influenced neuronal activity in a habituation condition-specific manner, increasing activity in the input layers of the PRh in the different habituation condition, and increasing activity in PRh output layers in the same habituation condition. Overall c-Fos expression was lower in mice of the same habituation condition.

Anterior insular cortex

Next, we investigated c-Fos expression and relative c-Fos-GAD67 co-expression in the aIC, dissociating its four subregions; the GI, DI, AID and AIV. Previous findings indicated that particularly the AID and AIV are involved in object recognition memory (Chen et al., 2018). In each subregion, we assessed the number of c-Fos-positive cells in both the input (layers II/III) and output (layers V/VI) regions (Figure 6A). A linear mixed model, including drug treatment and habituation condition as between-subject factors and both subregion and cortical layer as within-subject factors, revealed significant main effects of drug treatment ($F_{(2,49)} = 6.03$, $p = 0.005$), subregion ($F_{(3,146)} = 26.88$, $p < 0.001$) and layer ($F_{(1,195)} = 13.99$, $p < 0.001$), but no main effect of habituation condition ($F_{(1,49)} = 0.84$, $p = 0.36$). Furthermore, we found significant drug treatment X subregion ($F_{(6,146)} = 2.35$, $p = 0.03$) and subregion X layer interaction effects ($F_{(3,195)} = 7.55$, $p < 0.001$). Since we found no significant interactions between drug treatment and layer ($F_{(2,195)} = 0.06$, $p = 0.94$) or drug treatment X subregion X layer ($F_{(6,,195)} =1,19$, $p = 0.31$), we averaged counts over both layers for follow-up analysis to facilitate data comprehension (Figure 6B). See Figure S4 for the counts per layer.

Based on the significant drug treatment X subregion interaction effect, we next used two-way ANOVAs to investigate drug treatment effects in each of the four subregions separately. In the GI, we found no drug treatment ($F_{(2,48)} = 2.93$, $p = 0.06$), habituation condition ($F_{(1,48)} = 0.03$, $p = 0.86$) or drug treatment X habituation condition interaction effect ($F_{(2,48)} = 1.63$, $p = 0.21$). In the DI, a main effect of drug treatment was observed ($F_{(2,49)} = 3.66$, $p = 0.03$), in the absence of a habituation condition ($F_{(1,49)} = 0.008$, $p = 0.93$) or drug treatment X habituation condition interaction effect ($F_{(2,49)} = 0.45$, $p = 0.64$). Follow up analysis indicated that this drug treatment effect was caused by yohimbine ($F_{(1,34)} = 8.72$, $p = 0.006$) and not corticosterone ($F_{(1,33)} = 0.59$, $p = 0.45$). Yohimbine significantly increased c-Fos expression in the same habituation condition ($t_{(16)} = 2.15$, $p = 0.048$), whereas this effect just failed to reach significance in the different habituation condition ($t_{(18)} = 1.99$, $p = 0.06$). In the AID, we also found a main effect of drug treatment ($F_{(2,49)} = 8.39$, $p = 0.001$), but no habituation condition ($F_{(1,49)} = 3.50$, $p = 0.07$) or drug treatment X habituation condition interaction effect ($F_{(2,49)} = 0.26$, $p = 0.77$). This drug treatment effect was also caused by yohimbine ($F_{(1,34)} = 16.11$, $p < 0.001$) and not corticosterone ($F_{(1,33)} =$

0.02, $p = 0.31$). Yohimbine increased c-Fos expression in both the different ($t_{(18)} = 3.30$, $p = 0.004$) and same habituation conditions ($t_{(16)} = 2.57$, $p = 0.02$). In the AIV, we also found a main effect of drug treatment ($F_{(2,49)} = 6.04$, $p = 0.004$), but no habituation condition ($F_{(1,49)} = 2.31$, $p = 0.14$) or drug treatment X habituation condition interaction effect ($F_{(2,49)} = 0.52$, $p = 0.61$). Again, this drug treatment effect was caused by yohimbine ($F_{(1,34)} = 14.25$, $p = 0.001$) and not corticosterone ($F_{(1,33)} = 0.13$, $p = 0.72$). Yohimbine significantly increased c-Fos expression in the different habituation condition ($t_{(18)} = 5.96$, $p < 0.001$), whereas it failed to reach significance in the same habituation condition ($t_{(16)} = 1.71$, $p = 0.11$) (Figure 6B).

Assessment of relative c-Fos-GAD67 co-expression in the aIC revealed significant main effects of drug treatment ($F_{(2,49)} = 4.92$, $p = 0.01$) and subregion ($F_{(3,146)} = 13.43$, $p < 0.001$). Further, we found a significant drug treatment X subregion interaction effect ($F_{(6,146)} = 3.13$, $p = 0.006$), but no drug treatment X habituation condition ($F_{(2,49)} = 1.23$, $p = 0.30$) or drug treatment X habituation condition X subregion interaction effects ($F_{(6,146)} = 1.73$, $p = 0.12$; Figure 6C). We further found significant main effects of habituation condition ($F_{(1,49)} = 8.48$, $p = 0.005$), which was caused by a lower relative c-Fos-GAD67 co-expression in the same habituation condition. Similar to the c-Fos analyses, we subsequently explored drug treatment and habituation condition effects on relative c-Fos-GAD67 co-expression in each of the four subregions separately. The total number of GAD67-expressing neurons within these subregions is reported in Figure S4. In the GI, we found no drug treatment ($F_{(2,48)} = 0.14$, $p = 0.87$), habituation condition ($F_{(1,48)} = 2.74$, $p = 0.10$) or drug treatment X habituation condition interaction effect ($F_{(2,48)} = 0.56$, $p = 0.57$). In the DI, we found main effects of drug treatment ($F_{(2,49)} = 3.97$, $p = 0.03$) and habituation condition ($F_{(1,49)} = 8.28$, $p = 0.006$), in the absence of a drug treatment X habituation condition interaction effect ($F_{(2,49)} = 1.37$, $p = 0.26$). Follow up analysis revealed a near significant effect of yohimbine ($F_{(1,34)} = 3.93$, $p = 0.055$) but no effect of corticosterone ($F_{(1,33)} = 0.22$, $p = 0.64$). In the AID, we found significant main effects of drug treatment ($F_{(2,49)} = 11.87$, $p < 0.001$) and habituation condition ($F_{(2,49)} = 9.84$, $p = 0.003$), but no drug treatment X habituation condition interaction effect ($F_{(2,49)} = 2.26$, $p = 0.12$). Again, this drug treatment effect was caused by yohimbine ($F_{(1,34)} = 21.10$, $p < 0.001$) and not corticosterone ($F_{(1,33)} = 0.35$, $p = 0.56$). *Post hoc* analyses confirmed that yohimbine increased relative c-Fos-GAD67 co-expression in the different ($t_{(18)} = 3.39$, $p = 0.003$) and same habituation condition ($t_{(16)} = 3.36$, $p = 0.004$). In the AIV, we found no drug treatment ($F_{(2,49)} = 2.79$, $p = 0.07$) or drug treatment X habituation condition interaction effect ($F_{(2,49)} = 1.52$, $p = 0.23$), but we did find a significant effect of habituation condition ($F_{(1,49)} = 4.31$, $p = 0.04$) (Figure 6C).

Hence, yohimbine increased the total number of c-Fos-positive cells as well as increased relative c-Fos-GAD67 co-expression following object-in-context training within several subregions of the aIC. These effects were largely similar in both habituation conditions.

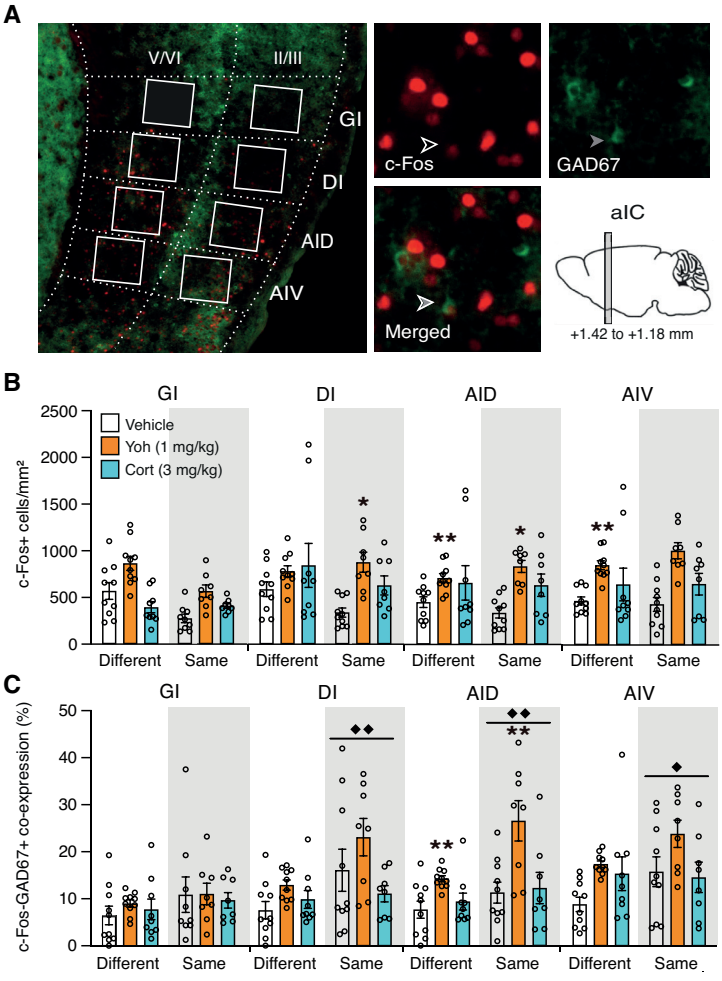

Figure 6. Effect of posttraining yohimbine and corticosterone administration on neuronal activity in the anterior insular cortex in the two habituation conditions.

A. Diagram illustrating the different regions of interest within the anterior insular cortex (aIC): granular insular cortex (GI), dysgranular insular cortex (DI), agranular insular cortex dorsal part (AID) and the agranular insular cortex ventral part (AIV). The areas drawn show the exact regions in which the number of c-Fos-expressing cells and c-Fos-GAD67 co-expressing cells were counted. **B.** Posttraining yohimbine administration increased the number of c-Fos-expressing cells in the DI, AID and AIV, whereas it had no effect the GI. Corticosterone administration did not affect the number of c-Fos-expressing cells in the aIC. **C.** Posttraining yohimbine administration significantly increased relative c-Fos-GAD67 co-expession in the AID, whereas this effect just failed to reach significance in the DI and AIV. Corticosterone did not affect relative-c-Fos-GAD67 co-expression in the aIC. Relative c-Fos-GAD67 co-expression was higher in the different compared to the same habituation condition. Different habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 10$; same habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 9$. Data are shown as mean ± SEM, dots represent individual data points. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. VEH; $^{\blacklozenge}p < 0.05$, $^{\blacklozenge\blacklozenge}p < 0.01$ different vs. same habituation condition.

Total c-Fos activity in the aIC did not depend on the habituation condition, whereas relative GABAergic activity was lower in the same habituation condition. Corticosterone had no effect on c-Fos activity or relative c-Fos-GAD67 co-expression within the aIC.

Basolateral amygdala

We also analyzed the number of c-Fos-expressing cells within the BLA, a potent modulator of stress hormone effects on memory (McGaugh, 2000; Roozendaal & McGaugh, 2011). Here, we found no main effects of drug treatment ($F_{(2,52)}$ = 0.03, p = 0.97), habituation condition ($F_{(1,52)}$ = 0.29, p = 0.59), or a drug treatment X habituation condition interaction effect ($F_{(2,52)}$ = 0.71, p = 0.50, Figure 7).

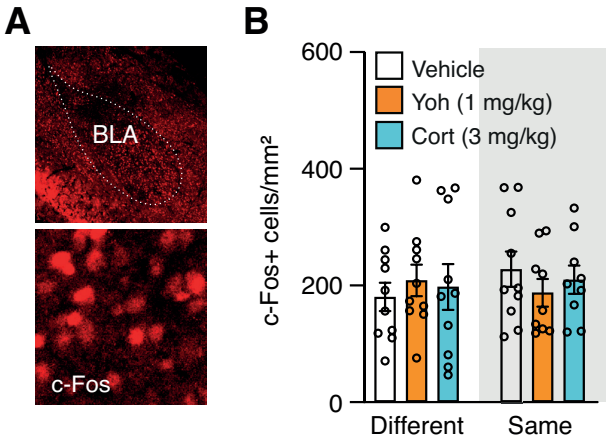

Figure 7. Effect of posttraining yohimbine and corticosterone administration on neuronal activity in the basolateral amygdala in the two habituation conditions.

A. Diagram illustrating the basolateral amygdala (BLA). The area drawn shows the exact region in which the number of c-Fos-expressing cells was counted. **B.** Posttraining yohimbine and corticosterone administration did not affect the number of c-Fos-expressing cells in the BLA. Different habituation condition, VEH: n = 10, YOH 1 mg/kg: n = 10, CORT 3 mg/kg: n = 10; same habituation condition, VEH: n = 10, YOH 1 mg/kg: n = 10, CORT 3 mg/kg: n = 9. Data are shown as mean ± SEM, dots represent individual data points.

Correlations in activity across memory-related brain regions

We next investigated whether drug treatment and habituation condition might also influence correlations in activity across these memory-related brain regions as a proxy for their functional connectivity. Similar to the analyses performed on the hippocampal subregions (Figure 4), we calculated Pearson correlations for c-Fos-expression data between these different (sub)areas for each of the three drug treatment groups and two habituation conditions (Figure 8). We mainly observed strong positive correlations in the number of c-Fos-expressing cells across the different subregions of the aIC as

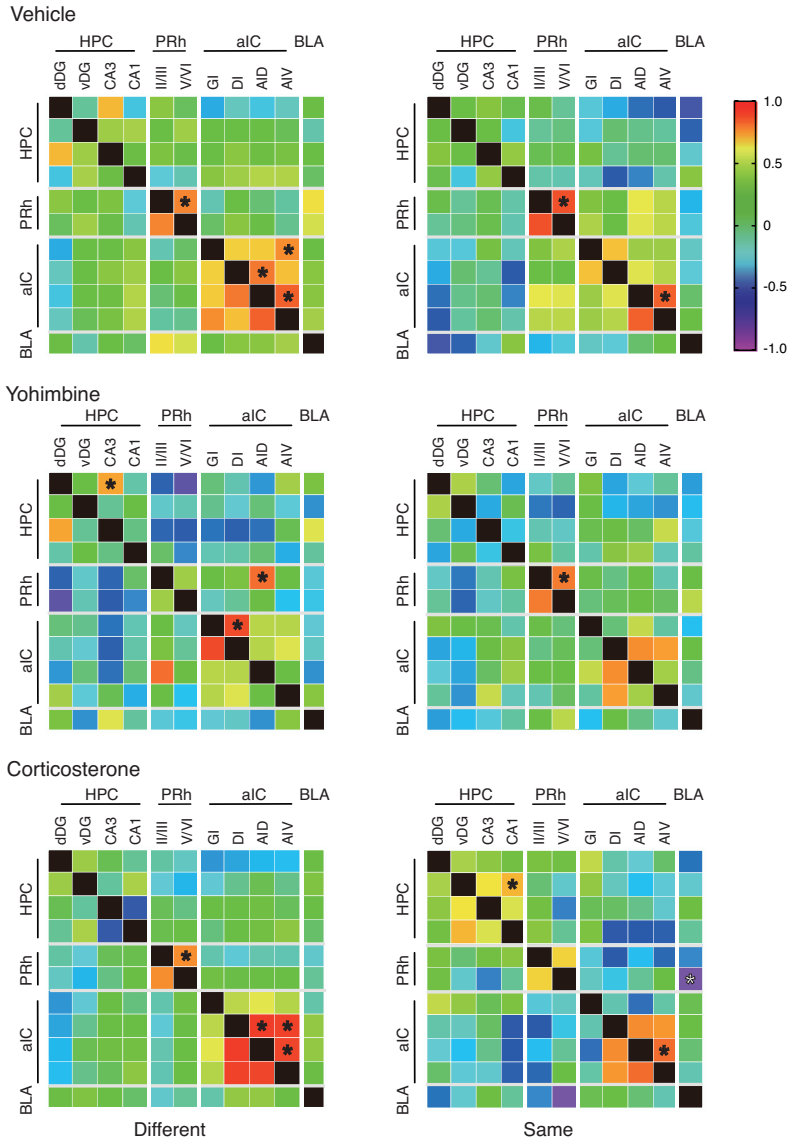

Figure 8. Across-animal correlations in the number of c-Fos-expressing cells in memory-related brain regions.

In the different habituation condition, there were significant positive correlation within aIC in all groups, a significant positive correlation between AID and PRh was observed in yohimbine group. Fewer significant correlations were observed in the same habituation condition. Different habituation condition, VEH: n = 10, YOH 1 mg/kg: n = 10, CORT 3 mg/kg: n = 10; same habituation condition, VEH: n = 10, YOH 1 mg/kg: n = 10, CORT 3 mg/kg: n = 9. *$p < 0.01$

well as across the cortical layers of the PRh. These findings thus suggest strong within-brain region connectivity. These within-brain regions correlations were seen in vehicle-treated mice and appear similar across drug treatment and habituation condition, with the exception of the yohimbine group in the different habituation condition. This group showed fewer significant correlations between aIC subregions and no correlation across the cortical layers of the PRh. Instead, this yohimbine group showed a significant positive correlation in c-Fos activity between the AID and PRh layer II/III ($r = 0.86$, $p < 0.001$), which was not found in any of the other groups.

Discussion

In this study, we examined the effect of posttraining administration of the noradrenergic stimulant yohimbine and corticosterone on episodic-like memory in an object-in-context task, a hippocampus-dependent memory task in which two object presentation events during the training session are distinguished by the contexts in which they appear. Particularly, we were interested in investigating whether prior context habituation alters these stress hormone effects on object-in-context memory. We found that yohimbine administered immediately after the training session dose-dependently enhanced object-in-context memory independent of the habituation condition. In contrast, the corticosterone effect on object-in-context memory critically depended on whether mice were previously familiarized with the training contexts. Corticosterone impaired object-in-context memory when the training took place in unfamiliar contexts, whereas it dose-dependently enhanced this memory when mice were previously habituated to the training contexts. At the neural level, we found that yohimbine-treated animals of the different habituation condition displayed a positive correlation in neural activity between the dDG and CA3 as well as an increased total activity within the hippocampal CA1 cell layer (reflecting mainly glutamatergic activity) during the posttraining consolidation period. Corticosterone-treated animals of the different habituation condition did not show this correlation between the dDG and CA3 or an increased total activity within the CA1, but rather displayed an increased GABAergic activity in the CA1 *stratum radiatum* and vDG. Prior habituation to the training contexts was associated with an absence of inter-subregion correlations of activity as well as an overall lower hippocampal activity posttraining.

Our finding that yohimbine and corticosterone induced opposite effects on object-in-context memory in mice that were not habituated to the training contexts is consistent with the findings of previous studies examining stress hormone effects on episodic-like memory in a dual-event inhibitory avoidance task. In both the dual-event inhibitory avoidance and object-in-context task, the animals are trained on two events close in

time. In the dual-event inhibitory avoidance task, animals explore two similar inhibitory avoidance apparatuses with a brief interval, but footshock is delivered in only one of the two training contexts (Atucha & Roozendaal, 2015). Yohimbine administered systemically after the training session was found not only to enhance the animals' memory of the shock experience *per se*, but also to increase their ability to successfully discriminate in which of the two training contexts they had previously received footshock (Atucha & Roozendaal, 2015; Roozendaal & Mirone, 2020). In contrast, corticosterone administration induced a generalized strengthening of memory with rats displaying increased retention latencies in both the Shock box and Non-Shock box (Roozendaal & Mirone, 2020). Although the object-in-context task does not allow to assess memory strength for the objects, successful discrimination of the acquired object-context association likely also requires the creation of at least two different memories. The animals need to form a memory for the two training objects *per se*, and this object information then needs to be integrated into the contexts (Balderas et al., 2008; Langston & Wood, 2010; Balderas et al., 2015), generating an episodic-like contextual memory (Mumby et al., 2002; Langston & Wood, 2010). The present findings are thus consistent with the reported opposite effects of yohimbine and corticosterone on episodic-like memory. However, this was only true when the mice were not previously habituated to the two training contexts. When mice were previously familiarized with the training contexts, both yohimbine and corticosterone induced a similar enhancement of object-in-context memory. Previous studies have not investigated whether context habituation in the dual-event inhibitory avoidance task might also result in similar enhancing effects of yohimbine and corticosterone on episodic-like memory.

Experiments investigating the neurobiological mechanisms underlying these stress hormone effects on episodic-like memory in the dual-event inhibitory avoidance task revealed some interesting findings which might also be relevant for object-in-context memory. Memories of events that occur close in time are often linked by directing storage into overlapping neuronal ensembles in the hippocampal CA1 region (Tanila, 1999; Silva et al., 2009; Cai et al., 2016). Norepinephrine administration after training on the dual-event inhibitory avoidance task enhanced episodic-like memory by a hippocampal mechanism that facilitates the separation of memory of the two training events into two distinct memory representations. This process was found to be dependent on pattern separation and a consolidation process within the dDG that was modulated by microRNA-134 (Atucha et al., under revision), a main regulator of the cAMP response element-binding (CREB) and brain-derived neurotrophic factor (BDNF) pathways (Schratt et al., 2006; Gao et al., 2010; Schratt, 2011), two canonical memory mechanisms that play critical roles in memory consolidation (Silva et al., 1998; Mizuno et al., 2000). Previous findings furthermore critically implicated *de novo* synthesis of BDNF within the dDG in the consolidation of pattern-separated memories (Bekinschtein et al., 2013b). In support of the view that

yohimbine also enhanced object-in-context memory by facilitating the separation of memory of the two training events, we found that yohimbine-treated animals displayed a positive correlation between the number of c-Fos-expressing neurons in the dDG and CA3. Computational models and empirical studies have indicated that the dDG-CA3 pathway is critically involved in pattern separation (Rolls, 1989; Mizumori et al., 1990; Rolls, 1996; Schaaf et al., 1998; Gilbert et al., 2001; Leutgeb et al., 2007). Pattern separation is performed by granule cells of the dDG using competitive learning on overlapping representations from the entorhinal cortex (Leutgeb et al., 2007). Mossy fibers of the dDG provide a strong excitatory input to CA3 pyramidal and interneurons (Swanson & Cowan, 1977; Witter, 1993), which can drive activity in the CA3 necessary to form new pattern separated representations to reduce interference and support new learning. Critically, lesions of the dDG input to the CA3 were found to impair object-in-context memory, but did not affect memory for the object *per se* (Dees & Kesner, 2013). Our finding that yohimbine did not increase the total number of c-Fos-positive neurons within either the dDG or CA3 would be in agreement with the evidence that the firing activity of dDG cells is sparse (Jung & Mcnaughton, 1993; Leutgeb et al., 2007), which via the mossy fibers can be transformed into also sparse firing activity in CA3 (Rolls, 1987; Rolls, 2013). We found, however, that yohimbine treatment increased the total number of c-Fos-positive neurons within the CA1 pyramidal cell layer. A main function of the CA1 is to encode and store memory of the temporal order of events, such that one event may get stored separated from another event in time (Skaggs et al., 1996; Gilbert et al., 2001; Fortin et al., 2002; Kesner et al., 2002). Thus, it can be proposed that the yohimbine-induced increase in the total number of c-Fos-positive cells in the CA1 reflects an enhanced storage of memory of the temporal order of the two training events into segregated, non-overlapping populations of hippocampal principal CA1 neurons (Tronson et al., 2009). However, it should be noted that we did not causally test this prediction, and therefore the functional significance of these neural activity changes should be interpreted with caution.

Corticosterone administration after a 7-min training session impaired object-in-context memory of mice that were not familiarized with the training contexts. Corticosterone administration following 5 min of training did not impair memory, but this training session was too short to induce any memory in vehicle-treated control mice. Our finding that corticosterone impaired object-in-context memory is also consistent with previous findings on the dual-event inhibitory avoidance task indicating that posttraining systemic corticosterone administration induced a generalized strengthening of memory, even if control animals were able to discriminate (Roozendaal & Mirone, 2020). These findings suggest that corticosterone treatment also induces two separable actions. First, corticosterone facilitates a linking of memory of the two training events. Second, it enhances the strength of memory for the object or fooshock *per se* (Okuda et al., 2004; Roozendaal et al., 2006; Roozendaal et al., 2010). We found that corticosterone-treated

animals did not display a positive correlation in neural activity between the dDG and CA3. Interestingly, this proxy for the strength of dDG-CA3 crosstalk was significantly weakened by corticosterone as compared to that of yohimbine-treated mice, whereas the comparison to vehicle-treated mice just failed to reach significance. Previous findings that glucocorticoids decrease BDNF expression within the dDG (Schaaf et al., 1998; Schaaf et al., 2009) support the view that corticosterone treatment can actively suppress pattern separation in the dDG-CA3 pathway. We further found that corticosterone increased GABAergic activity within the CA1 *stratum radiatum*, which provides inhibitory control to CA1 pyramidal cells. An inhibition of the CA1 pyramidal cells might direct the storage of memories of the two training events into an overlapping population of hippocampal principal CA1 neurons (He et al., 2002; Beer et al., 2018). As such, the 'generalizing' effect of corticosterone on episodic-like memory across training episodes in either the object-in-context or dual-event inhibitory avoidance task might be established through different mechanisms than its effect on context generalization in case of a single learning event as reported by others (Kaouane et al., 2012; dos Santos Corrêa et al., 2019; Lesuis et al., 2021).

Both yohimbine and corticosterone administration were found to enhance object-in-context memory after training in a familiar context. Potentially, this is caused by the fact that repeated exposure to the two training contexts during the habituation procedure is already sufficient to create (two separate) memories of the two training contexts. Thereby, posttraining yohimbine and corticosterone administration no longer influences this hippocampal mechanism of either separating or linking memory of the two training events but only enhances the strength of the memory for the training objects *per se*. Supporting this, both norepinephrine and corticosterone have been shown to enhance memory for an object in an object recognition task (Okuda et al., 2004; Roozendaal et al., 2006; Roozendaal et al., 2010; Song et al., 2020). Interestingly, the hippocampus is not involved in regulating memory for objects *per se* (Balderas et al., 2008; Roozendaal et al., 2010; Dees & Kesner, 2013), suggesting that other brain regions mediate the effects of yohimbine and corticosterone administration on enhancing memory for the objects *per se*. This finding thus seemingly confirms that habituation to the training contexts makes the stress hormone enhancing effects on the object-in-context task independent on the hippocampus, which is in line with our immunohistochemical findings showing that prior habituation to the training context reduced overall hippocampal activity. Moreover, it fits our observation that in this habituation condition no effect of posttraining yohimbine administration was seen on the dDG-CA3 correlation. In Chapter 4 of this thesis we will directly this test hypothesis.

We next investigated yohimbine and corticosterone effects on neuronal activity in other brain regions that could potentially mediate the enhancement of object memory.

Extensive evidence indicates that the PRh and aIC are importantly involved in regulating memory for an object (Ennaceur & Aggleton, 1997; Norman & Eacott, 2005; Bermudez-Rattoni et al., 2005; Balderas et al., 2008; Albasser et al., 2009; Roozendaal et al., 2010; Banks et al., 2014; Bermudez-Rattoni, 2014; Olarte-Sánchez et al., 2015), and that their function is modulated by noradrenergic and glucocorticoid activity (Roozendaal et al., 2010; Perugini et al., 2012; Laing and Bashir, 2014; Beldjoud et al., 2015; Chen et al., 2018). Interestingly, recent findings have suggested that the PRh and aIC might play different roles in (object) recognition memory. Whereas the PRh appears to be primarily involved in the discrimination of overlapping representations and the detection of novelty aspects of an object, the aIC is mostly involved in the detection of familiarity features of an object (Kafkas & Montaldi, 2014; Miranda et al., 2017; Molas et al., 2017; Chen et al., 2018; Chen et al., 2022). In the PRh, we found that both yohimbine and corticosterone treatment increased total c-Fos expression, whereas only yohimbine also enhanced relative c-Fos-GAD67 co-expression (likely reflecting changes in GABAergic activity). Further, corticosterone influenced neuronal activity in a habituation condition-specific manner. In the aIC, we observed that yohimbine administration increased both glutamatergic and GABAergic activity, whereas corticosterone did not have any effect. No habituation effects were observed in the aIC. Further, we found that whereas yohimbine induced a positive correlation in c-Fos activity between the aIC (agranular subdivision) and PRh, corticosterone primarily induced correlations in activity within each of these brain regions. Thus, yohimbine and corticosterone appear to induce different effects on the PRh and aIC. We previously found that yohimbine enhanced the detailedness of object memory in an object discrimination task by coordinated actions on both the PRh (improving novelty detection) and aIC (improving familiarity detection) (Song et al., unpublished findings). The present findings would thus suggest that corticosterone enhances object memory via a different regulation of novelty and familiarity discrimination, but this has not been investigated. We found no effects of yohimbine or corticosterone administration on neuronal activity within the BLA. This finding is surprising as norepinephrine administration directly into the BLA was found to enhance both object-in-context memory (Barsegyan et al., 2014) and object recognition memory (Roozendaal et al., 2010). Further, extensive evidence indicates that the BLA interacts with both the PRh and aIC in regulating stress hormone effects on object recognition memory (Langston & Wood, 2010; Laing & Bashir, 2014; Chen et al., 2018). The present findings, however, are consistent with those of an earlier study indicating that yohimbine administration after object training also did not affect neuronal activity within the BLA (Song et al., unpublished findings).

In summary, we found that yohimbine and corticosterone induced opposite effects on object-in-context memory, but this effect was critically dependent on the habituation condition. We propose that the opposite effects of yohimbine and corticosterone on object-in-context memory in the different habituation condition could be explained by a hippocampal mechanism inducing either a separation or linking of memory of the two training events. However, after repeated habituation to the training contexts, memories of the two training contexts have already been formed, and hence the enhancing effect of yohimbine and corticosterone on object-in-context memory could be explained by their similar influence on strengthening memory for the object *per se* by the involvement of other brain regions.

Acknowledgment

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Supplementary Materials

Posttraining yohimbine and corticosterone effects on total number of GAD67-expressing cells

Hippocampus

In the dDG granule cell layer, a two-way ANOVA for the number of GAD67-expressing cells revealed no main effects of drug treatment ($F_{(2,53)}$ = 0.38 p = 0.68), habituation condition ($F_{(1,53)}$ = 1.50, p = 0.23), or a drug treatment X habituation condition interaction effect ($F_{(2,53)}$ = 1.25, p = 0.30). In the vDG, the number of GAD67-expressing cells was affected by drug treatment ($F_{(2,53)}$ = 3.18, p = 0.049), but not habituation condition ($F_{(1,53)}$ = 1.36, p = 0.25), or a drug treatment X habituation condition interaction effect ($F_{(2,53)}$ = 0.23, p = 0.80). Yet, follow up tests on the source of the drug treatment effect revealed no significant differences between yohimbine- and vehicle-treated mice ($F_{(1,36)}$ = 0.61, p = 0.44), nor between corticosterone- and vehicle-treated mice ($F_{(2,35)}$ = 1.53, p = 0.11), but there is significant yohimbine- and corticosterone- treated mice ($F_{(2,35)}$ = 63.79, p < 0.001). In the CA3 pyramidal cell layer, the number of GAD67-expressing cells was not affected by drug treatment ($F_{(2,53)}$ = 1.49 p = 0.23), habituation condition ($F_{(1,53)}$ = 3.63, p = 0.06), or drug treatment X habituation condition interaction ($F_{(2,53)}$ = 0.17, p = 0.84). In the CA3 *stratum radiatum*, a main effect of drug treatment was observed on the number of GAD67-expressing cells ($F_{(2,53)}$ = 3.34, p = 0.04), without significant effects of habituation condition ($F_{(1,53)}$ = 0.01, p = 0.92) or drug treatment X habituation condition interaction ($F_{(2,53)}$ = 1.15, p = 0.32). This drug treatment effect was driven by a significant effect of corticosterone administration ($F_{(1, 35)}$ = 5.04, p = 0.03), but not yohimbine ($F_{(1,36)}$ = 0.73, p = 0.40). *Post hoc* tests revealed a significantly lower number of GAD67-expressing cells in corticosterone-treated mice in the different habituation condition ($t_{(18)}$ = 2.50, p = 0.02), without an effect in the same habituation condition ($t_{(17)}$ = 0.67, p = 0.52). In the CA1 pyramidal cell layer, the number of GAD67-expressing cells was not affected by a main effect of drug treatment ($F_{(2,53)}$ = 0.45 p = 0.64), but there was a significant main effect of habituation condition ($F_{(1,53)}$ = 22.15, p < 0.001), caused by reduced levels in the same habituation condition. No significant drug treatment X habituation condition interaction effect was observed ($F_{(2,53)}$ = 0.65, p = 0.53). In the CA1 *stratum radiatum*, the number of GAD67-expressing cells was not affected by drug treatment ($F_{(2,53)}$ = 0.05 p = 0.96), habituation condition ($F_{(1,53)}$ = 0.88, p = 0.35), or a drug treatment X habituation condition interaction effect ($F_{(2,53)}$ = 0.35, p = 0.71). Thus, yohimbine treatment after object-in-context training did not change the number of GAD67-expressing cells within the hippocampus, whereas corticosterone reduced the number of GAD67-expressing cells in the CA3 *stratum radiatum* in the different habituation condition.

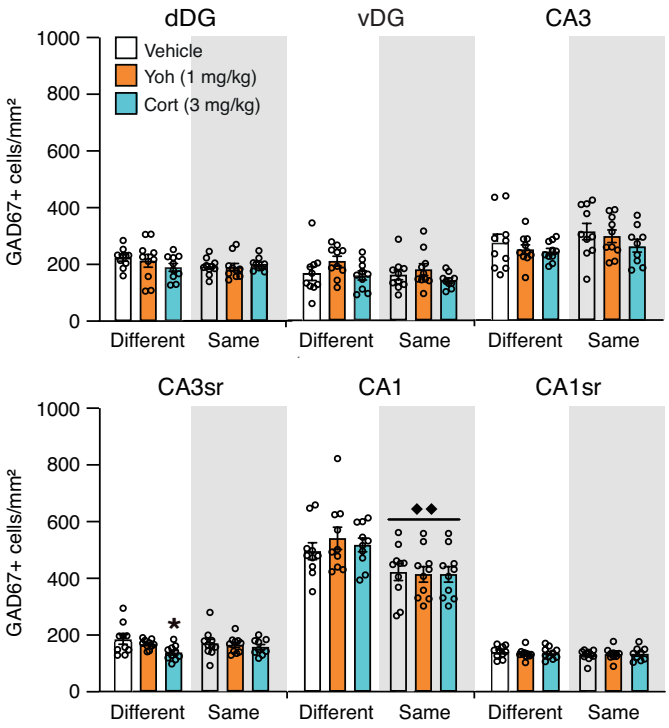


Figure S1. Effect of posttraining yohimbine and corticosterone administration on the number of GAD67-expressing cells in the hippocampus in the two habituation conditions.

Posttraining administration of corticosterone decreased GAD67+ cells in the CA3sr in the different habituation condition. In CA1, the number of GAD67-expressing cells was lower in the same habituation condition. Different habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 10$; same habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 9$. Data are shown as mean ± SEM, dots represent individual data points. $^{*}p < 0.05$ vs. VEH; $^{◆◆}p < 0.01$ different vs. same habituation condition.

Perirhinal cortex

A linear mixed model analyzing the number of GAD67+ cells in the PRh revealed no significant main effects of drug treatment ($F_{(2,51)} = 0.91$, $p = 0.41$), habituation condition ($F_{(1,51)} = 0.14$, $p = 0.71$), or cortical layer ($F_{(1,51)} = 0.005$, $p = 0.95$), nor any significant interactions between these factors (all p's > 0.21, Figure S2). Thus, both yohimbine and corticosterone administration after object-in-context training did not change the number of GAD67-expressing cells within the Prh.

