# Noradrenergic effects on the neural mechanisms underlying memory detailedness

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# Noradrenergic effects on the neural mechanisms underlying memory detailedness

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# CHAPTER 1

# General introduction

## 1. Introduction

Stressful and emotionally arousing experiences are typically well remembered (McGaugh, 2003, 2013). Such enhanced memory for emotional experiences is considered a highly adaptive phenomenon that is essential for our survival, as it helps us to remember both dangerous and favorable situations. However, emotional memories seem not only stronger than memories for non-emotional everyday experiences, they seem also be remembered with more detail and for a longer period of time. For example, most people are still able to remember exactly where they were, and what they were doing, when they first heard of the terrorist attacks on the Twin Towers in New York on 9/11 in 2001. Human behavioral studies indeed indicate that emotional memories are also of altered quality. However, the findings are conflicting: Some studies observed that emotional arousal enhances the accuracy of memory (Porter *et al.*, 2008; Hoscheidt *et al.*, 2014), whereas other studies found that emotional memories are remembered in a more generalized manner (Morgan III *et al.*, 2004), recalled with overconfidence (Talarico & Rubin, 2003) and subject to incorporation of misinformation (Payne *et al.*, 2002; Sharot *et al.*, 2004; Rimmele *et al.*, 2011).

Extensive evidence indicates that noradrenergic activation, as induced by emotional arousal, is crucially involved in strengthening the consolidation of long-term memory of emotional experiences (McGaugh, 2004; Aston-Jones & Cohen, 2005; Sara, 2009; Roozendaal & McGaugh, 2011; Takeuchi *et al.*, 2016). However, whether noradrenergic activity can also affect the quality of memory and/or whether it is involved in the long-term maintenance of memory remains largely elusive. In the past years, our laboratory has started a new research line investigating the role of the noradrenergic system in memory accuracy. Initial studies have shown that noradrenergic activation not only increases the strength, but also accuracy, of episodic-like memories (Atucha & Roozendaal, 2015; Atucha *et al.*, 2017; Roozendaal & Mirone, 2020), that depend on the hippocampus (Eichenbaum, 2017). Moreover, it was found that noradrenergic activation during initial memory consolidation keeps these memories accurate over time by maintaining long-term hippocampal involvement in the memory (Atucha *et al.*, 2017). However, nothing is currently known of whether noradrenergic activation can also enhance accuracy or detailedness of other forms of memories, such as recognition memory, that depend on cortical brain regions (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 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). In this thesis, I will present a series of experiments in mice, aimed at investigating whether noradrenergic activation enhances the detailedness of object recognition memory and how this memory detailedness is maintained over time.

In the following sections, I will first give a short overview of half a century of research examining the role of norepinephrine and epinephrine in modulating the consolidation of memory of different types of experiences and some of the major brain mechanisms that were found to mediate such adrenergic effects on memory consolidation. Then, I will define the key concepts of memory quality and summarize some recent findings on the effects of noradrenergic activation on increasing the accuracy and the persistence of hippocampus-dependent episodic-like memory. Finally, I will present the scope of this thesis and give a brief description of each of the experimental chapters.

## 2. Role of adrenergic catecholamines in enhancing memory consolidation

Not all experiences are equally well remembered. Particularly stressful or emotionally arousing experiences are well retained in memory, whereas everyday experiences are easily forgotten or not remembered at all. This observation suggests that emotional arousal may induce an endogenous mechanism that serves to regulate the strength of memories (Gold & Van Buskirk, 1975). Early in 1967, Livingston investigated the influence of emotional arousal on memory and suggested that stimulation of the limbic system and brainstem reticular formation might promote the storage of recently activated brain events by initiating a “neurohormonal influence favoring future repetitions of the same neural activities” (Livingston, 1967). Extensive evidence from subsequent studies backed this general hypothesis and provided strong support for the view that emotional arousal induces the release of stress hormones in the periphery and of norepinephrine, together with several other neurotransmitters and peptides, in the brain. Together, these stress hormones, neurotransmitters and neuropeptides have been shown to be critically involved in mediating emotional arousal effects on memory consolidation (McGaugh, 2004; Sara, 2009; Roozendaal & McGaugh, 2011; Takeuchi *et al.*, 2016). Stress and emotional arousal rapidly induce activation of the autonomic nervous system which results in the release of catecholamines, such as epinephrine and norepinephrine, from the adrenal medulla, and sympathetic nerve terminals (Smith & Vale, 2006; Ulrich-Lai & Herman, 2009). These affective conditions also induces activation of the hypothalamic-pituitary-adrenocortical axis, culminating in the release of glucocorticoids (cortisol in humans, corticosterone in rodents) (Ulrich-Lai & Herman, 2009). These stress hormone systems play a crucial role in appropriately responding to threatening situations. For example, activation of adrenal catecholamines is critical for immediate behavioral responses to threat, mediating the ‘flight or fight’ reaction by inducing a multitude of peripheral changes, e.g., increasing heart rate,

energy metabolism, blood pressure and respiration. However, both the adrenomedullary and adrenocortical hormones also induce long-term adaptive changes by enhancing the consolidation of memory of such emotional experiences. In the following section, I will focus on how peripherally released epinephrine and norepinephrine enhance memory consolidation by stimulating the release of norepinephrine in the brain (for glucocorticoid effects on memory, see de Quervain et al., 2017). I will focus on the effect of norepinephrine and emotional arousal on regulating memory consolidation, but it is well established that it also influences the encoding and retrieval of memory (Cahill *et al.*, 1996; Izquierdo *et al.*, 1997; de Quervain *et al.*, 1998; Cahill *et al.*, 2003; Richardson *et al.*, 2004; Smeets *et al.*, 2008; Rasch *et al.*, 2009; Barsegyan *et al.*, 2015).

## 2.1 The concept of memory consolidation

What exactly is memory consolidation? Müller and Pilzecker in 1900 were the first to propose that memory traces are initially fragile after learning and become stabilized over time (Müller & Pilzecker, 1900). This stabilization of the memory trace in the hours following initial learning became known as memory consolidation (McGaugh, 2000; Kandel, 2004). Short-term memory is characterized by the capacity of holding a limited amount of information in mind in a very accessible state (Cowan, 2008), and does not require protein synthesis (Schwartz *et al.*, 1971). Rather, it involves second messenger-mediated covalent modifications of previously synthesized proteins (Kandel & Schwartz, 1982) that modulate membrane properties of nerve cells and their synaptic connections (Kandel & Schwartz, 1982; Byrne, 1987; Tweedie-Cullen *et al.*, 2009).

For the memory to be retained for a longer period of time, memory consolidation needs to occur that can be divided into two main stages. First, within the first hours after a learning experience, changes in gene transcription induce protein synthesis, which is critical for establishing long-lasting modifications in order to replace degraded proteins, increase levels of present proteins, or express novel proteins (Steward & Schuman, 2001), necessary to induce structural modifications, such as the formation of new synaptic spines, at the local network level (Squire & Alvarez, 1995; Kandel, 2004). Second, at a longer interval after learning, memory consolidation also requires systems-level processes involving cross-talk between multiple brain regions (Nadel & Moscovitch, 1997; Frankland & Bontempi, 2005; Winocur *et al.*, 2010; Sutherland & Lehmann, 2011). Initially, a memory for an experience is characterized by rich episodic details (on the when, what, where?) and known to depend on an intact hippocampus, which functions as a main integrator of cortical information. However, with the progression of time, these episodic memories undergo a neural reorganization,

where they become more dependent on cortical networks and less on the hippocampus (Frankland *et al.*, 2004; Miller *et al.*, 2010). This systems consolidation and neural reorganization process is associated with a gradual reduction in its accuracy or detailedness of the memory, which is more remembered in a generalized, semantic manner, lacking episodic detail (Wiltgen & Tanaka, 2013).

### 2.2 Peripheral epinephrine and norepinephrine affect memory consolidation

Numerous studies have shown that a systemic administration of epinephrine or norepinephrine after a training experience enhances memory retention on a wide variety of tasks, ranging from inhibitory avoidance to a one-trial appetitive task and object recognition task (Gold & Van Buskirk, 1975; Gold & Van Buskirk, 1976; Williams *et al.*, 1998; Dornelles *et al.*, 2007; Jurado-Berbel *et al.*, 2010). Gold and Van Buskirk (Gold & Van Buskirk, 1975) were the first to show that the systemic administration of epinephrine immediately after inhibitory avoidance training enhanced memory of the training experience, and that this effect was lost when epinephrine was given 2 hours after the training. This time-limited effect provided direct evidence that the epinephrine modulated the consolidation phase of memory processing (McGaugh, 1966). Subsequent studies have shown effects of epinephrine and norepinephrine on many different types of training experiences (Roozendaal and McGaugh, 2011), including non-arousing tasks. Both object recognition memory (Dornelles *et al.*, 2007; Jurado-Berbel *et al.*, 2010) and object location memory (Jurado-Berbel *et al.*, 2010) have been found to be improved by systemic epinephrine administration, indicating noradrenergic improvement of both the "what" (object identity) and "where" (object location) components of recognition memory.

A well-established characteristic is the dose-dependency of this memory-enhancing effect: moderate doses of epinephrine were shown to enhance memory, whereas both lower and higher doses were ineffective (Hunt & Krivanek, 1966; Krivanek & McGaugh, 1968; Hunt & Bauer, 1969). Moreover, the optimal dose that produces maximal enhancement, was shown to depend on the experimental conditions used. Whereas the administration of epinephrine shortly after a mild footshock enhanced memory, the same dose of epinephrine impaired memory when administered following a high-intensity footshock, which was associated with higher endogenous epinephrine levels (Gold *et al.*, 1977), suggesting the existence of ‘optimal’ epinephrine levels for superior memory performance.

## 3. Role of central norepinephrine in memory consolidation

Since catecholamines cannot readily cross the blood-brain barrier (Weil-Malherbe *et al.*, 1959), it was initially unclear how peripherally released or administered epinephrine and norepinephrine were able to modulate the brain processes underlying memory consolidation. Extensive evidence indicated that these catecholamines initially bind to β-adrenoceptors in the periphery (Schreurs *et al.*, 1986), which in turn affect noradrenergic activity and corresponding activation of β-adrenoceptors in the brain (Introini-Collison *et al.*, 1992). We now know that peripheral epinephrine and norepinephrine activate β-adrenoceptors on the ascending branch of the vagus nerve, which induces activation of two norepinephrine-containing nuclei in the brain: the nucleus of the solitary tract (NTS) and locus coeruleus (LC) (Schreurs *et al.*, 1986; Introini-Collison *et al.*, 1992; Williams & McGaugh, 1993; Clayton & Williams, 2000).

### 3.1 Nucleus of the solitary tract

The vagus nerve directly innervates the NTS. Fluorescence-labeling studies revealed that norepinephrine is the major neurotransmitter in projection neurons from the NTS (Packard & Teather, 1998). Local drug administration studies showed that posttraining infusions of the local anesthetic lidocaine into the NTS not only impaired retention of inhibitory avoidance, but also blocked the memory-enhancing effects of posttraining systemic injections of epinephrine (Watabe *et al.*, 2000), suggesting that central noradrenergic neurons arising in the NTS may mediate the effects of peripheral epinephrine on memory consolidation. Conversely, posttraining infusion of the β-adrenoceptor agonist clenbuterol into the NTS improved retention performance in a Y-maze discrimination task in rats (Williams & Clayton, 2001). The NTS might exert its effects by innervating noradrenergic projections to forebrain structures involved in learning and memory, such as the amygdala, but also by influencing central norepinephrine release via its projections to the LC (Ricardo & Koh, 1978; Williams & McGaugh, 1993).

### 3.2 Locus coeruleus

Noradrenergic cells of the LC have widespread projections throughout the brain (including the cerebral cortex, amygdala, hippocampus, thalamus, midbrain, brain stem, cerebellum and spinal cord), and these projections are well conserved across species (Swanson, 1976; Aston-Jones *et al.*, 1984; Valentino, 1995; Rosenzweig *et al.*, 2002) (Figure 1). The LC supplies the major source of norepinephrine to most forebrain regions and exhibits regional and laminar specificity in its efferent projections (Morrison *et al.*, 1982). Especially brain areas that are associated with attentional processing receive dense noradrenergic

innervation (Morrison & Foote, 1986; Rosenzweig *et al.*, 2002). Interestingly, approximately 85% of noradrenergic fibers innervating the amygdala originate from the LC, suggesting that manipulation of LC activity should induce marked effects on memory consolidation (Liang, 2001).

Extensive findings have indicated that LC activity triggered by stress can be observed in both tonic (Valentino & Van Bockstaele, 2008; Arnsten, 2009) and phasic firing patterns (Bouret & Sara, 2004; Rajkowski *et al.*, 2004; Vazey *et al.*, 2018). Classic studies have also indicated an increased firing rate in noradrenergic neurons in LC (Foote *et al.*, 1980; Aston-Jones & Bloom, 1981), as well as a firing frequency that positively correlated with their synchrony, when animals are aroused (Alvarez *et al.*, 2002). Recently, the causal role of LC firing was further demonstrated via the use of optogenetics and chemogenetics in mediating anxiety-like and aversive behaviors in mice (McCall *et al.*, 2015; Zerbi *et al.*, 2019). Furthermore, a very recent study in mice using chemogenetics showed that selective activation of the LC rapidly induces these anxiety-like behaviors by a rapid-shift in network connectivity towards salience and fear processing (Zerbi *et al.*, 2019). These effects support the notion that the LC optimizes cognitive processes by rearranging neural activity in large-scale neuronal networks (Aston-Jones & Cohen, 2005; Seeley *et al.*, 2007; Bullmore & Sporns, 2009; Van Den Heuvel & Pol, 2010).

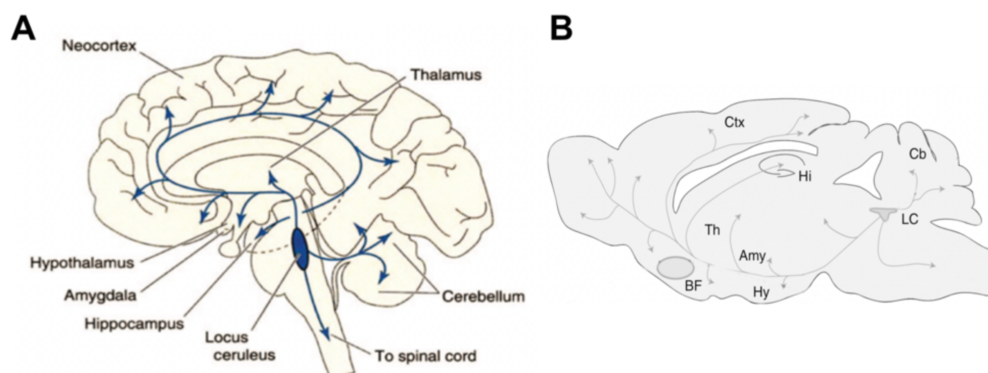


**Figure 1.** Projections of the locus coeruleus in (A) humans (Rosenzweig *et al.*, 2002) and (B) rodents (Swanson, 1976) are highly similar, indicative of their conservation across species.

### 3.3 Pharmacological and genetic evidence of central norepinephrine enhancement of memory

Extensive evidence supports the view that centrally released norepinephrine is critically involved in modulating memory consolidation (Ferry *et al.*, 1999). For example, the centrally acting β-adrenoceptor antagonist propranolol administered systemically impairs memory consolidation (Cohen & Hamburg, 1975; Cahill *et al.*, 2000). Furthermore, systemic administration of dipivefrin and clenbuterol, which are well known as β-adrenoceptor agonists, dose-dependently enhance memory consolidation, and these effects can be blocked by the administration of centrally acting β-adrenoceptor antagonists (Introini-Collison *et al.*, 1991; Introini-Collison *et al.*, 1992).

Moreover, an early study using genetically selected rat lines revealed that the Maudsley reactive rats exhibit a higher noradrenergic response to acute stress in the LC than Maudsley non-reactive rats, and that their behavioral performance was similar to rats treated with drugs that enhance noradrenergic function and improve long-term memory performance (Sara *et al.*, 1994). Another genetic study reported that mice with a genetically encoded reduction in tyrosine hydroxylase, i.e., the enzyme mediating the rate-limiting step in the biosynthesis of catecholamines, exhibit a moderate reduction in norepinephrine accumulation and release in the brain, as well as an impaired long-term memory in different behavioral paradigms, including active avoidance, cued fear conditioning and conditioned taste aversion. Furthermore, memory performance in these mice was rescued by posttraining stimulation of norepinephrine activity (Kobayashi & Kobayashi, 2001).

## 4. Norepinephrine in the amygdala on memory consolidation

The amygdala has a central role in modulating norepinephrine effects on memory consolidation (Liang *et al.*, 1986). Findings in both animals and humans have indicated that damage to the amygdala selectively impairs memory of emotionally arousing experiences (Bermudez-Rattoni & McGaugh, 1991; Cahill *et al.*, 1996), but not memory of emotionally neutral information (Cahill *et al.*, 1996), providing strong support for the view that amygdala activity mediates the enhancing effects of emotional arousal on memory processing (McGaugh, 2000; Roozendaal & McGaugh, 2011).

Many studies have examined how norepinephrine affects memory consolidation by influencing amygdala activity. For example, a memory-enhancing dose of epinephrine administered systemically was found to induce norepinephrine release within the amygdala (Williams *et al.*, 2000). Studies using electrophysiology further indicated that the firing rate

of amygdala neurons is increased after electrical stimulation of the vagus nerve (Radna & MacLean, 1981) or the NTS (Radna & MacLean, 1981; Rogers & Fryman, 1988). An adrenoceptor antagonist administered into the amygdala immediately after inhibitory avoidance training was found to impair retention performance, whereas norepinephrine administered concurrently restored the memory (Gallagher *et al.*, 1977). Moreover, posttraining administration of norepinephrine or the β-adrenoceptor agonist clenbuterol into the amygdala has been shown to induce a dose-dependent enhancement of retention (Liang *et al.*, 1990; Introini-Collison *et al.*, 1991). These findings strongly suggest that noradrenergic activation in the amygdala plays a critical role in memory enhancement.

The amygdala is a complex brain structure consisting of different nuclei. Several studies showed that the basolateral amygdala (BLA) is the critical nucleus of the amygdala that mediates the effects of stress and emotional arousal on memory consolidation (Quirarte *et al.*, 1997; Roozendaal & McGaugh, 1997; Roozendaal *et al.*, 1999; McGaugh, 2000). Early studies using targeted pharmacological manipulations have consistently shown that norepinephrine or noradrenergic agonists administered into the BLA (or the amygdala complex in the earliest studies) enhance the consolidation of memory on a wide variety of emotionally arousing training tasks, including inhibitory avoidance, active avoidance discrimination learning, contextual fear conditioning, water-maze spatial learning and appetitive tasks, but also in the emotionally neutral object recognition memory task (Liang *et al.*, 1986; Introini-Collison *et al.*, 1991; LaLumiere *et al.*, 2003). On the other hand, administration of a β-adrenoceptor antagonist has been found to impair memory consolidation and to block the effects of norepinephrine through the blockade of noradrenergic activity in the BLA (Liang *et al.*, 1986; Liang *et al.*, 1995; Salinas & McGaugh, 1995; Hatfield & McGaugh, 1999). Also the endogenous release of norepinephrine in the BLA after inhibitory avoidance training has been found to correlate with retention latencies 24 h later (McIntyre et al., 2002).
However, many different studies have shown that the BLA is not the storage site of these enhanced memories (Packard *et al.*, 1994; Cahill & McGaugh, 1998), but rather modulates neural plasticity elsewhere in the brain. As such, stress hormones require an intact BLA in order to exert their actions on other brain regions (McGaugh *et al.*, 1996; Roozendaal *et al.*, 1996; Ikegaya *et al.*, 1997; Setlow *et al.*, 2000; Roozendaal *et al.*, 2001; McReynolds *et al.*, 2010; Holloway-Erickson *et al.*, 2012) (Figure 2). Most of these prior experiments have investigated how noradrenergic activation of the BLA facilitates the consolidation of spatial, contextual or episodic memory by influencing neural plasticity and information storage processes within the hippocampus (Packard *et al.*, 1994; Pare *et al.*, 1995; Akirav & Richter-

Levin, 1999; Roozendaal *et al.*, 1999; Almaguer-Melian *et al.*, 2003; McIntyre *et al.*, 2005; Pape *et al.*, 2005; Atucha *et al.*, 2017). However, although only sparsely investigated, BLA noradrenergic activity has also been found to modulate the consolidation of other forms of memory via interactions with other memory systems, such as the medial prefrontal cortex, insular cortex (IC) and perirhinal cortex (PRh) (Roozendaal *et al.*, 1999; McIntyre *et al.*, 2005; Laing & Bashir, 2014; McReynolds *et al.*, 2014; Beldjoud *et al.*, 2015; Chen *et al.*, 2018; Barsegyan *et al.*, 2019).