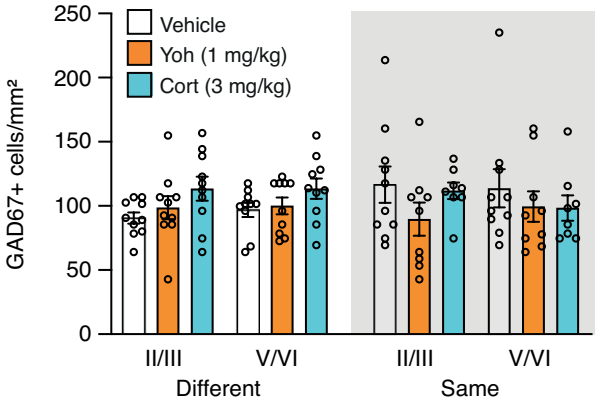


Figure S2. Effect of posttraining yohimbine and corticosterone administration on the number of GAD67-expressing cells in perirhinal cortex in the two habituation conditions.

Posttraining administration of yohimbine and corticosterone treatment did not affect the total GAD67-expressing cells in the two habituation conditions. Different habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 10$; same habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 9$. Data are shown as mean ± SEM, dots represent individual data points.

Anterior insular cortex

The number of GAD67-expressing cells in the anterior insular cortex was affected by a main effect of drug treatment ($F_{(2,49)} = 8.85$, $p = 0.001$), habituation condition ($F_{(1,49)} = 30.38$, $p < 0.001$) and subregion ($F_{(1,146)} = 39.38$, $p < 0.001$). All interactions between these factors failed to reach significance (all p's > 0.33, Figure S3). Similar to the analyses of c-Fos expression, we followed up on these effects by testing each of the four subregions separately. In the GI, we found a significant effect of drug treatment ($F_{(2,48)} = 7.49$, $p = 0.001$), as well as an effect of habituation condition ($F_{(1,48)} = 32.64$, $p < 0.001$), but no drug treatment X habituation condition interaction effect ($F_{(2,48)} = 1.02$, $p = 0.37$). Yohimbine treatment was associated with higher numbers of GAD67-expressing cells than vehicle treatment ($F_{(1, 33)} = 16.55$, $p < 0.001$), whereas corticosterone had no such effect ($F_{(1,32)} = 0.11$, $p = 0.75$). *Post hoc* analyses showed that yohimbine treatment increased the number of GAD67-expressing cells in the different habituation condition ($t_{(18)} = 4.43$, $p < 0.001$), but that this effect just failed to reach significance in the same habituation condition ($t_{(15)} = 1.94$, $p = 0.07$). The main effect of habituation condition was caused by a lower number of GAD67-expressing cells in the same habituation condition.

In the DI, we also observed a main effect of drug treatment ($F_{(2,49)}$ = 5.85, p = 0.005) and habituation condition ($F_{(1,49)}$ = 23.17, p < 0.001), in the absence of drug treatment X habituation condition interaction effect ($F_{(2,49)}$ = 0.95, p = 0.39). Yohimbine treatment increased the number of GAD67-expressing cells ($F_{(1, 34)}$ = 10.19, p = 0.003) whereas no effects were observed for corticosterone treatment ($F_{(1,33)}$ = 1.49, p = 0.23). *Post hoc* tests showed that yohimbine significantly increased the number of GAD67-expressing cells in the different habituation condition ($t_{(18)}$ = 2.97, p = 0.008), but not in the same habituation condition ($t_{(16)}$ = 1.56, p = 0.14). Again, the main effect of habituation condition was caused by a lower number of GAD67-expressing cells in the same habituation condition.

Also in the AID, main effects of drug treatment ($F_{(2,49)}$ = 6.42, p = 0.003) and habituation condition ($F_{(1,49)}$ = 20.12, p < 0.001) were observed, in the absence of a drug treatment X habituation condition interaction effect ($F_{(2,49)}$ = 1.01, p = 0.37). Yohimbine increased the number of GAD67+-expressing cells compared to vehicle ($F_{(1, 34)}$ = 10.42, p = 0.003), whereas corticosterone had no such effect ($F_{(1,33)}$ = 0.41, p = 0.55). *Post hoc* tests showed that yohimbine increased the number of GAD67-expressing cells in the different habituation condition ($t_{(18)}$ = 2.70, p = 0.014), but that this effect failed to reach significance in the same habituation condition ($t_{(16)}$ = 1.86, p = 0.08). Similar to the GI and DI, the main effect of habituation condition was caused by a lower number of GAD67-expressing cells in the same habituation condition.

Finally, in the AIV also main effects of drug treatment ($F_{(2,48)}$ = 11.17, p < 0.001) and habituation condition ($F_{(1,48)}$ = 11.17, p < 0.001) were observed, in the absence of drug treatment X habituation condition interaction effect ($F_{(2,48)}$ = 0.87, p = 0.43). Yohimbine treatment again increased the number of GAD67-expressing cells ($F_{(1, 34)}$ = 18.58, p < 0.001), whereas corticosterone did not ($F_{(1,32)}$ = 0.27, p = 0.60). *Post hoc* tests indicated that yohimbine increased the number GAD67-expressing cells both in the different ($t_{(18)}$ = 3.92, p = 0.001) and same habituation condition ($t_{(16)}$ = 2.28, p = 0.04).

Hence, yohimbine increased the total number of GAD67-expressing cells following object-in-context training within several subregions of the aIC. These effects were largely similar in both habituation conditions. Corticosterone had no effect of aIC neuronal activity whatsoever.

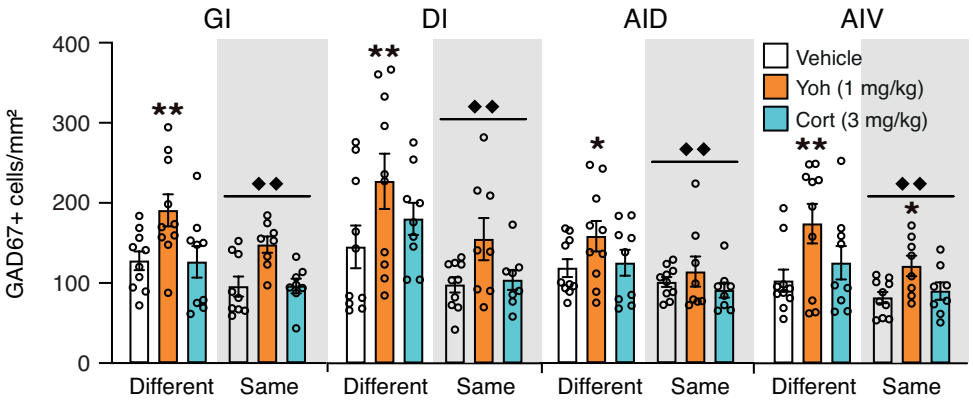
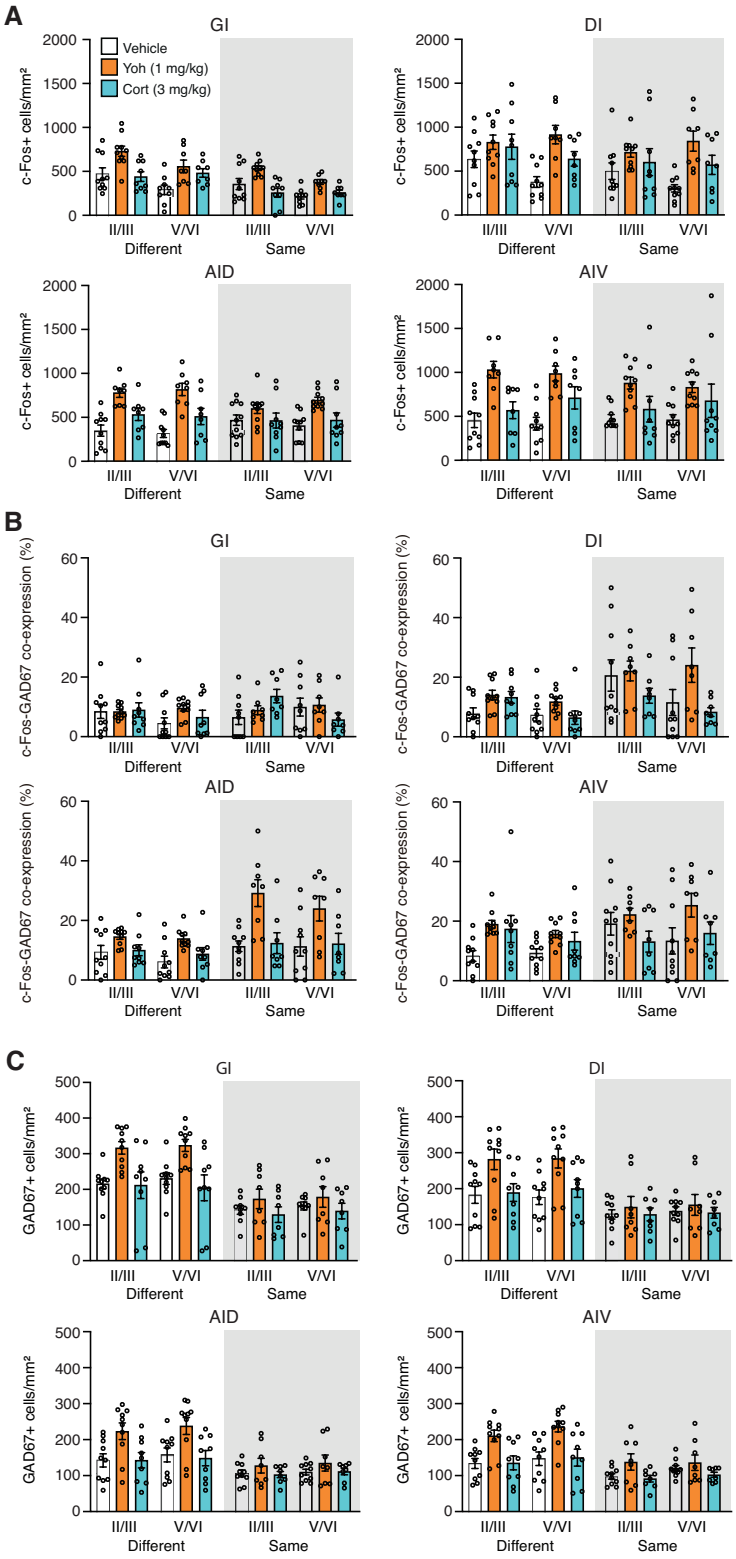


Figure S3. Effect of posttraining yohimbine and corticosterone administration on the number of GAD67-expressing cells in anterior insular cortex in the two habituation conditions.

Posttraining administration of yohimbine increased the total number of GAD67-expressing cells whereas corticosterone had no effect. Overall, the number of GAD67-expressing cells was lower in the same habituation condition VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 10$; same habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 9$. Data are shown as mean ± SEM, dots represent individual data points. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. VEH; $^{◆◆}p < 0.01$ different vs. same habituation condition.

Figure S4. Effect of posttraining yohimbine and corticosterone administration on the number of c-Fos, cFos-GAD67 co-expressing, and GAD67-expressing cells in the different layers of the anterior insular cortex in the two habituation conditions.

A. Effect of posttraining administration of yohimbine and corticosterone of the number of c-Fos cells in different layers of the aIC subregions in the two habituation conditions. **B.** Effect of posttraining administration effect of yohimbine and corticosterone on relative cFos-GAD67 co-expression in the different layers of the aIC subregions in the two habituation conditions. **C.** Effect of posttraining administration of yohimbine and corticosterone of the number of GAD67-expressing cells in the different layers of the aIC subregions in the two habituation conditions. Different habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 10$; same habituation condition, VEH: $n = 10$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 9$. Data are shown as mean ± SEM, dots represent individual data points.


A
GI
DI
AID
AIV
Vehicle
Yoh (1 mg/kg)
Cort (3 mg/kg)
c-Fos+ cells/mm²
B
c-Fos-GAD67 co-expression (%)
C
GAD67+ cells/mm²
II/III
V/VI
Different
Same

CHAPTER 3

Prior context habituation renders noradrenergic enhancement of object-in-context memory independent of the hippocampus

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Abstract

Stress and emotional arousal are well known to strengthen the consolidation of memory. Yet, how they affect the quality of these strengthened memories remains largely elusive. In Chapter 2, we reported that the noradrenergic stimulant yohimbine enhanced the episodic-like quality of memory in an object-in-context task, which was associated with an increased hippocampal activity during the post-learning consolidation period. We further found that yohimbine also enhanced object-in-context memory of mice that had been habituated to the two training contexts prior to training, but that this memory enhancement effect was not accompanied by a similar increase in hippocampal activity. We proposed that yohimbine in non-habituated mice enhances object-in-context memory by regulating a hippocampal mechanism that facilitates the separation of memory of the two training events into two distinct memories. However, once animals have been habituated to the training contexts, separate memories of the two training contexts have already been created, and the yohimbine effect on object-in-context memory might reflect its ability to strengthen memory for the objects themselves, which is not dependent of the hippocampus. The objective of this chapter was to offer concrete proof for the hypothesis that prior context habituation renders noradrenergic enhancement of object-in-context memory independent of the hippocampus. To achieve this, we employed DREADD technology to inactivate the hippocampus of either non-habituated or habituated mice 1 h prior to training on the object-in-context task. All mice received systemic administration of a memory-enhancing dose of yohimbine immediately after the training session. In both habituation conditions, mice injected with a control virus into the hippocampus displayed object-in-context memory on a 24-h retention test. However, and most importantly, DREADD inactivation of the hippocampus of non-habituated mice blocked this object-in-context memory, whereas hippocampal inactivation of habituated animals did not induce any memory impairment. These findings thus provide direct support for the hypothesis that prior context habituation renders noradrenergic effects on object-in-context memory independent of the hippocampus.

Key words: DREADD; hippocampus; yohimbine; object-in-context memory.

Introduction

Extensive evidence indicates that stressful and emotionally arousing experiences create strong and lasting memories (McGaugh, 2000; Sara, 2009; Joëls et al., 2011; Roozendaal & McGaugh, 2011; Takeuchi et al., 2016; de Quervain et al., 2017; Bahtiyar et al., 2020; Schwabe et al., 2022). However, it is still debated whether and how stress and emotional arousal impact the quality of these memories (Morgan et al., 2004b; Porter et al., 2008; Hoscheidt et al., 2014). Recent experiments from our group have provided evidence that norepinephrine, a major stress hormone that is released in both the brain and periphery during stressful and emotional experiences (Mason, 1968; McIntyre et al., 2002), not only increases the strength of memory but also enhances the episodic-like quality of memory. Both systemic administration of the noradrenergic stimulant yohimbine as well as local administration of norepinephrine into the basolateral amygdala was found to enhance episodic-like memory for the association of shock exposure with the training context on a dual-event inhibitory avoidance task in rats (Atucha & Roozendaal, 2015; Atucha et al., 2017; Roozendaal & Mirone, 2020). Moreover, norepinephrine administration into the basolateral amygdala was found to increase hippocampal activity after the training as well as maintain long-term involvement of the hippocampus in the expression of the memory (Atucha et al., 2017).

In Chapter 2 of this thesis, we investigated the effect of yohimbine on episodic-like memory in an object-in-context task in mice; a hippocampus-dependent task in which two object presentation events during the training session are distinguished by the contexts in which they appear. Like the yohimbine effect on memory in the dual-event inhibitory avoidance task, we found that systemic yohimbine administration enhanced episodic-like memory for the association of the object presentation with the training context. This yohimbine-induced enhancement of object-in-context memory was paralleled by an increased neuronal activity within the hippocampal CA1 subregion during the post-learning consolidation period. Additionally, we found that yohimbine-treated mice displayed more strongly correlated neural activity between the dorsal blade of the dentate gyrus (dDG) and CA3 subregions of the hippocampus, a pathway that has been critically implicated in pattern separation (Rolls, 1989; Mizumori et al., 1990; Rolls, 1996; Schaaf et al., 1998; Gilbert et al., 2001; Leutgeb et al., 2007).

Based on findings of early studies indicating that familiarization to the training context prior to training can reduce hippocampal involvement in contextual fear conditioning (Young et al., 1994), we also examined whether prior context habituation would alter the effect of yohimbine administration on object-in-context memory and hippocampal activity. Although the behavioral effect of yohimbine on enhancing object-in-context memory was similar in mice that had been habituated to either the two training contexts

or two different contexts, posttraining hippocampal activity was overall much lower in mice that had been habituated to the training contexts. Further, no significant correlation in activity between the dDG and CA3 was found after yohimbine administration to habituated animals. These findings made us propose that noradrenergic activity in non-habituated animals enhances object-in-context memory by facilitating a hippocampal mechanism that regulates the separation of memory for the two training events into two distinct memories, but that after repeated habituation to the training contexts, separate memories of the two training contexts might already have been formed, obviating the need for hippocampal pattern separation. Hence, the yohimbine effect on enhancing object-in-context memory following context habituation could be explained by its strengthening effect on memory for the objects *per se*, which does not depend on the hippocampus (Balderas et al., 2008; Roozendaal et al., 2010; Dees & Kesner, 2013).

In this chapter, we aimed to provide causal evidence for our hypothesis that the effect of yohimbine on enhancing object-in-context memory in non-habituated animals does require the hippocampus, but that prior habituation to the training contexts renders this effect hippocampus independent. Therefore, we combined posttraining systemic administration of yohimbine with Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetics to selectively silence the hippocampus during both the training on the object-in-context task and the post-learning consolidation period. Mice received bilateral intracranial injections into the hippocampus of an adeno-associated virus that induces the expression of an excitatory DREADD receptor selectively in inhibitory GABAergic neurons (AAV9-hDlx-GqDREADD-dTomato-Fishell-4) or its control virus (AAV9-mDlx-GFP-Fishell-1) (Dimidschstein et al., 2016; Krueger et al., 2020). Following viral transfection, mice received three habituation sessions to either the two training contexts or two different contexts. On the training day, we inactivated the hippocampus chemogenetically, with a low dose of clozapine injected 1 h prior to the training session, and yohimbine was administered immediately posttraining. Successful DREADD-mediated inhibition of hippocampal activity was verified by determining the spread of the virus within the hippocampus, and the local expression of c-Fos, a well-established molecular marker for activated cells (Minatohara et al., 2016), 1 h after the training session and yohimbine treatment. The effect of DREADD-mediated inhibition of hippocampal activity on the effects of yohimbine administration on object-in-context memory in the two habituation conditions was tested in a retention test 1 day later in another group of mice. Specifically, we aimed to test the hypothesis that hippocampal inactivation would impair object-in-context memory of yohimbine-treated mice that had been habituated to two different contexts prior to training, but that hippocampal inactivation would have no effect in mice that had been habituated to the two training contexts.

Material and methods

Animals

Ninety-two male C57BL/6J mice (7-11 weeks old at the time of surgery) from Charles River Breeding Laboratories (Kisslegg, Germany) were kept in a temperature-controlled (22 °C) vivarium room and maintained on a 12:12-h day:night regimen (7:00 – 19:00 h lights on). The vivarium room had a light intensity of 47 lux and humidity of 72%. Mice had *ad libitum* access to food and water. They were single housed starting at 3-7 days prior to surgery and remained single housed throughout the experiment to avoid potential stress or fighting induced by hierarchical status and to prevent testing order effects. Training and testing was performed during the light phase of the cycle, between 10:00 and 16:00 h, at the nadir of the diurnal cycle of corticosterone. All experimental procedures were in compliance with European Union Directive 2010/63/EU and approved by the Central Authority for Scientific Procedures on Animals (CCD), The Hague, The Netherlands. All efforts were made to minimize animal suffering and to reduce the number of animals.

Viral injection

Mice were anesthetized with isoflurane (5.0% for induction and 1.5-2.0% for maintenance) and placed in a stereotaxic frame (Neurostar, Tubingen, Germany). For analgesia, animals received 10 mg/kg carprofen (2-(6-chloro-9H-carbazol-2-yl)propanoic acid; Zoetis, The Netherlands) via the drinking water from 24 h before the surgery until 48 h after the surgery. Intra-operative analgesia was applied by a 2% lidocaine solution injected subcutaneously at the incision site. AAV9-hDlx-GqDREADD-dTomato-Fishell-4 (300 nL per injection site, 2.3×1013 GC/mL; Addgene, Cambridge, MA, USA, Cat# 83897-AAV9) or its control AAV9-mDlx-GFP-Fishell-1 (300 nL per site, 2.1×1013 GC/mL; Addgene, Cat# 839000-AAV9) was delivered bilaterally into the hippocampus (anteroposterior (AP), -1.70 mm from Bregma; mediolateral (ML), ±1.5 mm from midline; dorsoventral (DV), three injection sites at 1.7, 1.9 and 2.1 mm below the skull surface) using a 10-µL microsyringe with a 26 G needle (Nanofil; WPI, Sarasota, FL, USA) (Figure 1A). After each injection, the needle was left in place for another 1 min. Mice recovered for 14 days after the surgery to allow for virus expression.

Object-in-context task

Prior to training, mice were first handled for 2 min each on 4 consecutive days to become accustomed to the experimenter. Subsequently, the animals received three days of context habituation to reduce novelty stress, which is required to guarantee sufficient exploration of the objects on the training session (Stefanko et al., 2009). Some experimental groups were habituated to the same two contexts as those used for training (i.e., two gray, round plastic boxes (40 cm diameter, 40 cm height) with different modifications, placed next to one another) for 3 consecutive days. Other experimental

groups were habituated to two different contexts (two square boxes (40 cm width, 40 cm length, 40 cm height) with different modifications) for 3 days (Figure 2A). In each condition (either the round or square boxes), one box was gray with sawdust bedding and the other one had white stripes and dots on the walls and had corncob bedding. The experimental room was dimly illuminated (47 lux). During the habituation, the animals could explore each context, without any objects, for 5 min in a randomized order across animals (Figure 2A).

One hour prior to the training session, the mice received clozapine intraperitoneally to activate the DREADD receptors. On the training session, they were placed in the first box (context A or B), for 5 min where they were able to explore one set of two identical objects - either two transparent glass jars (5.5 cm diameter, 5 cm height) or two white light bulbs (6 cm diameter, 11 cm length) - secured to the floor of the boxes with Velcro tape. Objects were placed 5 cm away from the edge of the box. Immediately after the first context exposure, mice were placed in the second box (context B or A, respectively) for 5 min, containing the other set of two identical objects. The sequence of the two context exposures and the object-context combinations was counterbalanced across animals. To avoid the presence of olfactory trails, feces were removed, bedding was stirred, and the objects were thoroughly cleaned with 70% ethanol in between animals. On the 24-h retention test, the animals were placed in one of the two training contexts (context A or B) for 5 min with one exemplar of both training objects placed in the same location as the objects during the training trial. The context used on the retention test, as well as the location of the novel object, was counterbalanced to reduce potential biases due to preference for particular locations or objects.

Mice' behavior during the training and retention test was recorded with a video camera mounted above the experimental apparatus. Videos were analyzed offline by a trained observer blind to treatment and habituation condition, and the time spent exploring each object was manually scored. Object exploration was defined as actual active interaction with an object, i.e., pointing the nose to the object at a distance of <1 cm and/or touching it with the nose (Okuda et al., 2004; Leger et al., 2013; Song et al., 2021). Turning around, climbing or sitting on an object *per se* was not included in exploration time as the animals then often do not actively engage in exploring the object but rather exhibit grooming behavior or are using the object as platform to scan the environment (Bianchi et al., 2006; Roozendaal et al., 2006; Li et al., 2011; Wimmer et al., 2012; Leger et al., 2013; Vogel-Ciernia & Wood, 2014; Pezze et al., 2017). In order to analyze memory performance, a discrimination index (DI%) was calculated as the difference in time exploring the novel and familiar object-in-context combination, expressed as the ratio of the total time spent exploring both objects (i.e. [Time Novel - Time Familiar] / [Time

Novel + Time Familiar] x 100%). Two mice showing a total exploration time of <2 s during training and/or testing were removed from further analyses (Okuda et al., 2004).

Systemic drug administration

All animals received an intraperitoneal injection of a very low dose of clozapine (0.05 mg/kg; Sigma-Aldrich), in a volume of 0.005 mL/g of body weight, 1 h before the training session (Zerbi et al., 2019). Clozapine was first dissolved in 1 M hydrochloric acid, and then 10 µL of this solution was mixed with 3 mL 0.1 M phosphate-buffered saline (PBS) to reach a final concentration of 0.33% hydrochloric acid in PBS. We decided to use clozapine instead of clozapine N-oxide (CNO) because CNO is unable to cross the blood-brain barrier and is metabolized peripherally into clozapine (Manvich et al., 2018).

The noradrenergic stimulant yohimbine (1 mg/kg; 17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride; Sigma-Aldrich), an α_2-adrenoceptor antagonist that increases norepinephrine levels in the periphery and brain (Szemeredi et al., 1991), was dissolved in saline. The yohimbine solution was administered intraperitoneally, in a volume of 0.01 mL/g of body weight to all animals immediately after the training session. This yohimbine dose was selected based on its memory-enhancing effect in Chapter 2. Drug solutions were freshly prepared before each experiment.

Immunohistochemistry

To examine the effect of the DREADD manipulation on hippocampal activity during the post-learning consolidation period, some mice were anesthetized with an overdose of sodium pentobarbital (40-50 mg/kg) 1 h after training and posttraining yohimbine treatment, followed by transcardial perfusion with 10 mL of ice-cold PBS and 10 mL of ice-cold 4% paraformaldehyde (PFA) (pH 7.4). Brains were extracted, post-fixed in 4% PFA in 0.1 M PBS (pH 7.4) for 24 h, and then transferred to a 30% sucrose solution in 0.1 M PBS at 4 °C for 4 days . Coronal slices of 30 µm thickness were cut on a cryostat, collected in 0.1 M PBS with 0.01% sodium azide, and stored at 4 °C.

Three to four hippocampal sections (AP, -1.70 to -2.06 mm) per animal were selected according to the Franklin and Paxinos mouse brain atlas (Franklin & Paxinos, 2007). All procedures were performed in the dark. Sections were first rinsed in 0.5% Triton in PBS for 30 min at room temperature (RT), washed three times in PBS for 10 min per wash, and then blocked in 5% Normal Donkey Serum (NDS, Jackson ImmunoResearch Laboratories) and 1% Bovine Serum Albumin (BSA, Thermo Fisher) in PBS for 1 h at RT. Next, sections were incubated with a c-Fos primary antibody (guinea pig anti-c-Fos, 1:750, #226 004, Synaptic Systems) in PBS containing 2% NDS and 0.1% acetylated BSA (BSA-c, Aurion) overnight at RT. Afterwards, sections were washed three times in PBS for 10 min per wash,

followed by incubation with a fluorophore-conjugated secondary antibody (donkey anti-guinea pig Alexa Fluor 647, 1:750, Jackson ImmunoResearch) in 2% NDS and 0.1% BSA-c in PBS for 3 h at RT. Subsequently, sections were incubated with 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI, 1:5,000) in 0.1% BSA-c in PBS for 15 min, then washed three times in PBS for 10 min per wash, mounted on gelatin-coated slides, left to dry, and cover slipped with Fluorsave mounting medium (Sigma-Aldrich). The slides were stored in the dark at 4 °C.

Mice subjected to the retention test were also sacrificed by transcardial perfusion with PBS and 4% PFA following the retention test. To verify the viral transfection of the hippocampus in these mice, two sections containing the hippocampus (AP, -1.58 to -2.06 mm) of each animal were mounted on gelatin-coated slides, air-dried and coverslipped with Fluorsave mounting medium.

Imaging and quantification

Images were acquired on an automated high-content fluorescence microscope (Leica, DMI 6000B) with a 20x magnification. ImageJ software was used to count labeled cells and measure surface areas (Rueden et al., 2017). For the analyses of c-Fos-positive neurons, the hippocampus was divided into its four main subregions: the cellular layer of the dentate gyrus dorsal blade (dDG), cellular layer of the dentate gyrus ventral blade (vDG), cellular layer of the cornu ammonis 3 (CA3) and cornu ammonis 1 (CA1). Each subregion was manually drawn according to the Allen Mouse Brain Atlas (http://portal.brain-map.org/) (Figure 1B), and the number of c-Fos-positive cells was counted manually by a researcher blind to the treatment condition, and then converted to number of cells per mm^2.

For verification of viral transfection, images were acquired at 10x magnification using an automated high-content fluorescence microscope (Leica DMI 6000B). Viral transfection was considered successful in case of abundant dTomato (for DREADD virus) or GFP (for control virus) expression within all hippocampal subfields in both hemispheres (>50% of the area size). Two animals with insufficient viral transfection based on this criterion were excluded from analyses.

Statistics

Statistical analyses were performed using IBM SPSS statistics version 25. Total object exploration time in each of the two contexts during the training session was analyzed using linear mixed models with hippocampal manipulation (DREADD or control virus) and habituation condition (different or same) as between-subject parameters. Object exploration time in the first and second context, the object explored (bulb or jar), and training context (A or B) were added as within-subject parameters. Noteworthy,

the latter within-subject variables (i.e., object and context) were counterbalanced across animals. The DI% and total object exploration time at the retention test were analyzed with two-way ANOVAs with hippocampal manipulation (DREADD or control virus) and habituation condition (different or same) as between-subject parameters. When appropriate, Tukey *post hoc* analyses were used to determine the source of the significance. One-sample *t*-tests were used to determine whether the DI% was different from zero (i.e., chance level) and thus whether object-in-context memory formation had occurred.

Immunohistochemistry data were first analyzed by a linear mixed model with hippocampal manipulation (DREADD or control virus) and habituation condition (different or same) as between-subject variables and hippocampal subregion (dDG, vDG, CA3 or CA1) as within-subject variable. This was followed by two-way ANOVAs for each of the four hippocampal subregions separately with hippocampal manipulation and habituation condition as factors. If appropriate, Tukey *post hoc* analyses were conducted to determine the source of significance. For all statistical tests, $p < 0.05$ was accepted as statistical significance. Data are expressed as mean ± standard error of the mean (SEM). The number of mice per group is indicated in the figure legends.

Results

Effect of pretraining DREADD manipulation on hippocampal activity of animals in the different and same habituation condition

In this experiment, we set out to investigate whether prior habituation to the training context altered the involvement of the hippocampus in mediating yohimbine-induced enhancement of object-in-context memory. We first examined whether the DREADD manipulation successfully inhibited hippocampal activity during and shortly after the training session. For this, we habituated the mice to either the same or different contexts, inactivated the hippocampus by clozapine (0.05 mg/kg, i.p.) 1 h prior to the training session and administered a memory-enhancing dose of yohimbine (1 mg/kg, i.p.) to all mice immediately after the training session. Mice were sacrificed 1 h post-injection and we examined the number of c-Fos-expressing neurons within the cell layers of each of the four hippocampal subregions (Figure 1A).

Total object exploration time during training was not affected by the hippocampal manipulation ($F_{(1,14)} = 0.44$, $p = 0.52$), habituation condition ($F_{(1,14)} = 2.10$, $p = 0.17$), or hippocampal manipulation X habituation condition interaction ($F_{(1,14)} = 0.01$, $p = 0.98$) (Table I). Moreover, the training session (first vs. second, $F_{(1,14)} = 0.67$, $p = 0.43$), training context (A vs. B, $F_{(1,14)} = 1.51$, $p = 0.24$), and type of object (jars vs. bulbs, $F_{(1,14)} = 2.98$, $p =$

0.11) did not affect total object exploration time during training, nor did these factors significantly interact with our factors of interest in influencing training exploration times (all p's > 0.07).

Table I. Total object exploration time during training of the experimental groups used for immunohistochemistry

Hippocampal manipulation	Habituation condition	First context (s)	Second context (s)
Control (n = 6)	Different	4.8 ± 0.7	4.8 ± 0.4
DREADD (n = 5)	Different	5.1 ± 0.7	5.0 ± 0.5
Control (n = 6)	Same	4.9 ± 0.6	5.3 ± 0.6
DREADD (n = 6)	Same	5.6 ± 0.8	5.4 ± 0.6

Data represent mean ± SEM

A linear mixed model analyzing c-Fos expression revealed significant main effects of hippocampal manipulation ($F_{(1,19)}$ = 227.29, p < 0.001) and subregion ($F_{(3,57)}$ = 94.03, p < 0.001), but not of habituation condition ($F_{(1,19)}$ = 3.81, p = 0.66) (Figure 2B and C). Further, we found significant hippocampal manipulation X subregion ($F_{(3,57)}$ = 104.10, p < 0.001) and hippocampal manipulation X habituation condition interaction effects ($F_{(1,19)}$ =4.66, p = 0.04), as well as a trend-level effect for hippocampal manipulation X habituation condition X subregion interaction ($F_{(3,57)}$ = 2.56, p = 0.064), whereas the habituation condition X subregion interaction effect was not significant ($F_{(3,57)}$ = 1.94, p = 0.13).

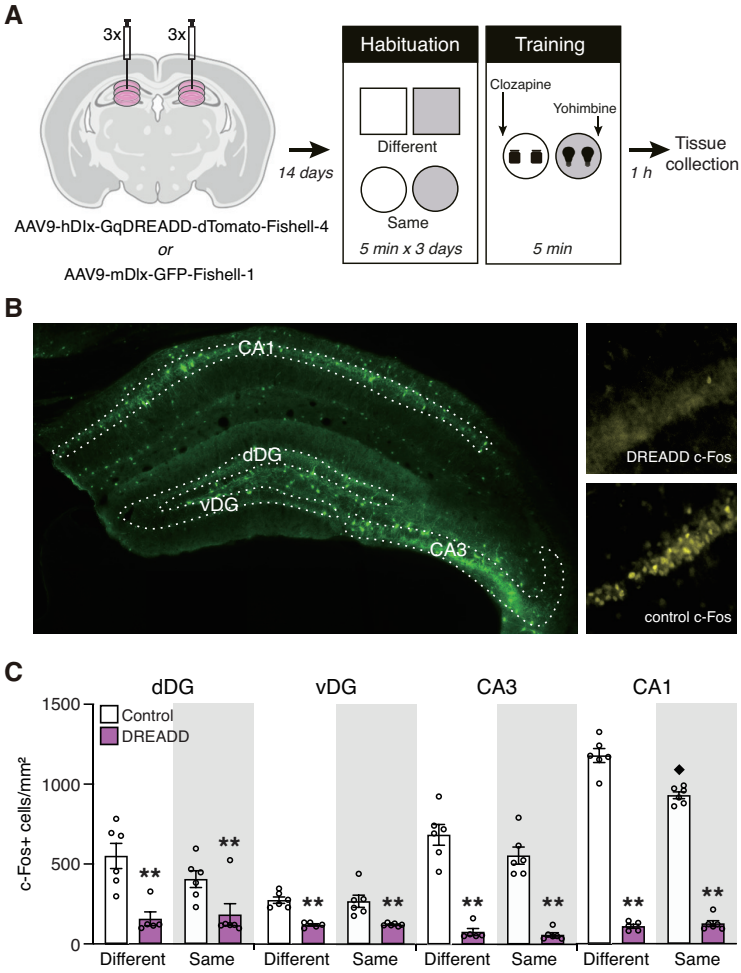

Figure 1. Effect of pretraining DREADD manipulation on hippocampal activity of animals in the different and same habituation condition.

A. Mice were injected bilaterally into the hippocampus with an excitatory DREADD virus selectively transfecting GABAergic neurons (AAV9-hDlx-GqDREADD-dTomato-Fishell-4), whereas control mice were injected with a control virus (AAV9-mDlx-GFP-Fishell-1). After a 14-day incubation period, mice were habituated for 5 min to the two training contexts (same) or two different contexts (different) on three consecutive days. A low dose of clozapine (0.05 mg/kg, i.p.) to activate the DREADD receptors was injected 1 h before training. On the training session, mice were placed in a first context for 5 min where they could freely explore one set of two identical objects, after which they were placed in a second context for 5 min where they could explore another set of two identical objects. Immediately after the training session, all mice were administered a memory-enhancing dose of yohimbine (1 mg/kg, i.p.). Mice were sacrificed 1 h later for tissue collection. **B.** Representative image illustrating the spread of viral transfection (green) throughout all hippocampus subregions: dorsal blade of the dentate gyrus granule cell layer (dDG), ventral blade of the dentate gyrus granule cell layer (vDG), CA3 pyramidal cell layer (CA3), CA1 pyramidal cell layer (CA1). The areas drawn show the exact regions in which the number of c-Fos-positive cells were counted. Right panels: higher magnification images of the CA1 showing inhibition of c-Fos expression of animals injected with the DREADD virus vs. control virus. **C.** The DREADD manipulation effectively reduced c-Fos expression within all hippocampal subregions. Data are shown as mean ± SEM, dots represent individual data points (Different habituation condition, Control: $n = 6$, DREADD: $n = 6$; Same habituation condition, Control: $n = 6$, DREADD: $n = 5$). $^{**}p < 0.01$ vs. control virus; $^{\blacklozenge}p < 0.05$ effect of habituation condition.

Based on these significant interaction effects, we analyzed the treatment effects on c-Fos expression in each of the four hippocampal subregions separately. In the dDG granule cell layer, a two-way ANOVA for c-Fos expression revealed a main effect of hippocampal manipulation ($F_{(1,19)} = 23.07$, $p < 0.001$), but no effect of habituation condition ($F_{(1,19)} = 0.85$, $p = 0.37$) or hippocampal manipulation X habituation condition interaction ($F_{(1,19)} = 1.77$, $p = 0.20$). The DREADD manipulation similarly reduced c-Fos expression in the dDG of animals in both the different ($t_{(9)} = 8.15$, $p < 0.001$) and same habituation condition ($t_{(10)} = 8.87$, $p < 0.001$). Comparably, in the vDG granule cell layer, a main effect of hippocampal manipulation was found ($F_{(1,19)} = 43.25$, $p < 0.001$), without any effect of habituation condition ($F_{(1,19)} = 0.02$, $p = 0.90$) or hippocampal manipulation X habituation condition interaction ($F_{(1,19)} = 0.04$, $p = 0.85$). Also here, the DREADD manipulation significantly reduced c-Fos expression of animals in both the different ($t_{(9)} = 6.81$, $p < 0.001$) and same habituation condition ($t_{(10)} = 3.81$, $p = 0.003$). In the CA3 pyramidal cell layer, we again observed a significant main effect of hippocampal manipulation ($F_{(1,19)} = 143.66$, $p < 0.01$), but no effect of habituation condition ($F_{(1,19)} = 2.57$, $p = 0.13$) or hippocampal manipulation X habituation condition interaction ($F_{(1,19)} = 1.49$, $p = 0.24$), with the DREADD manipulation significantly reducing c-Fos expression of animals in both the different ($t_{(9)} = 8.15$, $p < 0.001$) and same habituation condition ($t_{(10)} = 8.87$, $p < 0.001$). In contrast, in the CA1 pyramidal cell layer, we found main effects of both hippocampal manipulation ($F_{(1,19)} = 1127.23$, $p < 0.001$) and habituation condition ($F_{(1,19)} = 17.30$, $p = 0.001$), as well as a significant hippocampal manipulation X habituation condition interaction effect ($F_{(1,19)} = 22.64$, $p < 0.001$). *Post hoc* analyses revealed that the DREADD manipulation significantly reduced c-Fos expression in the CA1 pyramidal cell layer of animals in both the different ($t_{(9)} = 21.54$, $p < 0.001$) and same habituation condition ($t_{(10)} = 28.41$, $p < 0.001$), with both habituation conditions not differing from each other as a consequence of the manipulation ($t_{(9)} = 0.72$, $p = 0.49$). Yet, we found that c-Fos expression within the CA1 of control animals was significantly higher in animals of the different habituation group than in those of the same habituation group ($t_{(10)} = 5.10$, $p < 0.001$), explaining the significant interaction effect.

These findings thus indicate that the DREADD manipulation resulted in an effective inactivation of all four hippocampal subregions, regardless of habituation condition. However, consistent with our findings in Chapter 2, we found that control animals in the different habituation condition displayed significantly higher c-Fos expression within the CA1 pyramidal cell layer during the post-learning consolidation period than animals in the same habituation condition, whereas no differences across habituation conditions were observed in any of the other hippocampal subregions (also consistent with the findings in Chapter 2).

Effect of pretraining DREADD inactivation of the hippocampus on object-in-context memory of animals in the different and same habituation condition

Next, we investigated whether prior habituation to the training contexts altered the effect of the DREADD inactivation of the hippocampus on yohimbine-induced improvement of object-in-context memory formation. Therefore, we habituated mice to contexts that were either the same or different to the ones used during training, injected clozapine (0.05 mg/kg, i.p.) to either inhibit the hippocampus (DREADD virus) or not (control virus) 1 h before training on the object-in-context task, and administered a memory-enhancing dose of yohimbine (1 mg/kg, i.p.) to all mice immediately after the training session. Retention of the memory was tested 24 h later (Figure 2A).

Total object exploration time during training was not affected by the hippocampal manipulation ($F_{(1,54)}$ = 0.08, p = 0.78), habituation condition ($F_{(1,54)}$ = 0.26, p = 0.61) or hippocampal manipulation X habituation condition interaction ($F_{(1,54)}$ = 0.02, p = 0.90) (Figure 2B, Table II). Moreover, training session (first vs. second, $F_{(1,54)}$ = 1.01, p = 0.75), training context (A vs. B, $F_{(1,54)}$ = 1.15, p = 0.29) and the type of object (jars vs. bulbs, $F_{(1,54)}$ = 0.02, p = 0.89) did not affect object exploration time during training, or interact with our factors of interest in influencing training exploration times (all p's > 0.11).

Table II. Total object exploration time during training and retention test

Hippocampal manipulation	Habituation condition	First context (s)	Second context (s)	Retention test (s)
Control (n = 17)	Different	11.2 ± 1.4	10.2 ± 1.3	8.5 ± 1.9
DREADD (n = 17)	Different	10.7 ± 1.2	10.9 ± 1.8	9.1 ± 2.4
Control (n = 18)	Same	10.6 ± 1.8	11.5 ± 2.0	7.2 ± 2.5
DREADD (n = 17)	Same	10.5 ± 1.7	11.7 ± 1.8	9.6 ± 2.9[1]

Data represent mean ± SEM
[1]p < 0.01 vs control virus group

At the 24-h retention test, a two-way ANOVA for the DI% indicated significant main effects of hippocampal manipulation ($F_{(1,65)}$ = 15.94, p < 0.001) and habituation condition ($F_{(1,65)}$ = 5.33, p = 0.024), as well as a significant hippocampal manipulation X habituation condition interaction effect ($F_{(1,65)}$ = 7.29, p = 0.009, Figure 2C). Hippocampal inactivation of mice in the different habituation condition significantly impaired the DI% ($t_{(32)}$ = -5.52, p < 0.001). One-sample t-tests indicated that in this condition, the control virus group displayed successful memory recall, with the DI% being significantly greater than zero ($t_{(16)}$ = 5.17, p

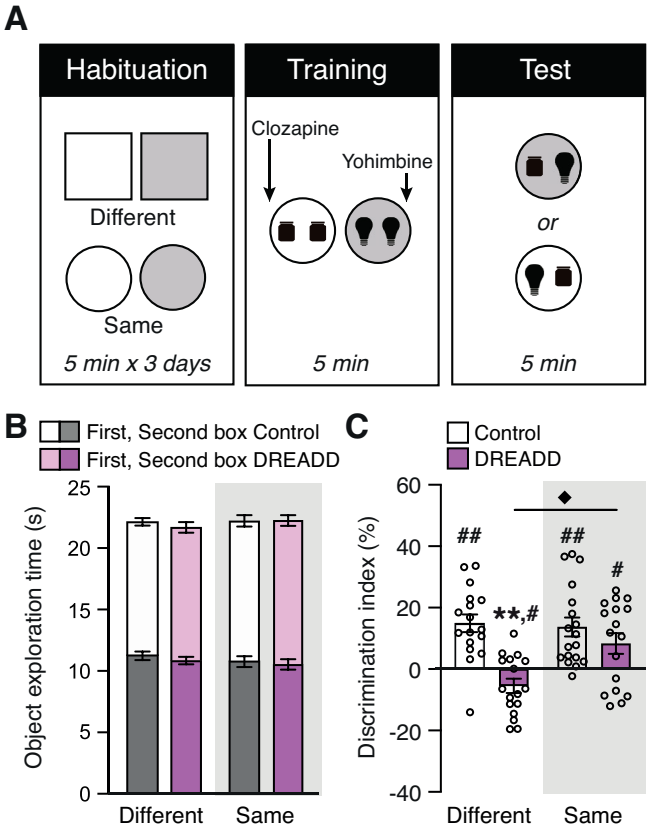

Figure 2. Effect of pretraining DREADD inactivation of the hippocampus on object-in-context memory of animals in the different and same habituation condition.

A. Experimental design of the object-in-context task. Mice were habituated for 5 min to the two training contexts (same) or two different contexts (different) on three consecutive days. Mice received a low dose of clozapine (0.05 mg/kg, i.p.) to activate the DREADD receptors 1 h before training. During the training session, mice were placed in a first context for 5 min where they could freely explore one set of two identical objects, after which they were placed in a second context for 5 min where they could explore another set of two identical objects. Immediately after the training session, all mice were administered a memory-enhancing dose of yohimbine (1 mg/kg, i.p.). Retention was tested 24 h later, during which the mice could explore one exemplar of each of the two training objects in one of the two training contexts. **B.** Hippocampal inactivation or habituation condition did not affect total object exploration time during the training session (Different habituation condition, Control: $n = 17$, DREADD: $n = 17$; Same habituation condition, Control: $n = 18$, DREADD: $n = 17$). **C.** Hippocampal inactivation selectively impaired object-in-context memory of mice in the different habituation condition, whereas it did not affect memory in the same habituation condition. Data are shown as mean ± SEM, dots represent individual data points (Different habituation condition, Control: $n = 17$, DREADD: $n = 17$; Same habituation condition, Control: $n = 18$, DREADD: $n = 17$). $^{**}p < 0.01$ vs. control virus; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. chance level, $^{\blacklozenge}p < 0.05$ effect of habituation condition.

< 0.001), whereas the DREADD virus group showed poor memory performance, with the DI% being significantly lower than zero ($t_{(16)}$ = -2.37, p = 0.03). In contrast, hippocampal inactivation of mice in the same habituation condition did not affect the DI% ($t_{(33)}$ = 0.82, p = 0.42). One-sample *t*-tests further indicated that mice of both the DREADD and control virus groups showed successful memory recall in the same habituation condition, with the DI%s being significantly greater than zero (control: $t_{(17)}$ = 4.46, p < 0.001, DREADD: $t_{(16)}$ = 2.62, p = 0.002). Moreover, whereas the DI% of the control virus group did not differ between the different and same habituation condition groups ($t_{(33)}$ = 0.28, p = 0.78), we found that the DI% of the DREADD virus group was significantly smaller in the different habituation condition than in the same habituation condition ($t_{(32)}$ = -5.95, p = 0.02).

A two-way ANOVA for total object exploration time during the retention test indicated a significant main effect of hippocampal manipulation ($F_{(1,65)}$ = 6.91, p = 0.01) with the DREADD virus group displaying more object exploration than the control virus group (Table II). We found no significant effect of either habituation condition ($F_{(1,65)}$ = 0.51, p = 0.48) or hippocampal manipulation X habituation condition ($F_{(1,65)}$ = 2.69, p = 0.11).

These findings indicate that DREADD-induced inactivation of the hippocampus during the training session in combination with posttraining yohimbine treatment selectively impaired object-in-context memory of mice that had no prior knowledge of the training context, whereas the hippocampal inactivation did not impair memory of mice that were previously habituated to the training context.

Discussion

In this chapter, we set out to test the hypothesis that the effect of systemic yohimbine administration on enhancing object-in-context memory in an unfamiliar training context requires the hippocampus, but that prior context habituation renders this memory enhancement hippocampus independent. This hypothesis originated from our findings in Chapter 2 where we demonstrated that posttraining yohimbine administration enhanced object-in-context memory of both habituated and non-habituated mice, but that the yohimbine effect on post-learning hippocampal activity differed between the two habituation conditions. Whereas yohimbine administration to non-habituated mice increased correlated activity between the dDG and CA3, reflecting pattern separation (Rolls, 1989; Mizumori et al., 1990; Rolls, 1996; Schaaf et al., 1998; Gilbert et al., 2001; Leutgeb et al., 2007), as well as increased neuronal activity within the hippocampal CA1 subregion, yohimbine administration to habituated mice did not produce dDG – CA3 correlated activity. Moreover, prior context habituation induced a general reduction in hippocampal activity during the post-learning consolidation period. Therefore, we

proposed that yohimbine administration to non-habituated animals enhances object-in-context memory by facilitating a hippocampal mechanism that supports the separation of memory of the two training events into two distinct memories. Following context habituation, separate memories for the two training contexts may have already been created, such that yohimbine enhanced memory for the objects *per se*, which does not require the hippocampus (Balderas et al., 2008; Roozendaal et al., 2010; Dees & Kesner, 2013).

Consistent with these prior findings, we here observed that yohimbine-treated mice of the control virus group displayed object-in-context memory in both the different and same habituation condition. Previously, we found that 5 min of training in each context is not sufficient to form long-term memory in saline-treated control animals (Chapter 2), and that posttraining yohimbine administration enhances the consolidation of this memory. Also consistent with the findings of Chapter 2, we found that prior context habituation reduced c-Fos expression in the CA1 region of mice of the control virus group, thus supporting the view that habituation lessens hippocampal involvement in the task. To induce hippocampal inactivation, we increased local inhibitory GABAergic activity by chemogenetic manipulation 1 h prior to the training session (Dimidschstein et al., 2016). This approach had been previously successfully implemented in a contextual fear conditioning task by others (Krueger et al., 2020), inducing decreased c-Fos expression in pyramidal neurons. We also observed reduced c-Fos expression in the cell layer of all hippocampal subregions during the post-learning consolidation period as a consequence of this manipulation. Most importantly, DREADD-mediated inactivation of the hippocampus successfully impaired object-in-context memory of yohimbine-treated mice that had not been previously habituated to the two training contexts, whereas hippocampal inactivation did not impair object-in-context memory of mice that had been familiarized with the two training contexts. These findings thus provide direct support for our hypothesis that prior context habituation renders the yohimbine effect on enhancing object-in-context memory hippocampus independent. In Chapter 2, we described that successful performance on the object-in-context task requires the creation of at least two different memories since the animals need to form memories of the two training contexts as well as of the training objects *per se*. Previously, we found that noradrenergic activation via a pattern separation mechanism within the hippocampus enhances the formation of distinct memories of the two training contexts (Atucha et al., under revision). However, following context habituation, separate memories for the two training contexts have already been created, such that yohimbine might only enhance memory for the objects *per se*. Several studies have reported that object memory is primarily dependent on cortical regions (Bermudez-Rattoni et al., 2005; Forwood et al., 2005; Balderas et al., 2008; Assini et al., 2009; Roozendaal et al., 2010; Haettig et al., 2011; Vogel-Ciernia & Wood, 2014), and that direct stimulation or inhibition of noradrenergic

activity in these regions regulates the strength of object memory (Roozendaal et al., 2010; Chen et al., 2022). It should be noted, however, that no consensus has been reached on whether or not this mnemonic process is entirely independent from the hippocampus (Clark et al., 2000; Broadbent et al., 2004; Hammond et al., 2004; de Lima et al., 2006; Rossato et al., 2007; Cohen & Stackman Jr., 2015; Stackman et al., 2016).

Noteworthy, we injected the DREADD agonist clozapine 1 h prior to training such that the hippocampus was inactivated both during the training session and post-learning consolidation period. This is important as we wanted to ensure that habituated animals could also not use their hippocampus to retrieve contextual information at the time of training (Nakazawa et al., 2016). Whereas such pretraining manipulation could potentially affect the animals' behavior during the training session itself, we found that hippocampal inactivation of neither habituated nor non-habituated animals significantly changed the time spent exploring the objects during the training session. This finding is consistent with previous reports indicating that bilateral hippocampal lesions prior to learning do not affect the acquisition of object-place or object-in-context memories (Cohen et al., 2013).

An interesting observation in our study is that hippocampal inactivation not merely blocked object-in-context memory in the different habituation condition, i.e., performance at chance level, but actually induced a statistically significant negative DI%, suggestive of a preference to explore the familiar object-in-context configuration. These findings might support the view that alternative elemental learning strategies are recruited in the absence of hippocampal function (Maren et al., 1997; Winocur, 1997; Gerlai, 1998; Good et al., 1998). There is now extensive evidence indicating that the hippocampus and dorsal striatum are two parallel memory systems and that their interaction can either be cooperative or competitive in nature (Packard et al., 1989; White et al., 2013). The dorsal striatum is critically involved in habit and procedural memory (Cohen & Squire, 1980; McDonald & White, 1993). Thus, it seems conceivable that inactivation of the hippocampus prior to training may induce a shift towards the use of a striatal memory system during training on the object-in-context task (Packard & McGaugh, 1996; Schroeder et al., 2002), enhancing habit memory, which could favor exploration of the familiar object-in-context configuration on the retention test.

We only examined the effect of hippocampal inactivation on the effect of yohimbine on object-in-context memory in the two habituation conditions, but the findings are also highly relevant for understanding corticosterone effects on object-in-context memory. In Chapter 2, we showed that the corticosterone effect on object-in-context memory was opposite for mice in the two habituation conditions. Corticosterone impaired object-in-context memory of non-habituated mice, whereas it enhanced this memory when mice

were previously habituated to the training contexts. Consistent with the findings of the present study, we hypothesized that corticosterone impairs object-in-context memory in the different habituation condition by a hippocampus-dependent mechanism of linking of memory of the two training events, whereas corticosterone enhances object-in-context memory of habituated mice by a hippocampus-independent strengthening memory for the objects *per se*. However, as corticosterone treatment already induces impairment of this hippocampal mechanism, it would not be possible to use the same approach of DREADD-mediated inactivation of the hippocampus to test this hypothesis.

In conclusion, these findings provide direct evidence that noradrenergic activity improves object-in-context memory in a hippocampus-dependent manner when trained in unfamiliar contexts, and in a hippocampus-independent manner in familiar contexts. Thereby, these findings support our hypothesis that noradrenergic activity in non-habituated animals enhances object-in-context memory by regulating a hippocampal mechanism that facilitates the separation of memory of the two training events. However, once animals have been habituated to the training contexts, separate memories of the two training contexts have already been created, and the noradrenergic effect on object-in-context memory may be attributed to its ability to strengthen memory for the objects themselves, which is not dependent on the hippocampus.

Acknowledgments

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CHAPTER 4

Both norepinephrine and glucocorticoids enhance spatial memory in an object location task independent of context habituation

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Abstract

The stress hormones norepinephrine and corticosterone are well known to strengthen the consolidation of memory for emotional experiences. Yet, how these two hormones affect the quality of these strengthened memories remains largely elusive. In Chapter 2, we reported that the noradrenergic stimulant yohimbine enhanced the episodic-like quality of memory in an object-in-context task, whereas corticosterone impaired this memory. However, both yohimbine and corticosterone enhanced object-in-context memory of mice that had been habituated to the training contexts prior to training. We proposed that yohimbine and corticosterone induce opposite effects on object-in-context memory by an opposite regulation of a hippocampal mechanism that facilitates the separation or linking of memory of multiple training events, respectively, which would only be required in novel contexts. This then raises the question of whether yohimbine and corticosterone solely induce opposite effects on hippocampus-dependent memory after training on a task that necessitate the separation of overlapping memory representations for multiple training events. To address this question, in the present study yohimbine and corticosterone were administered after training on an object location task. Spatial memory formation on the object location task also critically depends on the hippocampus, but the training comprises a single event obviating the need for separating overlapping memory representations. We found that posttraining administration of both yohimbine and corticosterone induced a very similar enhancement of object location memory, which was associated with a very similar increase in neuronal activity of the hippocampal CA1 region during the post-learning consolidation phase. Prior habituation to the training context did not alter these effects of yohimbine or corticosterone. These findings, together with those of Chapter 2, provide support for the view that yohimbine and corticosterone induce opposite effects on hippocampal memory and activity selectively when there is a need to separate overlapping memory representations for multiple training events.

Keywords: norepinephrine; glucocorticoids; object location memory; hippocampus.

Introduction

It is well known that the stress hormones norepinephrine and corticosterone, in a predominantly synergistic manner, enhance the consolidation of memory of emotional experiences (McGaugh, 2003; Roozendaal & McGaugh, 2011; McGaugh, 2013; Schwabe et al., 2022), but it is less clear how these two stress hormones affect the quality of these strengthened memories (Payne et al., 2002; Talarico & Rubin, 2003; Morgan et al., 2004b; Kensinger et al., 2007b; Schwabe & Wolf, 2009; Rimmele et al., 2011; Segal et al., 2012; Roozendaal & Mirone, 2020). In Chapter 2, we examined the effect of norepinephrine and corticosterone on episodic-like quality of memory in an object-in-context task, a hippocampus-dependent task in which two object presentation events during the training session are distinguished by the contexts in which they appear (Dix & Aggleton, 1999; Eacott & Norman, 2004; Balderas et al., 2008; Barsegyan et al., 2014). We reported that systemic administration of the noradrenergic stimulant yohimbine after the training session enhanced object-in-context memory, whereas corticosterone impaired this memory. Similar opposite effects of yohimbine and corticosterone on episodic-like memory were found earlier in a dual-event inhibitory avoidance task in rats (Atucha & Roozendaal, 2015). Yohimbine administered after the training session not only strengthened memory of the shock experience *per se*, but also enhanced episodic-like memory for the specific association of the shock experience with the training context. In contrast, corticosterone administration impaired the episodic-like component of memory and induced a generalized strengthening of memory (Atucha & Roozendaal, 2015; Roozendaal & Mirone, 2020). However, in Chapter 2, the effect of posttraining systemic corticosterone administration on object-in-context memory was critically dependent on prior context habituation, as corticosterone was found to improve object-in-context memory of mice that were habituated to the two training contexts prior to the training session.

In both the object-in-context task and dual-event inhibitory avoidance task, animals are sequentially trained, with either no or a brief delay, on two events. We proposed that yohimbine and corticosterone induce opposite effects on episodic-like memory for these two learning tasks by facilitating either a separation or linking of memory of the two training events, respectively. We suggested that yohimbine facilitates a separation of memory of the two training events by supporting pattern separation within the dentate gyrus (DG) - CA3 pathway in the hippocampus (Atucha et al., under revision), whereas corticosterone promotes a linking of memory of the two training events by inhibiting this pattern separation process. We further suggested that after repeated habituation to the training contexts, separate memories of the two training contexts had already been formed at the time of training, obviating the need for this hippocampal mechanism; hence, explaining the divergent effect of corticosterone administration in habituated

vs. non-habituated animals. The memory-enhancing effects of posttraining yohimbine and corticosterone administration in habituated animals might be explained by a strengthening of memory for the objects *per se* (Roozendaal et al., 2008; Roozendaal et al., 2010; Song et al., 2020).

This then raises the question of whether the opposing effects of yohimbine and corticosterone on hippocampus-dependent memory are solely observed in case of overlapping information of multiple training events experienced close in time. To examine this, in the present study yohimbine and corticosterone were administered after training on an object location task. In this task, spatial memory is formed by associating an object with a specific location within the training context, which also critically depends on the hippocampus (Balderas et al., 2008; Roozendaal et al., 2010; Barsegyan et al., 2019). However, the training experience comprises a single event, and thus the animals do not have to separate overlapping memory representations. Different doses of the noradrenergic stimulant yohimbine or corticosterone were administered systemically immediately after the training session. At a 24-h retention test, the mice were re-exposed to the same context with the same two objects, but one of the objects had been moved to a novel location. The animal's preference to explore the object in the novel location was interpreted as a measure of spatial memory (Roozendaal et al., 2010; Vogel-Ciernia & Wood, 2014; Song et al., 2020). To investigate whether prior context habituation influenced the effect of yohimbine and corticosterone administration on object location memory, animals received three habituation sessions to either the training context or a differently looking context prior to the training session. Additionally, we examined the effects of stress hormone manipulation and context habituation on posttraining neuronal activity in the hippocampus by analyzing local c-Fos expression, a well-established molecular marker for activated cells (Minatohara et al., 2016), 1 h after the training. We additionally assessed the co-expression of c-Fos with GAD67, a GABAergic marker, to dissociate excitatory vs inhibitory activity in the hippocampus. Both the effects on neuronal activity in the hippocampal subregions *per se* as well as correlations in activity between subregions were assessed as a proxy for hippocampal function.

Material and methods

Animals

Two-hundred-eighty-seven male CB57BL/6J mice (10-14 weeks old at time of behavioral experiments) from Charles River Breeding Laboratories (Kisslegg, Germany) were kept in a temperature-controlled (22 °C) vivarium room and maintained on a 12:12 h day:night regimen (7:00 – 19:00 h lights on). The vivarium room had a light intensity of 47 lux and humidity of 72%. Mice had *ad libitum* access to food and water. Mice were single housed

7 days prior to the start of the experiment and remained single housed throughout to avoid potential stress induced by hierarchical status or fighting and prevent testing order effects. Training and testing was performed during the light phase of the cycle, between 10:00 and 16:00 h, at the nadir of the diurnal cycle of corticosterone. All experimental procedures were in compliance with European Union Directive 2010/63/EU and approved by the Central Authority for Scientific Procedures on Animals (CCD), The Hague, The Netherlands. All efforts were made to minimize animal suffering and to reduce the number of animals.

Object location task

The experimental apparatus used for the object location task was a gray, square open-field box (40 x 40 x 40 cm) with the floor covered with corncob bedding. One side of the box was marked with white dots taped to the wall, serving as an internal cue. The objects that were used were two white glass light bulbs (6 cm diameter, 11 cm length) or two transparent glass vials (5.5 cm diameter, 5 cm height), secured to the floor of the box with Velcro tape. The behavior of the animals was videotaped by a camera connected to a laptop computer above the box.

Prior to training, mice were first handled for 2 min each on 4 consecutive days to become accustomed to the experimenter. Subsequently, the animals received three habituation sessions to reduce novelty stress, which is required to guarantee sufficient exploration of the objects on the training session (Stefanko et al., 2009). Some experimental groups were habituated to the same context as that used for training (a gray square box with the floor covered with corncobs) for 3 min on 3 consecutive days. Other experimental groups were habituated to a different context (a gray round box (40 cm diameter x 40 cm height) with the floor covered with sawdust) for 3 min (Figure 1A). By habituating animals to either the same or different context as the training context, we were able to investigate the effect of prior context encoding on new memory formation, which was observed to be a critical determinant of corticosterone effects on object-in-context memory (Chapter 2 of this thesis). During this habituation phase, mice could freely explore the apparatus without the objects.

Training and testing on the object location task was performed according to Song et al. (2020) with slight modifications. On the training trial, mice were placed individually in the experimental apparatus and allowed to explore two identical objects for 3 min. To avoid the presence of olfactory trails, feces were removed, bedding was stirred, and the objects were thoroughly cleaned with 70% ethanol in between trials. Immediately after the training session, the animals received a systemic drug injection and were placed back into their home cage. Some mice were sacrificed at 1 h after training and drug treatment for immunohistochemical assessment of training-induced neuronal activity. Other

mice were left undisturbed until the retention test 24 h later. For retention testing, the mice were placed in the same experimental apparatus and allowed to explore the two previously seen objects for 5 min, yet one object was moved to a novel location (Figure 1A). The objects and locations were used in a balanced manner across animals to reduce potential biases due to preference for particular objects or locations.

Behavioral videos of the training and test sessions were analyzed offline by a trained observer blinded to treatment condition, and the time spent exploring each object was scored. Object exploration was defined as actual active interaction with an object, i.e., pointing the nose to the object at a distance of <1 cm and/or touching it with the nose (Okuda et al., 2004; Leger et al., 2013; Song et al., 2020). Turning around, climbing or sitting on an object *per se* was not included in exploration time as the animals then often do not actively engage in exploring the object, but rather exhibit grooming behavior or are using the object as platform to scan the environment (Bianchi et al., 2006; Roozendaal et al., 2006; Li et al., 2011; Wimmer et al., 2012; Leger et al., 2013; Vogel-Ciernia & Wood, 2014; Pezze et al., 2017). In order to analyze memory performance, a discrimination index (DI%) was calculated as the difference in time exploring the object in the novel and familiar location, expressed as the ratio of the total time spent exploring both objects (i.e., [Time Novel - Time Familiar] / [Time Novel + Time Familiar] x 100%). Six mice exploring the objects for less than 1 s during training or testing were removed from analyses to guarantee sufficient memory encoding and robust assessment of DI%.

Systemic drug injection

For the behavioral experiments, the noradrenergic stimulant yohimbine (0.3, 1 or 3 mg/kg; 17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride; Sigma-Aldrich), an α_2-adrenoceptor antagonist that increases norepinephrine levels in the periphery and brain (Szemeredi et al., 1991), was dissolved in saline, whereas the control group received saline only. Corticosterone (1, 3 or 10 mg/kg, Sigma-Aldrich) was first dissolved in 100% ethanol and subsequently diluted in saline to get a 5% ethanol solution, and the control group was injected with a vehicle containing 5% ethanol in saline. Doses of yohimbine and corticosterone were selected based on previous studies (Chapter 2; Cai et al., 2006; Song et al., 2020). For the immunohistochemical experiments, we used only the behaviorally effective dose of yohimbine (1 mg/kg) and corticosterone (3 mg/kg), and both drugs were dissolved in a vehicle containing 5% ethanol in saline, and controls were injected with this vehicle. Drugs were administered intraperitoneally, in a volume of 0.01 mL/g body weight, immediately after the training session. Drug solutions were prepared freshly before each experiment.

Immunohistochemistry

Mice were anesthetized with an overdose of sodium pentobarbital (40-50 mg/kg) 1 h after training and drug treatment, followed by transcardial perfusion with 10 mL of ice-cold phosphate-buffered saline (PBS) and 10 mL of ice-cold 4% paraformaldehyde (PFA) (pH 7.4). Brains were extracted, post-fixed in 4% PFA in 0.1 M PBS (pH 7.4) for 24 h, and then transferred to a 30% sucrose solution in 0.1 M PBS for 4 days at 4 °C. Coronal slices of 30 µm thickness were cut on a cryostat, collected in 0.1 M PBS with 0.1% sodium azide, and stored at 4 °C. Three to four sections of the hippocampus (anteroposterior, -1.58 to -2.06 mm from Bregma) of each animal were selected according to the Franklin and Paxinos mouse brain atlas (Franklin & Paxinos, 2007). Sections were rinsed in 0.5% Triton in PBS for 30 min at room temperature (RT), washed three times in PBS for 10 min per wash, and then blocked in 5% Normal Donkey Serum (NDS, Jackson ImmunoResearch Laboratories) and 1% Bovine Serum Albumin (BSA, Thermo Scientific) in PBS for 1 h at RT. Next, sections were incubated with primary antibodies (c-Fos; guinea pig anti-c-Fos, 1:750, #226 004 Synaptic Systems, glutamic acid decarboxylase 67 (GAD67); mouse anti-GAD67, 1:500, #MAB5406-25ug Sigma-Aldrich) in PBS with 2% NDS and 0.1% acetylated BSA (BSA-c, Aurion) overnight at RT. Afterwards, sections were washed three times in PBS for 10 min per wash, followed by incubation with fluorophore-conjugated secondary antibodies donkey anti-guinea pig Alexa Fluor 647 (1:750, #706-605-148 Jackson ImmunoResearch) and donkey anti-mouse Alexa Fluor 488 (1:500, #A21202 Invitrogen) in PBS with 2% NDS and 0.1% BSA-c for 3 h at RT. All procedures starting from the secondary antibody incubation onwards were performed in the dark. Subsequently, sections were incubated with 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI, 1:5,000, #62248 Thermo Scientific) in PBS with 0.1% BSA-c for 15 min, then washed three times in PBS for 10 min per wash, mounted on gelatin-coated slides, left to dry, and coverslipped with Fluorsave mounting medium (Sigma-Aldrich). The slices were stored at 4 °C in the dark.

Imaging and quantification

Images were acquired on an Automated High-Content Fluorescence Microscope (Leica, DMI 6000B, Germany) with 20x magnification. The regions of interest were identified with a stereotactic mouse brain atlas (Franklin & Paxinos, 2007). The hippocampus was divided into its four main subregions and c-Fos expression in the cell layers analyzed: the granule cell layer of the dentate gyrus dorsal blade (dDG), granule cell layer of the dentate gyrus ventral blade (vDG), pyramidal cell layer of the cornu ammonis 3 (CA3) and cornu ammonis 1 (CA1). For the analysis of c-Fos and GAD67 double-positive neurons, we additionally looked in the *striatum radiatum* of the CA3 (CA3sr) and CA1 (CA1sr), i.e., a main regulatory site of activity within the pyramidal cell layers. Using ImageJ software (Rueden et al., 2017), the surface area of each subregion was assessed, and the number of c-Fos-positive and GAD67-positive cells and double-positive neurons was counted manually by a researcher blinded to the treatment condition, and then converted to

number of cells per mm^2. Relative GABAergic activity was calculated as the number of neurons showing co-localization of c-Fos and GAD67, expressed as the percentage of the total number of GAD67-positive neurons.

Statistics

Data are expressed as mean ± SEM. Statistical analyses were performed using IBM SPSS statistics version 25. Total object exploration time during the training and testing session and the DI% were analyzed with two-way ANOVAs with drug treatment (saline, yohimbine 0.3, 1, or 3 mg/kg, and vehicle, corticosterone 1, 3, or 10 mg/kg, respectively), and habituation condition (different vs same) as between-subject parameters. When appropriate, Tukey *post hoc* analyses were used to determine the source of the significance. One-sample *t*-tests were used to determine whether the DI% was different from zero (i.e., chance level) and thus whether learning had occurred.

Similarly, immunohistochemistry data for the hippocampal subregions was analyzed by two-way ANOVAs, with drug treatment (vehicle, yohimbine 1 mg/kg, corticosterone 3 mg/kg) and habituation condition (different, same) as between-subject variables. Hippocampal subregions were analyzed in separate models based on their differential role in memory processing (Kesner & Rolls, 2015). Significant effects of drug treatment were followed up by tests for yohimbine and corticosterone treatment separately. *Post hoc* independent-samples *t*-tests between appropriate groups were conducted to determine the source of significance. Finally, Pearson correlations were calculated to determine correlations between c-Fos expression data across hippocampal subregions. For all statistical tests, $p < 0.05$ was accepted for statistical significance, except for the Pearson correlations where we kept a more stringent threshold of $p < 0.01$. The figures only display significant *post hoc* comparisons unless stated otherwise. The number of mice per group is indicated in the figure legends.

Results

Posttraining noradrenergic stimulation dose-dependently enhances object location memory independent of the habituation condition

In the first experiment, we examined whether systemic administration of the noradrenergic stimulant yohimbine (0.3, 1 or 3 mg/kg) following training would enhance object location memory 24 h later and whether this effect depends on the habituation condition (being the same or different to the training context). Total object exploration time during training was different for the two habituation conditions ($F_{(1,110)} = 17.22$, $p < 0.001$), with animals showing more object exploration in the different habituation condition. However, critically, object exploration time did not differ between drug

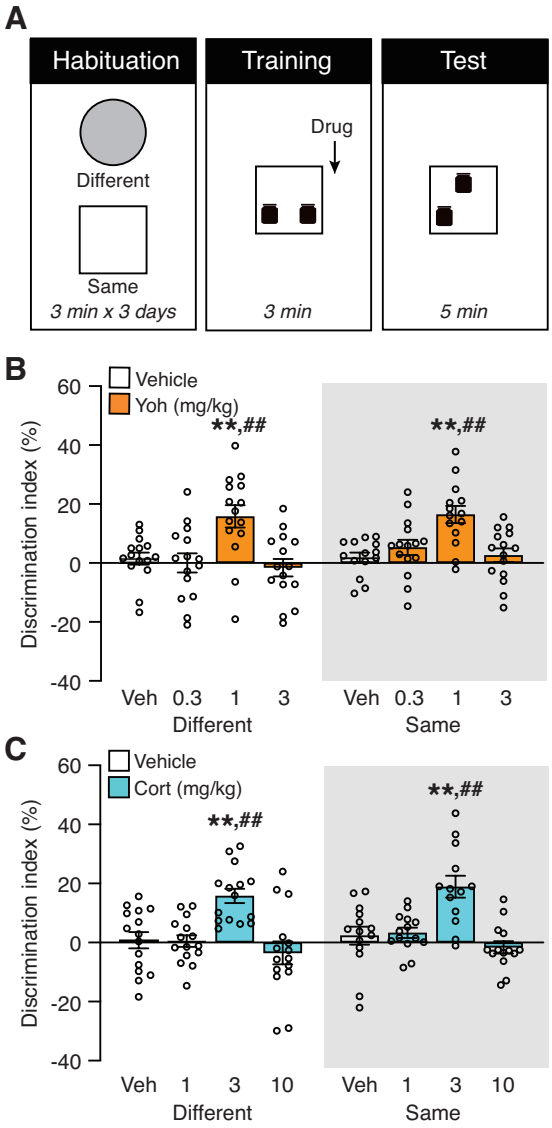

Figure 1. Effect of posttraining yohimbine and corticosterone administration on object location memory under the different and same habituation conditions.