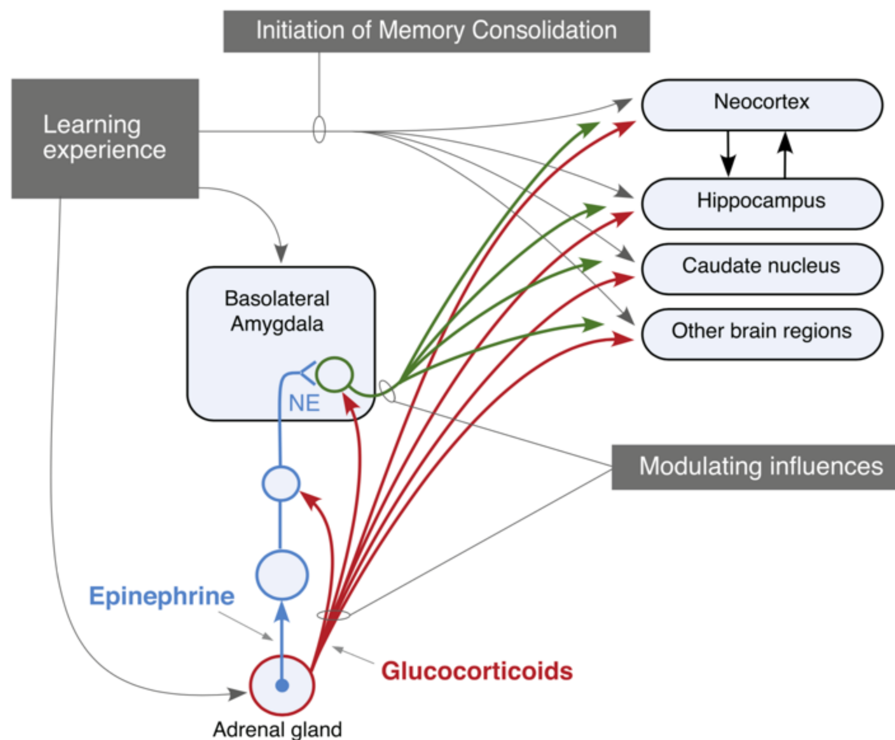


**Figure 2.** Noradrenergic activation of the BLA is crucially involved in enhancing the consolidation of long-term memory via its modulatory influences on neuronal plasticity and information storage processes in other brain structures (McGaugh, 2000).

Object recognition memory is one type of the non-hippocampal-dependent memory, which primarily depends on cortical regions such as the IC and the PRh (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 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).

### 4.1 Insular cortex

The IC appears to be a large and functionally diverse brain region, as anatomical studies in several species highlighted it to be one of the most complex anatomical hubs in the mammalian brain (Cechetto & Saper, 1987; Allen *et al.*, 1991; Yasui *et al.*, 1991; Menon & Uddin, 2010; Cauda *et al.*, 2012). The IC can be generally divided into the anterior IC (aIC) and the posterior IC (pIC) by the central sulcus in humans (and the cerebral artery in rodents). In both humans and rodents, independent of the aIC and pIC subdivision, the IC has diverging cytoarchitecture, changing from its granular to dysgranular to agranular divisions (Figure 3).

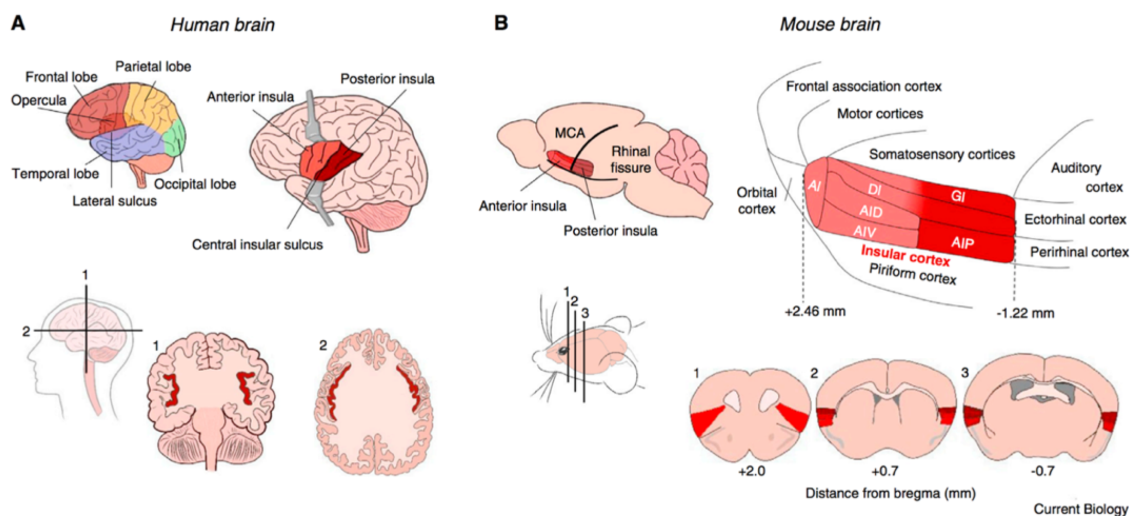


**Figure 3.** Location and architecture of the insular cortex in (A) the human and (B) mouse brain (Gogolla *et al.*, 2014).

The IC has been implicated in an overwhelming variety of functions as well, such as learning and memory (Bermudez-Rattoni *et al.*, 2005), processing of external and bodily sensory information (Kurth *et al.*, 2010; Gogolla, 2017), self-awareness (Craig, 2009, 2011), emotion regulation (Etkin *et al.*, 2015), feelings, complex social-affective functions like empathy (Damasio & Carvalho, 2013), and switching between large-scale brain networks (Menon & Uddin, 2010). Recent rodent studies further demonstrated roles for the IC in multisensory (Rodgers *et al.*, 2008; Gogolla *et al.*, 2014) and pain processing (Tan *et al.*, 2017), representation of valence (Wang *et al.*, 2018), social interactions (Rogers-Carter *et al.*, 2018), and aversive states (Escobar & Bermúdez-Rattoni, 2000; Rodriguez-Ortiz *et al.*, 2005; Stehberg *et al.*, 2011; Livneh *et al.*, 2017; Gehrlach *et al.*, 2019; Livneh *et al.*, 2020).

Interestingly, in humans, most neuroimaging studies have observed increased aIC activity during emotional awareness (Craig, 2009; Menon & Uddin, 2010), as well as during the encoding and recall of emotionally salient learning tasks (Büchel *et al.*, 1998; King *et al.*, 2009). On the other hand, pIC activity has typically found to be increased during the experience of pain or during somatosensory and auditory information processing tasks (Kurth *et al.*, 2010). As such, the aIC and pIC seem to be two largely different functional divisions. However, animal studies typically do not dissociate between the aIC and the pIC (Bermúdez-Rattoni *et al.*, 1997; Bermúdez-Rattoni *et al.*, 2004; Bermudez-Rattoni *et al.*, 2005; Balderas *et al.*, 2008; Miranda *et al.*, 2008; Roozendaal *et al.*, 2010; Stehberg *et al.*, 2011; Chen *et al.*, 2018).

There are dense mutual connections between the IC and BLA (McDonald & Jackson, 1987; Shi & Cassell, 1998; Kayyal *et al.*, 2019; Gehrlach *et al.*, 2020; McGinnis *et al.*, 2020). As mentioned above, the BLA influences memory consolidation of emotionally arousing training experiences by regulating neuroplasticity and information storage processes in many other brain regions. Therefore, it is possible that the IC and BLA share a functional commonality and cooperate in regulating memory consolidation. In support of this idea, functional interactions between the BLA and IC have been observed in regulating memory of conditioned taste aversion, a recognition task that heavily relies on the IC (Bermudez-Rattoni, 2014). It was shown that a synthetic cAMP analog administered into the IC enhanced memory of inhibitory avoidance and conditioned taste aversion, but that the memory enhancement was blocked by the concurrent administration of propranolol into the BLA (Miranda & McGaugh, 2004. Furthermore, an electrophysiological study showed that long-term potentiation in the BLA-IC pathway strengthens long-term conditioned taste aversion memory, whereas long-term depression in this pathway facilitated extinction of this memory (Rodríguez-Durán *et al.*, 2017).

More recent animal studies started to suggest that the IC is also involved in object recognition memory (Bermudez-Rattoni *et al.*, 2005; Balderas *et al.*, 2008; Roozendaal *et al.*, 2010; Chen *et al.*, 2018). These studies showed that local drug manipulation into the IC enhances the consolidation of object recognition memory. Very interestingly, our very recent study indicated that blockade of noradrenergic activity within the BLA prevented enhancement of object recognition memory induced by administration of the histone deacetylase inhibitor sodium butyrate into the aIC (Chen *et al.*, 2018). However, such studies with combined drug administration into the BLA and IC do not provide evidence for the

involvement of direct pathways between both regions in regulating emotional arousal effects on object recognition memory.

### 4.2 Perirhinal cortex

The PRh - along with the entorhinal and posthinal cortices - is considered an integral part of the para-hippocampal formation (Kealy & Commins, 2011). In humans, the PRh extends lateral to the full extent of the rhinal sulcus and includes cortex areas 35 and 36 of Brodmann (Brodmann, 1909). Similarly, in the rodent brain, the PRh is located along the rhinal sulcus and is composed of Brodmann's areas 35 and 36. It is bordered rostrally by the posterior agranular insular cortex and the visceral area, caudally by the posthinal cortex, dorsally by the ventral temporal association cortex and ventrally by the lateral entorhinal cortex (Burwell *et al.*, 1995; Burwell, 2001) (Figure 4).

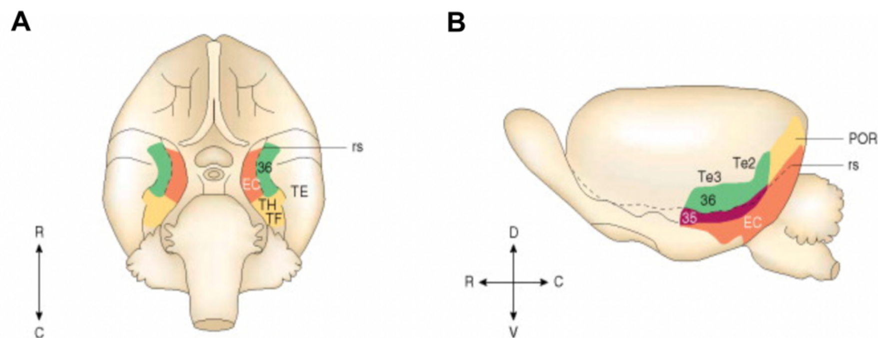


**Figure 4.** Location of the perirhinal cortex in (A) the human and (B) mouse brain (Brown & Eldrige, 2009)

The PRh is critically involved in recognition memory, since extensive studies have shown that lesions of the PRh disrupt object recognition memory in different phases, including encoding, consolidation and retrieval (Mumby & Pinel, 1994; Suzuki, 1996; Liu & Bilkey, 2001; Mumby *et al.*, 2002; Winters & Bussey, 2005; Albasser *et al.*, 2009). However, the exact role of the PRh in object recognition memory remains elusive. Findings in rodents indicated that the PRh is critically involved in the discrimination of familiarity (Ennaceur & Aggleton, 1997; Norman & Eacott, 2005; Balderas *et al.*, 2008; Albasser *et al.*, 2009; Olarte-Sánchez *et al.*, 2015) and overlapping emotional arousal memories (Miranda *et al.*, 2017), while neuroimaging study in humans indicated that the PRh is more involved in novelty detection (Kafkas & Montaldi, 2018).

Although not extensively studied, there is evidence for functional interactions between the BLA and the PRh. For example, electrical stimulation of the amygdala was found to reduce the threshold for the induction of LTP in the PRh (Perugini *et al.*, 2012), whereas repeated stimulation of the amygdala activated the PRh deep layers, and combined stimulation of both regions initiated signal propagation from the PRh to the entorhinal-hippocampal circuit (Kajiwara *et al.*, 2003). Furthermore, systemic administration of the β-adrenoceptor agonist isoprenaline was found to induce a long-lasting potentiation of synaptic plasticity within the amygdala-PRh pathway in response to a concurrent subthreshold electrical stimulation of the input (Laing & Bashir, 2014), supporting the notion of amygdala-mediated noradrenergic enhancement of memory processing within the PRh.

## 5. Memory quality

Although a rich literature of >50 years of research has provided convincing evidence for the view that noradrenergic activation by emotional arousal can strengthen memories, we know considerably less of whether such noradrenergic activation is also associated with changes in the quality of memory.

Although in humans, a wealth of studies has investigated the effect of emotional arousal on memory quality, the effects remain highly controversial. Segal and colleagues found that emotional enhancement, measured by increased salivary amylase levels, facilitated accurate memories (Segal *et al.*, 2012), while other studies reported a tendency of emotional memories to be remembered in a generalized, gist-like manner (Loftus, 1979; Morgan III *et al.*, 2004; Payne *et al.*, 2006; Qin *et al.*, 2012). In animal studies, memory quality, especially memory accuracy, has been mostly investigated with respect to hippocampus-dependent episodic-like memory. In some recent studies, our laboratory showed that systemic administration of the noradrenergic stimulant yohimbine not only strengthened memory of an inhibitory avoidance experience, but that it also enhanced the episodic-like accuracy of the memory (Roozendaal & Mirone, 2020). In these studies, vehicle-treated animals were unable to discriminate the context in which they had previously received a footshock, from a safe training context, whereas yohimbine-treated animals showed an accurate association of the shock experience with the correct training context. Moreover, we showed in an earlier study that noradrenergic activation maintained long-term accuracy of the memory by preventing the time-dependent reorganization of memory from the hippocampus to neocortical networks (Atucha *et al.*, 2017). However, little is known with respect to whether

such noradrenergic activation is also able to maintain long-term accuracy of other types of memory, which already depend on cortical networks, such as object recognition memory.

## 6. Scope and outline of this thesis

Thus, the aim of the research in this thesis was to investigate the effects of noradrenergic activation on the neuronal mechanisms underlying the accuracy or detailedness of object recognition memory.

In **Chapter 2**, I investigated the effect of noradrenergic activation on object recognition memory in mice. Previous studies into the effects of noradrenergic activation on object recognition memory have been restricted to rats, whereas mice, showing different cognitive abilities and higher endogenous arousal levels compared to rats have not yet been tested. Importantly, due to their wide variety of available transgenic lines, mice are preferred as experimental animal model for neural circuitry-based investigations (by means of optogenetics or chemogenetics), as well as those targeting specific neuronal subclasses. Thus, we performed a first experiment to replicate the memory-enhancing effect of noradrenergic activation on object memory in mice, as a fundamental step for the following studies. Results indicated that posttraining noradrenergic activation can also enhance object recognition memory in mice.

In **Chapter 3,** I investigated the effects of training duration and noradrenergic activation on the detailedness of object recognition memory and its maintenance over time. As the standard object recognition task (using only two objects during training) does not allow for the investigation of memory detailedness, merely longevity, I first developed a modified version of this task. In this new paradigm, i.e., the object discrimination task, multiple testing objects that vary in their resemblance with the training objects are used, in order to enable the examination of noradrenergic activation modulation of memory detailedness. Effects of training duration as well as noradrenergic activation were investigated over time, implementing several retention intervals. Both increased training duration and posttraining noradrenergic activation appeared to enhance memory detailedness in this task. Moreover, to better understand the underlying neural mechanisms, we assessed the effects of noradrenergic activity on neuronal activity during retention testing and found particularly neuronal activity in the aIC and PRh to be increased.

In **Chapter 4**, I further investigated the underlying neural circuitry of the memory-enhancing effect of noradrenergic activation, using TRAP and chemogenetic techniques. First, I employed a newly developed transgenic mice, the FosTRAP2 × tdTomato mice, in which an injection of the estrogen receptor agonist 4-hydroxytamoxifen (4-OHT) induces the permanent labeling of active (i.e., immediate-early gene c-Fos-expressing) neurons in a specific time window. Use of this transgenic line enabled me to tag the activated neuronal representations activated during training/early consolidation, and compare this to later retention-induced neuronal activity. I first validated whether posttraining noradrenergic stimulation in these mice enhanced memory detailedness on the object discrimination task. Next, I examined the neuronal activity changes underlying this behavior, by systemic injection of yohimbine as well as 4-OHT posttraining and exposing the mice later to either three familiar objects, three similar or three dissimilar objects to trigger memory recall. This approach allowed for the dissociation of neuronal activity encoding familiarity and novelty. In a second experiment, we investigated whether the norepinephrine-induced recruitment of BLA projections to the aIC is responsible for the yohimbine effect on enhanced memory detailedness. For this, we inactivated the BLA-aIC pathway during memory consolidation by an inhibitory chemogenetic manipulation and determined that this manipulation selectively impaired the yohimbine effect on memory detailedness.

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

## References


Akirav, I., & Richter-Levin, G. (1999). Biphasic modulation of hippocampal plasticity by behavioral stress and basolateral amygdala stimulation in the rat. *Journal of Neuroscience, 19*(23), 10530-10535.

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*(1), 115.

Allen, G. V., Saper, C. B., Hurley, K. M., & Cechetto, D. F. (1991). Organization of visceral and limbic connections in the insular cortex of the rat. *Journal of Comparative Neurology, 311*(1), 1-16.

Almaguer-Melian, W., Martinez-Marti, L., Frey, J. U., & Bergado, J. (2003). The amygdala is part of the behavioural reinforcement system modulating long-term potentiation in rat hippocampus. *Neuroscience, 119*(2), 319-322.

Alvarez, V. A., Chow, C. C., Van Bockstaele, E. J., & Williams, J. T. (2002). Frequency-dependent synchrony in locus ceruleus: Role of electrotonic coupling. *Proceedings of the National Academy of Sciences, 99*(6), 4032-4036.

Arnsten, A. F. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature reviews neuroscience, 10*(6), 410-422.

Aston-Jones, G., & Bloom, F. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *Journal of Neuroscience, 1*(8), 876-886.

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*, 403-450.

Aston-Jones, G., Shaver, R., & Dinan, T. (1984). Cortically projecting nucleus basalis neurons in rat are physiologically heterogeneous. *Neuroscience Letters, 46*(1), 19-24.

Atucha, E., & Roozendaal, B. (2015). The inhibitory avoidance discrimination task to investigate accuracy of memory. *Frontiers in Behavioral Neuroscience, 9*, 60.

Atucha, E., Vukojevic, V., Fornari, R. V., Ronzoni, G., Demougin, P., Peter, F., Atsak, P., Coolen, M. W., Papassotiropoulos, A., & McGaugh, J. L. (2017). Noradrenergic activation of the basolateral amygdala maintains hippocampus-dependent accuracy of remote memory. *Proceedings of the National Academy of Sciences of the United States of America, 114*(34), 9176-9181.

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.

Banks, P., Warburton, E., Brown, M., & Bashir, Z. (2014). Mechanisms of synaptic plasticity and recognition memory in the perirhinal cortex *Progress in molecular biology and translational science* (Vol. 122, pp. 193-209): Elsevier.

Barsegyan, A., Atsak, P., Hornberger, W. B., Jacobson, P. B., Van Gaalen, M. M., & Roozendaal, B. (2015). The vasopressin 1b receptor antagonist a-988315 blocks stress effects on the retrieval of object-recognition memory. *Neuropsychopharmacology, 40*(8), 1979-1989.

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 of the United States of America, 116*(14), 7077-7082.

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*(108), 108.

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

Bermúdez-Rattoni, F., Introini-Collison, I., Coleman-Mesches, K., & McGaugh, J. L. (1997). Insular cortex and amygdala lesions induced after aversive training impair retention: Effects of degree of training. *Neurobiology of Learning and Memory, 67*(1), 57-63.

Bermudez-Rattoni, F., & McGaugh, J. L. (1991). Insular cortex and amygdala lesions differentially affect acquisition on inhibitory avoidance and conditioned taste aversion. *Brain Research, 549*(1), 165-170.

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

Bermúdez-Rattoni, F., Ramírez-Lugo, L., Gutiérrez, R., & Miranda, M. I. (2004). Molecular signals into the insular cortex and amygdala during aversive gustatory memory formation. *Cellular and Molecular Neurobiology, 24*(1), 25-36.

Bouret, S., & Sara, S. J. (2004). Reward expectation, orientation of attention and locus coeruleus-medial frontal cortex interplay during learning. *European Journal of Neuroscience, 20*(3), 791-802.

Brodmann, K. (1909). 2006. Localization in the cerebral cortex, translated by garey lj: New York: Springer.