A. Experimental design of the object location task. Mice were initially habituated to the training context (Same) or a different context for 3 min on three consecutive days. Afterwards, they were trained on the object location task for 3 min during which they could freely explore two identical objects, followed immediately by an intraperitoneal injection of yohimbine (YOH, 0.3, 1 or 3 mg/kg), corticosterone (CORT, 1, 3, or 10 mg/kg) or their respective vehicle solutions. Retention was tested 24 h later in a 5-min retention test during which one of the training objects was moved to a novel location. **B.** Posttraining yohimbine administration dose-dependently enhanced object location memory in both habituation conditions. Different habituation condition, VEH: $n = 15$, YOH 0.3 mg/kg: $n = 15$, YOH 1 mg/kg: $n = 15$, YOH 3 mg/kg: $n = 15$; same habituation condition, VEH: $n = 14$, YOH 0.3 mg/kg: $n = 15$, YOH 1 mg/kg: $n = 14$, YOH 3 mg/kg: $n = 15$. **C.** Posttraining corticosterone administration dose-dependently enhanced object location memory in both habituation conditions. Different habituation condition, VEH: $n = 15$, CORT 1 mg/kg: $n = 15$, CORT 3 mg/kg: $n = 15$, CORT 10 mg/kg: $n = 15$; same habituation condition, VEH: $n = 14$, CORT 1 mg/kg: $n = 14$, CORT 3 mg/kg: $n = 13$, CORT 10 mg/kg: $n = 14$. Data are shown as mean ± SEM, dots represent individual data points. $^{**}p < 0.01$ vs. VEH; $^{\#\#}p < 0.01$ vs. chance level.

treatment groups ($F_{(3,110)}$ = 0.54, p = 0.66), and was not modulated by a drug treatment X habituation condition interaction ($F_{(3,110)}$= 0.38, p = 0.77, Table I). Total object exploration time during the 24-h retention test also differed across the two habituation conditions ($F_{(1,110)}$ = 5.34, p = 0.02), with animals showing more object exploration in the same habituation condition. Again, total object exploration time did not differ between drug treatment groups ($F_{(3,110)}$ = 1.96, p = 0.12), and was not influenced by a drug treatment X habituation condition interaction ($F_{(3,110)}$ = 1.47, p = 0.23, Table I).

Table I. Object exploration time during training and the retention test

Treatment group	Habituation condition	Training (s)	Retention test (s)
VEH (n = 15)	Different	7.5 ± 0.9	8.3 ± 1.8
YOH 0.3 mg/kg (n = 15)	Different	7.0 ± 1.5	7.3 ± 2.2
YOH 1 mg/kg (n = 15)	Different	7.1 ± 1.0	7.0 ± 2.1
YOH 3 mg/kg (n = 15)	Different	7.0 ± 0.9	7.4 ± 1.8
VEH (n = 14)	Same	5.8 ± 1.8	9.7 ± 4.2
YOH 0.3 mg/kg (n = 15)	Same	5.3 ± 2.1	7.6 ± 3.5
YOH 1 mg/kg (n = 14)	Same	6.2 ± 2.5	8.3 ± 2.1
YOH 3 mg/kg (n = 15)	Same	5.8 ± 2.6	7.7 ± 2.7
VEH (n = 15)	Different	5.9 ± 1.1	6.4 ± 1.6
CORT 1 mg/kg (n = 15)	Different	5.7 ± 1.2	7.4 ± 1.5
CORT 3 mg/kg (n = 15)	Different	5.8 ± 1.3	8.3 ± 2.1
CORT 10 mg/kg (n = 15)	Different	6.0 ± 0.7	7.9 ± 3.0
VEH (n = 14)	Same	7.0 ± 2.4	7.7 ± 2.4
CORT 1 mg/kg (n = 14)	Same	6.1 ± 1.6	7.8 ± 2.7
CORT 3 mg/kg (n = 13)	Same	5.1 ± 2.1	7.4 ± 3.3
CORT 10 mg/kg (n = 14)	Same	7.0 ± 2.4	7.9 ± 3.4

Data represent mean ± SEM

At the 24-h retention test, a two-way ANOVA for the DI% indicated a significant main effect of drug treatment ($F_{(3,110)}$ = 13.66, p < 0.001), but no effect of habituation condition ($F_{(1,110)}$ = 1.78, p = 0.19) or drug treatment X habituation condition interaction ($F_{(3,110)}$ = 0.40, P = 0.75, Figure 1B). Tukey's *post hoc* analyses revealed that, in both habituation conditions, the 1 mg/kg yohimbine group had a significantly greater DI% compared to the corresponding saline group (different: $t_{(28)}$ = -3.31, p = 0.003, same: $t_{(26)}$ = -4.34, p < 0.001). Further, one-sample t-tests indicated that, in both habituation conditions, the 1 mg/kg yohimbine groups showed successful object location memory recall, with the DI%

being significantly greater than zero (different: $t_{(14)} = 4.16$, $p = 0.001$, same: $t_{(13)} = 5.64$, $p < 0.001$). In contrast, mice treated with saline or the other doses of yohimbine in both habituation conditions did not show memory (different: saline: $t_{(14)} = 0.43$, $p = 0.46$, 0.3 mg/kg: $t_{(14)} = 0.73$, $p = 0.48$, 3 mg/kg: $t_{(14)} = -0.53$, $p = 0.61$; same: $t_{(13)} = 1.15$, $p = 0.27$, 0.3 mg/kg: $t_{(14)} = 2.05$, $p = 0.06$, 3 mg/kg: $t_{(14)} = 1.15$, $p = 0.27$). These findings indicate that yohimbine dose-dependently enhanced memory for the location of the objects in both habituation conditions.

Posttraining corticosterone dose-dependently enhances object location memory in both habituation conditions

In the second experiment, we examined whether systemic administration of corticosterone (1, 3 or 10 mg/kg) posttraining would enhance object location memory and whether this effect depends on the habituation condition. Total object exploration time during training did not differ between the two habituation conditions ($F_{(1,108)} = 2.28$, $p = 0.13$). Critically, total object exploration time also did not differ between drug treatment groups ($F_{(3,108)} = 2.64$, $p = 0.053$) and was not modulated by a drug treatment X habituation condition interaction ($F_{(3,108)} = 1.90$, $p = 0.14$, Table I). Total object exploration time during the 24-h retention test also did not differ between the drug treatment groups ($F_{(3,107)} = 0.64$, $p = 0.59$) or habituation conditions ($F_{(1,107)} = 0.22$, $p = 0.64$). Moreover, the interaction between the two factors did not influence total object exploration time during testing ($F_{(3,107)} = 0.99$, $p = 0.40$, Table I).

At the 24-h retention test, a two-way ANOVA for the DI% indicated a significant main effect of drug treatment ($F_{(3,107)} = 19.35$, $p < 0.001$), but no effect of habituation condition ($F_{(1,107)} = 1.40$, $p = 0.24$) or drug treatment X habituation condition interaction ($F_{(3,107)} = 0.03$, $p = 0.99$, Figure 1C). Tukey's *post hoc* analyses revealed that, in both habituation conditions, the 3 mg/kg corticosterone group had a significantly greater DI% compared to the vehicle group (different: $t_{(28)} = 4.08$, $p < 0.001$, same: $t_{(25)} = 3.49$, $p = 0.002$), and showed successful memory recall in both habituation conditions, with the DI% being significantly greater than zero (different: $t_{(14)} = 6.53$, $p < 0.001$, same: $t_{(12)} = 5.11$, $p < 0.001$). In contrast, mice treated with vehicle or the other doses of corticosterone in both habituation conditions did not show object location memory (different: vehicle: $t_{(14)} = 0.29$, $p = 0.77$, 1 mg/kg: $t_{(14)} = 0.26$, $p = 0.80$, 10 mg/kg: $t_{(14)} = -0.91$, $p = 0.38$; same: vehicle: $t_{(13)} = 0.77$, $p = 0.46$, 1 mg/kg: $t_{(13)} = 0.26$, $p = 0.80$, 10 mg/kg: $t_{(13)} = -0.76$, $p = 0.46$). These findings indicate that corticosterone dose-dependently enhanced memory for the location of the objects in both habituation conditions.

Effect of posttraining noradrenergic stimulation and corticosterone administration on neuronal activity in the hippocampus in the two habituation conditions

To examine the effect of posttraining yohimbine and corticosterone treatment on neuronal activity in the hippocampus, we assessed the number of neurons expressing the immediate-early gene c-Fos within the hippocampal subregions 1 h after training and drug treatment in the two habituation conditions (Figure 2A). Only the behaviorally effective dosage of yohimbine (1 mg/kg) and corticosterone (3 mg/kg) were used here and compared to vehicle control. To assess GABAergic activity, co-expression of c-Fos with the GABAergic cell marker GAD67 was assessed. Total counts on GAD67-expressing cells can be found in Figure S1.

Total object exploration times during the training session showed no differences for the drug treatment groups ($F_{(2,47)} = 2.06$, $p = 0.14$), habituation conditions ($F_{(1,47)} = 2.77$, $p = 0.10$) or drug treatment X habituation condition interaction ($F_{(2, 47)} = 0.97$, $p = 0.39$, Table II).

Table II. Object exploration time during training of the experimental groups used for immunohistochemistry

Treatment group	Habituation condition	Training (s)
VEH ($n = 8$)	Different	5.0 ± 2.4
YOH 1 mg/kg ($n = 10$)	Different	7.2 ± 1.9
CORT 3 mg/kg ($n = 8$)	Different	6.2 ± 1.9
VEH ($n = 9$)	Same	5.2 ± 1.5
YOH 1 mg/kg (n = 9)	Same	5.8 ± 1.9
CORT 3 mg/kg ($n = 10$)	Same	6.0 ± 2.8

Data represent mean ± SEM

In the dDG, a two-way ANOVA for the number of c-Fos-expressing cells revealed no significant effects of drug treatment ($F_{(2,47)} = 1.77$, $p = 0.18$), habituation condition ($F_{(1,47)} = 3.77$, $p = 0.06$), or drug treatment X habituation condition interaction effect ($F_{(2,47)} = 1.63$, $p = 0.21$, Figure 2B). Similarly, in the vDG, no significant main effect of drug treatment ($F_{(2,47)} = 0.82$, $p = 0.45$) or drug treatment X habituation condition interaction effect ($F_{(2,47)} = 1.99$, $p = 0.15$) was observed, but there was a significant effect of habituation condition ($F_{(2,47)} = 5.26$, $p = 0.03$), which was caused by fewer c-Fos-expressing cells in the same habituation condition. In the CA3, we observed a significant main effect of drug treatment ($F_{(2,48)} = 3.89$, $p = 0.03$), but no effect of habituation condition ($F_{(2,48)} = 0.81$, $p = 0.45$) or drug treatment X habituation condition interaction $F_{(2,48)} = 2.48$, $p = 0.12$). Follow-up analyses

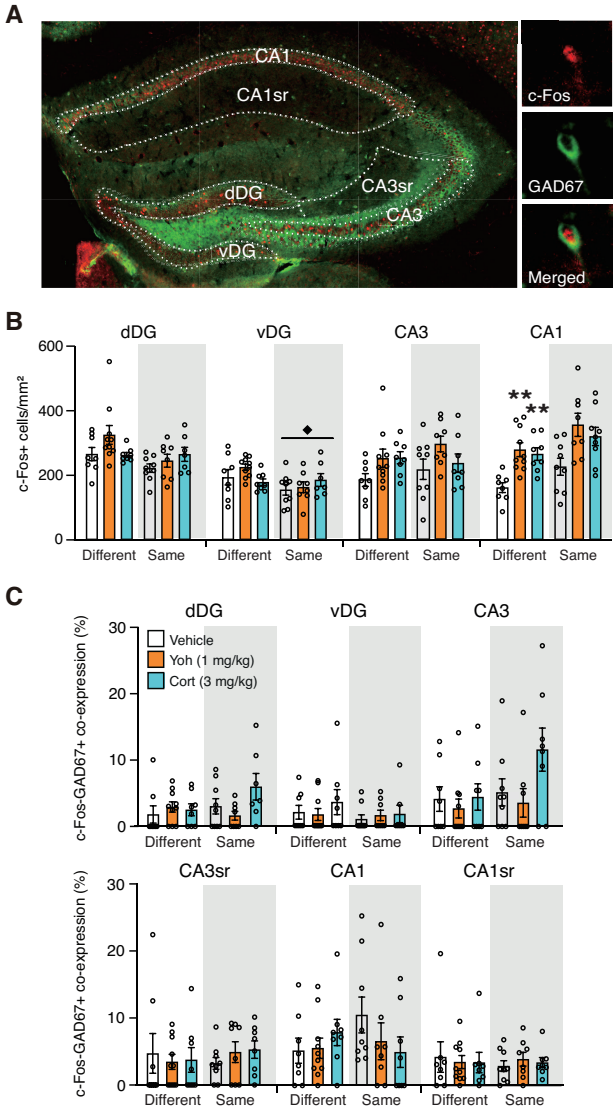

Figure 2. Effect of posttraining yohimbine and corticosterone administration on neuronal activity in the hippocampus in the two habituation conditions.

A. Diagram illustrating the different regions of interest: dorsal blade of the dentate gyrus granule cell layer (dDG), ventral blade of the dentate gyrus granule cell layer (vDG), CA3 pyramidal cell layer (CA3), CA3 *stratum radiatum* (CA3sr), CA1 pyramidal cell layer (CA1), CA1 *stratum radiatum* (CA1sr). The areas drawn show the exact regions in which the number of c-Fos-expressing cells and c-Fos+GAD67-expressing cells were counted. **B.** Posttraining yohimbine and corticosterone administration both increased the number of c-Fos-expressing cells in the CA1 region of the hippocampus. Moreover, yohimbine administration increased the number of c-Fos-expressing cells in the hippocampal CA3, but *post hoc* comparisons for the two habituation conditions failed to reach significance. Different habituation condition, VEH: $n = 8$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 8$; same habituation condition, VEH: $n = 9$, YOH 1 mg/kg: $n = 9$, CORT 3 mg/kg: $n = 10$. **C.** Posttraining yohimbine and corticosterone administration did not affect relative c-Fos-GAD67 co-expression in the hippocampus. Different habituation condition, VEH: $n = 8$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 8$; same habituation condition, VEH: $n = 9$, YOH 1 mg/kg: $n = 9$, CORT 3 mg/kg: $n = 10$. Data are shown as mean ± SEM, dots represent individual data points. $^{**}p < 0.01$ vs. VEH; $^{\blacklozenge}p < 0.05$ different vs. same habituation group.

indicated that the drug treatment effect was caused by yohimbine ($F_{(1,33)}$ = 7.03, p = 0.01) and not corticosterone ($F_{(1,31)}$ = 2.65, p = 0.11), increasing the number of c-Fos-expressing cells compared to the vehicle condition. *Post hoc* analyses for yohimbine in the two habituation conditions separately failed to reveal significance (different: $t_{(16)}$ = 1.84, p = 0.08, same: $t_{(17)}$ = 1.93, p = 0.07). Similarly, in the CA1, we found a main effect of drug treatment ($F_{(2,48)}$ = 5.40, p = 0.008), with no effect of habituation condition ($F_{(1,48)}$ = 3.78, p = 0.06) or drug treatment X habituation condition interaction effect ($F_{(2,48)}$ = 0.21, p = 0.81). Follow-up analyses indicated that both yohimbine ($F_{(1,33)}$ = 9.49, p = 0.004) and corticosterone ($F_{(1,31)}$ = 6.92, p = 0.01) significantly increased the number of c-Fos-expressing cells compared to the vehicle condition. *Post hoc* tests revealed a significant effect of yohimbine in the different ($t_{(16)}$ = 4.24, p = 0.001), but not the same habituation condition ($t_{(17)}$ = 1.60, p = 0.13). Corticosterone similarly increased c-Fos expression in the different ($t_{(14)}$ = 4.20, p = 0.001), but not the same habituation condition ($t_{(17)}$ = 1.11, p = 0.28).

Next, we continued by testing whether the observed drug-induced differences in overall c-Fos expression were related to alterations in relative GABAergic activity. In none of the hippocampal subregions, we found a significant effect of drug treatment (all p's > 0.37), habituation condition (all p's > 0.07) or drug treatment X habituation condition interaction effect (all p's > 0.12) on relative GABAergic activity (Figure 2C). Thus, relative GABAergic activity in the hippocampus after training on the object location task was not affected by either yohimbine or corticosterone administration.

We next investigated whether drug treatment and habituation condition might also influence correlations in activity across the hippocampal subregions as a proxy for their functional connectivity. Therefore, we calculated Pearson correlations for the number of c-Fos-expressing cells between each of the four hippocampal subregions for each of the three drug treatment groups and two habituation conditions (Figure 3). In the different habituation condition, we found no significant correlations for any of the drug treatment groups. In the same habituation condition, we observed a positive correlation between the number of c-Fos-expressing cells in the dDG and CA3 (r = 0.84; p = 0.003), and between the CA1 and CA3 (r = 0.79; p = 0.006) in mice treated with vehicle, and further a positive correlation between the number of c-Fos-expressing cells in the dDG and CA1 (r = 0.92; p = 0.0004) in mice treated with yohimbine. No significant correlations were found in animals treated with corticosterone.

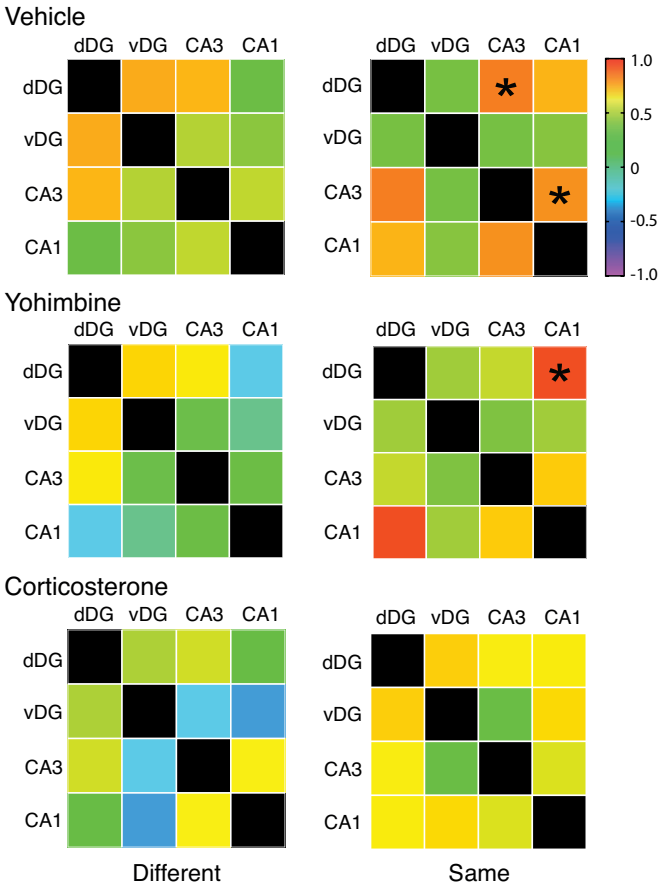

Figure 3. Across-animal correlations in the number of c-Fos+ cells in the cell layers of the hippocampal subregions.

No significant correlations across subregional hippocampal activity following training were observed in the different habituation condition. In the same habituation condition, the vehicle group displayed a significant positive correlation between the dDG and CA3 and between the CA3 and CA1, whereas the yohimbine group showed a significant correlation between the dDG and CA1. Different habituation condition, VEH: $n = 8$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 8$; same habituation condition, VEH: $n = 9$, YOH 1 mg/kg: $n = 9$, CORT 3 mg/kg: $n = 10$. *$p < 0.01$

Thus, we found that both yohimbine and corticosterone treatment induced a similar increase in the number of c-Fos-positive neurons within the CA1 cell layer (reflecting mainly glutamatergic activity) during the post-learning consolidation period. Although this effect only reached significance in the different habituation condition, a similar trend was seen after yohimbine and corticosterone treatment in the same habituation condition. Yohimbine also showed a main effect on increasing c-Fos expression in the CA3 cell layer, but *post hoc* analyses failed to reach significance. Yohimbine or corticosterone

treatment did not affect relative GABAergic activity in either habituation condition. Moreover, none of the drug treatment groups displayed a positive correlation in neural activity between the dDG and CA3. These findings indicate that both yohimbine and corticosterone treatment enhanced hippocampal activity and thus fit with the similar effects of yohimbine and corticosterone on enhancing object location memory.

Discussion

In this study, we examined the effect of posttraining administration of the noradrenergic stimulant yohimbine and corticosterone on spatial memory in an object location task, and whether prior habituation to the training context would alter these stress hormone effects. The interest from this question stems from the findings of Chapter 2 indicating that yohimbine enhances, whereas corticosterone impairs, episodic-like memory in an object-in-context task (when mice were not previously habituated to the training context). We proposed that these two stress hormones induce opposite effects on object-in-context memory by an opposite regulation of a hippocampal mechanism that facilitates the separation of overlapping memory representations of the two training events. To test this hypothesis, in the present study yohimbine and corticosterone were administered after training on an object location task, in which memory performance also critically depends on the hippocampus (Balderas et al., 2008; Roozendaal et al., 2010; Barsegyan et al., 2019), but which comprises a single event. In support of our hypothesis, we found that both yohimbine and corticosterone enhanced object location memory, independent of the habituation condition. Further, the memory-enhancing effects of both yohimbine and corticosterone were paralleled by an increased CA1 pyramidal cell activity after the training session, whereas yohimbine additionally induced an increase in CA3 pyramidal cell activity.

Our finding that both yohimbine and corticosterone administration enhanced object location memory is consistent with previous findings. Systemic administration of both epinephrine (Jurado-Berbel et al., 2010) and yohimbine (Song et al., 2021) to mice after object training was previously shown to enhance object location memory. Interestingly, other work has shown that object training induces a significant increase in norepinephrine levels in the hippocampus and other structures of the medial temporal lobe in adult, but not juvenile, rats, and that yohimbine administration to juvenile rats increased norepinephrine levels in the hippocampus and enhanced their object location memory (Nirogi et al., 2012). Corticosterone administration to mice or rats was also shown to enhance object location memory (Roozendaal et al., 2010). Moreover, consistent with extensive evidence of synergistic actions between both stress hormone systems (Okuda et al., 2004; Roozendaal & McGaugh, 2011), previous findings

indicated that the glucocorticoid and noradrenergic systems interact in regulating object location memory, likely by shared neural substrates. The enhancing effect of systemic corticosterone administration on object location memory was shown to critically depend on an activation of the cAMP response element-binding (CREB) protein pathway within the hippocampus (Roozendaal et al., 2010), and noradrenergic stimulation and emotional arousal are known to induce an activation of the CREB pathway (Okuda et al., 2004). Another study indicated that local glucocorticoid administration into the prefrontal cortex also enhanced object location memory as well as increased c-Fos expression within the CA1 region during the post-learning consolidation period (Barsegyan et al., 2019). Interestingly, concurrent administration of the b-adrenoceptor antagonist propranolol into the basolateral amygdala blocked this glucocorticoid effect on both object location memory and posttraining hippocampal activity (Barsegyan et al., 2019). These findings thus also demonstrate that the effects of these two stress hormones on object location memory are not limited to direct actions on the hippocampus but require the participation of other brain regions as well.

Consistent with the comparable effects of yohimbine and corticosterone administration on enhancing object location memory, we found that both treatments increased neuronal activity within the CA1 region of the hippocampus during the post-learning consolidation period. Both yohimbine and corticosterone selectively increased total c-Fos expression within the CA1 pyramidal cell layer (reflecting glutamatergic activity), but had no effect on GABAergic activity, thus suggesting that yohimbine and corticosterone both increase CA1 pyramidal cell activity after object location training. Extensive evidence has indicated that pyramidal cells within the CA1 are critically involved in spatial navigation and spatial memory. Seminal studies using recordings in freely moving rats have provided striking evidence that pyramidal cells in CA1 fire specifically in certain regions (place fields) of the local environment (O'Keefe & Dostrovsky, 1971; O'Keefe, 1976), and that the hippocampus participates in forming spatial representations (O'Keefe & Dostrovsky, 1971; O'Keefe & Conway, 1978; Muller & Kubie, 1987; Wilson & McNaughton, 1993; Henriksen et al., 2010). Moreover, selective inactivation of the hippocampal CA1 region by administration of the local anesthetic lidocaine before object training was previously found to impair object location memory (Assini et al., 2009). Yohimbine additionally increased CA3 pyramidal cell activity, which is in agreement with the findings of several studies indicating the CA3 subregion of the hippocampus may support mnemonic processes critical to the formation and retrieval of spatial memories (Gilbert & Brushfield, 2009).

We further found that the effect of yohimbine and corticosterone on enhancing object location memory was not affected by prior habituation to the training context. However, context habituation seemed to reduce the effect of yohimbine and corticosterone in increasing neuronal activity within the CA1. This latter finding is consistent with that of

a previous study indicating that administration of the noradrenergic agonist clenbuterol into the basolateral amygdala after object training enhanced hippocampal expression of the immediate early gene activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) of rats that had not received any habituation to the training context, but that the same clenbuterol administration did not increase Arc expression in the hippocampus of rats that had received extensive habituation prior to the training (McReynolds et al., 2014). Arc protein expression in the hippocampus plays a functional role in long-term plasticity and memory (Guzowski et al., 2000; McIntyre et al., 2005). Interestingly, similar to our observations on yohimbine administration, clenbuterol administration to either non-habituated or well-habituated rats enhanced memory of the object training. Thus, these findings support the view that norepinephrine, and possibly corticosterone, increases neuronal activity within the CA1 particularly in novel situations when endogenous emotional arousal is elevated. Alternatively, the habituation to the training context could have limited the requirement for long-lasting changes in the hippocampus following the object exposure trial for the establishment of a memory-enhancing effect.

Most importantly, the present findings, together with those of Chapter 2, indicate that the effects of yohimbine and corticosterone administration on hippocampus-dependent memory as well as hippocampal activity are critically dependent on the specific learning task. Whereas yohimbine enhanced both object-in-context and object location memory, corticosterone impaired object-in-context memory, but enhanced object location memory. These opposite effects of corticosterone administration on memory for these two tasks was paralleled by opposite effects on posttraining neuronal activity within the CA1 region. Corticosterone administration increased GABAergic activity within the CA1 after training on the object-in-context task (Chapter 2), whereas it increased CA1 pyramidal cell activity when administered after object location training. These findings thus provide strong support for our hypothesis that yohimbine and corticosterone induce opposite effects on hippocampus-dependent memory and hippocampal activity after training on a learning task when overlapping information of two similar events is present, but that they induce similar effects on hippocampus-dependent memory when the training comprises a single event and no separation of memory representations is needed. Our hypothesis that the opposite effects of yohimbine and corticosterone on object-in-context memory are caused by an opposite influence on facilitating either a separation or linking of memory of the two training events, respectively, is supported by our finding that yohimbine and corticosterone administration after training on the object-in-context task induced opposite effects on the strength of the correlation in activity between the dDG and CA3 (Chapter 2), a pathway critically involved in pattern separation (Rolls, 1989; Mizumori et al., 1990; Rolls, 1996; Gilbert et al., 2001; Leutgeb et al., 2007), but that these stress hormones did not affect the strength of this correlation when administered after a single object exposure. Similarly, we found previously that

norepinephrine administration selectively influenced a pattern separation process within the dDG after training on the dual-event inhibitory avoidance task, but that it did not recruit this mechanism within the dDG when administered after training on a single-event inhibitory avoidance task (Atucha et al., under revision).

In conclusion, the present findings indicate that both noradrenergic and glucocorticoid activity enhance spatial memory on an object location task independent of the prior habituation condition and that this effect was paralleled by an increased neuronal activity within the CA1 during the post-learning consolidation phase. Together with the findings of Chapter 2, these findings provide support for the view that yohimbine and corticosterone induce opposite effects on episodic-like memory when there is a need to separate overlapping memory representations of multiple training events specifically.

Acknowledgment

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Supplementary materials

Effect of posttraining noradrenergic stimulation and corticosterone administration on the number of GAD67-expressing cells in the hippocampus in the two habituation conditions

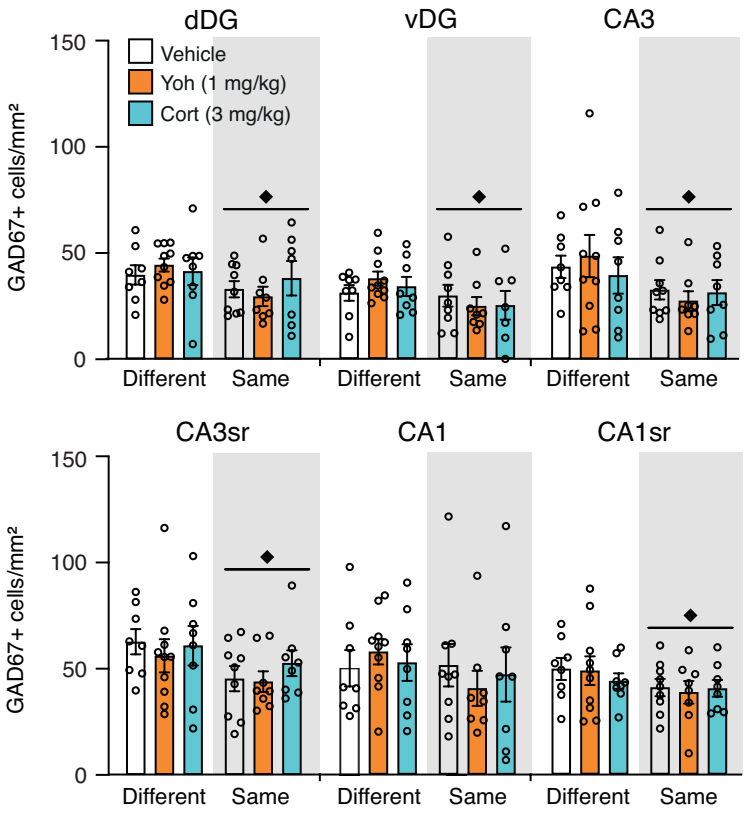

Figure S1. Effect of posttraining yohimbine and corticosterone administration on the number of GAD67-expressing cells in the hippocampus in the two habituation conditions.

Posttraining administration of yohimbine and corticosterone treatment did not affect the total number of GAD67-expressing cells in the two habituation conditions. The number of GAD67-expressing cells was overall lower in the same habituation condition compared to the different habituation condition in nearly all hippocampal subregions. Different habituation condition, VEH: $n = 8$, YOH 1 mg/kg: $n = 10$, CORT 3 mg/kg: $n = 8$; same habituation condition, VEH: $n = 9$, YOH 1 mg/kg: $n = 9$, CORT 3 mg/kg: $n = 10$. Data are shown as mean ± SEM, dots represent individual data points. ◆$p < 0.05$ different vs. same habituation condition.

In the dDG, the total number of GAD67-expressing neurons was not affected by drug treatment ($F_{(2,47)}$ = 0.07, p = 0.94) or drug treatment X habituation condition interaction ($F_{(2,47)}$ = 0.68, p = 0.51). However, there was a significant main effect of habituation condition ($F_{(2,47)}$ = 5.59, p = 0.02), which was caused by fewer GAD67-expressing cells in the same habituation condition (Figure S1). Similarly, in the vDG, the total number of GAD67-expressing neurons was not affected by drug treatment ($F_{(2,47)}$ = 0.31, p = 0.74) or drug treatment X habituation condition interaction ($F_{(2,47)}$ = 0.53, p = 0.59), but was affected by habituation condition ($F_{(1,47)}$ = 5.83, p = 0.02), with again fewer GAD67-expressing cells in the same habituation condition. In the CA3 pyramidal cell layer, the total number of GAD67-expressing neurons was also not affected by drug treatment ($F_{(2,48)}$ = 0.05, p = 0.95) or a drug treatment X habituation condition interaction ($F_{(2,48)}$ = 0.56, p = 0.57). However, there was again a significant main effect of habituation condition ($F_{(1,48)}$ = 6.11, p = 0.02), caused by fewer GAD67-expressing cells in the same habituation condition. Similarly, in the CA3 *stratum radiatum*, the total number of GAD67-expressing neurons was not affected by drug treatment ($F_{(2,48)}$ = 0.12, p = 0.89), or drug treatment X habituation condition interaction ($F_{(2,48)}$ = 0.32, p = 0.73), but was affected by habituation condition ($F_{(1,48)}$ = 4.59, p = 0.04), which was caused by fewer GAD67-expressing cells in the same habituation condition. In the CA1 pyramidal cell layer, the total number of GAD67-expressing neurons was not affected by drug treatment ($F_{(2,48)}$ = 0.006, p = 0.99), habituation condition ($F_{(1,48)}$ = 1.33, p = 0.26) or drug treatment X habituation condition interaction ($F_{(2,48)}$ = 0.33, p = 0.72). In the CA1 *stratum radiatum*, the total number of GAD67-expressing neurons was also not affected by drug treatment ($F_{(2,48)}$ = 0.41, p = 0.68) or drug treatment X habituation condition interaction ($F_{(2,48)}$ = 0.13, p = 0.88), but was affected again by habituation condition ($F_{(1,48)}$ = 4.39, p = 0.04), with fewer GAD67-expressing neurons in the same habituation condition.

CHAPTER 5

General discussion

In this thesis, I investigated the hypothesis that the two stress hormones norepinephrine and corticosterone induce opposite effects on episodic-like memory on a hippocampus-dependent task that requires the separation of memory representations of multiple training events, but that norepinephrine and corticosterone induce similar effects on hippocampus-dependent memory under training conditions that do not require the separation of memory of different training events.

Numerous studies have demonstrated that both norepinephrine and corticosterone, in a predominantly synergistic fashion, mediate the effects of stress and emotional arousal on the strengthening of memory by facilitating neural plasticity and information storage processes in several brain regions (Roozendaal et al., 2002; Joëls et al., 2011; Roozendaal & McGaugh, 2011; Schwabe et al., 2012; de Quervain et al., 2017; Schwabe et al., 2022). Besides this memory-strengthening effect, stress and emotional arousal also influence several qualitative aspects of memory, like its accuracy, specificity and detailedness. However, the directionality of this modulation is still debated (Loftus, 1979; Payne et al., 2002; Talarico & Rubin, 2003; Kensinger et al., 2007b; Schwabe & Wolf, 2009; Rimmele et al., 2011; Segal et al., 2012). While the effects of stress and emotional arousal on such qualitative aspects of memory have been extensively studied in humans, until recently this topic had received much less attention in animal research, mainly due to the difficulty of assessing these aspects of memory with existing behavioral tasks. Recent studies using newly developed behavioral tasks for rodents have allowed for the investigation of stress and stress hormone effects on memory quality as well as the underlying neurobiological mechanisms. Interestingly, these studies suggest that the two stress hormones norepinephrine and corticosterone exert opposing effects on episodic-like specificity of memory on a dual-event inhibitory avoidance task (Atucha & Roozendaal, 2015; Roozendaal & Mirone, 2020) further experiments have suggested that norepinephrine enhances episodic-like specificity on this task by facilitating a hippocampal mechanism that supports the separation of memory of the two training events into two discrete memories (Atucha et al., under revision). However, it had not been investigated whether corticosterone impairs this episodic-like specificity by exerting an opposite influence on this hippocampal mechanism.

Summary of main findings

In **Chapter 2,** I examined the effect of norepinephrine and corticosterone on object-in-context memory, an episodic-like memory task in which two object presentation events during the training session are distinguished by the contexts in which they appear (Dix & Aggleton, 1999; Eacott & Norman, 2004; Barsegyan et al., 2014; Balderas et al., 2015). To manipulate the necessity of separating memory of the two training events, prior

to training mice received three days of habituation to either the same two contexts as those used for training or to two different contexts. In animals that were not familiarized habituated to the training contexts, I found that posttraining yohimbine administration dose-dependently enhanced object-in-context memory, whereas posttraining corticosterone administration impaired this memory. In contrast, both yohimbine and corticosterone administration were found to dose-dependently enhance object-in-context memory when mice were previously habituated to the training contexts.

I further examined the effect of yohimbine and corticosterone administration, and habituation condition, on hippocampal activity during the memory consolidation phase one hour after the training session. For this, I examined expression of the immediate early gene product c-Fos, as a molecular marker of recently activated cell (Minatohara et al., 2016), in both the total cell population as well as specifically in GABAergic neurons in the different hippocampal subregions. I found that yohimbine-treated animals of the different habituation condition displayed a positive correlation in neuronal activity between the dorsal blade of the dentate gyrus (dDG) and CA3 subregion of the hippocampus as well as an increased total neuronal activity within the hippocampal CA1 cell layer (reflecting mainly glutamatergic activity) during the posttraining consolidation period. Computational models and empirical studies have suggested the critical involvement of the dDG-CA3 pathway in pattern separation (Leutgeb et al., 2007; Yassa & Stark, 2011), a process essential for distinguishing similar memories (McHugh et al., 2007; A. M. Morris, 2011; E. Rolls, 2013). Further, the hippocampal CA1 region plays an important role in determining whether events experienced close in time become stored as distinct or overlapping memories (Vazdarjanova & Guzowski, 2004; Smith & Bulkin, 2014; Chowdhury et al., 2022). Corticosterone-treated animals of the different habituation condition did not show this correlation in neuronal activity between the dDG and CA3 or an increased total activity within the CA1, but rather displayed an increased GABAergic activity in the CA1 *stratum radiatum* and ventral blade of the DG (vDG). Prior habituation to the training contexts was generally associated with an absence of inter-subregion correlations of activity as well as an overall lower hippocampal neuronal activity posttraining. Thus, these findings provide support for the hypothesis that yohimbine enhanced object-in-context memory by facilitating a hippocampal mechanism that supports the separation of memory of the two training events into distinct memories, whereas corticosterone impaired this memory by suppressing this hippocampal mechanism. However, after context habituation, separate memories for the two training contexts may already have been formed (Young et al., 1994), making that both yohimbine and corticosterone induced similar effects on strengthening memory for the training objects *per se*, which does not depend on the hippocampus (Dees & Kesner, 2013).

In **Chapter 3**, I further tested the hypothesis that the effect of yohimbine on enhancing object-in-context memory of mice that were not habituated to the training contexts requires the hippocampus, but that prior habituation to the training contexts makes this effect hippocampus independent. Therefore, I employed a Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based approach to selectively silence the hippocampus during the training session and posttraining consolidation period. Yohimbine was administered to all mice immediately after the training. I found that hippocampal inactivation of mice that were not previously habituated to the training contexts prevented the yohimbine effect on enhancing object-in-context memory, while hippocampal inactivation of habituated mice did not have any effect. These findings thus provide causal evidence for the idea that prior context habituation renders the task independent from the hippocampus and that yohimbine under those conditions enhances object-in-context memory via an extra-hippocampal memory process.

In **Chapter 4**, I further investigated whether yohimbine and corticosterone solely induce opposite effects on hippocampus-dependent memory after training on a task that requires the separation of overlapping memory representations for multiple training events. Therefore, I investigated the effects of posttraining yohimbine and corticosterone administration on hippocampus-dependent memory for the spatial location of an object. Also in the object location task mice explore two objects in a certain context, but this memory task consists of only a single training event and thus there is no necessity of separating memory representations of different training events. Comparable to the procedure of the object-in-context task, mice were habituated to either the training context or a different context prior to training. Both yohimbine and corticosterone administered immediately after the training session enhanced object location memory, and prior habituation to the training context did not alter these effects. Further, I found that yohimbine or corticosterone administration after this single object exploration event did not affect dDG-CA3 correlated neuronal activity, but that both yohimbine and corticosterone induced a very similar increase in neuronal activity within the hippocampal CA1 cell layer during the post-learning consolidation phase.

Thus, these findings provide support for the view that norepinephrine and corticosterone induce opposite effects on episodic-like memory when there is a need to separate overlapping memory representations of multiple training events, but that these two stress hormones induce similar effects on hippocampus-dependent memory when the training conditions do not require such a separation of memories. In the following sections, I will discuss some of the most interesting findings in more details.

Episodic-like memory in the object-in-context and dual-event inhibitory avoidance task

In this thesis, I have used the object-in-context task to investigate the effect of posttraining yohimbine and corticosterone administration on episodic-like memory. In previous studies, our laboratory had used the dual-event inhibitory avoidance task to explore the impact of noradrenergic and glucocorticoid activity on episodic-like memory in rats. Yohimbine administration after training on the dual-event inhibitory avoidance task was also found to facilitate episodic-like specificity of memory, as reflected by longer retention latencies in the shock box, but shorter retention latencies in the non-shock box (Roozendaal & Mirone, 2020). In contrast, corticosterone administration impaired episodic-like specificity of memory, as reflected by longer retention latencies in both training boxes. These findings thus indicate very similar opposing effects of norepinephrine and corticosterone on episodic-like specificity of memory in both the object-in-context and dual-event inhibitory avoidance tasks. In both tasks, the animals are trained in two contexts close in time, and for each training context they need to form an association with a specific event. In the object-in-context task, each context exposure was associated with a specific set of objects whereas context exposure in the dual-event inhibitory avoidance task was associated with either the delivery or absence of footshock. Therefore, it is very likely that the opposite effects of norepinephrine and corticosterone on episodic-like memory found in both tasks are brought about by their influence on a common neural mechanism of either separating (enhancing memory specificity) or linking memory (impairing memory specificity) of the two training events.

In the present study, we decided to use the object-in-context task as preliminary findings in our lab indicated difficulties with training mice on the dual-event inhibitory avoidance task (Bahtiyar et al., unpublished findings). Moreover, a clear advantage of the object-in-context task over the dual-event inhibitory avoidance task is that both training events are of equal salience. This makes it possible to fully counterbalance the order of training on the two events, whereas on the dual-event inhibitory avoidance task the animals are always trained on the two events in a fixed order: They are trained first in the non-shock box and second in the shock box to prevent an influence of shock administration on new learning and behavior in the non-shock box. Therefore, the findings of the dual-event inhibitory avoidance task could not fully exclude the possibility that the drug administration might have preferentially enhanced memory of the event closest in time to the timepoint of drug administration. Although it should be mentioned that the order of training on the shock box and non-shock box was reversed in one experiment, and it was still found that yohimbine increased retention latencies in the shock box and decreased latencies in the non-shock box (Atucha et al. , under revision). Further, because training in the shock box is more salient than training in the non-shock box, it could also not be excluded that stress hormone administration selectively enhanced memory of the more

salient event, with the dominant representation of the shock box suppressing memory for the less salient representation of the non-shock box (Mather et al., 2016). However, and importantly, the two training events of the object-in-context task are of equal salience, and therefore this alternative explanation can be excluded. A disadvantage of the object-in-context task, however, is that it does not allow for a direct assessment of the strength of memory for the objects *per se*. However, successful discrimination of the acquired object-context associations necessitates also the formation of a memory for the training objects themselves.

Effects of norepinephrine and corticosterone on the dDG-CA3 pathway in regulating episodic-like specificity of memory

Extensive evidence indicates that the dDG-CA3 pathway is critically involved in pattern separation and the formation of distinct memories of related experiences (Leutgeb et al., 2007; Yassa & Stark, 2011). In Chapter 2, I found that the opposite effect of yohimbine and corticosterone administration on object-in-context memory of non-habituated mice was associated with an opposite regulation of correlated neuronal activity within the dDG-CA3 pathway 1 hour after the training session, implicating this pathway in mediating the opposing memory effects. In contrast, the enhancing effect of both yohimbine and corticosterone administration on object-in-context memory of habituated mice, which presumably already had formed separate memories of the two training contexts, was not associated with any change in dDG-CA3 correlated activity after the training session. The view that the dDG-CA3 pathway appears not to play a role in regulating stress hormone effects on object-in-context memory of habituated mice is further supported by the findings of Chapter 3 demonstrating that DREADD-based inactivation of the hippocampus during and after the training of habituated mice did not block the yohimbine effect on object-in-context memory. Also the memory-enhancing effect of either yohimbine or corticosterone administration on the object location task, which consists of only a single training event, was not associated with any change in dDG-CA3 correlated activity. Noteworthy, the only difference between training on the object location and object-in-context task is the existence of either one or two object exploration events. As such, my findings indicate that yohimbine and corticosterone administration selectively affect the dDG-CA3 pathway after training on a multi-event learning task that requires a separation of different memory representations, and further that yohimbine and corticosterone regulate this dDG-CA3 pathway in an opposite manner. In the sections below, I will discuss further how yohimbine and corticosterone might induce opposite effects on this pattern separation mechanism that result in either an enhancement or impairment of episodic-like specificity of memory, respectively.

Norepinephrine: The finding that yohimbine administration immediately after the training session dose-dependently enhanced object-in-context memory of mice that were not previously habituated to the training contexts is consistent with prior findings on the dual-event inhibitory avoidance task. Also there it was found that posttraining yohimbine administration enhanced memory specificity; yohimbine-treated mice displayed longer retention latencies in the shock box and shorter ones in the non-shock box (Roozendaal & Mirone, 2020). Similar behavioral effects on the dual-event inhibitory avoidance task were observed when norepinephrine was administered directly into the basolateral amygdala (BLA) (Atucha et al., 2017; Atucha et al., under revision). Preliminary findings further indicated that noradrenergic activation of the BLA induced memory specificity via a miR-134-regulated consolidation process within the dDG. Norepinephrine administration into the BLA after training on the dual-event inhibitory avoidance task induced a down-regulation of miR-134 within the dDG, which was found both sufficient to induce memory specificity by itself, and necessary for mediating the norepinephrine effect on memory specificity. It was further found that down-regulation of miR-134 had no effect on the strengthening of memory *per se*. Down-regulation of miR-134 elevated mRNA levels of both cAMP response element-binding (CREB) and brain-derived neurotrophic factor (BDNF) within the dDG; proteins that play critical roles in neural plasticity and the consolidation of pattern-separated memories (Bekinschtein et al., 2013; Mizuno et al., 2000; Silva et al., 1998; Bekinschtein et al., 2014). Several further experiments indicated that this norepinephrine-induced down-regulation of miR-134 within the dDG was only observed under training conditions that required the separation of memories for two training events. That is, norepinephrine administration into the BLA did not down-regulate miR-134 in untrained rats, if rats were trained in a single context or when they were trained twice in the same context (Atucha et al., under revision). This suggests that noradrenergic activity improves memory specificity by boosting pattern separation within the dDG; a process by which similar memory representations are stored in a distinct and non-overlapping (orthogonalized) manner to prevent memory interference and maintain the integrity of the distinct memories (Yassa & Stark, 2011).

Extensive research both in animal models and human neuroimaging studies has consistently identified the dDG as a key player in the mechanism of pattern separation (Clelland et al., 2009; Schreiber & Newman-Tancredi, 2014). The dDG receives non-spatial contextual information from the entorhinal cortex via the perforant path that is initially processed by a sparse group of granule cells. These granule cells create unique representations of mnemonic information, despite the high similarity of the input data (Clelland et al., 2009; Leutgeb et al., 2007; McHugh et al., 2007); a process that relies on

robust inhibition through GABAergic interneurons establishing local networks with excitatory granule cells (Acsády & Káli, 2007). This inhibitory control may promote sparse neural activity (Myers & Scharfman, 2009), which can enhance pattern separation (GoodSmith et al., 2017; Senzai & Buzsáki, 2017). Information within the dDG is then transmitted via the mossy fiber pathway to the CA3 region (Kesner, 2013). CA3 pyramidal neurons are thought to store the forwarded representations using auto-associative cellular networks, enabling memory retrieval when partial cues or incomplete elements of the memory are detected; a process known as pattern completion (Leutgeb et al., 2007) (Neunuebel et al., 2013). As such, dDG-CA3 connectivity has been associated with various cognitive functions, such as novelty detection (Yassa & Stark, 2011; Kesner, 2013).

My observation of increased correlated neuronal activity between the dDG and CA3 as induced by posttraining yohimbine administration under training conditions that require pattern separation further supports such a role. Noteworthy, similar to the down-regulation of miR-134 in the dDG, this yohimbine effect on dDG-CA3 correlated activity was not observed under conditions that do not require pattern separation; i.e., after training on the object-in-context task in familiarized contexts or after training on a single-event object location memory task. The finding that yohimbine increased dDG-CA3 correlated activity is also in line with other unpublished work from our lab on training-induced neuronal activity in the dual-event inhibitory avoidance task in rats (Roozendaal & Mirone, unpublished findings). There, analyses of the number of c-Fos-expressing cells following training and yohimbine administration (similar to our approach in Chapter 2) revealed a significant positive correlation in the number of cFos-expressing cells within the dDG and CA3, that was absent in vehicle-treated control rats (Figure 1B). Unlike our findings, that study also observed overall increases in training-induced neuronal activity in the dDG and CA3 following yohimbine administration (Figure 1A), but task-specific differences might be responsible for this.

As yohimbine was administered systemically in the work described in this thesis, we do not know where the elevated norepinephrine levels might have acted to influence dDG function and pattern separation. Our previous findings showing that local administration of norepinephrine into the BLA influences dDG function in regulating memory specificity (Atucha et al., under revision) indicates that noradrenergic actions in the BLA can indirectly influence dDG function. The BLA receives direct input of noradrenergic cell projections originating from the locus coeruleus (LC) (Schwarz et al., 2015; Kebschull et al., 2016). However, the dDG also receives dense, direct neuromodulatory input from the LC (Blackstad et al., 1967). The projections from the LC to the hippocampus exhibit differentiation, with notably dense inputs directed towards the dDG (Jones & Moore, 1977; Loy et al., 1980). Additionally, afferents from the LC terminate in the CA3 region (Loy et al., 1980), where mossy fibers originating from the dDG establish synapses. The

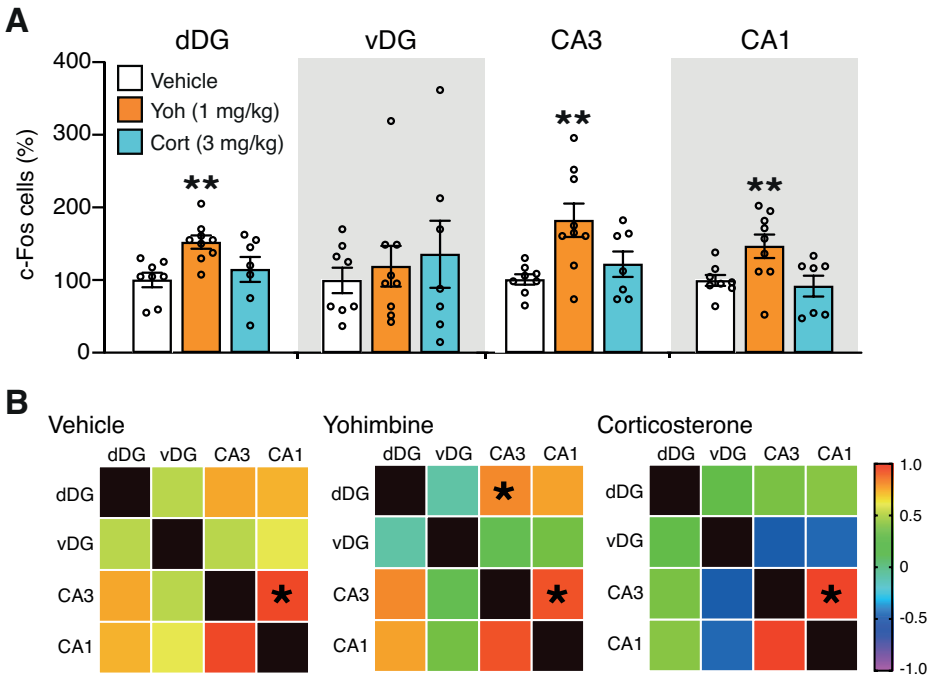

Figure 1. Effect of yohimbine and corticosterone administration after training on the dual-event inhibitory avoidance task on the number of c-Fos-expressing cells and their correlations across hippocampal subregions in rats.

A. Yohimbine administration after training on the dual-event inhibitory avoidance task (1-min interval between the two training events) increased the number of c-Fos-expressing cells (expressed as percentage relative to vehicle) in the dDG, CA3 and CA1 regions, but not in the vDG, assessed 1 hour later. Posttraining corticosterone administration did not significantly affect the number of c-Fos-expressing cells within the hippocampus. Vehicle: $n = 8$, Yoh 1 mg/kg: $n = 9$, Cort 3 mg/kg. Data are shown as mean ± SEM, dots represent individual data points. $^{**}p < 0.01$ vs. Vehicle. **B.** Across-animal correlations in the number of c-Fos-expressing cells in hippocampal subregions per treatment group. A significant positive correlation between the dDG and CA3 was observed in the yohimbine group, which was absent in the vehicle and corticosterone groups, similar to my findings. All treatment groups showed a positive correlation between CA3 and CA1. Vehicle: $n = 8$, Yoh 1 mg/kg: $n = 9$, Cort 3 mg/kg: $n = 7$; $^{*}p < 0.01$. Roozendaal & Mirone, unpublished findings.

dDG has a higher expression of both β1- and β2-adrenoceptors compared to the CA1 and CA3 regions, and appears more sensitive to noradrenergic control (Milner et al., 2000). Physiological studies suggest that norepinephrine affects perforant path synaptic plasticity via the modulation of the activity of DG granule cells and/or interneurons, both expressing noradrenergic receptors (Seidenbecher et al., 1997; Walling & Harley, 2004; Harley, 2007; Seo et al., 2021). High-frequency stimulation of the perforant path and glutamate-mediated release of norepinephrine within the DG was found to contribute to LTP in the DG (Bronzino et al., 2001; Mather et al., 2016).

A recent study manipulating LC-norepinephrine (LC-NE) projection neurons to the DG revealed that their activation resulted in contextual generalization, evidenced by a fear response to a different, yet similar, context than the conditioning context, whereas their inhibition promoted context discrimination (Seo et al., 2021). Those effects were reflected by context-specific responses of DG granule cell ensembles, and established by β-adrenergic-mediated modulation of hilar interneurons. However, importantly, the memory function assessed in the Seo et al. (2021) study, referred to by the authors as pattern separation, is quite different from the memory function assessed in this thesis. In their contextual conditioning task there was no need for the separation of memory of training events across time, as the exposures to the conditioning and safe context were at least 3 hours apart. Moreover, stimulation of LC-NE projection neurons to the DG also affected context generalization towards completely different contexts, disqualifying pattern separation as the sole mediating mechanism. As such, these findings might suggest that the direct modulation of the DG by locally released norepinephrine exerts a different role in modulating DG function compared to its indirect modulation by noradrenergic actions on DG-projection neurons in the BLA, with direct release of norepinephrine in the DG contributing to overall memory strength and release of norepinephrine in the BLA, affecting the DG, in memory specifcity. This idea is also consistent with other findings from our laboratory indicating that the direct administration of norepinephrine into the hippocampus after training on the dual-event inhibitory avoidance task did not enhance memory specificity, but undiscriminatingly increased retention latencies in both the shock box and non-shock box (Atucha et al., under revision).

Corticosterone: In contrast to yohimbine, corticosterone administration was found to impair object-in-context memory under training conditions that required pattern separation. This finding is also in line with prior reports on the dual-event inhibitory avoidance task in which posttraining corticosterone administration induced a generalized strengthening of memory across the two training events (Roozendaal & Mirone, 2020). Further, we found that corticosterone administration induced a significantly reduced correlation between neuronal activity in the dDG and CA3 relative to yohimbine-treated animals. Yet, the strength of this correlation in corticosterone-treated mice did not differ significantly from that of vehicle-treated animals ($p = 0.08$). This might be related to the fact that the effects of corticosterone on neuronal activity were assessed under training conditions (5-min training protocol) that did not generate a functional impairment in object-in-context memory compared to controls. Future studies should assess whether corticosterone significantly weakens dDG-CA3 correlated activity relative to controls under the extended training conditions (7-min training protocol) that are associated with a corticosterone-induced impairment of object-in-context memory. Our findings overall

hint towards an impairing role for corticosterone in modulating pattern separation within the dDG and dDG-CA3 pathway.

As corticosterone was administered systemically, we also do not know where corticosterone might have acted to induce such impairing effect on the pattern separation process within the hippocampus. Corticosterone binds to both the high-affinity mineralocorticoid receptor (MR) and the low-affinity glucocorticoid receptor (GR) in the brain (Reul & de Kloet, 1995), which are both highly expressed in all hippocampal regions (Morimoto et al., 1996) on both glutamatergic and GABAergic neurons, as well as non-neuronal cell types, e.g. glia cells. Typically, corticosterone exerts delayed effects, binding to intracellularly located receptors that serve as transcription factors (Datson et al., 2001), although more recently also rapid, nongenomic signaling was revealed (Karst et al., 2005a; Roozendaal et al., 2010). Whereas the effects of posttraining corticosterone administration on impairing object-in-context memory tested 24 hours later likely involves genomic actions, the finding that it also affected neuronal activity only 1 hour after the training implicates also rapid non-genomic actions (Olijslagers et al., 2008). Corticosterone, via genomic and non-genomic pathways, can affect many different intracellular pathways (Karst & Joëls, 2005b; Olijslagers et al., 2008; Krueger et al., 2020), and has been demonstrated to reduce BDNF expression within the dDG and CA3 (Cosi et al., 1993; Funakoshi et al., 1993; Chao & McEwen, 1994; Agasse et al., 2020; Blugeot et al., 2011). Recent findings have shown that BDNF plays a rapid and essential role in regulating hippocampal synaptic plasticity (Lakshminarasimhan & Chattarji, 2012). The observation that BDNF levels are highly dynamic in response to stress or corticosterone administration (Gray et al., 2013), implicates it as a mechanism modulating learning and memory after a stressful event. Thus, corticosterone effects on reducing BDNF levels within the dDG might potentially block pattern separation within the dDG-CA3 pathway and thereby impair episodic-like specificity of memory. Interestingly, Chen et al. (2012) reported that the enhancing effect of corticosterone on memory of single-event inhibitory avoidance training was associated with an increased activation of the BDNF-CREB pathway within the dorsal hippocampus. As this study examined the entire dorsal hippocampus, it is not known whether this upregulation of BDNF was also found in the dDG. However, this finding would be generally consistent with our conclusion that posttraining corticosterone administration can induce opposite effects on the hippocampus, both in terms of recruitment of specific neural mechanisms and hippocampal subregion, depending on the specific memory task (i.e., whether the task requires pattern separation or not) (Chen et al., 2012).

In addition to this weakening of dDG-CA3 correlated activity, we found that corticosterone increased GABAergic activity in the vDG without affecting the total number of c-Fos-

expressing neurons within the vDG. The effects of corticosterone therefore seem to target the vDG; the only region that was not affected by yohimbine. Interestingly, this corticosterone effect on increasing GABAergic activity within the vDG was also found in animals that were previously habituated to the training contexts. A recent study provided experimental evidence that adult-born granule cells that are under the control of BDNF (Bekinschtein et al., 2014), bidirectionally regulate dDG and vDG function in an antagonistic manner (Luna et al., 2019), exciting mature granule cells in the vDG and inhibiting mature granule cells in the dDG. Further, highly consistent with the present findings, that study proposed that stress might induce a shift in DG function from the dDG toward the vDG that would be associated with an impaired pattern separation and increased probability of similar contexts being represented as overlapping neural ensembles.

Task-specific effects of norepinephrine and corticosterone on the hippocampal CA1 region in regulating object-in-context and object location memory

The opposite effect of yohimbine and corticosterone administration on object-in-context memory of non-habituated mice was also associated with an opposite regulation of neuronal activity within the CA1 region. The yohimbine effect on enhancing object-in-context memory was associated with an increased total activity within the hippocampal CA1 cell layer, reflecting mainly glutamatergic activity, whereas the corticosterone effect on impairing this memory was associated with an increased GABAergic activity in the CA1 *stratum radiatum*, likely reflecting an inhibition of the CA1 region. These yohimbine and corticosterone effects on the CA1 were less prominent after context habituation, but no significant differences between the two habituation conditions could be detected. However, as DREADD inactivation of the hippocampus (including CA1) did not impair the yohimbine effect on object-in-context memory of habituated mice, the hippocampus appears not involved in regulating the enhancement of object-in-context memory after context habituation. The enhancing effect of both yohimbine and corticosterone administration on object location memory was associated with a similar increase in CA1 pyramidal cell layer activity. Thus, these findings indicate that both yohimbine and corticosterone administration influence CA1 neuronal activity after training on both the object-in-context and object location task, but that the effect of corticosterone on the CA1 is critically dependent on the specific memory task.

CA1 pyramidal neurons exhibit a dense arrangement in the rodent brain. They are large, triangular or ovoid neurons with distinct basilar dendrites extending into the *stratum oriens* and apical dendrites extending into the *stratum radiatum* and *stratum moleculare-lacunosum* (Duvernoy, 2013). CA1 activity is regulated by excitatory input originating from the DG, which' granule cells, through the mossy fibers, excite CA3 pyramidal cells

that in turn stimulate CA1 pyramidal cells (Duvernoy, 2013). Moreover, the activity of CA1 pyramidal neurons is regulated by inhibitory circuits (Andersen et al., 1964; Hounsgaard, 1978), with the parvalbumin-expressing basket cells being the main inhibitory circuit within the CA1. Basket cells form a dense inhibitory synapse basket around pyramidal cell somas, inducing a significant hyperpolarizing inhibitory postsynaptic potential that robustly regulates CA1 pyramidal cell activity (Miles, 1990). Recent studies, however, highlight great functional diversity in CA1 interneurons, proposing the existence of ten major GABAergic cell classes, including somatostatin, cholecystokinin (CCK), vasoactive intestinal polypeptide, and calretinin-expressing interneurons (Harris et al., 2018). These interneuron subclasses not only exhibit transcriptomic heterogeneity, but also display variation in spatial and contextual selectivity as well as temporal dynamics of their inhibitory actions (Pelkey et al., 2017; Harris et al., 2018; Geiller et al., 2020). Previous studies involving genetic, optogenetic, and pharmacological manipulations have shown that hippocampal interneurons play essential roles in spatial and episodic learning and memory (Jeong & Singer, 2022). Future studies should assess which GABAergic cell class is affected by corticosterone, whether this is due to direct local, or indirect mechanisms, and how this modulation is memory task specific.

Mishkin and colleagues have proposed a very influential model of episodic memory according to which spatial and non-spatial contextual information would be integrated into episodes at the level of the CA1 (Mishkin & Ungerleider, 1982; Mishkin et al., 1983). Specifically, the distal part of the CA1 was hypothesized to integrate non-spatial, contextual information from projections from the lateral entorhinal cortex (LEC) either directly to the CA1 or via the dDG, whereas the proximal part of the CA1 would integrate spatial information from direct projections from the medial entorhinal cortex (MEC), eventually via the vDG (Witter et al., 2000; Knierim et al., 2014; Beer et al., 2018). This then poses the hypothesis that norepinephrine and corticosterone generate their divergent behavioral effects on episodic-like object-in-context memory by exerting distinct influences on the LEC-distal CA1 pathway, whereas their similar enhancing effect on spatial object location memory might be mediated by joint actions on the MEC-proximal CA1 pathway. In the following sections, I will discuss how stress hormone effects on CA1 activity might be involved in regulating both episodic-like and spatial memory.

Object-in-context memory: Memories are often structured based on various factors, with time being a key element in their organization (D. J. Cai et al., 2016; Chowdhury & Caroni, 2018; de Sousa et al., 2021). An important function of the CA1 is to encode and store memories related to the temporal order of events. Memories of events occurring in close temporal proximity are frequently associated by directing their storage into overlapping neuronal ensembles within the hippocampal CA1 region (D. J. Cai et al., 2016; Silva et al., 2009; Tanila, 1999). According to the memory allocation hypothesis (Silva

et al., 2009; Rogerson et al., 2014), learning triggers a temporary increase in neuronal excitability that biases the storage of a subsequent memory in the neuronal ensemble that encoded the first memory, leading to an overlapping neuronal representation. Close temporal encoding of contextual memories, within hours but not days, thereby results in linked memories, where recalling one memory triggers others acquired within the same timeframe (D. J. Cai et al., 2016; Rashid et al., 2016). As such, one can speculate that the observed rise in the overall count of c-Fos-positive cells in the CA1 region induced by yohimbine in our study indicates an enhanced memory consolidation of the temporal order of two training events within separate, non-overlapping populations of principal CA1 neurons in the hippocampus (Tronson et al., 2009). Concurrently, corticosterone amplifies GABAergic activity in the CA1 *stratum radiatum*, exerting inhibitory control over CA1 pyramidal cells. This inhibition of CA1 pyramidal cells may potentially steer the storage of memories of the two training events into an intersecting population of hippocampal principal CA1 neurons (Beer et al., 2018; He et al., 2002).

The dynamic regulation of intracellular CREB activity in individual neurons has been implicated as a pivotal organizing principle for memory allocation (Tronson et al., 2009). The activation of CREB protein and the subsequent elevation in neuronal excitability are believed to initiate the molecular and cellular cascades that culminate in the linking of two memories (Zhou et al., 2009; Sano et al., 2014; Rashid et al., 2016). If training on a second event occurs during the period of heightened CREB activity as induced by the first event, the memory of the second event tends to be allocated to the overlapping neuronal ensemble. Conversely, when training transpires with a prolonged interval and no sustained increase in CREB activity from the first event, the memory of the second event is stored in a distinct neuronal ensemble. Notably, an inactivation of CREB in the hippocampus has been associated with impairments in contextual fear and spatial memory (Silva et al., 1998; Kida et al., 2002), as well as impairments in the integration of information encoded within overlapping representations (Schlichting et al., 2015; DeVito et al., 2010; Morton et al., 2017). This observation raises the intriguing possibility that the divergent effects of yohimbine and corticosterone administration on CA1 activity induce opposing outcomes in the separation versus linking of memory of the two training object exploration events. However, one important difference with the linking or separation based on CREB activation is that yohimbine administration induced separate memories, and corticosterone induced linked memories, of two training events that were experienced without any delay, thus likely before CREB activity could be elevated after the first training event. This temporal effect implies an interaction between a pattern separation process in the dDG-CA3 pathway and the subsequent storage of the two memories in non-overlapping neuronal ensembles within the CA1. Thus, it can be hypothesized that noradrenergic actions on the dDG-CA3 pathway separate memories of

the two training events and that the noradrenergic actions on the CA1 direct the storage of the two memories into two non-overlapping neuronal ensembles.

It should be noted that with systemic yohimbine administration, our findings do not allow any conclusions as to whether norepinephrine indeed directly acted on both the dDG-CA3 pathway and the CA1 region. It is possible that some of the neuronal activity changes we found are the indirect consequence of noradrenergic actions at other places in the neuronal circuit, e.g. LEC inputs to the dDG, regulating episodic-like memory processing and the separation of memory of multiple training events. As mentioned above, previous studies have shown that norepinephrine infusion directly into the BLA is also sufficient to facilitate the storage of memory of training on the dual-event inhibitory avoidance task into two separate memories (Atucha et al., under revision). However, it is well established that elevated levels of norepinephrine have a pronounced impact on increasing neuronal excitability in the CA1 (Mueller et al., 1981; Heginbotham & Dunwiddie, 1991; Dunwiddie et al., 1992; Jurgens et al., 2005) through the activation of local β-adrenoceptors (Kitchigina et al., 1997). Both β1 and β2-receptor subtypes are expressed in pyramidal cells within the CA1 (Booze et al., 1993; Milner et al., 2000; Guo & Li, 2007; Cox et al., 2008) and may thus be involved in mediating any direct effects of norepinephrine on the hippocampus.

Corticosterone administration resulted in an activation of GABAergic activity within the CA1. Given the important role of local GABAergic neurons in inhibiting CA1 pyramidal cell activity, one could hypothesize that corticosterone impairs CA1 pyramidal cell activity. However, we did not find such an impairing effect. Exactly how glucocorticoids increase GABAergic activity within the CA1, whether it predominantly affects a specific subclass of GABAergic neurons, and how this will affect pyramidal cell activity requires further investigation. Also here, our experimental design with systemic corticosterone administration does not allow any conclusion of whether corticosterone might directly act on the CA1 region or whether the effects are the result of upstream corticosterone effects in e.g. the LEC-dDG-CA3 pathway. GRs are expressed on both glutamatergic and GABAergic cells within the CA1 and several studies have shown that glucocorticoids interact with GABAergic mechanisms in influencing memory processing (Gunn et al., 2015). Consistent with our finding that corticosterone increases GABAergic activity, previous work has shown a rapid increase in the amplitude of evoked inhibitory postsynaptic currents by CCK-interneurons in the CA1 (Volkova et al., 2016). Evidence that these changes in GABAergic activity might mediate the behavioral effects of corticosterone was obtained from corticosterone administration studies into the infralimbic cortex, where corticosterone-mediated enhancement of fear memory extinction was blocked by co-administration of a GABAergic receptor antagonist (Omoumi et al., 2023).

Our findings prompt consideration for future studies to delve into whether the contrasting effects on CA1 activity as induced by yohimbine and corticosterone are sufficient to elicit the formation of either two distinct or a singular memory representation within the CA1 region or whether this information requires additional regulation of pattern separation within the dDG-CA3 pathway. This line of investigation could provide valuable insights into the nuanced mechanisms through which these stress hormones influence episodic-like memory and may pave the way for a more comprehensive understanding of their impact on episodic-like memory dynamics.

Object location memory: Our finding that yohimbine and corticosterone induce similar effects on object location memory is consistent with those of many other studies indicating that both noradrenergic and glucocorticoid agonists, whether administered systemically or directly into specific brain regions, enhance spatial memory for object location, radial arm and water-maze training (Sandi et al., 1997; Hatfield & McGaugh, 1999; Roozendaal et al., 2010; Song et al., 2021; Durán et al., 2023). Furthermore, previous studies have suggested an interaction between the glucocorticoid and noradrenergic systems in impacting object location memory, likely through shared neural pathways (Roozendaal et al., 2010; Roozendaal & Mirone, 2020). Seminal work conducted more than 50 years ago showed, using recordings in freely moving rats, that the majority of pyramidal cells in the CA1 fire specifically in certain regions (place fields) of a local environment (O'Keefe & Dostrovsky, 1971; O'Keefe, 1976). Since different cells were found to respond to different place fields, hippocampal neurons, as a population, provide an accurate dynamic representation of the animal's location in space (Wilson & McNaughton, 1993). Increased noradrenergic tone was observed to increase the firing rate of hippocampal place cells (Tanila, 2001), which is consistent with our findings that yohimbine increased CA1 pyramidal cell activity. Furthermore, acute stress, likely associated with increased corticosterone levels, was found to facilitate CA1 spatial coding (Tomar & McHugh, 2022). A potential link between the roles of the hippocampus in memory as well as to supporting the brain's representation of space is that the hippocampus organizes memories in space (Eichenbaum, 2017a). Hippocampal damage can lead to significant difficulties in learning and remembering spatial locations guided by distant visual cues (Morris, 1984; Sahgal, 1993). Mice with CA1 lesions display hyperactivity in novel environments, deficits in spatial working memory assessed by the Y-maze spontaneous alternation test, and impaired spatial learning in the 8-arm radial maze (Dillon et al., 2008). Place cells in the CA1 may receive positional information from the intrahippocampal associative network in area CA3 or directly from the entorhinal cortex (Witter et al., 2000). While complete hippocampal lesions disrupt performance in this task, there is no apparent impairment when the connection between CA3 and CA1 was disconnected (Eichenbaum & Buckingham, 1990). Instead, pyramidal cells in the CA1, after the removal of all input from CA3, still develop sharp and stable place fields (Brun et al., 2002), and rats showed normal acquisition of an

associative hippocampal-dependent spatial recognition task (Brun et al., 2002; Lisman & Grace, 2005). These experiments provided conclusive evidence that the CA1 can generate place-specific firing based on entorhinal input alone, without the involvement of further processing through the trisynaptic hippocampal circuitry. Thus, based on these findings, it could be hypothesized that the enhancing effects of yohimbine and corticosterone on spatial memory may be independent of the intrahippocampal network, but rely on direct projections from the MEC to the CA1. This would be consistent with our findings that yohimbine or corticosterone administration after training on the object location task was only associated with neuronal activity changes within the CA1, and not in any other hippocampal subregion. We also did not find any effect of yohimbine or corticosterone administration on correlated activity between any of the hippocampal subregions. Therefore, future studies should examine whether yohimbine and corticosterone directly regulate this MEC-CA1 pathway in enhancing spatial memory. This would then thus contrast the opposite effects of yohimbine and corticosterone on episodic-like object-in-context memory which might require coordinated actions in both the dDG and CA1.

Working model

Collectively, the findings presented in this thesis pose the hypothesis that norepinephrine and corticosterone generate their opposite effects on episodic-like specificity of memory for multiple training events by exerting an opposite influence on the LEC-dDG-CA3-distal CA1 pathway. We proposed that norepinephrine and corticosterone induce opposite effects on pattern separation within the dDG-CA3 pathway and additionally exert an opposite effect on the storage of this memory in either overlapping or distinct neuronal ensembles within the distal CA1. Contrarily, the similar effect of norepinephrine and corticosterone on enhancing spatial memory may be mediated by similar effects of these two stress hormones on the MEC-proximal CA1 pathway. The exact sites of action in establishing these modulatory effects remains to be determined, but the BLA is one logical candidate.