Büchel, C., Morris, J., Dolan, R. J., & Friston, K. J. (1998). Brain systems mediating aversive conditioning: An event-related fmri study. *Neuron, 20*(5), 947-957.

Bullmore, E., & Sporns, O. (2009). Complex brain networks: Graph theoretical analysis of structural and functional systems. *Nature reviews neuroscience, 10*(3), 186-198.

Burwell, R. D. (2001). Borders and cytoarchitecture of the perirhinal and postrhinal cortices in the rat. *Journal of Comparative Neurology, 437*(1), 17-41.

Burwell, R. D., Witter, M. P., & Amaral, D. G. (1995). Perirhinal and postrhinal cortices of the rat: A review of the neuroanatomical literature and comparison with findings from the monkey brain. *Hippocampus, 5*(5), 390-408.

Byrne, J. H. (1987). Cellular analysis of associative learning. *Physiological Reviews, 67*(2), 329-439.

Cahill, L., Gorski, L., & Le, K. (2003). Enhanced human memory consolidation with post-learning stress: Interaction with the degree of arousal at encoding. *Learning and Memory, 10*(4), 270-274.

Cahill, L., Haier, R. J., Fallon, J., Alkire, M. T., Tang, C., Keator, D., Wu, J., & Mcgaugh, J. L. (1996). Amygdala activity at encoding correlated with long-term, free recall of emotional information. *Proceedings of the National Academy of Sciences, 93*(15), 8016-8021.

Cahill, L., & McGaugh, J. L. (1998). Mechanisms of emotional arousal and lasting declarative memory. *Trends in Neurosciences, 21*(7), 294-299.

Cahill, L., Pham, C. A., & Setlow, B. (2000). Impaired memory consolidation in rats produced with β-adrenergic blockade. *Neurobiology of Learning and Memory, 74*(3), 259-266.

Cauda, F., Costa, T., Torta, D. M., Sacco, K., D'Agata, F., Duca, S., Geminiani, G., Fox, P. T., & Vercelli, A. (2012). Meta-analytic clustering of the insular cortex: Characterizing the meta-analytic connectivity of the insula when involved in active tasks. *Neuroimage, 62*(1), 343-355.

Cechetto, D. F., & Saper, C. B. (1987). Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat. *Journal of Comparative Neurology, 262*(1), 27-45.

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.

Clayton, E. C., & Williams, C. L. (2000). Adrenergic activation of the nucleus tractus solitarius potentiates amygdala norepinephrine release and enhances retention performance in emotionally arousing and spatial memory tasks. *Behavioural Brain Research, 112*(1-2), 151-158.

Cohen, R. P., & Hamburg, M. D. (1975). Evidence for adrenergic neurons in a memory access pathway. *Pharmacology Biochemistry and Behavior, 3*(3), 519-523.

Cowan, N. (2008). What are the differences between long-term, short-term, and working memory? *Progress in Brain Research, 169*, 323-338.

Craig, A. (2009). How do you feel--now? The anterior insula and human awareness. *Nature reviews neuroscience, 10*(1).

Craig, A. (2011). Significance of the insula for the evolution of human awareness of feelings from the body. *Annals of the New York Academy of Sciences, 1225*(1), 72-82.

Damasio, A., & Carvalho, G. B. (2013). The nature of feelings: Evolutionary and neurobiological origins. *Nature reviews neuroscience, 14*(2), 143-152.

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

Dornelles, A., de Lima, M. N., Grazziotin, M., Presti-Torres, J., Garcia, V. A., Scalco, F. S., Roesler, R., & Schroder, N. (2007). Adrenergic enhancement of consolidation of object recognition memory. *Neurobiology of Learning and Memory, 88*(1), 137-142.

Eichenbaum, H. (2017). Prefrontal–hippocampal interactions in episodic memory. *Nature reviews neuroscience, 18*(9), 547-558.

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.

Escobar, M. L., & Bermúdez-Rattoni, F. (2000). Long-term potentiation in the insular cortex enhances conditioned taste aversion retention. *Brain Research, 852*(1), 208-212.

Etkin, A., Büchel, C., & Gross, J. J. (2015). The neural bases of emotion regulation. *Nature reviews neuroscience, 16*(11), 693-700.

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.

Foote, S., Aston-Jones, G., & Bloom, F. (1980). Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. *Proceedings of the National Academy of Sciences, 77*(5), 3033-3037.

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nature reviews neuroscience, 6*(2), 119-130.

Frankland, P. W., Bontempi, B., Talton, L. E., Kaczmarek, L., & Silva, A. J. (2004). The involvement of the anterior cingulate cortex in remote contextual fear memory. *Science, 304*(5672), 881-883.

Gallagher, M., Kapp, B. S., Musty, R. E., & Driscoll, P. A. (1977). Memory formation: Evidence for a specific neurochemical system in the amygdala. *Science, 198*(4315), 423-425.

Gehrlach, D. A., Dolensek, N., Klein, A. S., Chowdhury, R. R., Matthys, A., Junghänel, M., Gaitanos, T. N., Podgornik, A., Black, T. D., & Vaka, N. R. (2019). Aversive state processing in the posterior insular cortex. *Nature Neuroscience, 22*(9), 1424-1437.

Gehrlach, D. A., Gaitanos, T. N., Klein, A. S., Weiand, C., Hennrich, A. A., Conzelmann, K.-K., & Gogolla, N. (2020). A whole-brain connectivity map of mouse insular cortex. *BioRxiv*.

Gogolla, N. (2017). The insular cortex. *Current Biology, 27*(12), R580-R586.

Gogolla, N., Takesian, A. E., Feng, G., Fagiolini, M., & Hensch, T. K. (2014). Sensory integration in mouse insular cortex reflects gaba circuit maturation. *Neuron, 83*(4), 894-905.

Gold, P. E., & Van Buskirk, R. (1976). Effects of posttrial hormone injections on memory processes. *Hormones and Behavior, 7*(4), 509-517.

Gold, P. E., Van Buskirk, R., & Haycock, J. W. (1977). Effects of posttraining epinephrine injections on retention of avoidance training in mice. *Behavioral Biology, 20*(2), 197-204.

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

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.

Holloway-Erickson, C. M., McReynolds, J. R., & McIntyre, C. K. (2012). Memory-enhancing intra-basolateral amygdala infusions of clenbuterol increase arc and camkiiα protein expression in the rostral anterior cingulate cortex. *Frontiers in Behavioral Neuroscience, 6*, 17.

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.

Hunt, E., & Krivanek, J. (1966). The effects of pentylenetetrazole and methylphenoxypropane on discrimination learning. *Psychopharmacologia, 9*(1), 1-16.

Hunt, E. B., & Bauer, R. H. (1969). Facilitation of learning by delayed injections of pentylenetetrazol. *Psychopharmacologia, 16*(2), 139-146.

Ikegaya, Y., Nakanishi, K., Saito, H., & Abe, K. (1997). Amygdala β-noradrenergic influence on hippocampal longterm potentiation in vivo. *Neuroreport, 8*(14), 3143-3146.

Introini-Collison, I., Saghafi, D., Novack, G. D., & McGaugh, J. L. (1992). Memory-enhancing effects of post-training dipivefrin and epinephrine: Involvement of peripheral and central adrenergic receptors. *Brain Research, 572*(1-2), 81-86.

Introini-Collison, I. B., Miyazaki, B., & McGaugh, J. L. (1991). Involvement of the amygdala in the memory-enhancing effects of clenbuterol. *Psychopharmacology, 104*(4), 541-544.

Izquierdo, I., Quillfeldt, J., Zanatta, M., Quevedo, J., Schaeffer, E., Schmitz, P., & Medina, J. (1997). Sequential role of hippocampus and amygdala, entorhinal cortex and parietal cortex in formation and retrieval of memory for inhibitory avoidance in rats. *European Journal of Neuroscience, 9*(4), 786-793.

Jurado-Berbel, P., Costa-Miserachs, D., Torras-Garcia, M., Coll-Andreu, M., & Portell-Cortes, 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.

Kafkas, A., & Montaldi, D. (2018). How do memory systems detect and respond to novelty? *Neuroscience Letters, 680*, 60-68.

Kajiwara, R., Takashima, I., Mimura, Y., Witter, M. P., & Iijima, T. (2003). Amygdala input promotes spread of excitatory neural activity from perirhinal cortex to the entorhinal–hippocampal circuit. *Journal of Neurophysiology, 89*(4), 2176-2184.

Kandel, E. R. (2004). The molecular biology of memory storage: A dialog between genes and synapses. *Bioscience Reports, 24*(4-5), 475-522.

Kandel, E. R., & Schwartz, J. H. (1982). Molecular biology of learning: Modulation of transmitter release. *Science, 218*(4571), 433-443.

Kayyal, H., Yiannakas, A., Chandran, S. K., Khamaisy, M., Sharma, V., & Rosenblum, K. (2019). Activity of insula to basolateral amygdala projecting neurons is necessary and sufficient for taste valence representation. *Journal of Neuroscience, 39*(47), 9369-9382.

Kealy, J., & Commins, S. (2011). The rat perirhinal cortex: A review of anatomy, physiology, plasticity, and function. *Progress in Neurobiology, 93*(4), 522-548.

King, A. P., Abelson, J. L., Britton, J. C., Phan, K. L., Taylor, S. F., & Liberzon, I. (2009). Medial prefrontal cortex and right insula activity predict plasma acth response to trauma recall. *Neuroimage, 47*(3), 872-880.

Kobayashi, K., & Kobayashi, T. (2001). Genetic evidence for noradrenergic control of long-term memory consolidation. *Brain and Development, 23*, S16-S23.

Krivanek, J., & McGaugh, J. L. (1968). Effects of pentylenetetrazol on memory storage in mice. *Psychopharmacologia, 12*(4), 303-321.

Kurth, F., Eickhoff, S. B., Schleicher, A., Hoemke, L., Zilles, K., & Amunts, K. (2010). Cytoarchitecture and probabilistic maps of the human posterior insular cortex. *Cerebral Cortex, 20*(6), 1448-1461.

Laing, M., & Bashir, Z. (2014). B-adrenoceptors and synaptic plasticity in the perirhinal cortex. *Neuroscience, 273*, 163-173.

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.

Liang, K., Chen, L. L., & Huang, T.-E. (1995). Memory formation: Involvement in the. *Chinese Journal of Physiology, 38*(2), 81-91.

Liang, K., 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.

Liang, K. C. (2001). Epinephrine modulation of memory: Amygdala activation and regulation of long-term memory storage.

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

Liu, P., & Bilkey, D. K. (2001). The effect of excitotoxic lesions centered on the hippocampus or perirhinal cortex in object recognition and spatial memory tasks. *Behavioral Neuroscience, 115*(1), 94.

Livingston, R. B. (1967). Reinforcement.

Livneh, Y., Ramesh, R. N., Burgess, C. R., Levandowski, K. M., Madara, J. C., Fenselau, H., Goldey, G. J., Diaz, V. E., Jikomes, N., & Resch, J. M. (2017). Homeostatic circuits selectively gate food cue responses in insular cortex. *Nature, 546*(7660), 611-616.

Livneh, Y., Sugden, A. U., Madara, J. C., Essner, R. A., Flores, V. I., Sugden, L. A., Resch, J. M., Lowell, B. B., & Andermann, M. L. (2020). Estimation of current and future physiological states in insular cortex. *Neuron, 105*(6), 1094-1111. e1010.

Loftus, E. (1979). Eyewitness reliability: American Association for the Advancement of Science.

McCall, J. G., Al-Hasani, R., Siuda, E. R., Hong, D. Y., Norris, A. J., Ford, C. P., & Bruchas, M. R. (2015). Crh engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. *Neuron, 87*(3), 605-620.

McDonald, A. J., & Jackson, T. R. (1987). Amygdaloid connections with posterior insular and temporal cortical areas in the rat. *Journal of Comparative Neurology, 262*(1), 59-77.

McGaugh, J. L. (1966). Time-dependent processes in memory storage. *Science, 153*(3742), 1351-1358.

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*: Columbia University Press.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience, 27*, 1-28.

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., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: Interaction with other brain systems. *Proceedings of the National Academy of Sciences, 93*(24), 13508-13514.

McGinnis, M. M., Parrish, B. C., & McCool, B. A. (2020). Withdrawal from chronic ethanol exposure increases postsynaptic glutamate function of insular cortex projections to the rat basolateral amygdala. *Neuropharmacology*, 108129.

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.

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: A network model of insula function. *Brain Structure and Function, 214*(5-6), 655-667.

Miller, C. A., Gavin, C. F., White, J. A., Parrish, R. R., Honasoge, A., Yancey, C. R., Rivera, I. M., Rubio, M. D., Rumbaugh, G., & Sweatt, J. D. (2010). Cortical DNA methylation maintains remote memory. *Nature Neuroscience, 13*(6), 664-666.

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).

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 and Memory, 15*(7), 468-476.

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

Morrison, J. H., & Foote, S. L. (1986). Noradrenergic and serotoninergic innervation of cortical, thalamic, and tectal visual structures in old and new world monkeys. *Journal of Comparative Neurology, 243*(1), 117-138.

Morrison, J. H., Foote, S. L., O'Connor, D., & Bloom, F. E. (1982). Laminar, tangential and regional organization of the noradrenergic innervation of monkey cortex: Dopamine-β-hydroxylase immunohistochemistry. *Brain Research Bulletin, 9*(1-6), 309-319.

Müller, G. E., & Pilzecker, A. (1900). *Experimentelle beiträge zur lehre vom gedächtniss* (Vol. 1): JA Barth.

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.

Mumby, D. G., & Pinel, J. P. (1994). Rhinal cortex lesions and object recognition in rats. *Behavioral Neuroscience, 108*(1), 11.

Nadel, L., & Moscovitch, M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex. *Current Opinion in Neurobiology, 7*(2), 217-227.

Norman, G., & Eacott, M. (2005). Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. *Behavioral Neuroscience, 119*(2), 557.

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.

Packard, M. G., Cahill, L., & McGaugh, J. L. (1994). Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. *Proceedings of the National Academy of Sciences, 91*(18), 8477-8481.

Packard, M. G., & Teather, L. A. (1998). Amygdala modulation of multiple memory systems: Hippocampus and caudate-putamen. *Neurobiology of Learning and Memory, 69*(2), 163-203.

Pape, H. C., Narayanan, R. T., Smid, J., Stork, O., & Seidenbecher, T. (2005). Theta activity in neurons and networks of the amygdala related to long-term fear memory. *Hippocampus, 15*(7), 874-880.

Pare, D., Dong, J., & Gaudreau, H. (1995). Amygdalo-entorhinal relations and their reflection in the hippocampal formation: Generation of sharp sleep potentials. *Journal of Neuroscience, 15*(3), 2482-2503.

Payne, J., Jackson, E., Ryan, L., Hoscheidt, S., Jacobs, J., & Nadel, L. (2006). The impact of stress on neutral and emotional aspects of episodic memory. *Memory, 14*(1), 1-16.

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

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.

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.

Qin, S., Hermans, E. J., van Marle, H. J., & Fernández, G. (2012). Understanding low reliability of memories for neutral information encoded under stress: Alterations in memory-related activation in the hippocampus and midbrain. *Journal of Neuroscience, 32*(12), 4032-4041.

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 of the United States of America, 94*(25), 14048-14053.

Radna, R. J., & MacLean, P. D. (1981). Vagal elicitation of respiratory-type and other unit responses in basal limbic structures of squirrel monkeys. *Brain Research, 213*(1), 45-61.

Rajkowski, J., Majczynski, H., Clayton, E., & Aston-Jones, G. (2004). Activation of monkey locus coeruleus neurons varies with difficulty and performance in a target detection task. *Journal of Neurophysiology, 92*(1), 361-371.

Rasch, B., Spalek, K., Buholzer, S., Luechinger, R., Boesiger, P., Papassotiropoulos, A., & de Quervain, D.-F. (2009). A genetic variation of the noradrenergic system is related to differential amygdala activation during encoding of emotional memories. *Proceedings of the National Academy of Sciences, 106*(45), 19191-19196.

Ricardo, J. A., & Koh, E. T. (1978). Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Research, 153*(1), 1-26.

Richardson, M. P., Strange, B. A., & Dolan, R. J. (2004). Encoding of emotional memories depends on amygdala and hippocampus and their interactions. *Nature Neuroscience, 7*(3), 278-285.

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

Rodgers, K. M., Benison, A. M., Klein, A., & Barth, D. S. (2008). Auditory, somatosensory, and multisensory insular cortex in the rat. *Cerebral Cortex, 18*(12), 2941-2951.

Rodríguez-Durán, L. F., Martínez-Moreno, A., & Escobar, M. L. (2017). Bidirectional modulation of taste aversion extinction by insular cortex ltp and ltd. *Neurobiology of Learning and Memory, 142*, 85-90.

Rodriguez-Ortiz, C. J., De la Cruz, V., Gutiérrez, R., & Bermudez-Rattoni, F. (2005). Protein synthesis underlies post-retrieval memory consolidation to a restricted degree only when updated information is obtained. *Learning and Memory, 12*(5), 533-537.

Rogers, R. C., & Fryman, D. L. (1988). Direct connections between the central nucleus of the amygdala and the nucleus of the solitary tract: An electrophysiological study in the rat. *Journal of the Autonomic Nervous System, 22*(1), 83-87.

Rogers-Carter, M. M., Varela, J. A., Gribbons, K. B., Pierce, A. F., McGoey, M. T., Ritchey, M., & Christianson, J. P. (2018). Insular cortex mediates approach and avoidance responses to social affective stimuli. *Nature Neuroscience, 21*(3), 404-414.

Roozendaal, B., Dominique, J.-F., Ferry, B., Setlow, B., & McGaugh, J. L. (2001). Basolateral amygdala–nucleus accumbens interactions in mediating glucocorticoid enhancement of memory consolidation. *Journal of Neuroscience, 21*(7), 2518-2525.

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. *Journal of Neuroscience, 30*(14), 5037-5046.

Roozendaal, B., & McGaugh, J. L. (1997). 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., & McGaugh, J. L. (2011). Memory modulation. *Behavioral Neuroscience, 125*(6), 797-824.

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

Roozendaal, B., Nguyen, B. T., Power, A. E., & McGaugh, J. L. (1999). 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., Portillo-Marquez, G., & McGaugh, J. L. (1996). Basolateral amygdala lesions block glucocorticoid-induced modulation of memory for spatial learning. *Behavioral Neuroscience, 110*(5), 1074.

Rosenzweig, M. R., Breedlove, S. M., & Leiman, A. L. (2002). *Biological psychology: An introduction to behavioral, cognitive, and clinical neuroscience*: Sinauer Associates.

Salinas, J. A., & McGaugh, J. L. (1995). Muscimol induces retrograde amnesia for changes in reward magnitude. *Neurobiology of Learning and Memory, 63*(3), 277-285.

Sara, S., Devauges, V., Biegon, A., & Blizard, D. (1994). The maudsley rat strains as a probe to investigate noradrenergic-cholinergic interaction in cognitive function. *Journal of Physiology-Paris, 88*(6), 337-345.

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

Schreurs, J., Seelig, T., & Schulman, H. (1986). Beta 2-adrenergic receptors on peripheral nerves. *Journal of Neurochemistry, 46*(1), 294-296.

Schwartz, J. H., Castellucci, V. F., & Kandel, E. R. (1971). Functioning of identified neurons and synapses in abdominal ganglion of aplysia in absence of protein synthesis. *Journal of Neurophysiology, 34*(6), 939-953.

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., Reiss, A. L., & Greicius, M. D. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *Journal of Neuroscience, 27*(9), 2349-2356.

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.

Setlow, B., Roozendaal, B., & McGaugh, J. L. (2000). Involvement of a basolateral amygdala complex–nucleus accumbens pathway in glucocorticoid-induced modulation of memory consolidation. *European Journal of Neuroscience, 12*(1), 367-375.

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

Shi, C. J., & Cassell, M. (1998). Cascade projections from somatosensory cortex to the rat basolateral amygdala via the parietal insular cortex. *Journal of Comparative Neurology, 399*(4), 469-491.

Smeets, T., Otgaar, H., Candel, I., & Wolf, O. T. (2008). True or false? Memory is differentially affected by stress-induced cortisol elevations and sympathetic activity at consolidation and retrieval. *Psychoneuroendocrinology, 33*(10), 1378-1386.

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience, 8*(4), 383.

Squire, L. R., & Alvarez, P. (1995). Retrograde amnesia and memory consolidation: A neurobiological perspective. *Current Opinion in Neurobiology, 5*(2), 169-177.

Stehberg, J., Moraga-Amaro, R., & Simon, F. (2011). The role of the insular cortex in taste function. *Neurobiology of Learning and Memory, 96*(2), 130-135.