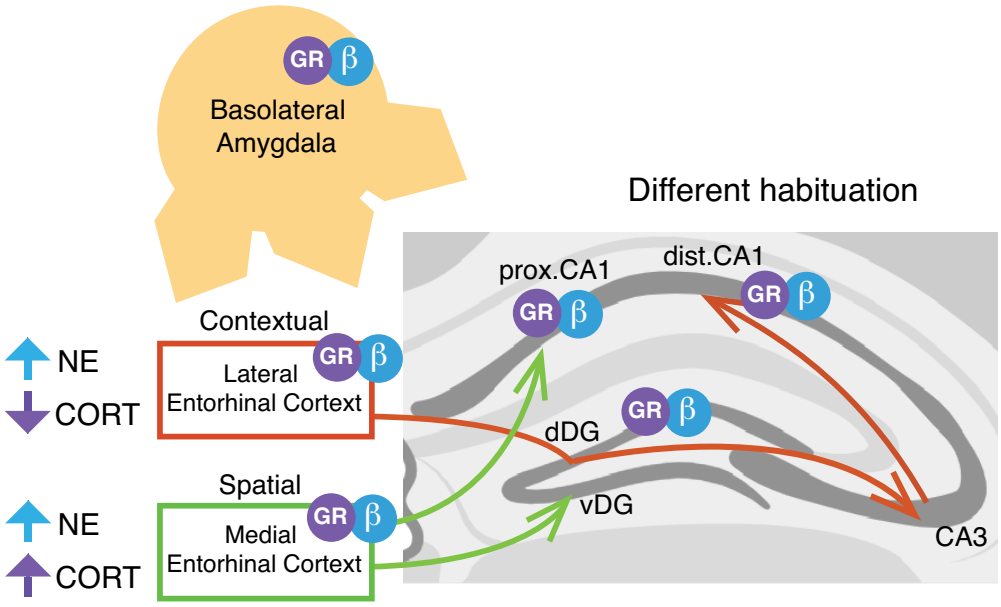

Figure 2. Proposed working model of how norepinephrine and corticosterone differentially affect episodic-like contextual and spatial aspects of hippocampus-dependent memory by acting on the lateral entorhinal cortex (LEC)-dDG-CA3-distal CA1 and medial entorhinal cortex (MEC)-proximal CA1 pathways under training conditions that require the separation of two training events (i.e., the different habituation condition).

Specifically, the LEC, which is essential for the processing of non-spatial contextual information, preferentially projects to the dDG, and then via the CA3 to the distal part of the hippocampal CA1 (shown in red). We propose that norepinephrine enhances object-in-context memory by boosting this pathway whereas corticosterone impairs object-in-context memory by suppressing this pathway. In contrast, the MEC, which is more sensitive to spatial content, predominantly projects directly to the proximal part of CA1 (shown in green). Norepinephrine and corticosterone may enhance object location memory by increasing the recruitment of this MEC-proximal CA1 pathway. Currently, we do not know exactly where in these circuits these hormones act. Possible sites of action are shown in the figure.

Prospects for future work

Future work needs to experimentally examine this working model. Most importantly, our model postulates that the two stress hormones norepinephrine and corticosterone induce opposite effects on the LEC-dDG-CA3-distal CA1 pathway after training on the object-in-context task and similar effects on the MEC-proximal CA1 pathway after training on the object location task. However, it is currently unclear where in the circuits norepinephrine and corticosterone act. We applied systemic manipulations of stress hormone levels throughout the work described in this thesis, and noradrenergic and glucocorticoid receptors are known to be expressed in different hippocampal subfields (in both GABAergic and glutamatergic neurons) as well as in the BLA and the entorhinal cortex (Morimoto et al., 1996; Milner et al., 2000; Joëls et al., 2012). Future work could target regional and even cell-type specific manipulations of noradrenergic and glucocorticoid signaling. This could be achieved by e.g. local administration of norepinephrine or corticosterone, local blockade of their effects by the administration of β-blocker or GR antagonist (Roozendaal et al., 2002), or specific knockdown of these receptors in specific cell types (Anacker et al., 2011; Roozendaal et al., 2002). Given the effect of corticosterone administration after object-in-context training on GABAergic activity, it would be important to determine whether corticosterone predominantly affects a specific subclass of GABAergic neurons. Whereas these manipulations should be feasible in the regions upstream of the hippocampus, i.e., the BLA, MEC and LEC, it will be quite challenging with local administrations to distinctly target different hippocampal subregions, e.g. the DG versus CA1, in the small mouse brain.

Proposedly, these experiments would implicate the BLA as site of action for the effects of norepinephrine and potentially also corticosterone, with BLA-projection neurons to the LEC/MEC being affected depending on the training conditions. Alternatively, the LEC and MEC themselves might be under direct influence of the stress hormones. To next prove the involvement of stress hormone-mediated differential recruitment of LEC and MEC projections to the hippocampus in establishing the behavioral memory effects, future work should also examine neuronal activity in these regions. Specifically, it would be interesting to examine activity in LEC-dDG and MEC-proximal CA1 projection neurons following training on the object-in-context and object location tasks and stress hormone administration. For this one could inject a retrograde virus in the dDG and another retrograde virus with distinct fluorophore in the proximal CA1. One could then examine whether yohimbine and corticosterone administration after training on the object-in-context task (under different habituation conditions) induce opposite effects on c-Fos activity in dDG-projecting LEC neurons, whereas both hormones after training on the object location task will increase c-Fos activity in MEC neurons that project directly to the proximal CA1 region. As a second step, to prove causality, one could exploit DREADD

technology to experimentally activate or silence these two pathways to determine whether this effectively prevents – proving necessity – or mimics – proving sufficiency – the effect of these stress hormones on object-in-context and object location memory.

Previous research demonstrated that noradrenergic stimulation of the BLA promotes the formation of separate and distinct memories for two events experienced close in time in the dual-event inhibitory avoidance task in rats through a consolidation process regulated by miR-134 within the dDG (Atucha et al., under revision). Future work should similarly investigate the role of miR-134 in mediating the stress hormone effects on episodic-like memory for object-in-context training in mice. First, it would be interesting to evaluate whether systemic manipulation of noradrenergic signaling recapitulates these previously reported effects of local BLA infusions on miR-134 signaling in the dDG of mice trained on the object-in-context task. Second, one should assess whether the memory-linking effects of corticosterone administration are mediated by an opposite regulation of miR-134 signaling. Direct inhibition of miR-134 in the hippocampus using a specific antagomir sequence has been demonstrated to upregulate BDNF expression, thereby improving memory specificity (Acheson et al., 1995; Yamada & Nabeshima, 2003; Bekinschtein et al., 2013; Atucha et al., under revision). Glucocorticoids are known to reduce dDG BDNF expression, but whether this corticosterone effect on BNDF involves miR-134 and plays a role in mediating the impairing effect of corticosterone object-in-context memory is currently unknown. Lastly, it would be relevant to show that the stress hormone effects on miR-134 signaling are only observed for object-in-context training following habituation in a different, but not the same context, as this would further confirm that prior context habituation renders the stress hormone effects on memory specificity hippocampus-independent. One could assess these questions by measuring miR-134 levels within the dDG by quantitative PCR, and show their causal involvement by manipulating them by either the local administration of the mimic (i.e., the exact copy) or antagomir (i.e., the exact complementary sequence) of miR-134.

Lastly, both yohimbine and corticosterone enhanced object-in-context memory of habituated animals. We hypothesized that habituation to the training contexts might have resulted in the creation of two separate memories of the two training contexts already prior to object-in-context training. As such, the enhancing effects of both yohimbine and corticosterone in this condition may reflect a strengthening of memory for the objects *per se*. However, we have not been able to provide any proof for this hypothesis in this thesis. It would be interesting to modify the retention test after object-in-context training to directly assess object memory and thus test this hypothesis. Animals could be trained on the object-in-context task as usual, but then on the retention be tested by a regular object recognition test, in which one of the previously encountered objects in the testing context is replaced by a completely novel object. If our hypothesis is correct, both

yohimbine and corticosterone administration to mice of the same habituation condition would enhance memory for the training object *per se*. Perhaps even more interesting would be to test the effect of yohimbine and corticosterone on object memory in mice of the different habituation condition. According to our hypothesis, whereas yohimbine and corticosterone administration have opposite effects on object-in-context memory, they should also here have similar enhancing effects on object memory *per se* under the exact same training conditions.

These additional experiments would shed further light on the nuanced interplay between stress hormones and the distinct hippocampal pathways that are recruited under different training conditions, providing a deeper understanding of how episodic-like and spatial aspects of memory are influenced by the orchestrated symphony of stress hormone actions within the hippocampus.

Conclusion and impact

In this thesis, I showed that norepinephrine and corticosterone induce opposite effects on episodic-like memory when there is a need to separate overlapping memory representations of multiple training events, but that these two stress hormones induce similar effects on hippocampus-dependent memory when the training conditions do not require such a separation of memories. My findings do not only provide a working model that could inspire future studies to further investigate the neural mechanisms underlying stress hormone effects on different types of hippocampal memory, but these findings also have important implications for human research. As described in previous parts of this thesis, human studies have reported opposite effects of stress or emotional arousal on qualitative aspects of hippocampus-dependent memories. Some studies reported that arousal improves the accuracy of memories, resulting in vivid recall of emotionally arousing experiences (Ochsner, 2000; Steidl et al., 2006; Segal et al., 2012), while other studies proposed that emotional memories are remembered in a more generalized manner, potentially leading to less accurate recollection of specific details (Morgan et al., 2004a; Richards & Gross, 2006; Levine & Edelstein, 2009). However, these human studies have employed many different types of (hippocampus-dependent) memory tasks and different readout measures. Further, the experimental procedures also drastically varied in terms of their degree of stressfulness or emotionality: Some studies investigated differences in accuracy or generalization for emotionally arousing vs neutral encoded information, whereas other studies examined memory quality after exposing participants to an actual stress challenge. My studies indicate three important aspects that might explain these contradictory effects reported in the literature: 1) the effects of stress and emotional arousal depend on the type of hippocampus-dependent memory that is

assessed, 2) norepinephrine and corticosterone can differentially affect hippocampus-dependent memory, 3) the training conditions, i.e., whether learning occurs in a familiar or novel environment, are a critical denominator in the eventual memory effects. Understanding these different determining factors provides insights into how the brain sorts and distinguishes memories under stress. Therefore, it seems imperative that future human studies take these findings into consideration. Particularly, it would be crucial to measure stress hormone levels in these studies and consider whether the memory task is episodic or not.

My findings are also important for understanding memory processing in stress-related disorders. Decontexualization and overgeneralization of memory is a characteristic hallmark of post-traumatic stress disorder (PTSD) and other fear- and stress-related disorders (Askelund et al., 2019; Bahtiyar et al., 2020a; Petzold & Bunzeck, 2022). Interestingly, these disorders are often associated with changes in stress hormone signaling. Particularly, a hypersensitive hypothalamus-pituitary-adrenal axis with low circulating levels of cortisol are described in PTSD (Yehuda, 2002; Yehuda, 2009; Pitman et al., 2012). Thus, my findings showing that glucocorticoids reduce episodic-like memory, but enhance other forms of hippocampus-dependent memory, and the putative neural pathways that might mediate these effects, might provide novel perspectives on the molecular/cellular foundations of maladaptive memory formation in the diseased brain.

References

Acheson, A., Conover, J. C., Fandl, J. P., DeChiara, T. M., Russell, M., Thadani, A., Squinto, S. P., Yancopoulos, G. D., & Lindsay, R. M. (1995). A BDNF autocrine loop in adult sensory neurons prevents cell death. *Nature*, *374*(6521), 450–453.

Acsády, L., & Káli, S. (2007). Models, structure, function: the transformation of cortical signals in the dentate gyrus. *Progress in Brain Research*, *163*, 577–599.

Agasse, F., Mendez-David, I., Christaller, W., Carpentier, R., Braz, B. Y., David, D. J., Saudou, F., & Humbert, S. (2020). Chronic corticosterone elevation suppresses adult hippocampal neurogenesis by hyperphosphorylating huntingtin. *Cell Reports*, *32*(1).

Albasser, M. M., Davies, M., Futter, J. E., & Aggleton, J. P. (2009). Magnitude of the object recognition deficit associated with perirhinal cortex damage in rats: Effects of varying the lesion extent and the duration of the sample period. *Behavioral Neuroscience*, *123*, 115–124.

Anacker, C., Zunszain, P. A., Carvalho, L. A., & Pariante, C. M. (2011). The glucocorticoid receptor: pivot of depression and of antidepressant treatment? *Psychoneuroendocrinology*, *36*(3), 415–425.

Andersen, P., Bie, B., & Ganes, T. (1982). Distribution of GABA sensitive areas on hippocampal pyramidal cells. *Experimental Brain Research*, *45*(3), 357–363.

Andersen, P., Eccles, J. C., & Lyning, Y. (1964). LOCATION OF POSTSYNAPTIC INHIBITORY SYNAPSES ON HIPPOCAMPAL PYRAMIDS. *Journal of Neurophysiology*, *27*(4), 592–607.

Askelund, A. D., Schweizer, S., Goodyer, I. M., & van Harmelen, A.-L. (2019). Positive memory specificity is associated with reduced vulnerability to depression. *Nature Human Behaviour*, *3*(3), 265–273.

Assini, F. L., Duzzioni, M., & Takahashi, R. N. (2009). Object location memory in mice: Pharmacological validation and further evidence of hippocampal CA1 participation. *Behavioural Brain Research*, *204*(1), 206–211.

Akirav, I., & Richter-Levin, G. (2002). Mechanisms of amygdala modulation of hippocampal plasticity. *Journal of Neuroscience, 22*(22), 9912–9921.

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: Adaptive Gain and Optimal Performance. *Annual Review of Neuroscience, 28*(1), 403–450.

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., Pais, M., Ronzoni, G., Schoenmaker, C., Atsak, P., Roura, D., Lohkamp, K. ., Schelling, G., McGaugh, J., Glennon, J. ., Aschrafi, A., & Roozendaal, B. (under revision). *Basolateral amygdala activity facilitates memory specificity for overlapping events*.

Atucha, E., & Roozendaal, B. (2015). The inhibitory avoidance discrimination task to investigate accuracy of memory. *Frontiers in Behavioral Neuroscience*, 9.

Atucha, E., Vukojevic, V., Fornari, R. V, Ronzoni, G., Demougin, P., Peter, F., Atsak, P., Coolen, M. W., Papassotiropoulos, A., McGaugh, J. L., de Quervain, D. J.-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.

Atzori, M., Cuevas-Olguin, R., Esquivel-Rendon, E., Garcia-Oscos, F., Salgado-Delgado, R. C., Saderi, N., Miranda-Morales, M., Treviño, M., Pineda, J. C., & Salgado, H. (2016). Locus ceruleus norepinephrine release: a central regulator of CNS spatio-temporal activation? *Frontiers in Synaptic Neuroscience, 8*, 25.

Axelrod, J., & Reisine, T. D. (1984). Stress hormones: their interaction and regulation. *Science, 224*(4648), 452–459.

Bahtiyar, S., Gulmez Karaca, K., Henckens, M. J. A. G., & Roozendaal, B. (2020). Norepinephrine and glucocorticoid effects on the brain mechanisms underlying memory accuracy and generalization. *Molecular and Cellular Neuroscience*, *108*(July), 103537.

Balderas, I., Rodriguez-Ortiz, C. J., & Bermudez-Rattoni, F. (2015). Consolidation and reconsolidation of object recognition memory. *Behavioural Brain Research, 285*, 213–222.

Balderas, I., Rodriguez-Ortiz, C. J., Salgado-Tonda, P., Chavez-Hurtado, J., McGaugh, J. L., & Bermudez-Rattoni, F. (2008). The consolidation of object and context recognition memory involve different regions of the temporal lobe. *Learning and Memory, 15*(9), 618–624.

Balleine, B. W., & O'Doherty, J. P. (2010). Human and Rodent Homologies in Action Control: Corticostriatal Determinants of Goal-Directed and Habitual Action. *Neuropsychopharmacology, 35*(1), 48–69.

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.

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.

Barsegyan, A., Mirone, G., Ronzoni, G., Guo, C., Song, Q., van Kuppeveld, D., Schut, E. H. S., Atsak, P., Teurlings, S., McGaugh, J. L., Schubert, 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.

Banks, P. J., Warburton, E. C., Brown, M. W., & Bashir, Z. I. (2014). Chapter Seven - Mechanisms of Synaptic Plasticity and Recognition Memory in the Perirhinal Cortex. *In Progress in Molecular Biology and Translational Science*, *122*, 193–209.

Beer, Z., Vavra, P., Atucha, E., Rentzing, K., Heinze, H. J., & Sauvage, M. M. (2018). The memory for time and space differentially engages the proximal and distal parts of the hippocampal subfields CA1 and CA3. *PLoS Biology*, *16*(8), 1–21.

Bekinschtein, P., Kent, B. A., Oomen, C. A., Clemenson, G. D., Gage, F. H., Saksida, L. M., & Bussey, T. J. (2013). BDNF in the dentate gyrus is required for consolidation of "pattern-separated" memories. *Cell Reports, 5*(3), 759–768.

Bekinschtein, P., Kent, B. A., Oomen, C. A., Clemenson, G. D., Gage, F. H., Saksida, L. M., & Bussey, T. J. (2014). Brain-derived neurotrophic factor interacts with adult-born immature cells in the dentate gyrus during consolidation of overlapping memories. *Hippocampus, 24*(8), 905–911.

Beldjoud, H., Barsegyan, A., & Roozendaal, B. (2015). Noradrenergic activation of the basolateral amygdala enhances object recognition memory and induces chromatin remodeling in the insular cortex. *Frontiers in Behavioral Neuroscience*, *9*.

Bermudez-Rattoni, F. (2014). The forgotten insular cortex: Its role on recognition memory formation. *Neurobiology of Learning and Memory*, *109*, 207–216.

Bermudez-Rattoni, F., Okuda, S., Roozendaal, B., & McGaugh, J. L. (2005). Insular cortex is involved in consolidation of object recognition memory. *Learning & Memory*, *12*(5), 447–449.

Beldjoud, H., Barsegyan, A., & Roozendaal, B. (2015). Noradrenergic activation of the basolateral amygdala enhances object recognition memory and induces chromatin remodeling in the insular cortex. *Frontiers in Behavioral Neuroscience*, *9*.

Berridge, C. W. (2008). Noradrenergic modulation of arousal. *Brain Research Reviews, 58*(1), 1–17.

Bianchi, M., Fone, K. F. C., Azmi, N., Heidbreder, C. A., Hagan, J. J., & Marsden, C. A. (2006). Isolation rearing induces recognition memory deficits accompanied by cytoskeletal alterations in rat hippocampus. *European Journal of Neuroscience*, *24*(10), 2894–2902.

Blackstad, T. W., Fuxe, K., & Hökfelt, T. (1967). Noradrenaline nerve terminals in the hippocampal region of the rat and the guinea pig. *Zeitschrift Für Zellforschung Und Mikroskopische Anatomie*, *78*(4), 463–473.

Blugeot, A., Rivat, C., Bouvier, E., Molet, J., Mouchard, A., Zeau, B., Bernard, C., Benoliel, J.-J., & Becker, C. (2011). Vulnerability to depression: from brain neuroplasticity to identification of biomarkers. *Journal of Neuroscience*, *31*(36), 12889–12899.

Booze, R. M., Crisostomo, E. A., & Davis, J. N. (1993). Beta-adrenergic receptors in the hippocampal and retrohippocampal regions of rats and guinea pigs: Autoradiographic and immunohistochemical studies. *Synapse*, *13*(3), 206–214.

Bramham, C. R., & Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. *Progress in Neurobiology*, *76*(2), 99–125.

Broadbent, N. J., Squire, L. R., & Clark, R. E. (2004). Spatial memory, recognition memory, and the hippocampus. *Proceedings of the National Academy of Sciences*, *101*(40), 14515–14520.

Bronzino, J. D., Kehoe, P., Mallinson, K., & Fortin, D. A. (2001). Increased extracellular release of hippocampal NE is associated with tetanization of the medial perforant pathway in the freely moving adult male rat. *Hippocampus*, *11*(4), 423–429.

Bramham, C. R., & Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. *Progress in Neurobiology, 76*(2), 99–125.

Brun, V. H., Otnæss, M. K., Molden, S., Steffenach, H.-A., Witter, M. P., Moser, M.-B., & Moser, E. I. (2002). Place cells and place recognition maintained by direct entorhinal-hippocampal circuitry. *Science*, *296*(5576), 2243–2246.

Cai, D. J., Aharoni, D., Shuman, T., Shobe, J., Biane, J., Song, W., Wei, B., Veshkini, M., La-Vu, M., Lou, J., Flores, S. E., Kim, I., Sano, Y., Zhou, M., Baumgaertel, K., Lavi, A., Kamata, M., Tuszynski, M., Mayford, M., … Silva, A. J. (2016). A shared neural ensemble links distinct contextual memories encoded close in time. *Nature*, *534*(7605), 115–118.

Cai, W., Blundell, J., Han, J., Greene, R. W., & Powell, C. M. (2006). Postreactivation Glucocorticoids Impair Recall of Established Fear Memory. *The Journal of Neuroscience*, *26*(37), 9560–9566.

Cannon, W. B. (1932). *The wisdom of the body.* New York: WW Norton

Chao, H. M., & McEwen, B. S. (1994). Glucocorticoids and the expression of mRNAs for neurotrophins, their receptors and GAP-43 in the rat hippocampus. *Molecular Brain Research*, *26*(1), 271–276.

Chen, Y., Barsegyan, A., Nadif Kasri, N., & Roozendaal, B. (2018). Basolateral amygdala noradrenergic activity is required for enhancement of object recognition memory by histone deacetylase inhibition in the anterior insular cortex. *Neuropharmacology*, *141*, 32–41.

Chen, Y. F., Song, Q., Colucci, P., Maltese, F., Siller-Pérez, C., Prins, K., McGaugh, J. L., Hermans, E. J., Campolongo, P., Nadif Kasri, N., & Roozendaal, B. (2022). Basolateral amygdala activation enhances object recognition memory by inhibiting anterior insular cortex activity. *Proceedings of the National Academy of Sciences*, *119*(22), e2203680119.

Chen, D. Y., Bambah-Mukku, D., Pollonini, G., & Alberini, C. M. (2012). Glucocorticoid receptors recruit the CaMKIIα-BDNF-CREB pathways to mediate memory consolidation. *Nature Neuroscience, 15*(12), 1707–1714.

Chowdhury, A., & Caroni, P. (2018). Time units for learning involving maintenance of system-wide cFos expression in neuronal assemblies. *Nature Communications*, *9*(1), 4122.

Chowdhury, A., Luchetti, A., Fernandes, G., Almeida Filho, D., Kastellakis, G., Tzilivaki, A., Ramirez, E. M., Tran, M. Y., Poirazi, P., & Silva, A. J. (2022). A locus coeruleus-dorsal CA1 dopaminergic circuit modulates memory linking. *Neuron*, *110*(20), 3374–3388.

Clark, R. E., Zola, S. M., & Squire, L. R. (2000). Impaired Recognition Memory in Rats after Damage to the Hippocampus. *The Journal of Neuroscience*, *20*(23), 8853–8860.

Clelland, C. D., Choi, M., Romberg, C., Clemenson, G. D., Fragniere, A., Tyers, P., Jessberger, S., Saksida, L. M., Barker, R. A., Gage, F. H., & Bussey, T. J. (2009). A Functional Role for Adult Hippocampal Neurogenesis in Spatial Pattern Separation. *Science*, *325*(5937), 210–213.

Clewett, D., Dunsmoor, J., Bachman, S., Phelps, E., & Davachi, L. (2020). Survival of the salient: emotion rescues otherwise forgettable memories via neural reactivation and post-encoding hippocampal connectivity. *Biorxiv*, 2007–2020.

Cohen, N. J., & Squire, L. R. (1980). Preserved Learning and Retention of Pattern-Analyzing Skill in Amnesia: Dissociation of Knowing How and Knowing That. *Science*, *210*(4466), 207–210.

Cohen, S. J., Munchow, A. H., Rios, L. M., Zhang, G., Ásgeirsdóttir, H. N., & Stackman, R. W. (2013). The Rodent Hippocampus Is Essential for Nonspatial Object Memory. *Current Biology*, *23*(17), 1685–1690.

Cohen, S. J., & Stackman Jr., R. W. (2015). Assessing rodent hippocampal involvement in the novel object recognition task. A review. *Behavioural Brain Research*, *285*, 105–117.

Cosi, C., Spoerri, P. E., Comelli, M. C., Guidolin, D., & Skaper, S. D. (1993). Glucocorticoids depress activity-dependent expression of BDNF mRNA in hippocampal neurones. *Neuroreport*, *4*(5), 527–530.

Cox, D. J., Racca, C., & Lebeau, F. E. N. (2008). β-adrenergic receptors are differentially expressed in distinct interneuron subtypes in the rat hippocampus. *Journal of Comparative Neurology*, *509*(6), 551–565.

Dallman, M. F., Pecoraro, N. C., La Fleur, S. E., Warne, J. P., Ginsberg, A. B., Akana, S. F., Laugero, K. C., Houshyar, H., Strack, A. M., & Bhatnagar, S. (2006). Glucocorticoids, chronic stress, and obesity. *Progress in Brain Research*, *153*, 75–105.

Dandolo, L. C., & Schwabe, L. (2018). Time-dependent memory transformation along the hippocampal anterior-posterior axis. *Nature Communications*, *9*(1).

Datson, N. A., Van Der Perk, J., De Kloet, E. R., & Vreugdenhil, E. (2001). Identification of corticosteroid-responsive genes in rat hippocampus using serial analysis of gene expression. *European Journal of Neuroscience*, *14*(4), 675–689.

De Bosscher, K., Vanden Berghe, W., & Haegeman, G. (2003). The Interplay between the Glucocorticoid Receptor and Nuclear Factor-κB or Activator Protein-1: Molecular Mechanisms for Gene Repression. *Endocrine Reviews*, *24*(4), 488–522.

de Kloet, E. R. (1991). Brain corticosteroid receptor balance and homeostatic control. *Front. Neuroendocrinol.*, *12*, 95–164.

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.

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocrine Reviews*, *19*(3), 269–301.

de Lima, M. N., Luft, T., Roesler, R., & Schröder, N. (2006). Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. *Neuroscience Letters*, *405*(1), 142–146.

de Quervain, D. J.-F., Roozendaal, B., & McGaugh, J. L. (1998). Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature, 394*(6695), 787–790.

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.

de Sousa, A. F., Chowdhury, A., & Silva, A. J. (2021). Dimensions and mechanisms of memory organization. *Neuron*, *109*(17), 2649–2662.

Dees, R. L., & Kesner, R. P. (2013). The role of the dorsal dentate gyrus in object and object-context recognition. *Neurobiology of Learning and Memory*, *106*, 112–117.

DeVito, L. M., Lykken, C., Kanter, B. R., & Eichenbaum, H. (2010). Prefrontal cortex: role in acquisition of overlapping associations and transitive inference. *Learning & Memory*, *17*(3), 161–167.

Di, S., Malcher-Lopes, R., Halmos, K. C., & Tasker, J. G. (2003). Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *Journal of Neuroscience*, *23*(12), 4850–4857.

Diamond, D. M., Bennett, M. C., Fleshner, M., & Rose, G. M. (1992). Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus*, *2*(4), 421–430.

Dillon, G. M., Qu, X., Marcus, J. N., & Dodart, J.-C. (2008). Excitotoxic lesions restricted to the dorsal CA1 field of the hippocampus impair spatial memory and extinction learning in C57BL/6 mice. *Neurobiology of Learning and Memory*, *90*(2), 426–433.

Dimidschstein, J., Chen, Q., Tremblay, R., Rogers, S. L., Saldi, G.-A., Guo, L., Xu, Q., Liu, R., Lu, C., Chu, J., Grimley, J. S., Krostag, A.-R., Kaykas, A., Avery, M. C., Rashid, M. S., Baek, M., Jacob, A. L., Smith, G. B., Wilson, D. E., … Fishell, G. (2016). A viral strategy for targeting and manipulating interneurons across vertebrate species. *Nature Neuroscience*, *19*(12), 1743–1749.

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

dos Santos Corrêa, M., Vaz, B. dos S., Grisanti, G. D. V., de Paiva, J. P. Q., Tiba, P. A., & Fornari, R. V. (2019). Relationship between footshock intensity, post-training corticosterone release and contextual fear memory specificity over time. *Psychoneuroendocrinology*, *110*, 104447.

Dunsmoor, J. E., Kroes, M. C. W., Moscatelli, C. M., Evans, M. D., Davachi, L., & Phelps, E. A. (2018). Event segmentation protects emotional memories from competing experiences encoded close in time. *Nature Human Behaviour*, *2*(4), 291–299.

Dunwiddie, T. V, Taylor, M., Heginbotham, L. R., & Proctor, W. R. (1992). Long-term increases in excitability in the CA1 region of rat hippocampus induced by beta-adrenergic stimulation: possible mediation by cAMP. *Journal of Neuroscience*, *12*(2), 506–517.

Durán, E., Pandinelli, M., Logothetis, N. K., & Eschenko, O. (2023). Altered norepinephrine transmission after spatial learning impairs sleep-mediated memory consolidation in rats. *Scientific Reports*, *13*(1), 4231.

Duvernoy, H. M. (2013). *The human hippocampus: an atlas of applied anatomy*. JF Bergmann-Verlag.

Eacott, M. J., & Norman, G. (2004). Integrated Memory for Object, Place, and Context in Rats: A Possible Model of Episodic-Like Memory? *The Journal of Neuroscience*, *24*(8), 1948–1953.

Eichenbaum, H. (2017a). On the integration of space, time, and memory. *Neuron*, *95*(5), 1007–1018.

Eichenbaum, H. (2017b). Prefrontal–hippocampal interactions in episodic memory. *Nature Reviews Neuroscience*, *18*(9), 547–558.

Eichenbaum, H., & Buckingham, J. (1990). *Studies on hippocampal processing: Experiment, theory, and model*.

Ennaceur, A., & Aggleton, J. P. (1997). The effects of neurotoxic lesions of the perirhinal cortex combined to fornix transection on object recognition memory in the rat. *Behavioural Brain Research*, *88*(2), 181–193.

Ferry, B., Roozendaal, B., & McGaugh, J. L. (1999). Role of norepinephrine in mediating stress hormone regulation of long-term memory storage: a critical involvement of the amygdala. *Biological Psychiatry*, *46*(9), 1140–1152.

Finsterwald, C., & Alberini, C. M. (2014). Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: from adaptive responses to psychopathologies. *Neurobiology of Learning and Memory*, *112*, 17–29.

Flood, J. F., Vidal, D., Bennett, E. L., Orme, A. E., Vasquez, S., & Jarvik, M. E. (1978). Memory facilitating and anti-amnesic effects of corticosteroids. *Pharmacology Biochemistry and Behavior*, *8*(1), 81–87.

Fortin, N. J., Agster, K. L., & Eichenbaum, H. B. (2002). Critical role of the hippocampus in memory for sequences of events. *Nature Neuroscience*, *5*(5), 458–462.

Forwood, S. E., Winters, B. D., & Bussey, T. J. (2005). Hippocampal lesions that abolish spatial maze performance spare object recognition memory at delays of up to 48 hours. *Hippocampus*, *15*(3), 347–355.

Foy, M. R., Stanton, M. E., Levine, S., & Thompson, R. F. (1987). Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behavioral and Neural Biology*, *48*(1), 138–149.

Franklin, K. B. J., & Paxinos, G. (2007). *Paxinos and Franklin's the Mouse brain in stereotaxic coordinates, compact: The coronal plates and diagrams*. Academic press.

Funakoshi, H., Frisén, J., Barbany, G., Timmusk, T., Zachrisson, O., Verge, V. M., & Persson, H. (1993). Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *The Journal of Cell Biology*, *123*(2), 455–465.

Gao, J., Wang, W.-Y., Mao, Y.-W., Gräff, J., Guan, J.-S., Pan, L., Mak, G., Kim, D., Su, S. C., & Tsai, L.-H. (2010). A novel pathway regulates memory and plasticity via SIRT1 and miR-134. *Nature*, *466*(7310), 1105–1109.

Gerlai, R. (1998). Contextual learning and cue association in fear conditioning in mice: a strain comparison and a lesion study. *Behavioural Brain Research*, *95*(2), 191–203.

Geiller, T., Vancura, B., Terada, S., Troullinou, E., Chavlis, S., Tsagkatakis, G., Tsakalides, P., Ócsai, K., Poirazi, P., & Rozsa, B. J. (2020). Large-scale 3D two-photon imaging of molecularly identified CA1 interneuron dynamics in behaving mice. *Neuron*, *108*(5), 968–983.

Gerlai, R. (1998). Contextual learning and cue association in fear conditioning in mice: a strain comparison and a lesion study. *Behavioural Brain Research*, *95*(2), 191–203.

Ghosh, S., & Chattarji, S. (2015). Neuronal encoding of the switch from specific to generalized fear. *Nature Neuroscience*, *18*(1), 112–120.

Gilbert, P. E., & Brushfield, A. M. (2009). The role of the CA3 hippocampal subregion in spatial memory: A process oriented behavioral assessment. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*(5), 774–781.

Gilbert, P. E., Kesner, R. P., & Lee, I. (2001). Dissociating hippocampal subregions: A double dissociation between dentate gyrus and CA1. *Hippocampus*, *11*(6), 626–636.

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

Good, M., de Hoz, L., & Morris, R. G. M. (1998). Contingent versus incidental context processing during conditioning: Dissociation after excitotoxic hippocampal plus dentate gyrus lesions. *Hippocampus*, *8*(2), 147–159.

GoodSmith, D., Chen, X., Wang, C., Kim, S. H., Song, H., Burgalossi, A., Christian, K. M., & Knierim, J. J. (2017). Spatial representations of granule cells and mossy cells of the dentate gyrus. *Neuron*, *93*(3), 677–690.

Gray, J. D., Kogan, J. F., Marrocco, J., & McEwen, B. S. (2017). Genomic and epigenomic mechanisms of glucocorticoids in the brain. *Nature Reviews Endocrinology*, *13*(11), 661–673.

Gray, J. D., Milner, T. A., & McEwen, B. S. (2013). Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors. *Neuroscience*, *239*, 214–227.

Gunn, B. G., Cunningham, L., Mitchell, S. G., Swinny, J. D., Lambert, J. J., & Belelli, D. (2015). GABAA receptor-acting neurosteroids: a role in the development and regulation of the stress response. *Frontiers in Neuroendocrinology*, *36*, 28–48.

Guo, N.N., & Li, B.M. (2007). Cellular and subcellular distributions of β1-and β2-adrenoceptors in the CA1 and CA3 regions of the rat hippocampus. *Neuroscience*, *146*(1), 298–305.

Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., McGaugh, J. L., Worley, P. F., & Barnes, C. A. (2000). Inhibition of Activity-Dependent Arc Protein Expression in the Rat Hippocampus Impairs the Maintenance of Long-Term Potentiation and the Consolidation of Long-Term Memory. *The Journal of Neuroscience*, *20*(11), 3993–4001.

Haettig, J., Stefanko, D. P., Multani, M. L., Figueroa, D. X., McQuown, S. C., & Wood, M. A. (2011). HDAC inhibition modulates hippocampus-dependent long-term memory for object location in a CBP-dependent manner. *Learning & Memory*, *18*(2), 71–79.

Hammond, R. S., Tull, L. E., & Stackman, R. W. (2004). On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiology of Learning and Memory*, *82*(1), 26–34.

Harley, C. W. (2007). Norepinephrine and the dentate gyrus. *Progress in Brain Research*, *163*, 299–318.

Harris, K. D., Hochgerner, H., Skene, N. G., Magno, L., Katona, L., Bengtsson Gonzales, C., Somogyi, P., Kessaris, N., Linnarsson, S., & Hjerling-Leffler, J. (2018). Classes and continua of hippocampal CA1 inhibitory neurons revealed by single-cell transcriptomics. *PLoS Biology*, *16*(6), e2006387.

Hatfield, T., & McGaugh, J. L. (1999). Norepinephrine infused into the basolateral amygdala posttraining enhances retention in a spatial water maze task. *Neurobiology of Learning and Memory*, *71*(2), 232–239.

He, L., & Hannon, G. J. (2004). MicroRNAs: small RNAs with a big role in gene regulation. *Nature Reviews Genetics*, *5*(7), 522–531.