Steward, O., & Schuman, E. M. (2001). Protein synthesis at synaptic sites on dendrites. *Annual Review of Neuroscience, 24*(1), 299-325.

Sutherland, R., & Lehmann, H. (2011). Alternative conceptions of memory consolidation and the role of the hippocampus at the systems level in rodents. *Current Opinion in Neurobiology, 21*(3), 446-451.

Suzuki, W. A. (1996). The anatomy, physiology and functions of the perirhinal cortex. *Current Opinion in Neurobiology, 6*(2), 179-186.

Swanson, L. (1976). The locus coeruleus: A cytoarchitectonic, golgi and immunohistochemical study in the albino rat. *Brain Research, 110*(1), 39-56.

Takeuchi, T., Duszkiewicz, A. J., Sonneborn, A., Spooner, P. A., Yamasaki, M., Watanabe, M., Smith, C. C., Fernandez, G., Deisseroth, K., Greene, R. W., & Morris, R. G.

(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*(5), 455-461.

Tan, L. L., Pelzer, P., Heinl, C., Tang, W., Gangadharan, V., Flor, H., Sprengel, R., Kuner, T., & Kuner, R. (2017). A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. *Nature Neuroscience, 20*(11), 1591.

Tweedie-Cullen, R. Y., Reck, J. M., & Mansuy, I. M. (2009). Comprehensive mapping of post-translational modifications on synaptic, nuclear, and histone proteins in the adult mouse brain. *Journal of Proteome Research, 8*(11), 4966-4982.

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

Valentino, R. (1995). Physiological and anatomical determinants of locus coeruleus discharge. Behavioral and clinical implications. *Psychopharmacology. The fourth generation of progress*, 373-385.

Valentino, R. J., & Van Bockstaele, E. (2008). Convergent regulation of locus coeruleus activity as an adaptive response to stress. *European Journal of Pharmacology, 583*(2-3), 194-203.

Van Den Heuvel, M. P., & Pol, H. E. H. (2010). Exploring the brain network: A review on resting-state fmri functional connectivity. *European Neuropsychopharmacology, 20*(8), 519-534.

Vazey, E. M., Moorman, D. E., & Aston-Jones, G. (2018). Phasic locus coeruleus activity regulates cortical encoding of salience information. *Proceedings of the National Academy of Sciences, 115*(40), E9439-E9448.

Wang, L., Gillis-Smith, S., Peng, Y., Zhang, J., Chen, X., Salzman, C. D., Ryba, N. J., & Zuker, C. S. (2018). The coding of valence and identity in the mammalian taste system. *Nature, 558*(7708), 127.

Watabe, A. M., Zaki, P. A., & O'Dell, T. J. (2000). Coactivation of β-adrenergic and cholinergic receptors enhances the induction of long-term potentiation and synergistically activates mitogen-activated protein kinase in the hippocampal ca1 region. *Journal of Neuroscience, 20*(16), 5924-5931.

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

Williams, C. L., & Clayton, E. C. (2001). Contribution of brainstem structures in modulating memory storage processes. *Memory consolidation: Essays honoring James L. McGaugh*, 141-164.

Williams, C. L., & McGaugh, J. L. (1993). Reversible lesions of the nucleus of the solitary tract attenuate the memory-modulating effects of posttraining epinephrine. *Behavioral Neuroscience, 107*(6), 955.

Williams, C. L., Men, D., & Clayton, E. C. (2000). The effects of noradrenergic activation of the nucleus tractus solitarius on memory and in potentiating norepinephrine release in the amygdala. *Behavioral Neuroscience, 114*(6), 1131.

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.

Wiltgen, B. J., & Tanaka, K. Z. (2013). Systems consolidation and the content of memory. *Neurobiology of Learning and Memory, 106*, 365-371.

Winocur, G., Moscovitch, M., & Bontempi, B. (2010). Memory formation and long-term retention in humans and animals: Convergence towards a transformation account of hippocampal–neocortical interactions. *Neuropsychologia, 48*(8), 2339-2356.

Winters, B. D., & Bussey, T. J. (2005). Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *Journal of Neuroscience, 25*(1), 52-61.

Yasui, Y., Breder, C. D., Safer, C. B., & Cechetto, D. F. (1991). Autonomic responses and efferent pathways from the insular cortex in the rat. *Journal of Comparative Neurology, 303*(3), 355-374.

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 e705.

# CHAPTER

# 2

## Noradrenergic enhancement of object recognition and object location memory in mice

## Abstract

Extensive evidence indicates that noradrenergic activation is essentially involved in mediating the enhancing effects of emotional arousal on memory consolidation. Our current understanding of the neurobiological mechanisms underlying the memory-modulatory effects of the noradrenergic system is primarily based on pharmacological studies in rats, employing targeted administration of noradrenergic drugs into specific brain regions. However, the further delineation of the specific neural circuitry involved would benefit from experimental tools that are currently more readily available in mice. Previous studies have not, as yet, investigated the effect of noradrenergic enhancement of memory in mice, which show different cognitive abilities and higher endogenous arousal levels induced by a training experience compared to rats. In the present study, we investigated the effect of posttraining noradrenergic activation in male C57BL/6J mice on the consolidation of object recognition and object location memory. We found that the noradrenergic stimulant yohimbine (0.3 or 1.0 mg/kg) administered systemically immediately after an object training experience dose-dependently enhanced 24-h memory of both the identity and location of the object. Thus, these findings indicate that noradrenergic activation also enhances memory consolidation processes in mice, paving the way for a systematic investigation of the neural circuitry underlying these emotional arousal effects on memory.

**Keywords:** yohimbine; norepinephrine; stress hormones; memory consolidation; object recognition; object location

## Introduction

Emotionally arousing training conditions enhance noradrenergic activity (McGaugh, 2004). Animal studies have provided extensive evidence that such noradrenergic activation, arising from catecholaminergic cell bodies in the locus coeruleus (LC), is crucially involved in strengthening the consolidation of long-term memory (McGaugh, 2004; Sara, 2009; Roozendaal & McGaugh, 2011; Takeuchi *et al.*, 2016). Our current understanding of the neurobiological mechanisms underlying the memory-modulatory effects of the noradrenergic system is primarily based on pharmacological studies in rats, employing either systemic administration of noradrenergic drugs or targeted administration into specific brain regions. For example, norepinephrine or noradrenergic agents administered into the basolateral amygdala (BLA), or other brain regions such as the hippocampus or prefrontal cortex, were found to enhance long-term memory of emotionally arousing training experiences (Liang *et al.*, 1990; Introini-Collison *et al.*, 1991; Bevilaqua *et al.*, 1997; Ferry & McGaugh, 1999; Hatfield & McGaugh, 1999; LaLumiere *et al.*, 2003). Conversely, posttraining infusions of β-adrenoceptor antagonists into these brain regions were shown to impair retention and block the memory-enhancing effects of co-administered norepinephrine (Bevilaqua *et al.*, 1997; Hatfield & McGaugh, 1999; Berman *et al.*, 2000). Noteworthy, noradrenergic activation does not only enhance memory for highly arousing events that are known to induce the release of high levels of norepinephrine throughout the brain (Quirarte *et al.*, 1998; Hatfield & McGaugh, 1999; McIntyre *et al.*, 2002), but also for low-arousing experiences such as different forms of object recognition training (Roozendaal *et al.*, 2008; McReynolds *et al.*, 2014).

Human research supports the findings from animal studies that an activation of the noradrenergic system induces better memory (Cahill *et al.*, 1994; O'Carroll *et al.*, 1999; Southwick *et al.*, 2002; Cahill *et al.*, 2003). Accumulating evidence from human neuroimaging studies, however, indicates that emotional arousal and noradrenergic activation are associated with widespread changes in functional connectivity and the activation of large-scale neural networks (Seeley *et al.*, 2007; Murty *et al.*, 2010; Hermans *et al.*, 2011; Hermans *et al.*, 2014a; Hermans *et al.*, 2014b). A recent neuroimaging study in mice indicated that direct chemogenetic stimulation of the LC induces a highly comparable large-scale reconfiguration of neural network activity (Zerbi *et al.*, 2019). However, how such changes in network activity by norepinephrine could contribute to enhancement of memory for emotional experiences remains to be elucidated. A further delineation of this specific neural circuitry would benefit from novel experimental tools such as optogenetics and chemogenetics. Many laboratories are using mice for such circuitry-based investigations

because of the availability of a wide variety of transgenic lines. However, previous studies have not investigated whether noradrenergic activation by exogenous administration further enhances memory in this species, which shows different cognitive abilities and higher endogenous arousal levels induced by a training experience compared to rats (Hok *et al.*, 2016; Stepanichev *et al.*, 2016).

In the present study, we investigated the effect of posttraining noradrenergic activation in male C57BL/6J mice on object recognition memory (ORM) and object location memory (OLM) (Roozendaal *et al.*, 2010; Leger *et al.*, 2013; Vogel-Ciernia & Wood, 2014). Several findings suggest that memory performance in these two tasks is supported by distinct neural substrates. Whereas memory for the identity of objects primarily depends on neural plasticity within cortical structures (Balderas *et al.,* 2008; Barker & Warburton, 2011), memory for the location on an object relies on the hippocampus (Balderas *et al.,* 2008). Standard ORM and OLM have been successfully tested using mice (Vogel-Ciernia & Wood, 2014), but the effect of posttraining noradrenergic activation in mice on ORM and OLM has not yet been investigated. Here we found that, similar to rats, systemic posttraining injection of yohimbine, a noradrenergic stimulant which increases noradrenergic signaling (Szemeredi *et al.*, 1991; Nirogi *et al.*, 2012), induces dose-dependent enhancement of memory consolidation on both the ORM and OLM tasks. These findings thus pave the way for a systematic investigation of the neural circuitry underlying emotional arousal effects on memory.

## Material and methods

### Animals

One-hundred-and-five male CB57BL/6J mice (8 weeks old at the time of behavioral experiments) from Charles River Breeding Laboratories (Kisslegg, Germany) were group housed (3 animals per cage) in a temperature-controlled (22 °C) vivarium room with a regular 12-h/12-h light/dark cycle (lights on between 7:00 and 19:00 h). The vivarium room had a light intensity of 47 lux and humidity of 72%. Mice had *ad libitum* access to food and water. Object recognition memory differs between sexes (Sutcliffe *et al.*, 2007), is modulated by stress exposure in a sex-specific manner (Luine, 2002; Coutellier & Würbel, 2009), and varies with the estrous cycle phase in females (e.g., (Minni *et al.*, 2014; Graham & Scott, 2018; do Nascimento *et al.*, 2019; Kirry *et al.*, 2019). Since we aimed at replicating previous rat studies in our lab (e.g.,Roozendaal *et al.*, 2006; Barsegyan *et al.*, 2014; Atucha *et al.*, 2017; Chen *et al.*, 2018), in which only male rats were used, we restricted our studies to male mice only. Training and testing was performed during the light phase of the cycle, between 10:00 and 15:00 h. All experimental procedures were in compliance with European

Union Directive 2010/63/EU and approved by the Institutional Animal Care and Use Committee of Radboud University, Nijmegen, The Netherlands. All efforts were made to minimize animal suffering and to reduce the number of animals.

### Experimental apparatus and behavioral procedures



The experimental apparatus used for both the ORM and OLM tasks was a gray open-field box (40 cm × 40 cm × 40 cm) with the floor covered with sawdust. One side of the box was marked with a line of white tape through the midline of the wall, serving as an internal cue. The objects to be discriminated were white glass light bulbs (6 cm diameter, 11 cm length) and 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 mounted above the box, which was connected to a laptop computer.

Mice were first handled for 1 min each for 3 consecutive days. Subsequently, the animals underwent a 5-min habituation procedure to the experimental box for another 3 days prior to training. Habituation to the box is required to guarantee sufficient exploration of the objects by the mice, necessary to form long-term ORM (Stefanko *et al.*, 2009). During this habituation phase, mice could freely explore the training apparatus without the objects. Training and testing on the ORM and OLM tasks was according to Leger *et al.* (2013) and Vogel-Cierna *et al.* (2014) with slight modifications. On the training trial, the mouse was placed in the experimental apparatus and allowed to explore two identical objects (A1 and A2), placed 5 cm away from the corners of the apparatus, for 3 min. Drug administration occurred immediately after the training trial, after which the animals were placed back into their home cages. To avoid the presence of olfactory trails, sawdust was stirred, feces were removed, and the objects were thoroughly cleaned with 70% ethanol in between trials. Retention was tested 1 h or 24 h after the training trial. For the ORM task, one exemplar of the familiar object (A3) and a novel object (B) were placed at the same locations as during the training trial (Fig. 1A). For the OLM task, both objects were familiar (A3 and A4), yet one was placed at a novel location (Fig. 2A). All combinations of locations and objects were used in a balanced manner to reduce potential biases due to preference for particular location or object. For testing, the mouse was placed in the experimental apparatus and allowed to explore the objects for 5 min. Behavioral videos of the training and test sessions were analyzed offline by a trained observer blind to treatment condition, and the time spent exploring the novel and familiar object (or location) and the total time spent exploring both objects were scored. Part of the videos was analyzed by a second independent and blinded rater. Reliability of scoring was confirmed by high intra ($r_{(42)} = 0.804$, $p < 0.001$) and inter-

rater ($r_{(42)} = 0.670$, $p < 0.001$) correlations in object exploration times. 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). Turning around, climbing or sitting on an object *per se* was not included in exploration times as the animals then often are not actively engaged in exploring the object but rather exhibit grooming behavior or are using the object 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 was calculated as the difference in time exploring the novel and familiar object (or location), expressed as the ratio of the total time spent exploring both objects (i.e., [(time novel − time familiar)/(time novel + time familiar)] × 100%). Since low object exploration during training might result in poor long-term memory unrelated to the drug condition, mice showing a total exploration time of <4 s on the training trial (n = 3) were removed from analyses. Furthermore, three mice showing a clear preference for one of the objects or locations during the training trial (defined as a discrimination index deviating more than two standard deviations from the mean) were removed (Leger *et al.*, 2013; Vogel-Ciernia & Wood, 2014). Video analysis software (EthoVision XT, Noldus Information Technology, Wageningen, The Netherlands) was used to also measure total distance moved by the mice in the experimental apparatus during both training and retention testing.

### Systemic drug administration

Yohimbine (17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride; 0.3 or 1.0 mg/kg; Sigma-Aldrich), an $\alpha_2$-adrenoceptor antagonist which increases noradrenergic activity (Szemeredi *et al.*, 1991), was dissolved in saline and administered subcutaneously, in a volume of 0.1 mL/10 g of body weight, immediately after the training trial. The two doses were selected based on previous studies in rats (Roozendaal *et al.*, 2006) and pilot data in mice (Figure S1). Drug solutions were freshly prepared before each experiment.

### Statistics

Data are expressed as mean ± SEM. The discrimination index, total exploration time of the objects and total distance moved were analyzed with one-way ANOVAs with drug condition as between-subject variable. When appropriate, Tuckey *post-hoc* analyses were used to determine the source of significance in the ANOVA. One-sample *t*-tests were used to determine whether the discrimination index was different from zero (i.e., chance level) and thus whether learning had occurred. For all comparisons, $p < 0.05$ was accepted as statistical significance. The number of mice per group is indicated in the figure legends.

## Results

### Posttraining noradrenergic stimulation dose-dependently enhances object recognition memory

In this experiment, we first determined, in non-injected control mice, whether 3 min of object training was sufficient to induce successful acquisition of the identity of the training object in the ORM task. With these training conditions, we found that the discrimination index was significantly greater than zero at 1 h following training ($M$ = 20.02, $SEM$ = 6.14; $t_{(11)}$ = 3.26, $p < 0.01$), but not 24 h later ($M$ = 3.64, $SEM$ = 2.16; $t_{(10)}$ = 1.69, $p$ = 0.12, Figure S2). Thus, these findings indicate that 3 min of object training is sufficient to induce short-term, but not long-term, memory.

Next, we investigated whether yohimbine (0.3 or 1.0 mg/kg) administered immediately after a 3-min training trial enhanced 24-h memory for the object in the ORM task. Total exploration time of the two identical objects ($F_{(2,38)}$ = 0.85, $p$ = 0.69, Table S1) or the total distance moved ($F_{(2,38)}$ = 3.11, $p$ = 0.06, Table S1) during the training trial did not differ between later drug treatment groups. During the 24-h retention test, the discrimination index showed a significant effect of yohimbine treatment ($F_{(2,38)}$ = 3.95, $p$ = 0.03, Figure 1B). Tukey's *post-hoc* analysis revealed that mice treated with the higher dose of yohimbine (1.0 mg/kg) had a significantly greater discrimination index than that of the saline group ($p < 0.05$), whereas the discrimination index of mice treated with the lower dose of yohimbine (0.3 mg/kg) did not differ from that of saline-treated animals ($p$ = 0.65). The discrimination index of both saline-treated mice ($t_{(13)}$ = 0.47, $p$ = 0.65) and those treated with the lower dose of yohimbine ($t_{(13)}$ = 1.53; $p$ = 0.15) was not significantly different from zero, indicating that a 3-min training trial was not sufficient to induce long-term memory of the training object in these groups. Mice treated with the higher dose of yohimbine, however, exhibited a significant exploration preference for the novel object ($t_{(12)}$ = 3.35; $p < 0.01$). Yohimbine treatment did not affect total exploration time of the two objects ($F_{(2,38)}$ = 0.34, $p$ = 0.54, Figure 1C) or total distance moved in the apparatus during the retention test ($F_{(2,38)}$ = 0.65, $p$ = 0.53, Figure 1D).

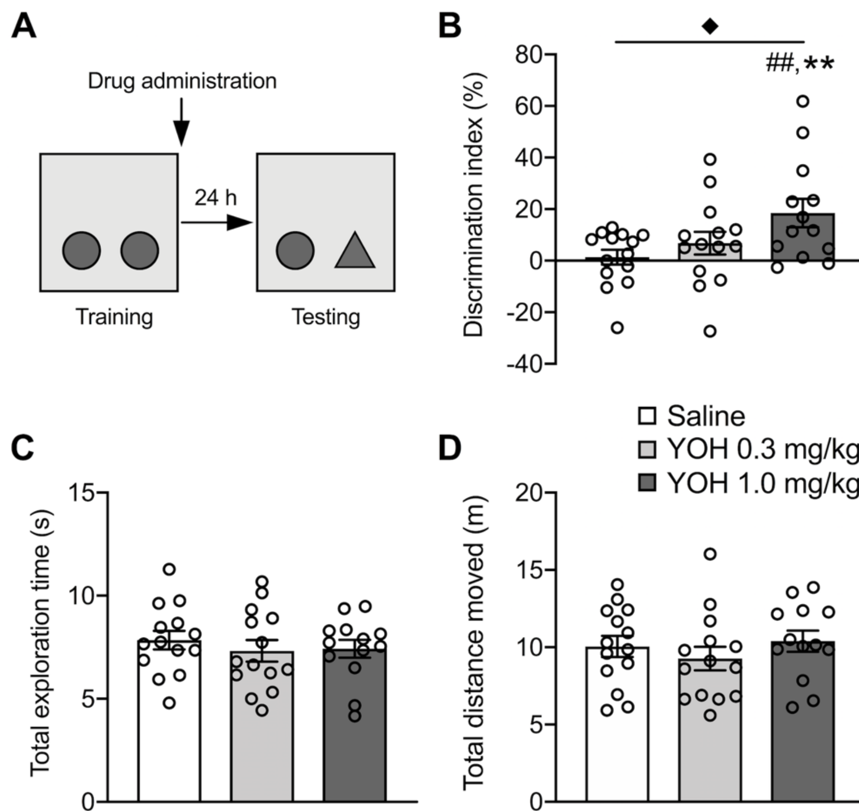


**Figure 1. Posttraining administration of the noradrenergic stimulant yohimbine dose-dependently enhances consolidation of object recognition memory.** Data are shown as mean ± SEM. **A,** Experimental design of the object recognition memory (ORM) task. Mice were trained for 3 min followed immediately by a subcutaneous injection of yohimbine (YOH, 0.3 or 1.0 mg/kg) or saline. Object recognition memory was tested 24 h later during which one of the objects was replaced by a novel object. **B,** The higher dose of yohimbine improved memory performance on the object recognition retention test compared to saline-treated animals. **C,** Yohimbine treatment did not affect total exploration time of the two objects during the retention test. **D,** Yohimbine treatment did not affect the total distance moved during the retention test. saline: $n$ = 14, YOH 0.3 mg/kg: $n$ = 14, YOH 1.0 mg/kg: $n$ = 13. ◆$p < 0.05$, main effect of drug administration; ## $p < 0.01$, difference from saline; **$p <$ 0.01, difference from chance level.