He, Z., He, Y. J., Day, A. L., & Simpkins, J. W. (2002). Proestrus levels of estradiol during transient global cerebral ischemia improves the histological outcome of the hippocampal CA1 region: Perfusion-dependent and-independent mechanisms. *Journal of the Neurological Sciences*, *193*(2), 79–87.

Heginbotham, L. R., & Dunwiddie, T. V. (1991). Long-term increases in the evoked population spike in the CA1 region of rat hippocampus induced by beta-adrenergic receptor activation. *Journal of Neuroscience*, *11*(8), 2519–2527.

Henckens, M. J. A. G., Pu, Z., Hermans, E. J., van Wingen, G. A., Joëls, M., & Fernández, G. (2012). Dynamically changing effects of corticosteroids on human hippocampal and prefrontal processing. *Human Brain Mapping*, *33*(12), 2885–2897.

Henriksen, E. J., Colgin, L. L., Barnes, C. A., Witter, M. P., Moser, M.-B., & Moser, E. I. (2010). Spatial Representation along the Proximodistal Axis of CA1. *Neuron*, *68*(1), 127–137.

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. *Neurobiology of Learning and Memory*, *112*, 237–247.

Hounsgaard, J. (1978). Presynaptic inhibitory action of acetylcholine in area CA1 of the hippocampus. *Experimental Neurology*, *62*(3), 787–797.

Jänig, W. (2014). Sympathetic nervous system and inflammation: A conceptual view. *Autonomic Neuroscience*, *182*, 4–14.

Jeong, N., & Singer, A. C. (2022). Learning from inhibition: Functional roles of hippocampal CA1 inhibition in spatial learning and memory. *Current Opinion in Neurobiology*, *76*, 102604.

Joëls, M. (2008). Functional actions of corticosteroids in the hippocampus. *European Journal of Pharmacology*, *583*(2), 312–321.

Joëls, M., Fernandez, G., & Roozendaal, B. (2011). Stress and emotional memory: a matter of timing. *Trends in Cognitive Sciences*, *15*(6), 280–288.

Joëls, M., Sarabdjitsingh, R. A., & Karst, H. (2012). Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. *Pharmacological Reviews*, *64*(4), 901–938.

John, S., Sabo, P. J., Thurman, R. E., Sung, M.-H., Biddie, S. C., Johnson, T. A., Hager, G. L., & Stamatoyannopoulos, J. A. (2011). Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. *Nature Genetics*, *43*(3), 264–268.

Jones, B. E., & Moore, R. Y. (1977). Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. *Brain Research*, *127*(1), 23–53.

Johnson, M. K., Raye, C. L., Mitchell, K. J., Greene, E. J., Cunningham, W. A., & Sanislow, C. A. (2005). Using fMRI to investigate. *Cognitive, Affective, & Behavioral Neuroscience, 5*(3), 339–361.

Jung, M. W., & McNaughton, B. L. (1993). Spatial selectivity of unit activity in the hippocampal granular layer. *Hippocampus*, *3*(2), 165–182.

Jurado-Berbel, P., Costa-Miserachs, D., Torras-Garcia, M., Coll-Andreu, M., & Portell-Cortés, I. (2010). Standard object recognition memory and "what" and "where" components: Improvement by post-training epinephrine in highly habituated rats. *Behavioural Brain Research*, *207*(1), 44–50.

Jurgens, C. W. D., Boese, S. J., King, J. D., Pyle, S. J., Porter, J. E., & Doze, V. A. (2005). Adrenergic receptor modulation of hippocampal CA3 network activity. *Epilepsy Research*, *66*(1–3), 117–128.

Kafkas, A., & Montaldi, D. (2014). Two separate, but interacting, neural systems for familiarity and novelty detection: A dual-route mechanism. *Hippocampus*, *24*(5), 516–527.

Kaouane, N., Porte, Y., Vallée, M., Brayda-Bruno, L., Mons, N., Calandreau, L., Marighetto, A., Piazza, P. V., & Desmedt, A. (2012). Glucocorticoids Can Induce PTSD-Like Memory Impairments in Mice. *Science*, *335*(6075), 1510–1513.

Karst, H., Berger, S., Erdmann, G., Schütz, G., & Joëls, M. (2010). Metaplasticity of amygdalar responses to the stress hormone corticosterone. *Proceedings of the National Academy of Sciences*, *107*(32), 14449–14454.

Karst, H., Berger, S., Turiault, M., Tronche, F., Schütz, G., & Joëls, M. (2005a). Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proceedings of the National Academy of Sciences*, *102*(52), 19204–19207.

Karst, H., & Joëls, M. (2005b). Corticosterone Slowly Enhances Miniature Excitatory Postsynaptic Current Amplitude in Mice CA1 Hippocampal Cells. *Journal of Neurophysiology*, *94*(5), 3479–3486.

Karst, H., & Joëls, M. (2016). Severe stress hormone conditions cause an extended window of excitability in the mouse basolateral amygdala. *Neuropharmacology*, *110*, 175–180.

Kebschull, J. M., da Silva, P. G., Reid, A. P., Peikon, I. D., Albeanu, D. F., & Zador, A. M. (2016). High-throughput mapping of single-neuron projections by sequencing of barcoded RNA. *Neuron*, *91*(5), 975–987.

Kensinger, E. A., Garoff-Eaton, R. J., & Schacter, D. L. (2007a). Effects of Emotion on Memory Specificity in Young and Older Adults. *The Journals of Gerontology: Series B*, *62*(4), 208–215.

Kensinger, E. A., Garoff-Eaton, R. J., & Schacter, D. L. (2007b). How negative emotion enhances the visual specificity of a memory. *Journal of Cognitive Neuroscience*, *19*(11), 1872–1887.

Kesner, R. P. (2013). An analysis of the dentate gyrus function. *Behavioural Brain Research*, *254*, 1–7.

Kesner, R. P., Gilbert, P. E., & Barua, L. A. (2002). The role of the hippocampus in memory for the temporal order of a sequence of odors. *Behavioral Neuroscience*, *116*, 286–290.

Kesner, R. P., & Rolls, E. T. (2015). A computational theory of hippocampal function, and tests of the theory: New developments. *Neuroscience & Biobehavioral Reviews*, *48*, 92–147.

Kida, S., Josselyn, S. A., de Ortiz, S. P., Kogan, J. H., Chevere, I., Masushige, S., & Silva, A. J. (2002). CREB required for the stability of new and reactivated fear memories. *Nature Neuroscience*, *5*(4), 348–355.

Kitchigina, V., Vankov, A., Harley, C., & Sara, S. J. (1997). Novelty-elicited, noradrenaline-dependent enhancement of excitability in the dentate gyrus. *European Journal of Neuroscience*, *9*(1), 41–47.

Knierim, J. J., Neunuebel, J. P., & Deshmukh, S. S. (2014). Functional correlates of the lateral and medial entorhinal cortex: objects, path integration and local–global reference frames. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1635), 20130369.

Koning, A.-S. C. A. M., Buurstede, J. C., van Weert, L. T. C. M., & Meijer, O. C. (2019). Glucocorticoid and Mineralocorticoid Receptors in the Brain: A Transcriptional Perspective. *Journal of the Endocrine Society*, *3*(10), 1917–1930.

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flügge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B., & Palanza, P. (2011). Stress revisited: a critical evaluation of the stress concept. *Neuroscience & Biobehavioral Reviews*, *35*(5), 1291–1301.

Krueger, J. N., Wilmot, J. H., Teratani-Ota, Y., Puhger, K. R., Nemes, S. E., Crestani, A. P., Lafreniere, M. M., & Wiltgen, B. J. (2020). Amnesia for context fear is caused by widespread disruption of hippocampal activity. *Neurobiology of Learning and Memory*, *175*, 107295.

Kuo, T., McQueen, A., Chen, T.-C., & Wang, J.-C. (2015). Regulation of glucose homeostasis by glucocorticoids. *Glucocorticoid Signaling: From Molecules to Mice to Man*, 99–126.

Laing, M., & Bashir, Z. I. (2014). β-Adrenoceptors and synaptic plasticity in the perirhinal cortex. *Neuroscience*, *273*, 163–173.

Lakshminarasimhan, H., & Chattarji, S. (2012). Stress leads to contrasting effects on the levels of brain derived neurotrophic factor in the hippocampus and amygdala. *PloS One*, *7*(1), e30481.

LaLumiere, R. T., Buen, T.-V., & McGaugh, J. L. (2003). Post-training intra-basolateral amygdala infusions of norepinephrine enhance consolidation of memory for contextual fear conditioning. *Journal of Neuroscience*, *23*(17), 6754–6758.

Langston, R. F., & Wood, E. R. (2010). Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus*, *20*(10), 1139–1153.

Le Menuet, D., & Lombès, M. (2014). The neuronal mineralocorticoid receptor: from cell survival to neurogenesis. *Steroids*, *91*, 11–19.

Lee, H., GoodSmith, D., & Knierim, J. J. (2020). Parallel processing streams in the hippocampus. *Current Opinion in Neurobiology*, *64*, 127–134.

Lee, M.-J., Gong, D.-W., Burkey, B. F., & Fried, S. K. (2011). Pathways regulated by glucocorticoids in omental and subcutaneous human adipose tissues: a microarray study. *American Journal of Physiology-Endocrinology and Metabolism*, *300*(3), 571–580.

Leger, M., Quiedeville, A., Bouet, V., Haelewyn, B., Boulouard, M., Schumann-Bard, P., & Freret, T. (2013). Object recognition test in mice. *Nature Protocols*, *8*(12), 2531–2537.

Lesuis, S. L., Brosens, N., Immerzeel, N., van der Loo, R. J., Mitrić, M., Bielefeld, P., Fitzsimons, C. P., Lucassen, P. J., Kushner, S. A., van den Oever, M. C., & Krugers, H. J. (2021). Glucocorticoids Promote Fear Generalization by Increasing the Size of a Dentate Gyrus Engram Cell Population. *Biological Psychiatry*, *90*(7), 494–504.

Lesuis, S. L., Timmermans, W., Lucassen, P. J., Hoogenraad, C. C., & Krugers, H. J. (2020). Glucocorticoid and β-adrenergic regulation of hippocampal dendritic spines. *Journal of Neuroendocrinology*, *32*(1), e12811.

Leutgeb, J. K., Leutgeb, S., Moser, M.-B., & Moser, E. I. (2007). Pattern Separation in the Dentate Gyrus and CA3 of the Hippocampus. *Science*, *315*(5814), 961–966.

Levine, L. J., & Edelstein, R. S. (2009). Emotion and memory narrowing: A review and goal-relevance approach. *Cognition and Emotion*, *23*(5), 833–875.

Li, J.-T., Su, Y.-A., Guo, C.-M., Feng, Y., Yang, Y., Huang, R.-H., & Si, T.-M. (2011). Persisting cognitive deficits induced by low-dose, subchronic treatment with MK-801 in adolescent rats. *European Journal of Pharmacology*, *652*(1), 65–72.

Liang, K. C., Juler, R. G., & McGAUGH, J. L. (1986). Modulating effects of posttraining epinephrine on memory: involvement of the amygdala noradrenergic system. *Brain Research*, *368*(1), 125–133.

Liang, K. C., McGaugh, J. L., & Yao, H.-Y. (1990). Involvement of amygdala pathways in the influence of post-training intra-amygdala norepinephrine and peripheral epinephrine on memory storage. *Brain Research*, *508*(2), 225–233.

Lis, S., Thome, J., Kleindienst, N., Mueller-Engelmann, M., Steil, R., Priebe, K., Schmahl, C., Hermans, D., & Bohus, M. (2020). Generalization of fear in post-traumatic stress disorder. *Psychophysiology*, *57*(1), e13422.

Lisman, J. E., & Grace, A. A. (2005). The hippocampal-VTA loop: controlling the entry of information into long-term memory. *Neuron*, *46*(5), 703–713.

Lissek, S., Bradford, D. E., Alvarez, R. P., Burton, P., Espensen-Sturges, T., Reynolds, R. C., & Grillon, C. (2014). Neural substrates of classically conditioned fear-generalization in humans: a parametric fMRI study. *Social Cognitive and Affective Neuroscience*, *9*(8), 1134–1142.

Loftus, E. (1979). Eyewitness Reliability: The Psychology of Person Identification. Brian R. Clifford and Ray Bull. Routledge and Kegan Paul, Boston, 1978. xiv, 254 pp. $20. *Science*, *205*(4404), 386–387.

Loy, R., Koziell, D. A., Lindsey, J. D., & Moore, R. Y. (1980). Noradrenergic innervation of the adult rat hippocampal formation. *Journal of Comparative Neurology*, *189*(4), 699–710.

Luna, V. M., Anacker, C., Burghardt, N. S., Khandaker, H., Andreu, V., Millette, A., Leary, P., Ravenelle, R., Jimenez, J. C., & Mastrodonato, A. (2019). Adult-born hippocampal neurons bidirectionally modulate entorhinal inputs into the dentate gyrus. *Science*, *364*(6440), 578–583.

Lupien, S. J., Wilkinson, C. W., Brière, S., Ménard, C., Kin, N. M. K. N. Y., & Nair, N. P. V. (2002). The modulatory effects of corticosteroids on cognition: studies in young human populations. *Psychoneuroendocrinology*, *27*(3), 401–416.

Manvich, D. F., Webster, K. A., Foster, S. L., Farrell, M. S., Ritchie, J. C., Porter, J. H., & Weinshenker, D. (2018). The DREADD agonist clozapine N-oxide (CNO) is reverse-metabolized to clozapine and produces clozapine-like interoceptive stimulus effects in rats and mice. *Scientific Reports*, *8*(1), 3840.

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behavioural Brain Research*, *88*(2), 261–274.

Maroun, M., & Akirav, I. (2008). Arousal and stress effects on consolidation and reconsolidation of recognition memory. *Neuropsychopharmacology*, *33*(2), 394–405.

Marr, D. (1971). A theory of archicortical function. *Proc R Soc Lond B Biol Sci*, *262*, 23–81.

Martinez Jr, J. L., Jensen, R. A., Messing, R. B., Vasquez, B. J., Soumireu-Mourat, B., Geddes, D., Liang, K. C., & McGaugh, J. L. (1980). Central and peripheral actions of amphetamine on memory storage. *Brain Research*, *182*(1), 157–166.

Mason, J. W. (1968). A Review of Psychoendocrine Research on the Sympathetic-Adrenal Medullary System. *Psychosomatic Medicine*, *30*(5), 631–653.

Massey, P. V, Phythian, D., Narduzzo, K., Warburton, E. C., Brown, M. W., & Bashir, Z. I. (2008). Learning-Specific Changes in Long-Term Depression in Adult Perirhinal Cortex. *The Journal of Neuroscience*, *28*(30), 7548–7554.

Mather, M., Clewett, D., Sakaki, M., & Harley, C. W. (2016). Norepinephrine ignites local hotspots of neuronal excitation: How arousal amplifies selectivity in perception and memory. *Behavioral and Brain Sciences*, *39*, e200.

McDonald, R. J., & White, N. M. (1993). A triple dissociation of memory systems: Hippocampus, amygdala, and dorsal striatum. *Behavioral Neuroscience*, *107*(1), 3–22.

McEwen, B. S., & Akil, H. (2020). Revisiting the stress concept: implications for affective disorders. *Journal of Neuroscience*, *40*(1), 12–21.

McEwen, B. S., Weiss, J. M., & Schwartz, L. S. (1968). Selective retention of corticosterone by limbic structures in rat brain. *Nature*, *220*(5170), 911–912.

McGaugh, J. L. (2000). Memory--a Century of Consolidation. *Science*, *287*(5451), 248–251.

McGaugh, J. L. (2003). Memory and emotion: The making of lasting memories. *Memory and Emotion: The Making of Lasting Memories*., 162–162.

McGaugh, J. L. (2013). Making lasting memories: Remembering the significant. *Proceedings of the National Academy of Sciences*, *110*(supplement_2), 10402–10407.

McGaugh, J. L., & Gold, P. E. (1989). Hormonal modulation of memory. *Psychoendocrinology*, 305–340.

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.

McHugh, T. J., Jones, M. W., Quinn, J. J., Balthasar, N., Coppari, R., Elmquist, J. K., Lowell, B. B., Fanselow, M. S., Wilson, M. A., & Tonegawa, S. (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science*, *317*(5834), 94–99.

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.

McReynolds, J. R., Anderson, K. M., Donowho, K. M., & McIntyre, C. K. (2014). Noradrenergic actions in the basolateral complex of the amygdala modulate Arc expression in hippocampal synapses and consolidation of aversive and non-aversive memory. *Neurobiology of Learning and Memory*, *115*, 49–57.

McReynolds, J. R., Donowho, K., Abdi, A., McGaugh, J. L., Roozendaal, B., & McIntyre, C. K. (2010). Memory-enhancing corticosterone treatment increases amygdala norepinephrine and Arc protein expression in hippocampal synaptic fractions. *Neurobiology of Learning and Memory*, *93*(3), 312–321.

Miles, R. (1990). Synaptic excitation of inhibitory cells by single CA3 hippocampal pyramidal cells of the guinea-pig in vitro. *The Journal of Physiology*, *428*(1), 61–77.

Milner, T. A., Shah, P., & Pierce, J. P. (2000). β-Adrenergic receptors primarily are located on the dendrites of granule cells and interneurons but also are found on astrocytes and a few presynaptic profiles in the rat dentate gyrus. *Synapse*, *36*(3), 178–193.

Minatohara, K., Akiyoshi, M., & Okuno, H. (2016). Role of Immediate-Early Genes in Synaptic Plasticity and Neuronal Ensembles Underlying the Memory Trace. *Frontiers in Molecular Neuroscience*, *8*(78).

Miranda, M. I., Quirarte, G. L., Rodriguez-Garcia, G., McGaugh, J. L., & Roozendaal, B. (2008). Glucocorticoids enhance taste aversion memory via actions in the insular cortex and basolateral amygdala. *Learning & Memory*, *15*(7), 468–476.

Miranda, M., Kent, B. A., Morici, J. F., Gallo, F., Weisstaub, N. V, Saksida, L. M., Bussey, T. J., & Bekinschtein, P. (2017). Molecular Mechanisms in Perirhinal Cortex Selectively Necessary for Discrimination of Overlapping Memories, but Independent of Memory Persistence. *Eneuro*, *4*(5), 0293.

Mishkin, M., & Ungerleider, L. G. (1982). Contribution of striate inputs to the visuospatial functions of parieto-preoccipital cortex in monkeys. *Behavioural Brain Research*, *6*(1), 57–77.

Mishkin, M., Ungerleider, L. G., & Macko, K. A. (1983). Object vision and spatial vision: two cortical pathways. *Trends in Neurosciences*, *6*, 414–417.

Mizumori, S. J. Y., Perez, G. M., Alvarado, M. C., Barnes, C. A., & McNaughton, B. L. (1990). Reversible inactivation of the medial septum differentially affects two forms of learning in rats. *Brain Research*, *528*(1), 12–20.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., & Nabeshima, T. (2000). Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *Journal of Neuroscience*, *20*(18), 7116–7121.

Molas, S., Zhao-Shea, R., Liu, L., DeGroot, S. R., Gardner, P. D., & Tapper, A. R. (2017). A circuit-based mechanism underlying familiarity signaling and the preference for novelty. *Nature Neuroscience*, *20*(9), 1260–1268.

Morgan, C. A., Hazlett, G., Doran, A., Garrett, S., Hoyt, G., Thomas, P., Baranoski, M., & Southwick, S. M. (2004a). Accuracy of eyewitness memory for persons encountered during exposure to highly intense stress. *International Journal of Law and Psychiatry, 27*(3), 265–279.

Morgan, C. A., Southwick, S., Hazlett, G., Rasmusson, A., Hoyt, G., Zimolo, Z., & Charney, D. (2004b). Relationships Among Plasma Dehydroepiandrosterone Sulfate and CortisolLevels, Symptoms of Dissociation, and Objective Performance in Humans Exposedto Acute Stress. *Archives of General Psychiatry, 61*(8), 819–825.

Morimoto, M., Morita, N., Ozawa, H., Yokoyama, K., & Kawata, M. (1996). Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study. *Neuroscience Research*, *26*(3), 235–269.

Morris, A. M. (2011). *Further Defining the Functional Properties of the Dentate Gyrus: Contributions to Spatial Representations*. Department of Psychology, University of Utah.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, *11*(1), 47–60.

Morton, N. W., Sherrill, K. R., & Preston, A. R. (2017). Memory integration constructs maps of space, time, and concepts. *Current Opinion in Behavioral Sciences*, *17*, 161–168.

Moscovitch, M., & Gilboa, A. (2021). *Systems consolidation, transformation and reorganization: Multiple trace theory, trace transformation theory and their competitors*.competitors. Preprint at PsyArXiv.

Mueller, A. L., Hoffer, B. J., & Dunwiddie, T. V. (1981). Noradrenergic responses in rat hippocampus: evidence for mediation by α and β receptors in the in vitro slice. *Brain Research*, *214*(1), 113–126.

Muller, R. U., & Kubie, J. L. (1987). The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *The Journal of Neuroscience*, *7*(7), 1951–1968.

Mumby, D. G., Glenn, M. J., Nesbitt, C., & Kyriazis, D. A. (2002). Dissociation in retrograde memory for object discriminations and object recognition in rats with perirhinal cortex damage. *Behavioural Brain Research*, *132*(2), 215–226.

Myers, C. E., & Scharfman, H. E. (2009). A role for hilar cells in pattern separation in the dentate gyrus: a computational approach. *Hippocampus*, *19*(4), 321–337.

Nakazawa, Y., Pevzner, A., Tanaka, K. Z., & Wiltgen, B. J. (2016). Memory retrieval along the proximodistal axis of CA1. *Hippocampus*, *26*(9), 1140–1148.

Nathan, S. V, Griffith, Q. K., McReynolds, J. R., Hahn, E. L., & Roozendaal, B. (2004). Basolateral amygdala interacts with other brain regions in regulating glucocorticoid effects on different memory functions. *Annals of the New York Academy of Sciences*, *1032*(1), 179–182.

Nederhof, E., & Schmidt, M. V. (2012). Mismatch or cumulative stress: toward an integrated hypothesis of programming effects. *Physiology & Behavior*, *106*(5), 691–700.

Neunuebel, J. P., Yoganarasimha, D., Rao, G., & Knierim, J. J. (2013). Conflicts between local and global spatial frameworks dissociate neural representations of the lateral and medial entorhinal cortex. *Journal of Neuroscience*, *33*(22), 9246–9258.

Nirogi, R., Goura, V., Shanmuganathan, D., Jayarajan, P., & Abraham, R. (2012). Comparison of manual and automated filaments for evaluation of neuropathic pain behavior in rats. *Journal of Pharmacological and Toxicological Methods*, *66*(1), 8–13.

Norman, G., & Eacott, M. J. (2005). Dissociable Effects of Lesions to the Perirhinal Cortex and the Postrhinal Cortex on Memory for Context and Objects in Rats. *Behavioral Neuroscience*, *119*, 557–566.

O'Keefe, J. (1976). Place units in the hippocampus of the freely moving rat. *Experimental Neurology*, *51*(1), 78–109.

O'Keefe, J., & Conway, D. H. (1978). Hippocampal place units in the freely moving rat: Why they fire where they fire. *Experimental Brain Research*, *31*(4), 573–590.

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map: Preliminary evidence from unit activity in the freely-moving rat. In *Brain Research* (Vol. 34, pp. 171–175). Elsevier Science.

Ochsner, K. N. (2000). Are affective events richly recollected or simply familiar? The experience and process of recognizing feelings past. *Journal of Experimental Psychology: General*, *129*(2), 242.

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.

Olarte-Sánchez, C. M., Amin, E., Warburton, E. C., & Aggleton, J. P. (2015). Perirhinal cortex lesions impair tests of object recognition memory but spare novelty detection. *European Journal of Neuroscience*, *42*(12), 3117–3127.

Olijslagers, J. E., De Kloet, E. R., Elgersma, Y., Van Woerden, G. M., Joëls, M., & Karst, H. (2008). Rapid changes in hippocampal CA1 pyramidal cell function via pre-as well as postsynaptic membrane mineralocorticoid receptors. *European Journal of Neuroscience*, *27*(10), 2542–2550.

Oliveira, A. M. M., Hawk, J. D., Abel, T., & Havekes, R. (2010). Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. *Learning & Memory*, *17*, 155–160.

Omoumi, S., Rashidy-Pour, A., Seyedinia, S. A., Tarahomi, P., Vafaei, A. A., & Raise-Abdullahi, P. (2023). Corticosterone injection into the infralimbic prefrontal cortex enhances fear memory extinction: Involvement of GABA receptors and the extracellular signal-regulated kinase. *Physiology & Behavior*, *265*, 114156.

Packard, M. G., Hirsh, R., & White, N. M. (1989). Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems. *Journal of Neuroscience*, *9*(5), 1465–1472.

Packard, M. G., & McGaugh, J. L. (1996). Inactivation of Hippocampus or Caudate Nucleus with Lidocaine Differentially Affects Expression of Place and Response Learning. *Neurobiology of Learning and Memory*, *65*(1), 65–72.

Payne, J. D., Nadel, L., Allen, J. J. B., Thomas, K. G. F., & Jacobs, W. J. (2002). The effects of experimentally induced stress on false recognition. *Memory*, *10*(1), 1–6.

Pelkey, K. A., Chittajallu, R., Craig, M. T., Tricoire, L., Wester, J. C., & McBain, C. J. (2017). Hippocampal GABAergic inhibitory interneurons. *Physiological Reviews*, *97*(4), 1619–1747.

Perugini, A., Laing, M., Berretta, N., Aicardi, G., & Bashir, Z. I. (2012). Synaptic plasticity from amygdala to perirhinal cortex: a possible mechanism for emotional enhancement of visual recognition memory? *European Journal of Neuroscience*, *36*(4), 2421–2427.

Petrovich, G. D., Canteras, N. S., & Swanson, L. W. (2001). Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Research Reviews*, *38*(1–2), 247–289.

Petzold, M., & Bunzeck, N. (2022). Impaired episodic memory in PTSD patients—A meta-analysis of 47 studies. *Frontiers in Psychiatry*, *13*, 909442.

Pezze, M. A., Marshall, H. J., Fone, K. C. F., & Cassaday, H. J. (2017). Role of the anterior cingulate cortex in the retrieval of novel object recognition memory after a long delay. *Learning & Memory*, *24*, 310–317.

Pitkänen, A., Pikkarainen, M., Nurminen, N., & Ylinen, A. (2000). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat: a review. *Annals of the New York Academy of Sciences*, *911*(1), 369–391.

Pitman, R. K., Rasmusson, A. M., Koenen, K. C., Shin, L. M., Orr, S. P., Gilbertson, M. W., Milad, M. R., & Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nature Reviews Neuroscience*, *13*(11), 769–787.

Porter, S., Taylor, K., & ten Brinke, L. (2008). Memory for media: Investigation of false memories for negatively and positively charged public events. *Memory*, *16*(6), 658–666.

Price, J. L. (2003). Comparative aspects of amygdala connectivity. *Annals of the New York Academy of Sciences*, *985*(1), 50–58.

Provençal, N., Arloth, J., Cattaneo, A., Anacker, C., Cattane, N., Wiechmann, T., Röh, S., Ködel, M., Klengel, T., Czamara, D., Müller, N. S., Lahti, J., team, P., Räikkönen, K., Pariante, C. M., Binder, E. B., Kajantie, E., Hämäläinen, E., Villa, P., & Laivuori, H. (2020). Glucocorticoid exposure during hippocampal neurogenesis primes future stress response by inducing changes in DNA methylation. *Proceedings of the National Academy of Sciences*, *117*(38), 23280–23285.

Quaedflieg, C. W. E. M., Schneider, T. R., Daume, J., Engel, A. K., & Schwabe, L. (2020). Stress Impairs Intentional Memory Control through Altered Theta Oscillations in Lateral Parietal Cortex. *The Journal of Neuroscience*, *40*(40), 7739–7748.

Quaedflieg, C. W. E. M., Schneider, T. R., Daume, J., Engel, A. K., & Schwabe, L. (2020). Stress Impairs Intentional Memory Control through Altered Theta Oscillations in Lateral Parietal Cortex. *The Journal of Neuroscience, 40*(40), 7739–7748.

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.

Rang, H. P., Ritter, J. M., Flower, R., & Henderson, G. (2014). Chapter 14: Noradrenergic transmission. Rang & Dale's Pharmacology. *Elsevier Health Sciences*, 177–196.

Rashid, A. J., Yan, C., Mercaldo, V., Hsiang, H.-L., Park, S., Cole, C. J., De Cristofaro, A., Yu, J., Ramakrishnan, C., & Lee, S. Y. (2016). Competition between engrams influences fear memory formation and recall. *Science*, *353*(6297), 383–387.

Raybuck, J. D., & Lattal, K. M. (2014). Differential effects of dorsal hippocampal inactivation on expression of recent and remote drug and fear memory. *Neuroscience Letters*, *569*, 1–5.

Reul, J. M. H. M., Kloet, & E R D E. (1985). Two Receptor Systems for Corticosterone in Rat Brain: Microdistribution and Differential Occupation. *Endocrinology*, *117*(6), 2505–2511.

Richards, J. M., & Gross, J. J. (2006). Personality and emotional memory: How regulating emotion impairs memory for emotional events. *Journal of Research in Personality*, *40*(5), 631–651.

Riedemann, T., Patchev, A. V, Cho, K., & Almeida, O. F. X. (2010). Corticosteroids: way upstream. *Molecular Brain*, *3*, 1–20.

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*, 553–562.

Rogerson, T., Cai, D. J., Frank, A., Sano, Y., Shobe, J., Lopez-Aranda, M. F., & Silva, A. J. (2014). Synaptic tagging during memory allocation. *Nature Reviews Neuroscience*, *15*(3), 157–169.

Rolls, E. (2013). The mechanisms for pattern completion and pattern separation in the hippocampus. *Frontiers in Systems Neuroscience*, *7*.

Rolls, E. T. (1987). Information Representation, Processing, and Storage in the Brain: Analysis at the Single Neuron Level. *The Neural and Molecular Bases of Learning*, 503–539.

Rolls, E. T. (1989). *Functions of Neuronal Networks in the Hippocampus and Neocortex in Memory*. 240–265.

Rolls, E. T. (1996). A theory of hippocampal function in memory. *Hippocampus*, *6*(6), 601–620.

Rolls, E. T. (2016). Pattern separation, completion, and categorisation in the hippocampus and neocortex. *Neurobiology of Learning and Memory*, *129*, 4–28.

Roozendaal, B., Carmi, O., & McGaugh, J. L. (1996a). Adrenocortical suppression blocks the memory-enhancing effects of amphetamine and epinephrine. *Proceedings of the National Academy of Sciences*, *93*(4), 1429–1433.

Roozendaal, B., & McGaugh, J. L. (1996b). Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task. *Neurobiology of Learning and Memory, 65*(1), 1–8.

Roozendaal, B., & McGaugh, J. L. (1996c). The memory-modulatory effects of glucocorticoids depend on an intact stria terminalis. *Brain Research*, *709*(2), 243–250.

Roozendaal, B., Portillo-Marquez, G., & McGaugh, J. L. (1996d). Basolateral amygdala lesions block glucocorticoid-induced modulation of memory for spatial learning. *Behavioral Neuroscience, 110*(5), 1074.

Roozendaal, B., Bohus, B., & McGaugh, J. L. (1996e). Dose-dependent suppression of adrenocortical activity with metyrapone: effects on emotion and memory. *Psychoneuroendocrinology*, *21*(8), 681–693.

Roozendaal, B., & McGaugh, J. L. (1997a). Basolateral amygdala lesions block the memory-enhancing effect of glucocorticoid administration in the dorsal hippocampus of rats. *European Journal of Neuroscience, 9*(1), 76–83.

Roozendaal, B., & McGaugh, J. L. (1997b). Glucocorticoid Receptor Agonist and Antagonist Administration into the Basolateral but Not Central Amygdala Modulates Memory Storage. *Neurobiology of Learning and Memory*, *67*(2), 176–179.

Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009a). Stress, memory and the amygdala. *Nature Reviews Neuroscience*, *10*(6), 423–433.

Roozendaal, B., McReynolds, J. R., Zee, E. A. Van der, Lee, S., McGaugh, J. L., & McIntyre, C. K. (2009b). Glucocorticoid Effects on Memory Consolidation Depend on Functional Interactions between the Medial Prefrontal Cortex and Basolateral Amygdala. *The Journal of Neuroscience, 29*(45), 14299–14308.

Roozendaal, B., Nguyen, B. T., Power, A. E., & McGaugh, J. L. (1999a). Basolateral amygdala noradrenergic influence enables enhancement of memory consolidation induced by hippocampal glucocorticoid receptor activation. *Proceedings of the National Academy of Sciences, 96*(20), 11642–11647.

Roozendaal, B., Williams, C. L., & McGaugh, J. L. (1999b). Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: involvement of the basolateral amygdala. *European Journal of Neuroscience, 11*(4), 1317–1323.

Roozendaal, B., Quirarte, G. L., & McGaugh, J. L. (2002). Glucocorticoids interact with the basolateral amygdala β-adrenoceptor–cAMP/cAMP/PKA system in influencing memory consolidation. *European Journal of Neuroscience, 15*(3), 553–560.

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. P*roceedings of the National Academy of Sciences, 103*(17), 6741–6746.

Roozendaal, B., Hernandez, A., Cabrera, S. M., Hagewoud, R., Malvaez, M., Stefanko, D. P., Haettig, J., & Wood, M. A. (2010). Membrane-Associated Glucocorticoid Activity Is Necessary for Modulation of Long-Term Memory via Chromatin Modification. *The Journal of Neuroscience, 30*(14), 5037–5046.

Roozendaal, B., & McGaugh, J. L. (2011). Memory modulation. Behavioral *Neuroscience, 125*, 797–824

Roozendaal, B., & Mirone, G. (2020). Opposite effects of noradrenergic and glucocorticoid activation on accuracy of an episodic-like memory. *Psychoneuroendocrinology, 114*, 104588.

Rossato, J. I., Bevilaqua, L. R. M., Myskiw, J. C., Medina, J. H., Izquierdo, I., & Cammarota, M. (2007). On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. *Learning & Memory*, *14*, 36–46.

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).

Sah, P., Faber, E. S. L., Lopez de Armentia, M., & Power, J. (2003). The amygdaloid complex: anatomy and physiology. *Physiological Reviews*, *83*(3), 803–834.

Sahgal, A. (1993). Behavioural neuroscience: *a practical approach*. IRL Press.

Sandi, C., Loscertales, M., & Guaza, C. (1997). Experience-dependent facilitating effect of corticosterone on spatial memory formation in the water maze. *European Journal of Neuroscience*, *9*(4), 637–642.

Sandi, C., & Pinelo-Nava, M. T. (2007). Stress and memory: behavioral effects and neurobiological mechanisms. *Neural Plasticity*, *2007*.

Sandi, C., & Rose, S. P. R. (1994). Corticosterone enhances long-term retention in one-day-old chicks trained in a weak passive avoidance learning paradigm. *Brain Research*, *647*(1), 106–112.

Sano, Y., Shobe, J. L., Zhou, M., Huang, S., Shuman, T., Cai, D. J., Golshani, P., Kamata, M., & Silva, A. J. (2014). CREB regulates memory allocation in the insular cortex. *Current Biology*, *24*(23), 2833–2837.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*(1), 55–89.

Sara, S. J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nature Reviews Neuroscience, 10*(3), 211–223.

Sazma, M. A., Shields, G. S., & Yonelinas, A. P. (2019). The effects of post-encoding stress and glucocorticoids on episodic memory in humans and rodents. *Brain and Cognition, 133*, 12–23.

Schaaf, M. J. M., de Jong, J., de Kloet, E. R., & Vreugdenhil, E. (1998). Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. *Brain Research, 813*(1), 112–120.

Schaaf, M. J. M., De Kloet, E. R., & Vreugdenhil, E. (2000). Corticosterone effects on bdnf expression in the hippocampus implications for memory formation. *Stress*, *3*(3), 201–208.

Schacter, D. L. (1999). The seven sins of memory: Insights from psychology and cognitive neuroscience. *American Psychologist, 54*(3), 182–203.

Schlichting, M. L., Mumford, J. A., & Preston, A. R. (2015). Learning-related representational changes reveal dissociable integration and separation signatures in the hippocampus and prefrontal cortex. *Nature Communications*, *6*(1), 8151.

Schratt, G. (2009). MicroRNAs at the synapse. *Nature Reviews Neuroscience*, *10*(12), 842–849.

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kiebler, M., & Greenberg, M. E. (2006). A brain-specific microRNA regulates dendritic spine development. *Nature*, *439*(7074), 283–289.

Schreiber, R., & Newman-Tancredi, A. (2014). Improving cognition in schizophrenia with antipsychotics that elicit neurogenesis through 5-HT1A receptor activation. *Neurobiology of Learning and Memory*, *110*, 72–80.

Schroeder, J. P., Wingard, J. C., & Packard, M. G. (2002). Post-training reversible inactivation of hippocampus reveals interference between memory systems. *Hippocampus*, *12*(2), 280–284.

Schwabe, L., Hermans, E. J., Joëls, M., & Roozendaal, B. (2022). Mechanisms of memory under stress. *Neuron*, *110*(9), 1450–1467.

Schwabe, L., Joëls, M., Roozendaal, B., Wolf, O. T., & Oitzl, M. S. (2012). Stress effects on memory: an update and integration. *Neuroscience & Biobehavioral Reviews*, *36*(7), 1740–1749.

Schwabe, L., & Wolf, O. T. (2009). Stress Prompts Habit Behavior in Humans. *The Journal of Neuroscience*, *29*(22), 7191–7198.

Schwarz, L. A., Miyamichi, K., Gao, X. J., Beier, K. T., Weissbourd, B., DeLoach, K. E., Ren, J., Ibanes, S., Malenka, R. C., & Kremer, E. J. (2015). Viral-genetic tracing of the input–output organization of a central noradrenaline circuit. *Nature*, *524*(7563), 88–92.

Segal, S. K., Stark, S. M., Kattan, D., Stark, C. E., & Yassa, M. A. (2012). Norepinephrine-mediated emotional arousal facilitates subsequent pattern separation. *Neurobiology of Learning and Memory*, *97*(4), 465–469.

Seidenbecher, T., Reymann, K. G., & Balschun, D. (1997). A post-tetanic time window for the reinforcement of long-term potentiation by appetitive and aversive stimuli. *Proceedings of the National Academy of Sciences*, *94*(4), 1494–1499.

Selye, H. (1936). A syndrome produced by diverse nocuous agents. *Nature, 138*(3479), 32.

Senzai, Y., & Buzsáki, G. (2017). Physiological properties and behavioral correlates of hippocampal granule cells and mossy cells. *Neuron*, *93*(3), 691–704.

Seo, D., Zhang, E. T., Piantadosi, S. C., Marcus, D. J., Motard, L. E., Kan, B. K., Gomez, A. M., Nguyen, T. K., Xia, L., & Bruchas, M. R. (2021). A locus coeruleus to dentate gyrus noradrenergic circuit modulates aversive contextual processing. *Neuron*, *109*(13), 2116-2130.e6.

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

Shohamy, D., & Adcock, R. A. (2010). Dopamine and adaptive memory. *Trends in Cognitive Sciences, 14*(10), 464–472.

Shors, T. J., Foy, M. R., Levine, S., & Thompson, R. F. (1990). Unpredictable and uncontrollable stress impairs neuronal plasticity in the rat hippocampus. *Brain Research Bulletin, 24*(5), 663–667.

Silva, A. J., Kogan, J. H., Frankland, P. W., & Kida, S. (1998). CREB and memory. *Annual Review of Neuroscience, 21*(1), 127–148.

Silva, A. J., Zhou, Y., Rogerson, T., Shobe, J., & Balaji, J. (2009). Molecular and Cellular Approaches to Memory Allocation in Neural Circuits. *Science*, *326*(5951), 391–395.

Skaggs, W. E., McNaughton, B. L., Wilson, M. A., & Barnes, C. A. (1996). Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. *Hippocampus*, *6*(2), 149–172.

Smith, D. M., & Bulkin, D. A. (2014). The form and function of hippocampal context representations. *Neuroscience & Biobehavioral Reviews*, *40*, 52–61.

Smith, M. A., Makino, S., Kvetnansky, R., & Post, R. M. (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *Journal of Neuroscience*, *15*(3), 1768–1777.

Song, Q., Bolsius, Y. G., Ronzoni, G., Henckens, M. J. A. G., & Roozendaal, B. (2021). Noradrenergic enhancement of object recognition and object location memory in mice. *Stress*, *24*(2), 181–188.

Swanson, L. W., & Cowan, W. M. (1977). An autoradiographic study of the organization of the efferet connections of the hippocampal formation in the rat. *Journal of Comparative Neurology*, *172*(1), 49–84.

Szemeredi, K., Komoly, S., Kopin, I. J., Bagdy, G., Keiser, H. R., & Goldstein, D. S. (1991). Simultaneous measurement of plasma and brain extracellular fluid concentrations of catechols after yohimbine administration in rats. *Brain Research*, *542*(1), 8–14.

Spencer, R. L., Young, E. A., Choo, P. H., & McEwen, B. S. (1990). Adrenal steroid type I and type II receptor binding: estimates of in vivo receptor number, occupancy, and activation with varying level of steroid. *Brain Research, 514*(1), 37–48.

Stackman, R. W., Cohen, S. J., Lora, J. C., & Rios, L. M. (2016). Temporary inactivation reveals that the CA1 region of the mouse dorsal hippocampus plays an equivalent role in the retrieval of long-term object memory and spatial memory. *Neurobiology of Learning and Memory*, *133*, 118–128.

Stefanko, D. P., Barrett, R. M., Ly, A. R., Reolon, G. K., & Wood, M. A. (2009). Modulation of long-term memory for object recognition via HDAC inhibition. *Proceedings of the National Academy of Sciences*, *106*(23), 9447–9452.

Steidl, S., Mohi-Uddin, S., & Anderson, A. K. (2006). Effects of emotional arousal on multiple memory systems: evidence from declarative and procedural learning. *Learning & Memory*, *13*(5), 650–658.

Sternberg, D. B., Isaacs, K. R., Gold, P. E., & McGaugh, J. L. (1985). Epinephrine facilitation of appetitive learning: Attenuation with adrenergic receptor antagonists. *Behavioral and Neural Biology*, *44*(3), 447–453.

Swanson, L. W., & Cowan, W. M. (1977). An autoradiographic study of the organization of the efferet connections of the hippocampal formation in the rat. *Journal of Comparative Neurology*, *172*(1), 49–84.

Szemeredi, K., Komoly, S., Kopin, I. J., Bagdy, G., Keiser, H. R., & Goldstein, D. S. (1991). Simultaneous measurement of plasma and brain extracellular fluid concentrations of catechols after yohimbine administration in rats. *Brain Research*, *542*(1), 8–14.

Takeuchi, T., Duszkiewicz, A. J., Sonneborn, A., Spooner, P. A., Yamasaki, M., Watanabe, M., Smith, C. C., Fernández, G., Deisseroth, K., Greene, R. W., & Morris, R. G. M. (2016). Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature*, *537*(7620), 357–362.

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

Tanila, H. (1999). Hippocampal Place Cells Can Develop Distinct Representations of Two Visually Identical Environments. *Hippocampus*, *9*(3), 235–246.

Tanila, H. (2001). Noradrenergic regulation of hippocampal place cells. *Hippocampus*, *11*(6), 793–808.

Tomar, A., & McHugh, T. J. (2022). The impact of stress on the hippocampal spatial code. *Trends in Neurosciences*, *45*(2), 120–132.

Tronson, N. C., Schrick, C., Guzman, Y. F., Huh, K. H., Srivastava, D. P., Penzes, P., Guedea, A. L., Gao, C., & Radulovic, J. (2009). Segregated Populations of Hippocampal Principal CA1 Neurons Mediating Conditioning and Extinction of Contextual Fear. *Journal of Neuroscience*, *29*(11), 3387–3394.

Tulving, E., & Markowitsch, H. J. (1998). Episodic and declarative memory: Role of the hippocampus. *Hippocampus*, *8*(3), 198–204.

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience*, *10*(6), 397–409.

Valentino, R. J., & Van Bockstaele, E. (2008). Convergent regulation of locus coeruleus activity as an adaptive response to stress. *European Journal of Pharmacology*, *583*(2), 194–203.

van Ast, V. A., Cornelisse, S., Meeter, M., Joëls, M., & Kindt, M. (2013). Time-Dependent Effects of Cortisol on the Contextualization of Emotional Memories. *Biological Psychiatry*, *74*(11), 809–816.

Vazdarjanova, A., & Guzowski, J. F. (2004). Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *Journal of Neuroscience*, *24*(29), 6489–6496.

Vogel-Ciernia, A., & Wood, M. A. (2014). Examining Object Location and Object Recognition Memory in Mice. *Current Protocols in Neuroscience*, *69*(1), 8.31.1-8.31.17.

Volkova, E. P., Rozov, A. V, Nadareishvili, G. G., & Bol'shakov, A. P. (2016). Corticosterone induces rapid increase in the amplitude of inhibitory response in hippocampal synapses with asynchronous GABA release. *Bulletin of Experimental Biology and Medicine*, *160*, 628–631.

Wajchenberg, B. L., Leme, C. E., Lerario, A. C., Toledo, I. T., Souza, E., Rodbard, H. W., & Rodbard, D. (1984). Insulin resistance in Cushing's disease: evaluation by studies of insulin binding to erythrocytes. *Diabetes*, *33*(5), 455–459.

Walling, S. G., & Harley, C. W. (2004). Locus ceruleus activation initiates delayed synaptic potentiation of perforant path input to the dentate gyrus in awake rats: a novel β-adrenergic-and protein synthesis-dependent mammalian plasticity mechanism. *Journal of Neuroscience*, *24*(3), 598–604.

Warburton, E. C., Koder, T., Cho, K., Massey, P. V, Duguid, G., Barker, G. R. I., Aggleton, J. P., Bashir, Z. I., & Brown, M. W. (2003). Cholinergic Neurotransmission Is Essential for Perirhinal Cortical Plasticity and Recognition Memory. *Neuron*, *38*(6), 987–996.

Waterhouse, E. G., An, J. J., Orefice, L. L., Baydyuk, M., Liao, G.-Y., Zheng, K., Lu, B., & Xu, B. (2012). BDNF Promotes Differentiation and Maturation of Adult-born Neurons through GABAergic Transmission. *The Journal of Neuroscience*, *32*(41), 14318 LP – 14330.

Weil-Malherbe, H., Axelrod, J., & Tomchick, R. (1959). Blood-brain barrier for adrenaline. *Science*, *129*(3357), 1226–1227.

White, N. M., Packard, M. G., & McDonald, R. J. (2013). Dissociation of memory systems: The story unfolds. *Behavioral Neuroscience*, *127*(6), 813–834.

Williams, C. L., Men, D., Clayton, E. C., & Gold, P. E. (1998). Norepinephrine release in the amygdala after systemic injection of epinephrine or escapable footshock: contribution of the nucleus of the solitary tract. *Behavioral Neuroscience*, *112*(6), 1414.

Wilson, M. A., & McNaughton, B. L. (1993). Dynamics of the Hippocampal Ensemble Code for Space. *Science*, *261*(5124), 1055–1058.

Wimmer, M. E., Hernandez, P. J., Blackwell, J., & Abel, T. (2012). Aging impairs hippocampus-dependent long-term memory for object location in mice. *Neurobiology of Aging*, *33*(9), 2220–2224.

Winocur, G. (1997). Hippocampal lesions alter conditioning to conditional and contextual stimuli. *Behavioural Brain Research*, *88*(2), 219–229.

Witter, M. P. (1993). Organization of the Entorhinal-Hippocampal System : A Review of Current Anatomical Data. *Hippocampus*, *3*(15), 33–44.

Witter, M. P., Naber, P. A., Van Haeften, T., Machielsen, W. C. M., Rombouts, S. A. R. B., Barkhof, F., Scheltens, P., & Lopes da Silva, F. H. (2000). Cortico-hippocampal communication by way of parallel parahippocampal-subicular pathways. *Hippocampus*, *10*(4), 398–410.

Yamada, K., & Nabeshima, T. (2003). Brain-derived neurotrophic factor/TrkB signaling in memory processes. *Journal of Pharmacological Sciences*, *91*(4), 267–270.

Yassa, M. A., & Stark, C. E. L. (2011). Pattern separation in the hippocampus. *Trends in Neurosciences*, *34*(10), 515–525.

Yehuda, R. (2002). Post-traumatic stress disorder. *New England Journal of Medicine*, *346*(2), 108–114.

Yehuda, R. (2009). Status of glucocorticoid alterations in post-traumatic stress disorder. *Annals of the New York Academy of Sciences*, *1179*(1), 56–69.

Young, S. L., Bohenek, D. L., & Fanselow, M. S. (1994). NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: Immunization against amnesia by context preexposure. *Behavioral Neuroscience*, *108*, 19–29.

Zerbi, V., Floriou-Servou, A., Markicevic, M., Vermeiren, Y., Sturman, O., Privitera, M., von Ziegler, L., Ferrari, K. D., Weber, B., De Deyn, P. P., Wenderoth, N., & Bohacek, J. (2019). Rapid Reconfiguration of the Functional Connectome after Chemogenetic Locus Coeruleus Activation. *Neuron*, *103*(4), 702-718.e5.

Zerbes, G., Kausche, F. M., & Schwabe, L. (2022). Stress-induced cortisol modulates the control of memory retrieval towards the dorsal striatum. European Journal of *Neuroscience, 55*(9–10), 2699–2713.

Zhou, M., Hoogenraad, C. C., Joëls, M., & Krugers, H. J. (2012). Combined β-adrenergic and corticosteroid receptor activation regulates AMPA receptor function in hippocampal neurons. *Journal of Psychopharmacology, 26*(4), 516–524.

Zhou, Y., Won, J., Karlsson, M. G., Zhou, M., Rogerson, T., Balaji, J., Neve, R., Poirazi, P., & Silva, A. J. (2009). CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. *Nature Neuroscience*, *12*(11), 1438–1443.

APPENDICES

Samenvatting

Summary

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Acknowledgement

Samenvatting

Stressvolle en emotionele ervaringen leiden tot sterke en blijvende herinneringen. Onderzoek bij zowel proefdieren als de mens heeft laten zien dat deze versterking van het geheugen afhankelijk is van stresshormonen en neurotransmitters. Aan de ene kant veroorzaakt stress snel de afgifte van noradrenaline in de hersenen, aan de andere kant is er een vertraagde activering van de hypothalamus-hypofyse-bijnieras, wat resulteert in de afgifte van glucocorticoïdhormonen (corticosteron in knaagdieren, cortisol in mensen). Zowel noradrenaline als corticosteron zorgen voor een verbetering van het geheugen, op een overwegend synergetische manier. Herinneringen kunnen echter onderhevig zijn aan veranderingen die verder gaan dan louter een versterking, en het is nog steeds onduidelijk hoe de emotionele impact van een ervaring de kwaliteit van het geheugen, zoals diens specificiteit, betrouwbaarheid en gedetailleerdheid, kan beïnvloeden. Onderzoek bij de mens heeft tegenstrijdige resultaten laten zien: sommige studies meldden dat de gedetailleerdheid van herinneringen verbetert onder emotionele omstandigheden, wat resulteert in een levendige herinnering aan emotionele ervaringen, terwijl andere studies juist lieten zien dat emotionele herinneringen op een meer gegeneraliseerde manier worden onthouden, wat mogelijk kan leiden tot een minder nauwkeurige herinnering van specifieke details. In dieronderzoek is dit onderwerp van geheugenkwaliteit pas onlangs in de belangstelling komen te staan.

Dit proefschrift presenteert een reeks experimenten met muizen waarin de invloed van de stresshormonen noradrenaline en corticosteron op de episodisch-achtige kwaliteit van het geheugen wordt onderzocht. Concreet onderzocht ik de hypothese dat noradrenaline en corticosteron tegengestelde effecten hebben op hippocampus-afhankelijk episodisch geheugen na het trainen op een leertaak die een separatie van geheugenrepresentaties van meerdere gebeurtenissen vereist. Daarentegen veronderstelde ik dat noradrenaline en corticosteron vergelijkbare effecten hebben op hippocampus-afhankelijk geheugen onder trainingsomstandigheden die geen separatie van trainingservaringen vereisen.

In **Hoofdstuk** 2 onderzocht ik het effect van systemische toediening van de noradrenerge stimulant yohimbine en corticosteron op episodisch-achtig geheugen in een object-in-context taak bij muizen, een hippocampus-afhankelijke taak waarin twee objectpresentaties tijdens de trainingssessie worden onderscheiden door de contexten waarin ze plaatsvinden. Om experimenteel de noodzaak te manipuleren om herinneringen voor de twee trainingsgebeurtenissen te scheiden, werden de dieren voor de trainingssessie ofwel gehabitueerd aan de twee trainingscontexten ofwel aan twee contexten die verschilden van die gebruikt werden tijdens de training. Ik ontdekte dat de toediening van yohimbine bij dieren die niet gehabitueerd waren aan de twee

trainingscontexten zorgde voor een verbetering van object-in-context-geheugen 24 uur later, terwijl de toediening van corticosteron dit geheugen juist verslechterde onder deze omstandigheden. Echter, zowel yohimbine als corticosteron verbeterden object-in-context-geheugen wanneer muizen vooraf gehabitueerd waren aan de twee trainingscontexten, waardoor de noodzaak voor het scheiden van herinneringen voor de twee trainingscontexten overbodig was geworden.

Vervolgens onderzocht ik hoe de toediening van yohimbine en corticosteron na training op deze leertaak de neuronale activiteit in verschillende hippocampale subgebieden beïnvloedde tijdens de consolidatieperiode, en of voorafgaande contexthabituatie deze stresshormooneffecten op de hippocampus veranderden. Ik ontdekte dat yohimbine bij niet-gehabitueerde muizen een positieve correlatie veroorzaakte in activiteit tussen het dorsale blad van de dentate gyrus (dDG) en CA3, een neurale verbinding die kritisch betrokken is bij 'pattern separation'; het proces dat bijdraagt aan het (onder)scheiden van twee trainingsgebeurtenissen. Daarnaast vond ik ook een verhoogde activiteit van de pyramidale cellen in het CA1 gebied. Niet-gehabitueerde muizen die behandeld waren met corticosteron vertoonden geen correlatie tussen de dDG en CA3 en ook geen verhoogde activiteit in het CA1 gebied, maar juist een verhoging van inhibitoire GABAerge activiteit in het CA1 gebied en het ventrale blad van de dentate gyrus. Voorafgaande contexthabituatie ging gepaard met het ontbreken van deze correlatie tussen de dDG en CA3 en met een algeheel lagere activiteit van de hippocampus. Deze bevindingen ondersteunen dus het idee dat yohimbine en corticosteron tegengestelde effecten heeft op object-in-context-geheugen doordat ze een tegengesteld effect hebben op een mechanisme in de hippocampus dat respectievelijk zorgt voor een scheiding of juist koppeling van het geheugen van de twee trainingsgebeurtenissen. Contexthabituatie lijkt de betrokkenheid van de hippocampus bij object-in-contextgeheugen te verminderen. De effecten van yohimbine en corticosteron op de verbetering van het geheugen bij deze dieren zouden kunnen worden veroorzaakt door een versterking van het geheugen voor de objecten zelf elders in het brein.

In **Hoofdstuk 3** probeerde ik causaal bewijs te leveren voor de hypothese dat de hippocampus noodzakelijk is voor het bewerkstelligen van het effect van noradrenaline op het verbeteren van object-in-context geheugen bij niet-gehabitueerde muizen, maar dat voorafgaande contexthabituatie dit noradrenaline-effect onafhankelijk maakt van de hippocampus. Om dit te onderzoeken heb ik een techniek gebruikt waarmee ik de hippocampus tijdens de trainingssessie kon inactiveren. Alle muizen kregen onmiddellijk na de trainingssessie yohimbine toegediend. Bij zowel gehabitueerde als niet-gehabitueerde muizen vond ik dat controle dieren met een intacte hippocampus goed object-in-context-geheugen lieten zien. Het inactiveren van de hippocampus bij niet-gehabitueerde muizen zorgde ervoor dat dit object-in-context-geheugen

verslechterde, terwijl inactivatie van de hippocampus bij gehabitueerde dieren geen enkel effect had. Deze bevindingen laten dus zien dat eerdere contexthabituatie het effect van noradrenaline op de verbetering van object-in-contextgeheugen inderdaad onafhankelijk maakt van de hippocampus.

Om verder te onderzoeken of noradrenaline en corticosteron enkel tegengestelde effecten hebben op hippocampus-afhankelijk geheugen waarbij een separatie van overlappende geheugenrepresentaties vereist is, heb ik in **Hoofdstuk 4** de dieren getraind op een objectlocatietaak. Bij deze taak wordt ruimtelijk geheugen gevormd door een object te associëren met de specifieke locatie waarin het zich bevindt binnen de trainingscontext. Ook deze taak is afhankelijk van de hippocampus, maar de training bestaat uit slechts één enkele gebeurtenis en de dieren hoeven dan dus ook geen overlappende geheugenrepresentaties te scheiden. Ik ontdekte dat zowel yohimbine als corticosteron zorgden voor een verbetering van dit type geheugen, en dat dit gepaard ging met een vergelijkbare toename van CA1-activiteit tijdens de consolidatiefase. Voorafgaande contexthabituatie veranderde niets aan deze effecten van yohimbine of corticosteron.

De resultaten van deze experimenten ondersteunen dus mijn hypothese dat noradrenaline en corticosteron tegengestelde effecten hebben op hippocampus-afhankelijk geheugen voor een leertaak waarbij een separatie van geheugenrepresentaties van meerdere trainingsgebeurtenissen vereist is, maar dat ze vergelijkbare effecten hebben op hippocampus-afhankelijk geheugen waarbij dit niet nodig is. Ik stel een model voor hoe deze twee stresshormonen op verschillende manieren episodische en ruimtelijke aspecten van hippocampus-afhankelijk geheugen kunnen beïnvloeden. De laterale entorhinale cortex, die essentieel is voor het verwerken van niet-ruimtelijke contextuele informatie, projecteert met name naar de dDG, en vervolgens via de CA3 naar de CA1. Ik stel voor dat noradrenaline object-in-context-geheugen verbetert door op deze route in te werken. Concreet zou het effect van noradrenaline op de versterking van dDG-CA3-connectiviteit de separatie van het geheugen van de twee trainingsgebeurtenissen kunnen vergemakkelijken, terwijl het effect van noradrenaline op de CA1 de opslag van deze twee afzonderlijke herinneringen in niet-overlappende neuronale ensembles ondersteunt. Verder stel ik voor dat corticosteron object-in-context-geheugen verslechtert door deze route juist te onderdrukken, wat resulteert in een koppeling van de twee trainingsgebeurtenissen. Ruimtelijk geheugen is afhankelijk van een ander circuit in de hippocampus, en dan vooral van directe projecties van de mediale entorhinale cortex naar de CA1. Daarom stel ik voor dat de effecten van noradrenaline en corticosteron op het verbeteren van dit geheugen worden veroorzaakt door vergelijkbare effecten van deze twee stresshormonen op het verhogen van de activiteit van deze mediale entorhinale cortex-CA1-route.

Mijn bevindingen bieden niet alleen een werkmodel dat toekomstige studies zou kunnen inspireren om de neurale mechanismen van verschillende soorten hippocampus-afhankelijk geheugen verder te onderzoeken, maar ze hebben ook belangrijke implicaties voor onderzoek bij de mens. Deze bevindingen zouden, althans gedeeltelijk, een verklaring kunnen bieden voor de tegenstrijdige bevindingen over de impact van stress en emotie op kwaliteitsaspecten van het geheugen. Verder kunnen maladaptieve vormen van geheugenverwerking zoals een sterk gegeneraliseerde verwerking van nare emotionele herinneringen, leiden tot stress-gerelateerde stoornissen, waaronder posttraumatische stressstoornis en fobieën. De vertaling van deze nieuwe conceptuele inzichten naar de mens zou dus ook inzicht kunnen verschaffen in individuele verschillen in stressbestendigheid en het risico op het ontwikkelen van psychopathologie.

Summary

Stressful and emotionally arousing experiences induce strong and lasting memories. A large body of literature on both animals and humans shows how this strengthening involves actions of hormones and neurotransmitters released during stressful experiences. On the one hand, stress rapidly triggers the release of norepinephrine in the brain and from the adrenal medulla and sympathetic nerve endings. On the other hand, stress induces a more delayed activation of the hypothalamic-pituitary-adrenocortical axis that culminates in the release of glucocorticoid hormones (corticosterone in rodents, cortisol in humans). An impressive body of literature indicates that both norepinephrine and glucocorticoids, in a predominantly synergistic manner, mediate the effects of stress and emotional arousal on the enhancement of memory consolidation. However, memories can be subject to multiple types of modifications beyond mere strengthening, and it is still debated how the emotional impact of an experience might influence quality aspects of memory, such as memory specificity, fidelity and detailedness. Literature from human studies shows contradictory results: Some studies reported that arousal improves the detailedness of memories, resulting in vivid recall of emotionally arousing experiences, while other studies proposed that emotional memories are remembered in a more generalized manner, potentially leading to less accurate recollection of specific details. In animal research, this topic of memory quality has only recently begun to attract attention.

This thesis presents a series of experiments in mice exploring the impact of the stress hormones norepinephrine and corticosterone on episodic-like quality of memory. Specifically, I investigated the hypothesis that norepinephrine and corticosterone induce opposite effects on hippocampus-dependent episodic-like memory after training on a task that requires the separation of memory representations of multiple training events. In contrast, I hypothesized that norepinephrine and corticosterone induce similar effects on hippocampus-dependent memory under training conditions that do not require the separation of memory of different training events experienced close in time.

In **Chapter 2**, I examined the effect of systemic administration of the noradrenergic stimulant yohimbine and corticosterone on episodic-like memory in an object-in-context task in mice, a hippocampus-dependent task in which two object presentation events during the training session are distinguished by the contexts in which they appear. To experimentally manipulate the necessity to separate memories for the two training events, animals received three habituation sessions to either the two training contexts or two different contexts prior to the training session. I found that yohimbine administered immediately after the training session dose-dependently enhanced object-in-context memory assessed 24 h later, whereas corticosterone impaired this memory of mice that were not previously habituated to the training contexts. However, both yohimbine

and corticosterone enhanced object-in-context memory when mice were previously familiarized with these contexts, obviating the need for separating memories for the two training contexts.

Next, I explored how yohimbine and corticosterone administration after object-in-context training influences neuronal activity within different hippocampal regions during the posttraining consolidation period, and whether prior context habituation alters these stress hormone effects on the hippocampus. I found that yohimbine-treated animals of the different habituation condition displayed a positive correlation in neural activity between the dorsal blade of the dentate gyrus (dDG) and CA3, a pathway critically involved in pattern separation, as well as an increased total activity within the hippocampal CA1 cell layer during the posttraining consolidation period. Corticosterone-treated animals of the different habituation condition did not show this correlation between the dDG and CA3 or an increased total activity within the CA1, but rather displayed an increased GABAergic activity in the CA1 *stratum radiatum* and the ventral blade of the dentate gyrus. Prior habituation to the training contexts was associated with an absence of inter-subregion correlations of activity as well as an overall lower hippocampal activity posttraining. These findings support the view that yohimbine and corticosterone administration induce opposite effects on object-in-context memory by regulating a hippocampal mechanism that facilitates either a separation or linking of memory of the two training events, respectively. Habituation to the training contexts appears to reduce the overall involvement of the hippocampus in object-in-context memory. The enhancing effects of both yohimbine and corticosterone in this condition might be mediated by a strengthening of memory for the objects *per se* by the involvement of other brain regions.

In **Chapter 3**, I aimed to provide causal evidence for the hypothesis that the effect of norepinephrine in enhancing object-in-context memory of the different habituation condition requires the hippocampus in order to separate the memory of the two training events, but that prior habituation to the training contexts renders this norepinephrine effect hippocampus independent. To achieve this, I employed Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetics to inactivate the hippocampus prior to training on the object-in-context task. All mice received systemic administration of a memory-enhancing dose of yohimbine immediately after the training session. In both habituation conditions, mice injected with a control virus into the hippocampus displayed object-in-context memory on a 24-h retention test. However, and most importantly, hippocampal inactivation of mice that had not been habituated to the training contexts prevented this object-in-context memory, whereas hippocampal inactivation of mice that had been habituated to the training contexts did not induce any memory-impairing effect. These findings thus provide direct support for

the hypothesis that prior context habituation renders noradrenergic effects on object-in-context memory independent of the hippocampus.

To further examine whether norepinephrine and corticosterone solely induce opposite effects on hippocampus-dependent memory after training on a task that requires the separation of overlapping memory representations for multiple training events, in **Chapter 4** yohimbine and corticosterone were administered after training on an object location task. In this task, spatial memory is formed by associating an object with a specific location within the training context, which also critically depends on the hippocampus. However, the training experience comprises a single event, and thus the animals do not have to separate overlapping memory representations. I found that both yohimbine and corticosterone induced a very similar enhancement of object location memory, which was associated with a very similar increase in hippocampal CA1 activity during the post-learning consolidation phase. Prior habituation to the training context did not alter these effects of yohimbine or corticosterone.

Thus, the findings of this series of experiments provide support for my hypothesis that norepinephrine and corticosterone induce opposite effects on hippocampus-dependent memory after training on a task that requires the separation of memory representations of multiple training events, but induce similar effects on hippocampus-dependent memory under training conditions that do not require such separation. I proposed a model of how these two stress hormones might differentially affect episodic-like and spatial aspects of hippocampus-dependent memory by differently influencing two distinct hippocampal circuits. The lateral entorhinal cortex, which is essential for the processing of non-spatial contextual information, preferentially projects to the dDG, and then via the CA3 to the CA1. I propose that norepinephrine enhances object-in-context memory by increasing activity of this pathway. Specifically, the norepinephrine effect on increasing dDG-CA3 connectivity might facilitate the separation of memories of the two training events, and the norepinephrine effect on increasing CA1 activity might then support the storage of these two different memories into two non-overlapping neuronal ensembles. In contrast, corticosterone administration might impair object-in-context memory by inhibiting this specific pathway, resulting in a linkage of memories of the two training events. Contrarily, spatial memory is known to depend on projections from the medial entorhinal cortex to the proximal part of the CA1 region of the hippocampus. Thus, I propose that the similar effects of norepinephrine and corticosterone on enhancing spatial memory are mediated by a similar influence of these two stress hormones on increasing the activity of the medial entorhinal cortex-CA1 pathway.

My findings do not only provide a working model that could inspire future studies to further investigate the neural mechanisms underlying stress hormone effects on different types of hippocampal memory, but they also have important implications for human research. These findings might offer, at least in part, an explanation for the conflicting findings from human experiments on the impact of stress and emotional arousal on quality aspects of memory. Further, aberrant memory processing of emotional information, often resulting in impaired contextualization and episodic specificity of memory, lies at the core of several stress-related disorders, including post-traumatic stress disorder and phobias. Thus, translation of these new conceptual insights to humans might also provide understanding of individual differences in stress resilience and risk for developing psychopathology.

Research data management

Type of data	Subject to privacy	Way of Anonymization	Storage
Behavioral data	No	N.A.	All behavioral videos are stored at the Cognitive Neuroscience department on secured servers from Donders Repository with regular back-up (\\project\ fileserver.dccn.nl\P:\ 4040000.05)
Microscope data	No	N.A.	All microscopy files are stored at the Cognitive Neuroscience department on secured servers from Donders Repository with regular back-up (\\project\ fileserver.dccn.nl\P:\ 4040000.05)
Documentation and files containing experimental data	No	N.A.	Data files are stored at the Cognitive Neuroscience department on secured servers from Donders Repository with regular back-up (\\project\ fileserver.dccn.nl\P:\ 4040000.05)
Documentation and files containing experimental protocol	No	N.A.	Documentation in form of electronic lab book is stored in the online lab journal system Labguru(https://radboudumc.labguru.com/knowledge/projects/)

PhD Portfolio

Name of PhD candidate	Chunan Guo
Graduate school	Donders Graduate School
Department	Cognitive Neuroscience
(Co-)Promotor(s)	Prof. Dr. Benno Roozendaal Dr. Marloes Henckens
Research period	September 2016 – April 2024

Professional courses and workshops

Subject	Course provider	Year	EC
Laboratory Animal Science	Radboudumc	2016	3.0
Advanced Conversation	Radboud University	2017	1.5
Achieving your Goals	Radboud University	2017	1.5
Art of Presenting Science	Radboud University	2017	1.5
Scientific Integrity for PhD candidates	Radboudumc	2017	1.0
Presentation Skills	Radboudumc	2017	1.5
Digital tools	Radboud University	2017	0.1
Stress and Cognition:From basic mechanisms to psychopathology	Radboud University	2017	2.0
Designing a PhD research project	Radboud University	2017	3.0
Scientific Writing	Radboud University	2018	3.0
Image Analysis with FIJI	RIMLS	2018	1.0
Lectures and others			12
			31.1

Symposia and Congresses

Subject	Location	Year
Radboud Summer School: Stress and cognition	Nijmegen, the Netherlands	2017
Dutch neuroscience meeting	Lunteren, the Netherlands	2017
Dutch neuroscience meeting	Lunteren, the Netherlands	2018
2nd Munich Winter Conference on Stress	Munich, Germany	2019
50th Society for Neuroscience meeting	Chicago, USA	2019
FENS Forum of Neuroscience	Online forum	2020

List of publications

Areg Barsergyan, Gabriele Mirone, Giacomo Ronzoni, **Chunan Guo**, Daan Kuppeveld, Evelien H.S. Schut, Piray Atsak, Selina Teurlings, James L. McGaugh, Dirk Schubert & Benno Roozendaal (2019). Glucocorticoid enhancement of recognition memory via basolateral amygdala-driven facilitation of prelimbic cortex interactions. *Proceedings of the National Academy of Sciences of the United States of America*, **116**(14), 7077-7082, doi: 10.1073/pnas.1901513116

Chunan Guo, Qin Zhang & Yuan Huang (2017). The complete mitochondrialgenome of the Oedaleusinfernalis sauss (Orthoptera: Oedipodidae). *Mitochondrial DNA Part A*, **28**(1), 89-90, doi: 10.3109/19401736.2015.1110812.

Qin Zhang, **Chunan Guo** & Yuan Huang (2016). The complete mitochondrialgenome of Gonistabicolor (Haan) (Orthoptera: Acrididae). *Mitochondrial DNA Part A*, **27**(6), 4578-4579, doi: 10.3109/19401736.2015.1101572.

About the author

Chunan Guo was born on 7th of October 1990 in Baotou, Inner Mongolia, China. In 2009, she

started her bachelor study at Hainan University in China, with a major in veterinary medicine. In 2013, she was admitted to a 3-year Master research program at Shaanxi Normal University, Shanxi, China. She performed her research project under the supervision of Prof. Dr. Huanyuan on zoology. In her third year, she applied for an international exchange program to study in Australia. It was during this time that she opened the new door to neuroscience research and became fascinated by the mysteries of learning and memory.

In 2016, she obtained her Master degree of Science and decided to apply for a PhD program. In the same year, she received a scholarship from the China Scholarship Council (CSC) to pursue her PhD at the Department of Cognitive Neuroscience, Radboud university medical center (Radboudumc), Nijmegen, the Netherlands. Under the supervision of Prof. Dr. Benno Roozendaal and Dr. Marloes Henckens, she carried out her PhD project on the stress hormone effects on the hippocampus in regulating episodic-like memory. Most of the scientific studies she performed during her PhD are described in this thesis.

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To my family, for your love and support.

To myself, the girl who lives and to be continued.

Donders Graduate School for Cognitive Neuroscience

For a successful research Institute, it is vital to train the next generation of young scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the

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The DGCN tracks the career of PhD graduates carefully. More than 50% of PhD alumni show a continuation in academia with postdoc positions at top institutes worldwide, e.g. Stanford University, University of Oxford, University of Cambridge, UCL London, MPI Leipzig, Hanyang University in South Korea, NTNU Norway, University of Illinois, North Western University, Northeastern University in Boston, ETH Zürich, University of Vienna etc..

Positions outside academia spread among the following sectors: specialists in a medical environment, mainly in genetics, geriatrics, psychiatry and neurology. Specialists in a psychological environment, e.g. as specialist in neuropsychology, psychological diagnostics or therapy. Positions in higher education as coordinators or lecturers. A smaller percentage enters business as research consultants, analysts or head of research and development. Fewer graduates stay in a research environment as lab coordinators, technical support or policy advisors. Upcoming possibilities are positions in the IT sector and management position in pharmaceutical industry. In general, the PhDs graduates almost invariably continue with high quality positions that play an important role in our knowledge economy.

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