## Posttraining noradrenergic stimulation dose-dependently enhances object location memory

Next, we investigated, in separate groups of mice, whether posttraining systemic yohimbine (0.3 or 1.0 mg/kg) treatment also enhanced 24-h retention for the location of the object in the OLM task. Total exploration time of the two identical objects ($F_{(2,26)}$ = 2.75, $p$ = 0.08, Table S1) and the total distance moved ($F_{(2,26)}$ = 1.80, $p$ = 0.19, Table S1) during the training trial did not differ between later drug treatment groups. The discrimination index during the retention test, however, indicated a significant effect of yohimbine on memory performance

($F_{(2,26)}$ = 8.52, $p$ < 0.001, Figure 2B). Tukey's *post-hoc* analysis revealed that the discrimination index of mice treated with either the 0.3 or 1.0 mg/kg dose of yohimbine was significantly greater than that of the saline group ($p$ < 0.01). Whereas the discrimination index of saline-treated mice did not significantly differ from zero ($t_{(9)}$ = 0.67, $p$ = 0.52), indicating that they did not express memory of the location of the training object, mice treated with either dose of yohimbine exhibited a significant exploration preference for the object located in the novel position (0.3 mg/kg: $t_{(7)}$ = 5.15; $p$ < 0.01; 1.0 mg/kg: $t_{(10)}$ = 4.77; $p$ < 0.001). Yohimbine treatment did not affect total exploration time of the two objects ($F_{(2,26)}$ = 2.27, $p$ = 0.12, Figure 2C) or total distance moved in the apparatus during the retention test ($F_{(2,26)}$ = 1.49, $p$ = 0.24, Figure 2D).

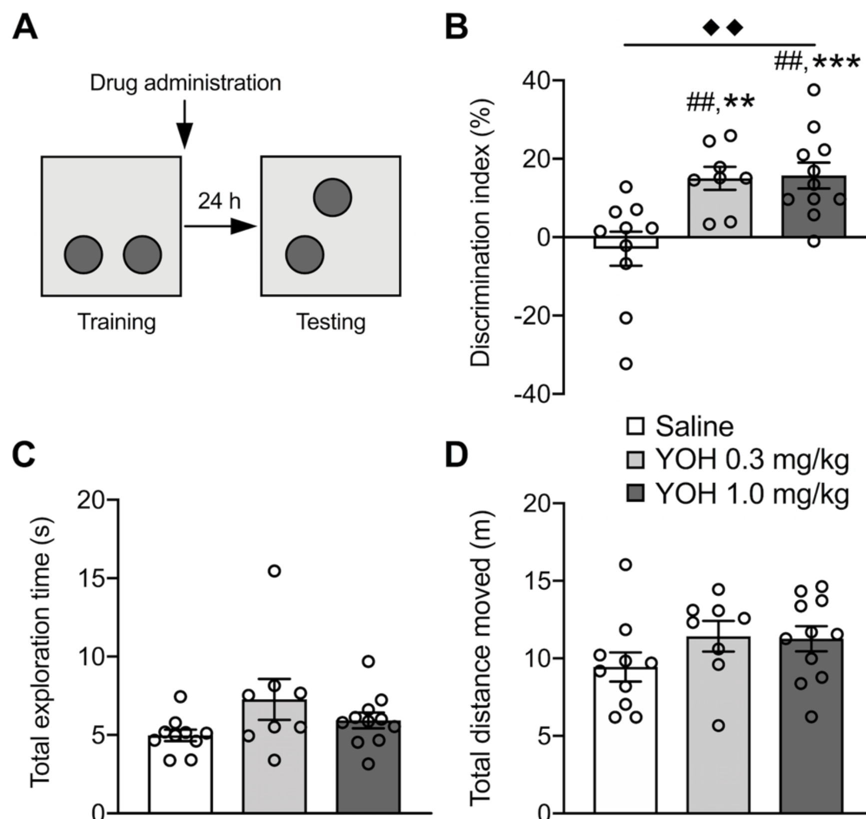


**Figure 2. Posttraining administration of the noradrenergic stimulant yohimbine dose-dependently enhances the consolidation of object location memory.** Data are shown as mean ± SEM**. A,** Experimental design of the object location memory (OLM) task. Mice were trained for 3 min followed immediately by a subcutaneous injection of yohimbine (YOH, 0.3 or 1.0 mg/kg) or saline. Object location memory was tested 24 h later during which one of the objects was relocated to a novel location. **B,** Both the higher and lower dose of yohimbine improved memory performance on the object location retention test compared to saline. **C,** Yohimbine treatment did not affect total exploration time of the two objects during the retention test. **D,** Yohimbine treatment did not affect the total distance moved during the retention test. saline: $n$ = 10, YOH 0.3 mg/kg: $n$ = 8, YOH 1.0

mg/kg: $n = 11$. ◆◆ $p < 0.01$, main effect of drug administration; ## $p < 0.01$, difference from saline; ** $p < 0.01$, *** $p < 0.001$, difference from chance level.

## Discussion

The current study successfully validated the memory-enhancing effect of posttraining noradrenergic stimulation with systemic yohimbine in both the ORM and OLM tasks in mice. As such, this study provides a fundamental proof-of-principle for future investigation of the neural circuits underlying the effects of noradrenergic arousal on long-term memory in this species.

Similar to previous findings in rats (Dornelles *et al.*, 2007; Jurado-Berbel *et al.*, 2010; Nirogi *et al.*, 2012), the main finding of the present study is that, in mice given 3 min of object training, noradrenergic activation immediately after the training trial induces dose-dependent enhancement of 24-h memory on both the ORM and OLM tasks. We found that 3 min of object training was insufficient to induce 24-h memory in saline-treated controls, but that such training conditions were sufficient to enable posttraining systemic yohimbine administration to enhance memory of both the identity and location of the object. Yohimbine is an $\alpha_2$-adrenoceptor antagonist which, by blocking receptors located on noradrenergic terminals, elevates norepinephrine levels and its metabolites in the brain and in blood (Szemeredi *et al.*, 1991). As the yohimbine was administered immediately after the training experience, the retention improvement effects cannot be attributed to memory-encoding effects or to non-specific influences on attentional or locomotor effects during the training trial. Furthermore, the yohimbine treatment did not affect total exploration of the two objects or total distance moved during the retention test. These findings are thus consistent with the view that the noradrenergic stimulation enhances consolidation processes on both versions of the object memory task.

Extensive evidence from pharmacological manipulation studies in rats indicates that (nor)adrenergic agonists administered systemically or directly into specific brain regions enhance memory consolidation on a wide variety of emotionally arousing training tasks, including inhibitory avoidance, active avoidance discrimination learning, contextual fear conditioning, water-maze spatial learning and appetitive tasks (Gold & Van Buskirk, 1975; Izquierdo & Dias, 1985; Sternberg *et al.*, 1985; Introini-Collison & McGaugh, 1986; Liang *et al.*, 1986; Liang *et al.*, 1990; Introini-Collison *et al.*, 1991; Costa-Miserachs *et al.*, 1994; Bevilaqua *et al.*, 1997; Ferry & McGaugh, 1999; Hatfield & McGaugh, 1999; LaLumiere *et al.*, 2003). Noradrenergic activation also enhances recognition memory in rats (Dornelles *et*

*al.*, 2007; Jurado-Berbel *et al.*, 2010). In the experiments by Dornelles *et al.* (2007), the adrenomedullary hormone epinephrine injected systemically immediately after the training session increased the retention delay at which memory was still present (Dornelles *et al.*, 2007). Another study confirmed the finding that posttraining systemic epinephrine administration improves long-term memory on both the ORM and OLM tasks (Jurado-Berbel *et al.*, 2010). Other studies support the view that posttraining systemic yohimbine administration increases norepinephrine levels in the medial temporal lobe (Nirogi *et al.*, 2012), and enhances memory on the ORM task (Roozendaal *et al.*, 2006; Nirogi *et al.*, 2012). Norepinephrine administration directly into the BLA also enhances the consolidation of ORM as well as of the association of an object with its context (Roozendaal *et al.*, 2008; Barsegyan *et al.*, 2014). With such targeted pharmacological manipulation studies in rats, considerable knowledge has been gained regarding the neural mechanisms by which norepinephrine facilitates long-term memory formation, particularly by its actions on the BLA (McGaugh, 2000; McGaugh, 2004; Roozendaal & McGaugh, 2011), subsequently modulating neural plasticity and information storage processes in its projection regions, including the hippocampus, perirhinal cortex, medial prefrontal cortex and insular cortex (Roozendaal *et al.*, 1999; McIntyre *et al.*, 2005; Laing & Bashir, 2014; McReynolds *et al.*, 2014; Beldjoud *et al.*, 2015; Chen *et al.*, 2018; Barsegyan *et al.*, 2019).

Neuroimaging studies in humans, however, have indicated that emotional arousal triggers dynamic shifts in network balance throughout the brain, leading to a large-scale neural network reconfiguration (Seeley *et al.*, 2007; Murty *et al.*, 2010). Moreover, exposure to emotional arousal induces complex temporal dynamics in neural activity. Emotional arousal, in a norepinephrine-dependent fashion, first rapidly increases salience network activity, while simultaneously suppressing central executive control network activity (Seeley *et al.*, 2007; Hermans *et al.*, 2011). Later, when the arousing situation subsides, resource allocation to these two networks reverses: the salience network shuts off and the central executive control network becomes active, which normalizes emotional reactivity and enhances higher-order cognitive processes (Hermans *et al.*, 2014b; Van Leeuwen *et al.*, 2018). Studies have shown that the LC noradrenergic system has the ability to rapidly rearrange neural activity within and between large-scale neural systems to optimize cognitive processes relevant for task performance or adaptive behaviors (Aston-Jones & Cohen, 2005; Bullmore & Sporns, 2009; Van Den Heuvel & Pol, 2010; Zerbi *et al.*, 2019). However, it remains unknown how noradrenergic activation by emotional arousal might achieve both spatial and temporal specificity in regulating large-scale neural network activity. Such effects might depend on brain region- and time-specific effects of norepinephrine on

excitatory and inhibitory subpopulations of neurons. Further, it is poorly understood how such changes in network activity by norepinephrine could contribute to enhancement of memory for emotional experiences.

Dedicated studies allowing for tight experimental control over neuronal subpopulations and neural circuit activity are required to elucidate these exact mechanistic underpinnings. New technologies such as optogenetics and chemogenetics could be optimally combined with the use of a variety of readily available transgenic lines of mice, to decipher these mechanisms. Validated behavioral tasks and effects of norepinephrine are a prerequisite to conduct such studies. The present findings indicating that noradrenergic activation enhances memory for ORM and OLM in mice, pave the way for a further investigation of the specific neural circuits and molecular mechanisms that regulate emotional arousal effects on memory consolidation.

## Disclosure statement

The authors declare no conflict of interest.


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## References


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*, 403-450.

Atucha, E., Vukojevic, V., Fornari, R. V., Ronzoni, G., Demougin, P., Peter, F., Atsak, P., Coolen, M. W., Papassotiropoulos, A., & McGaugh, J. L. (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.

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

Barsegyan, A., Mirone, G., Ronzoni, G., Guo, C., Song, Q., van Kuppeveld, D., 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.

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*(108), 108.

Berman, D. E., Hazvi, S., Neduva, V., & Dudai, Y. (2000). The role of identified neurotransmitter systems in the response of insular cortex to unfamiliar taste: Activation of erk1–2 and formation of a memory trace. *Journal of Neuroscience, 20*(18), 7017-7023.

Bevilaqua, L., Ardenghi, P., Shröder, N., Bromberg, E., Schmitz, P., Schaeffer, E., Quevedo, J., Bianchin, M., Walz, R., & Medina, J. (1997). Drugs acting upon the cyclic adenosine monophosphate/protein kinase a signalling pathway modulate memory consolidation when given late after training into rat hippocampus but not amygdala. *Behavioural Pharmacology, 8*(4), 331-338.

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

Bullmore, E., & Sporns, O. (2009). Complex brain networks: Graph theoretical analysis of structural and functional systems. *Nature reviews neuroscience, 10*(3), 186-198.

Cahill, L., Gorski, L., & Le, K. (2003). Enhanced human memory consolidation with post-learning stress: Interaction with the degree of arousal at encoding. *Learning and Memory, 10*(4), 270-274.

Cahill, L., Prins, B., Weber, M., & McGaugh, J. L. (1994). Β-adrenergic activation and memory for emotional events. *Nature, 371*(6499), 702-704.

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.

Costa-Miserachs, D., Portell-Cortés, I., Aldavert-Vera, L., Torras-García, M., & Morgado-Bernal, I. (1994). Long-term memory facilitation in rats by posttraining epinephrine. *Behavioral Neuroscience, 108*(3), 469-474.

Coutellier, L., & Würbel, H. (2009). Early environmental cues affect object recognition memory in adult female but not male c57bl/6 mice. *Behavioural Brain Research, 203*(2), 312-315.

Do Nascimento, E. B., Dierschnabel, A. L., de Macêdo Medeiros, A., Suchecki, D., Silva, R. H., & Ribeiro, A. M. (2019). Memory impairment induced by different types of prolonged stress is dependent on the phase of the estrous cycle in female rats. *Hormones and Behavior, 115*, 104563.

Dornelles, A., de Lima, M. N., Grazziotin, M., Presti-Torres, J., Garcia, V. A., Scalco, F. S., Roesler, R., & Schroder, N. (2007). Adrenergic enhancement of consolidation of object recognition memory. *Neurobiology of Learning and Memory, 88*(1), 137-142.

Ferry, B., & McGaugh, J. L. (1999). Clenbuterol administration into the basolateral amygdala post-training enhances retention in an inhibitory avoidance task. *Neurobiology of Learning and Memory, 72*(1), 8-12.

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

Graham, B. M., & Scott, E. (2018). Effects of systemic estradiol on fear extinction in female rats are dependent on interactions between dose, estrous phase, and endogenous estradiol levels. *Hormones and Behavior, 97*, 67-74.

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.

Hermans, E. J., Battaglia, F. P., Atsak, P., de Voogd, L. D., Fernandez, G., & Roozendaal, B. (2014a). How the amygdala affects emotional memory by altering brain network properties. *Neurobiology of Learning and Memory, 112*, 2-16.

Hermans, E. J., Henckens, M. J., Joels, M., & Fernandez, G. (2014b). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in Neurosciences, 37*(6), 304-314.

Hermans, E. J., Van Marle, H. J., Ossewaarde, L., Henckens, M. J., Qin, S., Van Kesteren, M. T., Schoots, V. C., Cousijn, H., Rijpkema, M., & Oostenveld, R. (2011). Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. *Science, 334*(6059), 1151-1153.

Hok, V., Poucet, B., Duvelle, E., Save, E., & Sargolini, F. (2016). Spatial cognition in mice and rats: Similarities and differences in brain and behavior. *Wiley Interdisciplinary Reviews: Cognitive Science, 7*(6), 406-421.

Introini-Collison, I. B., & McGaugh, J. L. (1986). Epinephrine modulates long-term retention of an aversively motivated discrimination. *Behavioral and Neural Biology, 45*(3), 358-365.

Introini-Collison, I. B., Miyazaki, B., & McGaugh, J. L. (1991). Involvement of the amygdala in the memory-enhancing effects of clenbuterol. *Psychopharmacology, 104*(4), 541-544.

Izquierdo, I., & Dias, R. D. (1985). Influence on memory of posttraining or pre-test injections of acth, vasopressin, epinephrine, and β-endorphin, and their interaction with naloxone. *Psychoneuroendocrinology, 10*(2), 165-172.

Jurado-Berbel, P., Costa-Miserachs, D., Torras-Garcia, M., Coll-Andreu, M., & Portell-Cortes, 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.

Kirry, A. J., Durigan, D. J., Twining, R. C., & Gilmartin, M. R. (2019). Estrous cycle stage gates sex differences in prefrontal muscarinic control of fear memory formation. *Neurobiology of Learning and Memory, 161*, 26-36.

Laing, M., & Bashir, Z. (2014). B-adrenoceptors and synaptic plasticity in the perirhinal cortex. *Neuroscience, 273*, 163-173.

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.

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.

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., 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.

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.

Luine, V. (2002). Sex differences in chronic stress effects on memory in rats. *Stress, 5*(3), 205-216.

McGaugh, J. L. (2000). Memory--a century of consolidation. *Science, 287*(5451), 248-251.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience, 27*, 1-28.

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.

Minni, A., de Medeiros, G., Helbling, J.-C., Duittoz, A., Marissal-Arvy, N., Foury, A., De Smedt-Peyrusse, V., Pallet, V., & Moisan, M.-P. (2014). Role of corticosteroid binding globulin in emotional reactivity sex differences in mice. *Psychoneuroendocrinology, 50*, 252-263.

Murty, V. P., Ritchey, M., Adcock, R. A., & LaBar, K. S. (2010). Fmri studies of successful emotional memory encoding: A quantitative meta-analysis. *Neuropsychologia, 48*(12), 3459-3469.

Nirogi, R., Abraham, R., Jayarajan, P., Medapati, R. B., Shanmuganathan, D., Kandikere, V., Irappanavar, S., Saralaya, R., Benade, V., Bhyrapuneni, G., & Muddana, N. (2012). Difference in the norepinephrine levels of experimental and non-experimental rats with age in the object recognition task. *Brain Research, 1453*, 40-45.

O'Carroll, R. E., Drysdale, E., Cahill, L., Shajahan, P., & Ebmeier, K. P. (1999). Stimulation of the noradrenergic system enhances and blockade reduces memory for emotional material in man. *Psychological Medicine, 29*(5), 1083-1088.

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.

Pezze, M. A., Marshall, H. J., Fone, K. C., & Cassaday, H. J. (2017). Role of the anterior cingulate cortex in the retrieval of novel object recognition memory after a long delay. *Learning and Memory, 24*(7), 310-317.

Quirarte, G. L., Galvez, R., Roozendaal, B., & McGaugh, J. L. (1998). Norepinephrine release in the amygdala in response to footshock and opioid peptidergic drugs. *Brain Research, 808*(2), 134-140.

Roozendaal, B., Castello, N. A., Vedana, G., Barsegyan, A., & McGaugh, J. L. (2008). Noradrenergic activation of the basolateral amygdala modulates consolidation of object recognition memory. *Neurobiology of Learning and Memory, 90*(3), 576-579.

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. *Journal of Neuroscience, 30*(14), 5037-5046.

Roozendaal, B., & McGaugh, J. L. (2011). Memory modulation. *Behavioral Neuroscience, 125*(6), 797-824.

Roozendaal, B., Nguyen, B. T., Power, A. E., & McGaugh, J. L. (1999). 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., Okuda, S., Van der Zee, E. A., & McGaugh, J. L. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences, 103*(17), 6741-6746.

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

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., Reiss, A. L., & Greicius, M. D. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *Journal of Neuroscience, 27*(9), 2349-2356.

Southwick, S. M., Davis, M., Horner, B., Cahill, L., Morgan III, C. A., Gold, P. E., Bremner, J. D., & Charney, D. C. (2002). Relationship of enhanced norepinephrine activity during memory consolidation to enhanced long-term memory in humans. *American Journal of Psychiatry, 159*(8), 1420-1422.

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.

Stepanichev, M., Markov, D., Pasikova, N., & Gulyaeva, N. (2016). Behavior and the cholinergic parameters in olfactory bulbectomized female rodents: Difference between rats and mice. *Behavioural Brain Research, 297*, 5-14.

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

Sutcliffe, J., Marshall, K., & Neill, J. (2007). Influence of gender on working and spatial memory in the novel object recognition task in the rat. *Behavioural Brain Research, 177*(1), 117-125.

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., Fernandez, G., Deisseroth, K., Greene, R. W., & Morris, R. G. (2016). Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature, 537*(7620), 357-362.

Van Den Heuvel, M. P., & Pol, H. E. H. (2010). Exploring the brain network: A review on resting-state fmri functional connectivity. *European Neuropsychopharmacology, 20*(8), 519-534.

Van Leeuwen, J., Vink, M., Fernández, G., Hermans, E., Joëls, M., Kahn, R., & Vinkers, C. (2018). At-risk individuals display altered brain activity following stress. *Neuropsychopharmacology, 43*(9), 1954-1960.

Vogel-Ciernia, A., & Wood, M. A. (2014). Examining object location and object recognition memory in mice. *Current Protocols in Neuroscience, 69*, 8.31.31-17.

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.

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 e705.

## Supplementary Materials

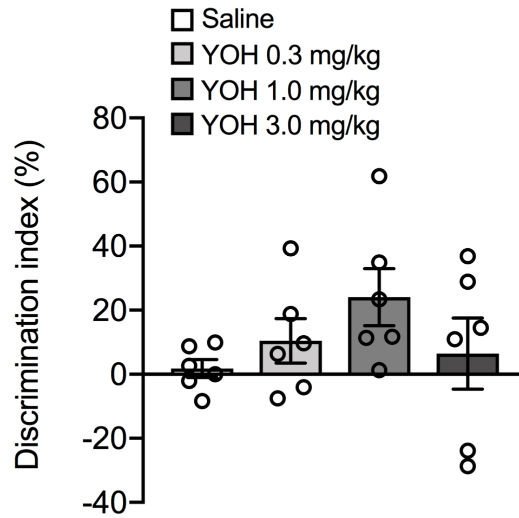



**Figure S1. Pilot experiment examining different doses of yohimbine on object recognition memory in a small sample size.** Data are shown as mean ± SEM**.** Mice were trained for 3 min followed immediately by a subcutaneous injection of yohimbine (YOH, 0.3, 1.0 or 3.0 mg/kg). Object recognition memory was tested 24 h later during which one of the objects was replaced by a novel object. YOH 0.3 mg/kg: *n* = 6, YOH 1.0 mg/kg: *n* = 6, YOH 3.0 mg/kg: *n* = 6.

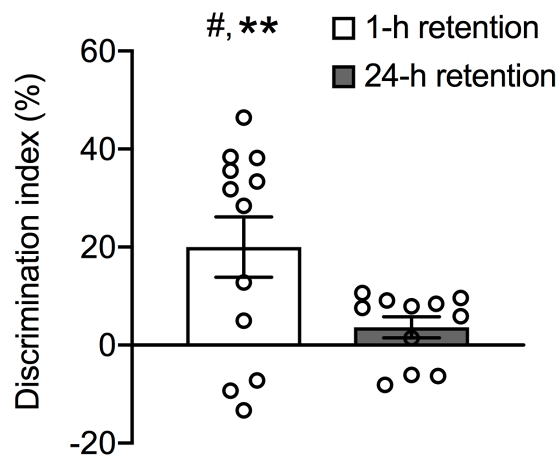



**Figure S2. Three minutes of object training induces short-term, but not long-term, object recognition memory.** Data are shown as mean ± SEM. Non-injected control mice were trained for 3 min on the object task. Object recognition memory was tested either 1 h or 24 h later during which one of the objects was replaced by a novel object. Findings indicate that 3 min of object training is sufficient to induce short-term, but not long-term, memory. 1-h retention: n = 12, 24-h retention: n = 11. # $p < 0.05$, difference from 24-h retention group; ** $p < 0.01$, difference from chance level.

**Table S1. Training data of object recognition memory (ORM) and object location memory (OLM)**

| Task | Training data items | Saline | YOH 0.3 mg/kg | YOH 1.0 mg/kg |
|---|---|---|---|---|
| ORM | Object exploration time (s) | 10.9 ± 0.8 | 11.8 ± 0.9 | 12.5 ± 1.0 |
| | Total distance moved (m) | 6.4 ± 0.5 | 8.2 ± 0.7 | 8.3 ± 0.6 |
| OLM | Object exploration time (s) | 8.4 ± 0.8 | 11.7 ± 1.2 | 10.0 ± 1.0 |
| | Total distance moved (m) | 7.8 ± 0.4 | 8.3 ± 0.7 | 9.1 ± 0.5 |

Data are shown as mean ± SEM.

# *CHAPTER*

# 3

## Effect of training duration and noradrenergic activation on memory detailedness and its neural correlates over time

## Abstract

Noradrenergic activation is well known to enhance the consolidation of long-term memory. However, little is known of how it affects the quality of these memories and their maintenance over time (i.e., their longevity). To be able to examine memory detailedness, we developed a new object discrimination task for mice. On the training session, mice could explore three identical objects. On the retention test, they were exposed to three objects that differed in similarity with the training object; one familiar object, and two objects that were either similar or very dissimilar to the familiar one. In the first experiment, we examined whether a longer training duration enhances the detailedness of the memory. On a 1-day retention test, mice that had received 3 min of training could discriminate the dissimilar, but not similar, object, whereas mice that had received 10 min of training were able to discriminate both the dissimilar and similar object from the familiar one, indicative of a more detailed memory. At a 7-day retention test, mice that had received 10 min of training still discriminated the two objects, whereas the 3-min training group was unable to discriminate either of them from the familiar object. Both the 3-min and 10-min training groups were not able to discriminate the dissimilar or similar object 14 days after training. Next, we examined the effect of the noradrenergic stimulant yohimbine (0.3 or 1.0 mg/kg) administered systemically immediately after training on the object discrimination task, and observed an enhanced detailedness of the memory at a 1-day retention test. This memory detailedness was paralleled by an increased retention-induced neuronal activity within the anterior insular cortex and perirhinal cortex. This yohimbine effect on memory detailedness and retention-induced neuronal activity was also gradually lost at the 7-day and 14-day retention interval. These findings indicate that noradrenergic activation does not only enhance the strength but also the detailedness of object memory, without strong effects on its longevity, paving the way for a systematic investigation of the underlying neural circuitry.

**Keywords:** norepinephrine; object recognition; memory detailedness; memory longevity; neuronal activity

## Introduction

Emotionally arousing or stressful experiences induce strong and lasting memories (McGaugh, 2000). This is typically a highly adaptive survival phenomenon as it allows an individual to prepare for similar situations in the future. However, we know considerably less of whether the emotional impact of an experience is also associated with changes in the quality of memory (Morgan III *et al.*, 2004; Porter *et al.*, 2008; Hoscheidt *et al.*, 2014). Findings from human studies show contradictory results: Some studies report on emotional arousal improving the accuracy of memories such that emotionally arousing experiences are remembered with greater detail (Segal *et al.*, 2012), while other studies report that emotional memories are remembered in a more generalized, gist-like manner (Morgan III *et al.*, 2004). Since strong, but less specific or overgeneralized, fear memories are believed to be a major risk factor for the development of post-traumatic stress disorder (PTSD) following trauma (Lopresto *et al.*, 2016), and involved in the etiology of anxiety disorders in general (Dunsmoor & Paz, 2015), it is of critical importance to understand how stress hormones also affect the accuracy and/or detailedness of memory of emotionally arousing experiences.

Extensive evidence indicates that emotionally arousing conditions induce the release of norepinephrine in the brain and periphery (Mason, 1968), and that this noradrenergic activation is crucially involved in strengthening the consolidation of long-term memory (McGaugh, 2004; Sara, 2009; Roozendaal & McGaugh, 2011; Takeuchi *et al.*, 2016). In a recent study, we showed that systemic administration of the noradrenergic stimulant yohimbine not only strengthened memory of an inhibitory avoidance experience, but also enhanced the episodic-like accuracy of the memory (Roozendaal & Mirone, 2020). Whereas vehicle-treated animals were unable to differentiate the context in which they had previously received a footshock, from a safe training context, generalizing their fear memory to both contexts, yohimbine-treated animals showed an accurate association of the shock experience with the correct training context. Moreover, whereas memories tend to generalize over time (Frankland & Bontempi, 2005; Wiltgen & Silva, 2007; Winocur & Moscovitch, 2011), we showed in another study that noradrenergic activation maintained long-term accuracy of the memory by preventing the time-dependent reorganization of memory from the hippocampus to neocortical networks (Atucha *et al.*, 2017). However, such norepinephrine effect on enhancing memory accuracy and altering the time-dependent reorganization of memory has yet only been investigated with respect to hippocampus-dependent episodic-like memory. Little is known with respect to whether noradrenergic activation is also able to maintain long-term accuracy of other types of memories, particularly

those that initially already depend on cortical networks.

Noradrenergic activation is also known to enhance object recognition memory (Roozendaal *et al.*, 2009; Song *et al.*, 2020), a task that primarily depends on cortical regions such as the insular cortex (IC) and perirhinal cortex (PRh) (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 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). In the present study, we investigated whether posttraining noradrenergic activation enhances the detailedness (and thus improves the accuracy) of object memory over time. We therefore setup a novel memory task, termed the object discrimination task, in which mice on the training session could explore three identical objects. On the retention test trial, three different objects were placed at the same locations as during training: 1) the previously explored training object (i.e., familiar object), 2) a novel object that was highly similar to the training object (i.e., similar object), and 3) a novel object that had a completely different shape and texture than the training object (i.e., dissimilar object). In this task, a preference to explore the similar compared to familiar object indicates that the mice successfully discriminate it from the familiar object and thus reflects having a detailed memory for the training object. A preference for only the dissimilar object compared to the familiar one is indicative of having a sparsely encoded memory, whereas no differences in exploration of all three objects may indicate that the training object is not remembered. In the first experiment, we examined whether the duration of the training session enhances the detailedness as well as longevity of the memory. Mice were trained for either 3 or 10 min and retention was tested 1 day, 7 days or 14 days later. In the second experiment, mice were administered the noradrenergic stimulant yohimbine (0.3 or 1.0 mg.kg) systemically immediately after a 3-min training experience, and retention was tested again 1 day, 7 days or 14 days later. We further determined retention-induced expression of the immediately early gene product c-Fos, a well-established molecular marker for activated cells (Minatohara *et al.*, 2016), within the IC, PRh as well as the basolateral amygdala (BLA) and prefrontal cortex (PFC) to examine whether object memory also undergoes a reorganization process over time and whether noradrenergic stimulation is able to modify this process.

## Material and methods

### Animals

One-hundred-and-forty-eight male CB57BL/6J mice (10 weeks old at the time of behavioral experiments) from Charles River Breeding Laboratories (Kisslegg, Germany) were single housed in a temperature-controlled (22 °C) vivarium room with a regular 12-h/12-h light/dark

cycle (lights on between 7:00 and 19:00 h). The vivarium room had a light intensity of 47 lux and humidity of 72%. Mice had *ad libitum* access to food and water. Training and testing was performed during the light phase of the cycle, between 10:00 and 15:00 h. 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 discrimination task

The experimental apparatus used for the object discrimination task was a gray round plastic box (40 cm diameter, 40 cm height) with the floor covered with sawdust. During both the training and retention test sessions, three objects were equally spaced along the perimeter of the apparatus, 5 cm away from the wall. The objects were secured to the floor of the box with Velcro tape. A camera was mounted above the box to videotape the behavior of the animals during the training and test sessions.

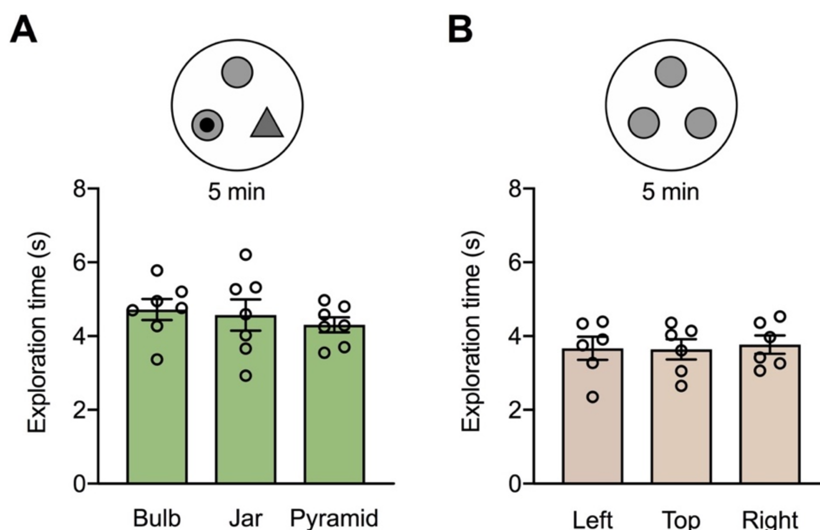


Mice were first handled for 1 min each on 4 consecutive days to become accustomed to the experimenter. Subsequently, the animals received three 5-min habituation sessions to the experimental box during which they could freely explore the experimental apparatus without the objects. This habituation procedure is required to guarantee sufficient exploration of the objects during the training session (Stefanko *et al.*, 2009). On the training trial, the mice were placed into the apparatus and allowed to explore three identical copies of either a glass light bulb (6 cm diameter, 11 cm length) or the similar looking glass vial (5.5 cm diameter, 5 cm height), randomized across animals. For the first experiment, examining the effect of training duration on object discrimination memory, animals were allowed to explore the objects for either 3 or 10 min. For the second experiment, all animals were allowed to explore the objects for 3 min, followed by immediate posttraining drug administration. To avoid the presence of olfactory trails, feces were removed, sawdust was stirred, and the objects were thoroughly cleaned with 70% ethanol in between animals. Retention of the memory was tested, in separate groups of animals, at 1 day, 7 days or 14 days after the training trial. During the retention test trial, three different objects were placed at the same locations as during training: the previously explored training object (familiar object), a novel object that was highly similar to the training object (glass jar or light bulb, similar object), and a novel object that had a completely different shape and texture than the training object (wooden pyramid, 7 cm × 7 cm × 7 cm, dissimilar object). All combinations and locations of objects were used in a balanced manner across animals to reduce potential confounding influences

due to preference for a particular object or location. Pilot experiments had indicated that the animals do not display an innate preference for any of the three objects or locations used (See Box I). For testing, the mice were placed in the apparatus and allowed to explore the objects for 5 min. After the retention test, the mice were left undisturbed in their home cage until sacrifice 1 h later.

Videos of the training and test sessions were analyzed offline by a trained observer blind to treatment condition, and the time spent exploring each object was scored. Video analysis software (EthoVision XT, Noldus Information Technology, Wageningen, The Netherlands) was used to measure the total distance moved by the mice in the experimental apparatus during both training and retention testing, which serves as a measure of exploration of the experimental apparatus. 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 are not actively engaged in exploring the object but rather exhibit grooming behavior or are using the object 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). Re-scoring of a subset of the behavioral videos by an independent second rater confirmed the reliability of scoring ($r_{(42)}$ = 0.888, $p$ < 0.001, n = 22 videos per rater).

***Box I: Validation of the objects and object locations for the object discrimination task***



*For the object discrimination task to work, we needed to ascertain that mice do not display an innate preference for any of the three objects used (glass jar, glass light bulb and wooden pyramid). Therefore, mice (n = 7) were placed in the experimental apparatus for 5 min and allowed to freely explore the three objects. Exploration times of the three objects did not differ ($F_{(2,18)}$ = 0.43, p = 0.65; panel A), indicating that the mice do not show a preference for any of the objects over the others. Next, to ascertain that mice also do not display a preference for exploring an object placed in any of the three locations, mice (n = 6) were allowed to freely explore three identical objects (counterbalanced across animals) located at the different positions. Exploration times of the objects located in the three different positions also did not differ ($F_{(2,15)}$ = 0.06, p = 0.95, panel B).*



To determine both robustness and detailedness of the memory, two different discrimination indexes (DI) were calculated, one as the difference in time exploring the dissimilar and familiar object, divided by the total time exploring these two objects ($DI_{dissimilar}$), and the other as the difference in time exploring the similar and familiar object, divided by the total time exploring these two objects ($DI_{similar}$).

$$DI_{dissimilar} = \frac{(time\ dissimilar\ -\ time\ familiar)}{(time\ dissimilar\ +\ time\ familiar)} \times 100\%$$

$$DI_{similar} = \frac{(time\ similar\ -\ time\ familiar)}{(time\ similar\ +\ time\ familiar)} \times 100\%$$

The $DI_{dissimilar}$ is indicative of whether the mice discriminated the familiar object as compared to the dissimilar novel object and thus reflects having memory for the training object during the retention test. The $DI_{similar}$ is indicative of whether the mice successfully discriminated the familiar object as compared to the highly similar novel object and thus reflects having a detailed memory for the training object.

### Systemic drug administration

Yohimbine (17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride; 0.3 or 1.0 mg/kg; Sigma-Aldrich), an $\alpha_2$-adrenoceptor antagonist which increases noradrenergic activity (Szemeredi *et al.*, 1991), was dissolved in saline and administered intraperitoneally, in a volume of 0.01 mL/g of body weight, immediately after the training trial. The two doses were selected based on their memory-enhancing effect in a previous object recognition study in mice (Song *et al.*, 2020). Drug solutions were freshly prepared before each experiment.

### Immunohistochemistry

One hour after the retention test, the mice were anesthetized with an overdose of sodium pentobarbital and perfused transcardially with ice-cold 0.1 M phosphate-buffered saline (PBS), pH 7.4, followed by ice-cold 4% paraformaldehyde (PFA). Brains were post-fixed overnight in 4% PFA and then cryoprotected in 30% sucrose for 72 h at 4 °C. Coronal slices of 35 µm thickness were cut on a cryostat, and collected in 0.1 M PBS with 0.1% sodium azide, and stored at 4 °C. For immunohistochemistry procedures, two sections of each of the brain regions investigated were selected according to the Franklin and Paxinos mouse brain atlas (Franklin and Paxinos, 3rd edition, 2007): anterior IC (aIC; anteroposterior (AP), +1.18 and +0.86 mm); posterior IC (pIC; AP, +0.02 and -0.46 mm); PRh (AP, -1.34 and -1.58 mm); BLA (AP, -1.34 and -1.58 mm) and PFC (AP, +1.94 and +1.70 mm). Sections were rinsed in 0.5% Triton X-100 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) in PBS for 1 h at RT. Sections were then incubated with primary antibodies (c-Fos (guinea pig anti-c-Fos; 1:500, Synaptic Systems), glutamic acid decarboxylase 67 (GAD67) (mouse anti-GAD67; 1:500, Merck)) overnight at RT. The incubation buffer contained 5% NDS and 0.1% acetylated bovine serum albumin (BSA-c; Aurion). 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, 1:500, Jackson ImmunoResearch; donkey anti-mouse Alexa Fluor 488, 1:500, Thermo Fisher) for 3 h at RT. All procedures starting from the secondary antibody incubation onwards were performed in the dark. Then, sections were briefly rinsed and incubated with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI, 1:5000) in PBS for 15 min at RT, then washed three times in PBS for 10 min per wash, mounted on gelatin-coated slides, air-dried and coverslipped with FluoroSave mounting medium (Sigma-Aldrich).

### Imaging and quantification

Fluorescent images of the regions of interest (ROIs) were taken at 20x magnification using an automated high-content fluorescence microscope (Leica DMI 6000B, Germany) and image processing was performed in FIJI (NIH, version 1.0) (Schindelin *et al.*, 2012). First, tiles of images were corrected for background and signal bleaching by the BaSiC plugin (Peng *et al.*, 2017) and were stitched to a single image by the Grid/StitchCollection plugin in FIJI, then a set of ROIs for each brain region was created based on the Allen Mouse Brain Atlas (https://portal.brain-map.org/) (Figure S1). For the agranular subdivisions of the aIC and pIC, squared areas (200 × 200 µm) were selected to cover layers II/III and layers V/VI. For the dysgranular and granular subdivisions of the aIC and pIC, a squared area (200 × 200 µm) covering layers II/III and a rectangular area (200 × 300 µm) covering layers V/VI

were selected. For the PRh, two squared areas (200 × 200 μm) covering layers II/III and V/VI were selected. For the BLA, a circular area (600 μm in diameter) was selected. For the prelimbic (PrL) and infralimbic (IL) regions of the PFC, a rectangular area (150 × 250 μm) covering layers II/III and a squared area (250 × 250 μm) covering layers V/VI were selected. For each ROI, the number of c-Fos-positive and GAD67-positive cells was counted manually by a researcher blind to the treatment condition, and double-labeled neurons were counted with the help of the multi-point tool in FIJI.

### Statistics

Data are expressed as mean ± SEM. The discrimination indexes were analyzed with a three-way mixed ANOVA with training duration (3 or 10 min) or drug condition (saline, 0.3 and 1.0 mg/kg of yohimbine), and retention interval (1 day, 7 days and 14 days) as between-subject variables, and type of object ($DI_{dissimilar}$ and $DI_{similar}$) as within-subject variable. Total exploration time of the objects and total distance moved were analyzed with a two-way ANOVA with training duration or drug condition, and retention interval as between-subject variables. Follow up testing of the discrimination indexes at each retention interval separately occurred by a two-way mixed ANOVA with training duration or drug condition as between-subject variable, and type of object as within-subject variable. Tukey *post-hoc* *t*-tests were used to determine the source of the significance in the ANOVAs. One-sample *t*-tests were used to determine whether the $DI_{dissimilar}$ or $DI_{similar}$ differed from zero (i.e. chance level).

Based on our behavioral observations, immunohistochemistry data were initially analyzed only for the 1-day retention interval with a mixed ANOVA with drug condition as between-subject variable, and cortical layers (layers II/III and layers V/VI) and subregion (in case of aIC, pIC and PFC) as within-subject variables. For brain regions that showed a significant drug effect on retention-induced c-Fos expression, we subsequently explored the effect of retention interval by adding it as a between-subject variable in the ANOVA. When appropriate, Tukey *post-hoc* analyses were used to determine the source of the significance in the ANOVAs. Finally, Pearson correlations were calculated to determine correlations between c-Fos expression data and the discrimination indexes. For all statistical tests, $p < 0.05$ was accepted for statistical significance. The number of mice per group is indicated in the figure legends.

## Results

### Effect of training duration on the detailedness of object memory over time

In this first experiment, we examined whether the duration of the training trial influences both the robustness and detailedness of object memory and how this changes over time. Therefore, mice were trained for either 3 or 10 min, and retention was tested, in separate groups of animals, 1 day, 7 days or 14 days later. As expected, mice that were trained for 10 min exhibited significantly more total object exploration time (independent samples *t*-test: $t_{(46)} = 10.40$, $p < 0.001$; Table S1) and moved a greater distance in the experimental apparatus ($t_{(46)} = 16.29$, $p < 0.001$; Table S1) during the training session compared to animals that were trained for 3 min.

A three-way mixed ANOVA for the two discrimination indexes at retention testing indicated significant main effects of training duration ($F_{(1,42)} = 10.10$, $p = 0.003$) and retention interval ($F_{(2,42)} = 7.59$, $p = 0.002$). Moreover, we observed significant training duration × retention interval ($F_{(2,42)} = 3.98$, $p = 0.03$) and type of object × retention interval interaction effects ($F_{(2,42)} = 9.76$, $p < 0.001$). All other effects were not significant (all *p*'s > 0.10). A two-way ANOVA for the total object exploration time and total distance moved during the retention test revealed no significant effects of training duration, retention interval, or training duration × retention interval interaction (all *p*'s > 0.17, Figure S2). To better understand the effect of training duration on memory robustness and detailedness over time, we analyzed the effect of training duration on memory performance at each retention interval separately.

At the 1-day retention test, a two-way mixed ANOVA for the two discrimination indexes indicated significant main effects of training duration ($F_{(1,14)} = 6.84$, $p = 0.02$) and type of object ($F_{(1,14)} = 15.33$, $p = 0.002$). There was no significant training duration × type of object interaction effect ($F_{(1,14)} = 0.51$, $p = 0.49$). The $DI_{dissimilar}$ of mice that were trained for 10 min did not differ significantly from that of mice that were trained for 3 min (independent samples *t*-test: $t_{(14)} = 2.05$, $p_{corr} = 0.06$, Figure 1B). Further, one-sample *t*-tests indicated that the $DI_{dissimilar}$ of mice of both training groups was significantly greater than zero (3 min: $t_{(7)} = 2.39$, $p = 0.048$; 10 min: $t_{(7)} = 4.23$, $p = 0.003$, Figure 1B). These findings thus indicate that mice of both the 3-min and 10-min training groups were able to identify the dissimilar object as a novel object. However, we found a significant effect of training duration on the mice' ability to identify the similar object as novel object (independent samples *t*-test: $t_{(14)} = 2.87$, $p_{corr} = 0.01$, Figure 1B). Whereas the $DI_{similar}$ of mice of the 3-min training group did not differ from chance ($t_{(7)} = 0.48$, $p = 0.65$, Figure 1B), the $DI_{similar}$ of mice of the 10-min training group was significantly greater than zero ($t_{(7)} = 3.12$, $p = 0.02$). These findings thus indicate that mice given 3 min of training were able to discriminate the dissimilar, but not similar, object from

the familiar object at the 1-day retention test, whereas mice given 10 min of training had a more detailed memory of the training object and were able to also discriminate the similar object from the familiar object.

At the 7-day retention test, a two-way mixed ANOVA for the two discrimination indexes indicated merely a significant main effect of training duration ($F_{(1,12)}$ = 4.73, $p$ = 0.05), but no significant main effect of type of object ($F_{(1,12)}$ = 0.08, $p$ = 0.79) or training duration × type of object interaction effect ($F_{(1,12)}$ = 0.10, $p$ = 0.76). The $DI_{dissimilar}$ of mice that had received 3 min of training was no longer different from zero ($t_{(6)}$ = 0.68, $p$ = 0.52, Figure 1B), whereas that of mice of the 10-min training group was still significantly greater than zero ($t_{(6)}$ = 2.89, $p$ = 0.03). Similarly, the $DI_{similar}$ of mice that had received 3 min of training did not differ from chance level ($t_{(6)}$ = 1.61, $p$ = 0.16), whereas the $DI_{similar}$ of mice that had received 10 min of training was still significantly greater than zero ($t_{(6)}$ = 4.37, $p$ = 0.005, Figure 1B). Further, we again found a significant effect of training duration on the mice' ability to identify the similar object as novel object (independent samples $t$-test: $t_{(12)}$ = 2.35, $p_{corr}$ = 0.04, Figure 1B). These findings thus indicate that extended training can induce a relatively long-term memory with sufficient level of detailedness after one week, whereas brief training is not sufficient to generate a memory that lasts for one week. Exploratory comparisons were performed between retention intervals within $DI_{dissimilar}$ or $DI_{similar}$. Comparison of the $DI_{dissimilar}$ and $DI_{similar}$ at the 7-day retention test vs 1-day retention test revealed no significant effect of retention interval in mice of either the 3-min or 10-min training groups (all $p_{uncorr}$'s > 0.37).

At 14 days, a two-way mixed ANOVA for the two discrimination indexes indicated no significant main effects of training duration ($F_{(1,12)}$ = 0.34, $p$ = 0.57) or type of object ($F_{(1,12)}$ = 3.60, $p$ = 0.08), and no training duration × type of object interaction effect ($F_{(1,12)}$ = 0.22, $p$ = 0.65). At this time point, the $DI_{dissimilar}$ of mice of both the 3-min and 10-min training groups did not differ from zero (3 min: $t_{(8)}$ = 0.34, $p$ = 0.74; 10 min: $t_{(8)}$ = 0.57, $p$ = 0.59, Figure 1B). Also the $DI_{similar}$ of mice from both the 3-min and 10-min training groups did not differ from zero (3 min: $t_{(8)}$ = 1.76, $p$ = 0.12; 10 min: $t_{(8)}$ = 0.41, $p$ = 0.69, Figure 1B). Exploratory comparisons were performed between retention intervals within $DI_{dissimilar}$ or $DI_{similar}$. Comparison of the $DI_{dissimilar}$ and $DI_{similar}$ at the 14-day retention test vs the other retention intervals revealed that both the $DI_{dissimilar}$ and $DI_{similar}$ of mice that had received 10 min of training were significantly smaller at 14 days than at 1 day ($p_{uncorr}$ < 0.001 and $p_{uncorr}$ = 0.002, respectively) and 7 days ($p_{uncorr}$ = 0.02 and $p_{uncorr}$ = 0.005, respectively, Figure 1B). We found no significant differences in the $DI_{dissimilar}$ or $DI_{similar}$ of mice that had received 3 min of training across the three retention intervals (all $p_{uncorr}$'s > 0.20). These findings indicate that although

extended training initially enhances memory detailedness, it does not seem to prevent loss of memory after two weeks.

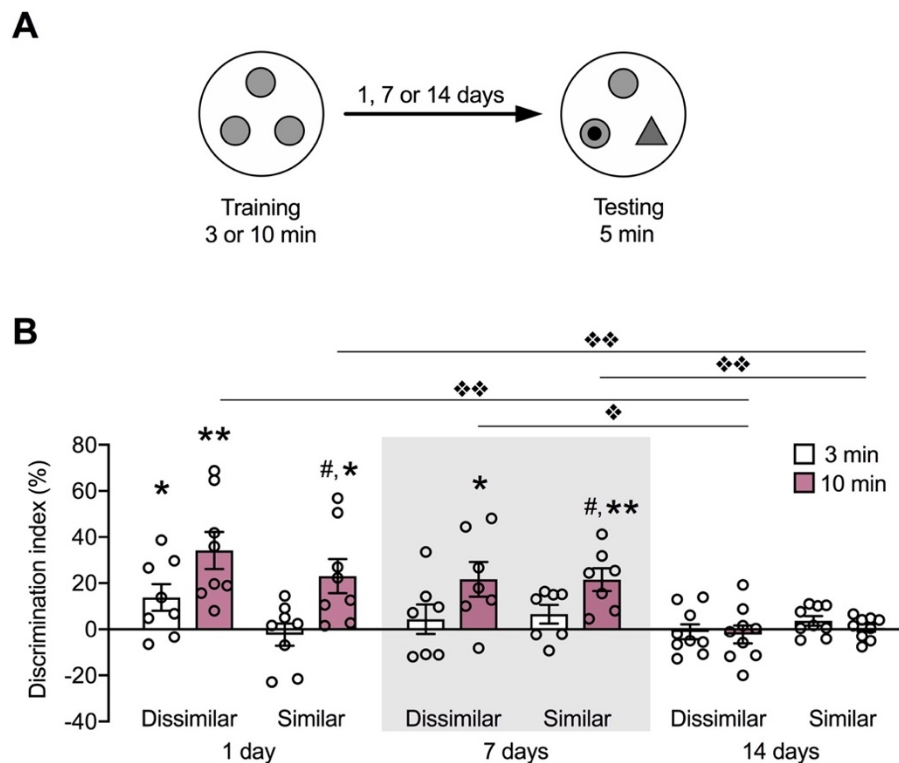



**Figure 1. Effect of training duration on the detailedness of object memory over time**. **A,** Experimental design of the object discrimination task. Mice could freely explore three identical objects for either 3 or 10 min, and retention was tested, in separate groups of animals, at 1 day, 7 days or 14 days after the training trial. On the retention test, one object was identical to the training object (i.e., familiar object), one object was highly similar to the training object and another object was dissimilar to the training object. **B,** At the 1-day retention test, mice trained for 3 min discriminated the dissimilar, but not similar, object from the familiar object, whereas mice trained for 10 min were able to accurately discriminate both the dissimilar and similar objects (3 min: $n$ = 8, 10 min: $n$ = 8). At 7 days, mice trained for 3 min no longer discriminated the dissimilar or similar object from the familiar object, whereas mice trained for 10 min still showed accurate discrimination of both the dissimilar and similar objects (3 min: $n$ = 7, 10 min: $n$ = 7). At 14 days, mice from both the 3-min and 10-min training groups failed to discriminate the dissimilar or similar object from the familiar object (3 min: $n$ = 9, 10 min: $n$ = 9). Data are shown as mean ± SEM, dots represent individual data points. * $p < 0.05$, ** $p < 0.01$ vs. chance level; # $p_{corr} < 0.05$ vs. 3-min group; ❖ $p_{uncorr} < 0.05$, ❖❖ $p_{uncorr} < 0.01$ between retention intervals.

### Effect of posttraining noradrenergic stimulation on the detailedness of object memory over time

In this experiment, we examined whether systemic yohimbine (0.3 or 1.0 mg/kg) administration after object training would enhance both the detailedness and longevity of the

memory. To examine such yohimbine-induced memory improvement, we implemented a 3-min training duration, and retention was tested 1 day, 7 days or 14 days later in different groups of animals. Table S2 shows that the posttraining yohimbine treatment groups did not differ in their total object exploration time ($F_{(2,84)} = 1.26$, $p = 0.29$) or total distance moved ($F_{(2,84)} = 0.28$, $p = 0.76$) during the training session.

A three-way mixed ANOVA for the two discrimination indexes at retention testing indicated significant main effects of type of object ($F_{(1,78)} = 5.36$, $p = 0.02$) and retention interval ($F_{(2,78)} = 11.67$, $p < 0.001$) as well as significant type of object × retention interval ($F_{(2,84)} = 11.14$, $p < 0.001$), drug condition × type of object ($F_{(2,78)} = 3.16$, $p = 0.048$) and drug condition × type of object × retention interval ($F_{(4,78)} = 2.87$, $p = 0.03$) interaction effects. All other effects were not significant (all $p$'s > 0.33). A two-way ANOVA for the total object exploration time and total distance moved during the retention test revealed no significant effects of drug condition, retention interval, or drug condition × retention interval interaction (all $p$'s > 0.06, Figure S3). To follow up on the three-way interaction effect, we investigated the effect of yohimbine treatment on the two discrimination indexes at the different retention intervals separately.

At the 1-day retention test, a two-way mixed ANOVA for the two discrimination indexes indicated a significant main effect for drug condition ($F_{(2,30)} = 3.37$, $p = 0.048$) and type of object ($F_{(1,30)} = 21.16$, $p < 0.001$), as well as a significant drug condition × type of object interaction effect ($F_{(2,30)} = 6.81$, $p = 0.004$). Mice treated with the higher dose of yohimbine (1.0 mg/kg) displayed a significantly greater $DI_{dissimilar}$ than those treated with saline ($p_{corr} = 0.046$) but not the lower dose of yohimbine (0.3 mg/kg) ($p_{corr} = 0.09$). The $DI_{dissimilar}$ of mice treated with the lower dose did not differ from that of saline-treated animals ($p_{corr} = 0.95$). Further, one-sample $t$-tests indicated that the $DI_{dissimilar}$ of all groups was significantly greater than zero (saline: $t_{(10)} = 6.12$, $p < 0.001$; 0.3 mg/kg: $t_{(10)} = 4.13$, $p = 0.002$; 1.0 mg/kg: $t_{(10)} = 9.19$, $p < 0.001$, Figure 2B). These findings indicate that the 3-min training session was sufficient for the mice to identify the dissimilar object as a novel object, and that this ability was enhanced by high-dose yohimbine treatment. There was also an effect of drug condition on the $DI_{similar}$, which was driven by a significantly greater $DI_{similar}$ of mice treated with the lower dose of yohimbine compared to those treated with saline ($p_{corr} = 0.01$), but not the higher dose of yohimbine (1.0 mg/kg) ($p_{corr} = 0.52$). The $DI_{similar}$ of mice treated with the higher dose did not differ from that of saline-treated animals ($p_{corr} = 0.15$). Further, one-sample $t$-tests indicated that the $DI_{similar}$ was significantly greater than zero in both yohimbine groups (0.3 mg/kg: $t_{(10)} = 6.24$, $p < 0.001$; 1.0 mg/kg: $t_{(10)} = 2.70$, $p = 0.02$, Figure 2B), but

not saline-treated mice ($t_{(10)}$ = 0.73, $p$ = 0.48, Figure 2B). These findings indicate that saline-treated animals were not able to identify the similar object as a novel object, and that yohimbine treatment created a more detailed memory.

At 7 days, a two-way mixed ANOVA for the two discrimination indexes indicated a significant main effect for type of object ($F_{(1,29)}$ = 4.41, $p$ = 0.04), but no significant main effect of drug condition ($F_{(2,29)}$ = 0.22, $p$ = 0.80), or drug condition × type of object interaction effect ($F_{(2,29)}$ = 2.08, $p$ = 0.14). One-sample $t$-tests indicated that the $DI_{dissimilar}$ of saline-treated mice did not significantly differ from zero ($t_{(9)}$ = 1.15, $p$ = 0.28, Figure 2B), indicating that they did not express memory for the familiar object 7 days after training. However, the $DI_{dissimilar}$ of mice treated with either dose of yohimbine was significantly greater than zero (0.3 mg/kg: $t_{(10)}$ = 2.86; $p$ = 0.02; 1.0 mg/kg: $t_{(10)}$ = 2.90; $p$ = 0.02, Figure 2B), indicative of memory. The $DI_{similar}$ of none of the groups differed from zero (saline: $t_{(9)}$ = 1.89, $p$ = 0.09; 0.3 mg/kg: $t_{(8)}$ = 2.03, $p$ = 0.07; 1.0 mg/kg: $t_{(10)}$ = 0.70; $p$ = 0.50, Figure 2B), indicating that yohimbine was not able to maintain the detailedness of object memory after 7 days. Exploratory comparisons were performed between retention intervals within $DI_{dissimilar}$ or $DI_{similar}$. Comparison of the discrimination indexes at the 7-day retention test vs 1-day retention test revealed that mice treated with the higher dose of yohimbine had a significantly smaller $DI_{dissimilar}$ at 7 days than at 1 day ($p_{uncorr}$ = 0.01, Figure 2B), and mice treated with the lower dose of yohimbine had a significantly smaller $DI_{similar}$ at 7 days than at 1 day ($p_{uncor}$ = 0.048, Figure 2B). There was no significant difference in the other discrimination indexes between both intervals (all $p_{uncor}$'s > 0.09).

At 14 days, a two-way mixed ANOVA for the two discrimination indexes indicated a significant main effect for type of object ($F_{(1,19)}$ = 7.16, $p$ = 0.02), but no significant main effect of drug condition ($F_{(2,19)}$ = 0.21, $p$ = 0.80) or drug condition × type of object interaction effect ($F_{(2,19)}$ = 0.28, $p$ = 0.76). The $DI_{dissimilar}$ of mice treated with saline ($t_{(7)}$ = 0.04, $p$ = 0.97) or those treated with either dose of yohimbine (0.3 mg/kg: $t_{(6)}$ = 0.91, $p$ = 0.07; 1.0 mg/kg: $t_{(6)}$ = 0.30; $p$ = 0.77, Figure 2B) did not significantly differ from zero, indicating that none of the drug treatment groups expressed any memory for the familiar object 14 days after training. As expected, the $DI_{similar}$ did not differ from zero either (saline: $t_{(7)}$ = 1.94, $p$ = 0.09; 0.3 mg/kg: $t_{(6)}$ = 1.80, $p$ = 0.12; 1.0 mg/kg: $t_{(6)}$ = 0.91; $p$ = 0.40, Figure 2B). These findings indicate that yohimbine was not able to enhance the detailedness of object discrimination memory after 14 days. Exploratory comparisons were performed between retention intervals within $DI_{dissimilar}$ or $DI_{similar}$. Comparison of the discrimination indexes at the 14-day retention test vs. the other retention intervals revealed that mice from all three groups had a significantly

smaller $DI_{dissimilar}$ at 14 days than at 1 day (saline: $p_{uncor} = 0.02$; 0.3 mg/kg: $p_{uncor} = 0.02$; 1.0 mg/kg: $p_{uncor} < 0.001$, Figure 2B), and mice treated with the lower dose of yohimbine had a significantly smaller $DI_{similar}$ at 14 days than at 1 day ($p_{uncor} = 0.04$, Figure 2B). There was no significant difference in the other discrimination indexes between retention intervals (all $p_{uncor}$'s > 0.07).

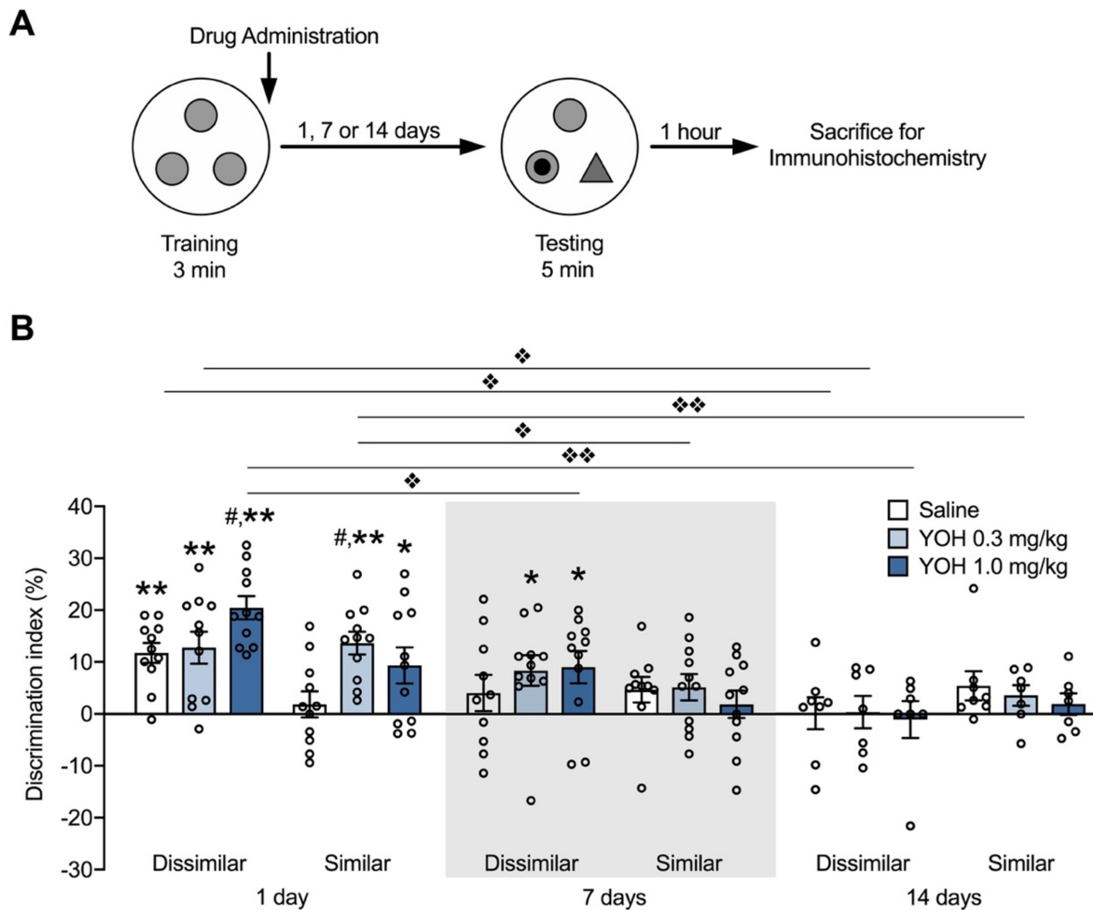



**Figure 2. Effect of posttraining noradrenergic activation on the detailedness of object memory over time**. **A,** Experimental design of the object discrimination memory task. Mice could freely explore three identical objects for 3 min, followed by an immediate posttraining intraperitoneal injection of yohimbine (YOH, 0.3 or 1.0 mg/kg) or saline. Retention was tested, in separate groups of animals, 1 day, 7 days or 14 days later. On the retention test, one object was identical to the training object (i.e., familiar object), one object was highly similar to the training object and another object was dissimilar to the training object. **B,** At the 1-day retention test, saline-treated mice discriminated the dissimilar, but not similar, object, whereas mice treated with either dose of yohimbine were able to discriminate both the dissimilar and similar object from the familiar object (Saline: *n* = 11, YOH 0.3 mg/kg: *n* = 11, YOH 1.0 mg/kg: *n* = 11). At the 7-day retention test, mice treated with saline no longer discriminated the dissimilar or similar object from the familiar object, whereas mice treated with either dose of yohimbine continued to display accurate discrimination of the dissimilar, but not similar, object (Saline: *n* = 10,

YOH 0.3 mg/kg: $n = 11$, YOH 1.0 mg/kg: $n = 11$). At the 14-day retention test, mice from the both the saline and yohimbine treatment groups failed to discriminate either the dissimilar or similar object (Saline: $n = 8$, YOH 0.3 mg/kg: $n = 7$, YOH 1.0 mg/kg: $n = 7$). Data are shown as mean ± SEM, dots represent individual data points. * $p < 0.05$, ** $p < 0.01$ vs. chance level; # $p_{corr} < 0.05$ vs. saline group; ❖ $p_{uncorr} < 0.05$, ❖❖ $p_{uncorr} < 0.01$ between retention intervals.

### Effect of posttraining noradrenergic stimulation on retention-induced neuronal activity

To examine the neural correlates of yohimbine-induced memory enhancement, we assessed neuronal activity within the brain regions of interest (i.e., aIC, pIC, PRh, BLA and PFC) by assessing the number of c-Fos-positive cells 1 h after the retention test. Since the behavioral effects of posttraining yohimbine treatment were most pronounced at the 1-day retention test, we started by analyzing retention-induced neuronal activity at this short-term retention interval.

For the aIC, we distinguished four anatomical subregions based on the Franklin and Paxinos mouse brain atlas (Franklin and Paxinos, 3rd edition, 2007): the anterior granular insular (aGI), anterior dysgranular insular (aDI), agranular insular dorsal part (AID) and agranular insular ventral part (AIV). For all subregions, the number of c-Fos-positive cells was counted in both the input (layers II/III) and output (layers V/VI) regions (Figure S1). A mixed ANOVA revealed a significant main effect of drug condition ($F_{(2,21)} = 15.10$, $p < 0.001$) and drug condition × subregion interaction ($F_{(2,21)} = 15.35$, $p < 0.001$), but no drug condition × subregion × layer interaction ($p > 0.73$). Follow-up analyses revealed a significant drug condition effect on retention-induced c-Fos expression within the AID ($F_{(2,21)} = 15.10$, $p < 0.001$), AIV ($F_{(2,21)} = 21.08$, $p < 0.001$) and aDI ($F_{(2,21)} = 4.40$, $p = 0.03$), but not within the aGI ($F_{(2,21)} = 2.34$, $p = 0.12$). Tukey's *post-hoc* tests revealed that drug condition effects in the AID and AIV were driven by significantly more c-Fos expression in both yohimbine groups compared to saline-treated animals (AID and AIV: $p_{corr}$'s < 0.001, Figure 3B), whereas the two yohimbine groups did not differ from each other (AID: $p_{corr} = 0.92$, AIV: $p_{corr} = 0.72$). In the aDI, Tukey's *post-hoc* tests revealed no drug condition effect between groups (all $p_{corr}$'s > 0.06, Figure 3B). None of these aIC subregions displayed a significant drug condition × layer interaction effect (all $p$'s > 0.44), indicating that the yohimbine increased retention-induced c-Fos expression in layers II/III and layers V/VI in a similar manner.

For the pIC, we analyzed the number of c-Fos-positive cells within layers II/III and layers V/VI of the posterior granular insular (pGI), posterior dysgranular insular (pDI) and agranular

insular posterior part (AIP). A mixed ANOVA revealed no significant effect of drug condition on retention-induced c-Fos expression in any of the pIC subregions (main effect of drug condition, drug condition × subregion, drug condition × layer, drug condition × subregion × layer interaction; all $p$'s > 0.32, Figure S4).

In the PRh, we found a significant effect of drug condition on retention-induced c-Fos expression ($F_{(2,21)} = 13.09$, $p < 0.001$), independent of layer ($F_{(1,22)} = 0.34$, $p = 0.71$). Both the lower ($p_{corr} = 0.001$) and higher-dose yohimbine groups ($p_{corr} = 0.001$, Figure 3B) displayed significantly more retention-induced c-Fos expression than the saline-treated mice in both layers II/III and layers V/VI, whereas they did not significantly differ from each other ($p_{corr} = 0.99$).

Yohimbine treatment did not affect the number of c-Fos-positive cells after the retention test within the other brain regions (PFC and BLA, all $p$'s > 0.41, Figure S4).

We next investigated whether the increased number of c-Fos-positive cells reflected a change in excitatory or inhibitory activity, the latter being identified by co-expression of c-Fos and GAD67, a marker for GABAergic inhibitory neurons (Ito *et al.*, 2015). For all brain regions, we found that yohimbine administration did not significantly alter the number of activated GABAergic neurons (all $p$'s > 0.70; data not shown). Additionally, to determine relative inhibitory tone, the number of cells co-expressing c-Fos and GAD67 was divided by the total number of c-Fos-positive cells. Inhibitory tone was overall very low (<1% ratio) and not affected by yohimbine administration (all $p$'s > 0.34; data not shown). Thus, these findings indicate that the increased number of c-Fos-positive cells within the aIC and PRh after the retention test likely reflects an increased excitatory activity.

Lastly, we performed Pearson correlation to determine how retention-induced c-Fos expression in these brain regions related to the robustness and detailedness of object memory. The $DI_{similar}$ positively correlated with the number of c-Fos-positive cells within the aDI ($r = 0.52$, $p = 0.009$), AID ($r = 0.42$, $p = 0.04$), AIV ($r = 0.58$, $p = 0.003$) and PRh ($r = 0.44$, $p = 0.03$) (Figure 3C), whereas no significant correlations were found between the $DI_{dissimilar}$ and c-Fos activity in any of the regions assessed (Figure S5). Thus, these findings support the view that a greater memory detailedness is associated with more retention-induced neuronal activity within the aIC and PRh.

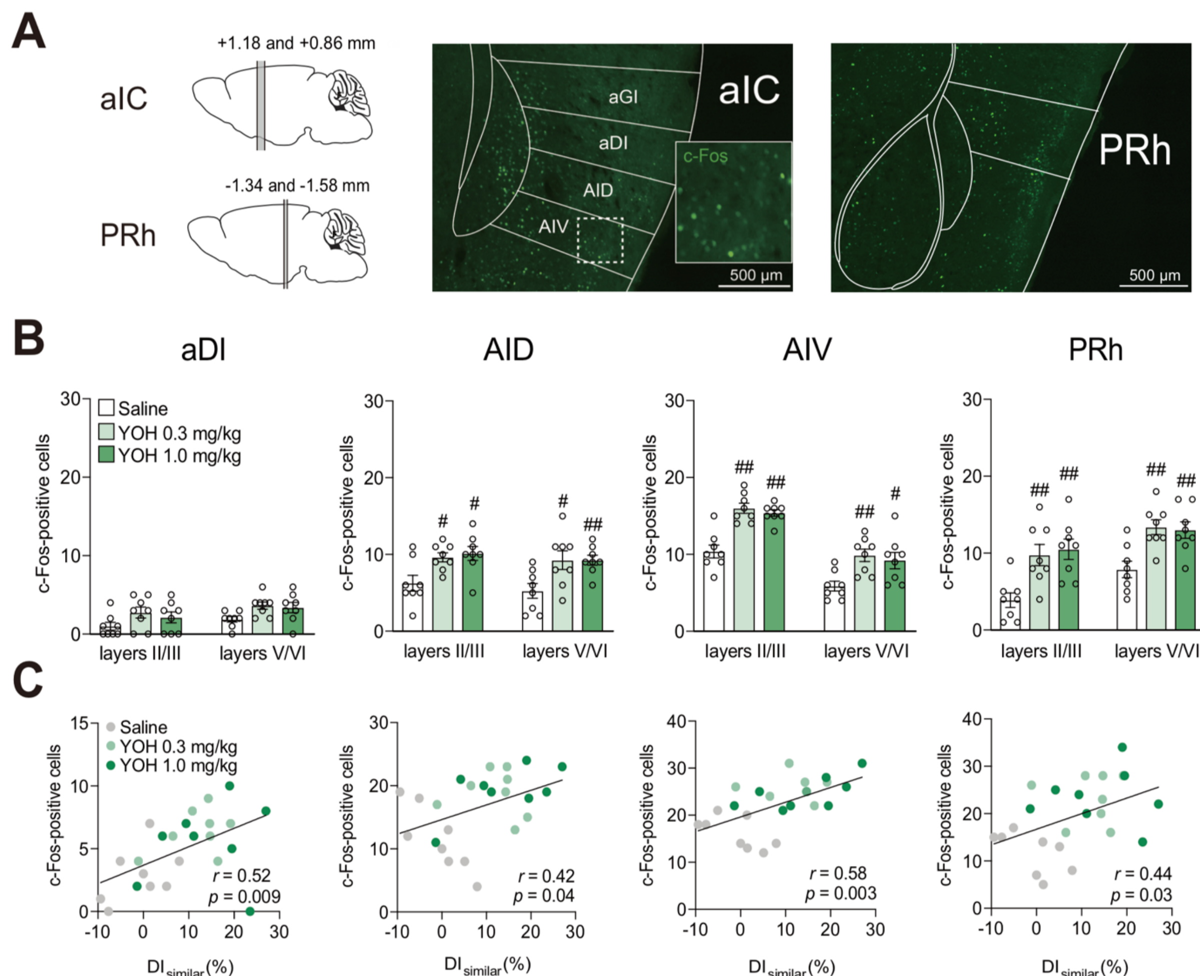



**Figure 3. Effect of posttraining noradrenergic stimulation yohimbine on 1-day retention test-induced neuronal activity. A,** Photomicrographs of the anterior insular cortex (aIC), which included the anterior dysgranular insular cortex (aDI), agranular insular cortex dorsal part (AID) and the agranular insular cortex ventral part (AIV), and the perirhinal cortex (PRh). Insert shows magnification of c-Fos-positive neurons. **B,** Yohimbine-treated animals showed significantly more retention-induced c-Fos-positive cells within layers II/III and layers V/VI of the AID, AIV and PRh at the 1-day retention test. No effect of yohimbine administration on retention-induced c-Fos expression was found in any other brain region examined (Figure S4) (Saline: $n$ = 8, YOH 0.3 mg/kg: $n$ = 8, YOH 1.0 mg/kg: $n$ = 8). **C,** The number of retention-induced c-Fos-positive cells in the aDI, AID, AIV and PRh positively correlated with the $DI_{similar}$, i.e., the animals' ability to discriminate the similar object, at the 1-day retention test. No significant correlations were found between c-Fos expression within these brain regions and the $DI_{dissimilar}$ (Figure S5). Data are shown as mean ± SEM, dots represent individual data points. # $p_{corr} < 0.05$, ## $p_{corr} < 0.01$ vs. saline group.

## Effect of posttraining noradrenergic stimulation on retention-induced neuronal activity at later retention intervals

Given our behavioral finding that posttraining yohimbine treatment enhances memory detailedness at the 1-day retention test, but that this memory detailedness is gradually lost during the later retention tests, we performed an exploratory analysis to examine whether this decay of memory detailedness was also reflected by a reduction in the retention-induced c-Fos expression within the aIC and PRh (i.e., the brain regions affected by yohimbine treatment at the 1-day interval) at the 7-day and 14-day retention intervals.

Within the aIC, a mixed ANOVA for c-Fos expression across all retention intervals revealed no main effect of drug condition ($F_{(2,52)}$ = 2.41, $p$ = 0.10), but significant main effects of retention interval ($F_{(2,52)}$ = 5.22, $p$ = 0.009), layer ($F_{(1,52)}$ = 8.58, $p$ = 0.005), and subregion ($F_{(1,52)}$ = 1033.40, $p$ = 0.005), as well as interaction effects between subregion × retention interval ($F_{(2,52)}$ = 9.22, $p$ < 0.001), subregion × layer ($F_{1,52)}$ = 67.58, $p$ < 0.001), and drug condition × subregion × retention interval ($F_{(4,52)}$ = 2.66, $p$ = 0.04). At both the 7-day and 14-day retention test intervals, we found that none of the aIC subregions showed a significant effect of drug condition (all $p$'s > 0.31; Figure 4). Interestingly, exploratory comparison of c-Fos expression between the retention intervals within layers II/III or layers V/VI indicated that this lack of drug condition effect at the later time intervals was not driven by a reduction in retention-induced c-Fos expression in the yohimbine treatment groups at the later retention intervals, but rather an increase in c-Fos expression in the saline group (layers II/III: 14 days vs 1 day: $p_{uncor}$ = 0.007, 7 days vs 1 day: $p_{uncor}$ = 0.02; layers V/VI: 14 days vs 1 day: $p_{uncor}$ = 0.03; Figure 4).

Within the PRh, a mixed ANOVA across all retention intervals also revealed no significant main effect of drug condition ($F_{(2,52)}$ = 0.49, $p$ = 0.62), but significant main effects of retention interval ($F_{(2,52)}$ = 7.62, $p$ = 0.001) and layer ($F_{(1,52)}$ = 90.65, $p$ < 0.001), as well as a significant drug condition × retention interval interaction effect ($F_{(4,52)}$ = 6.45, $p$ < 0.001). All other effects were not significant (all $p$'s > 0.20). Also here, we found no significant effect of drug condition at the 7-day and 14-day retention test intervals, and this lack of effect appeared again driven by an increased c-Fos expression in the saline group at these later retention intervals (layer II/III: 14 days vs 1 day: $p_{uncor}$ < 0.001, 7 days vs 1 day: $p_{uncor}$ = 0.01; layer V/VI: 14 days vs 1 day: $p_{uncor}$ < 0.001, 7 days vs 1 day: $p_{uncor}$ = 0.006; Figure 4).

The absence of any drug treatment effect at these later retention intervals thus matches the behavioral data, which also revealed no effects of yohimbine at the 7-day and 14-day retention test. There were also no correlations between the discrimination indexes and c-Fos activity at the 7-day or 14-day retention test (Figure S5).

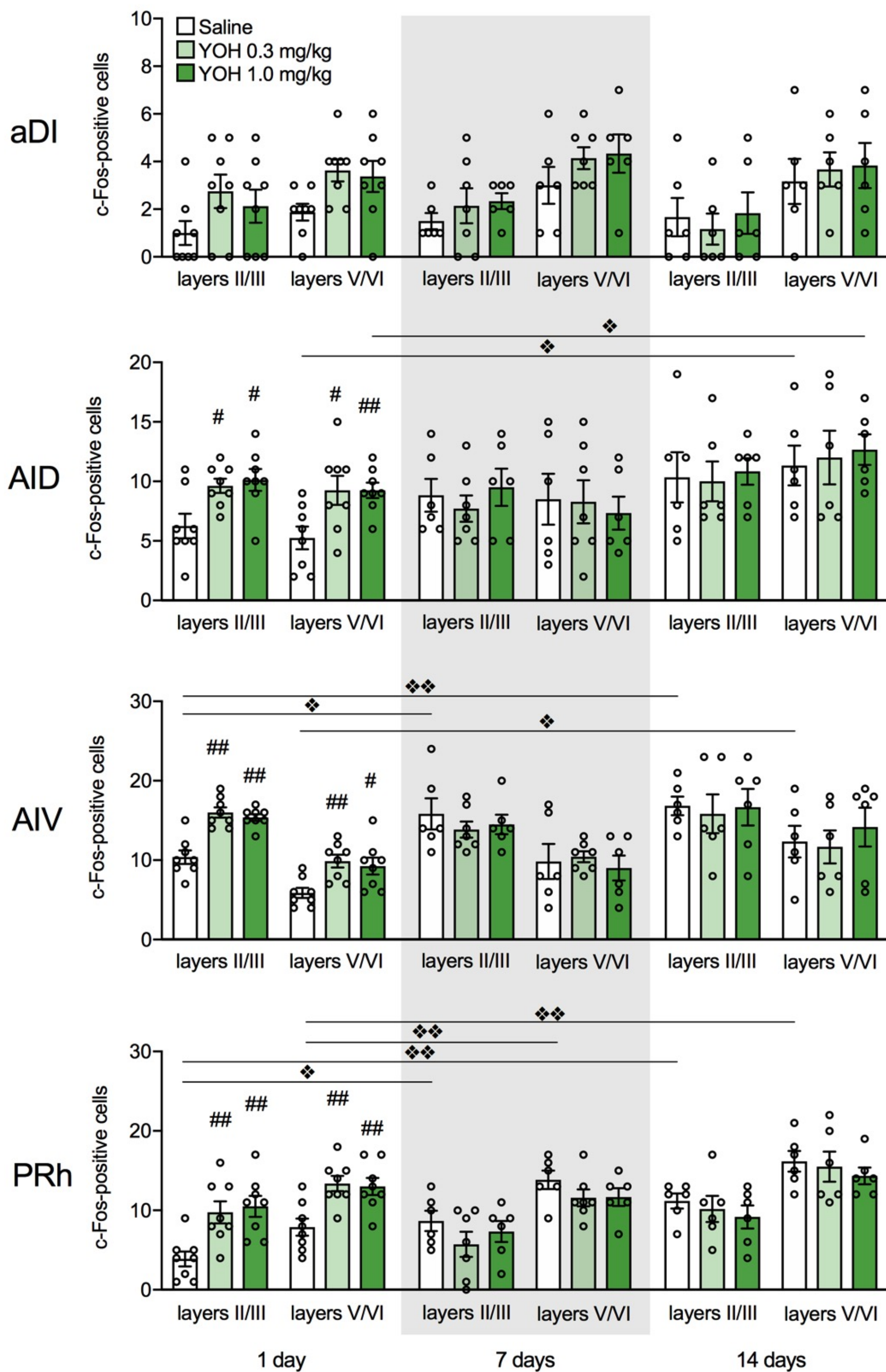



**Figure 4. Effect of posttraining noradrenergic stimulation yohimbine on retention-induced neuronal activity over time.** Yohimbine administration increased the number of c-Fos-positive cells within the layers II/III and layers V/VI of the anterior dysgranular insular cortex (aDI), agranular insular cortex dorsal part (AID), the agranular insular cortex ventral part (AIV) and the perirhinal cortex (PRh) at the 1-day retention test, but not at later retention intervals. Exploratory comparison between retention intervals indicated that this effect was not

caused by a decrease in c-Fos expression in yohimbine-treated animals over time, but an increased number of c-Fos-positive cells in saline-treated animals (1-day retention test: Saline: $n$ = 8, YOH 0.3 mg/kg: $n$ = 8, YOH 1.0 mg/kg: $n$ = 8; 7-days retention test: Saline: $n$ = 6, YOH 0.3 mg/kg: $n$ = 7, YOH 1.0 mg/kg: $n$ = 6; 14-days retention test: Saline: $n$ = 6, YOH 0.3 mg/kg: $n$ = 6, YOH 1.0 mg/kg: $n$ = 6). Data are shown as mean ± SEM, dots represent individual data points. # $p_{corr} < 0.05$, # # $p_{corr} < 0.01$ vs 1-day saline group; ❖ $p_{uncorr} < 0.05$, ❖❖ $p_{uncorr} < 0.01$ between retention intervals.

## Discussion

The current study investigated whether extended training and noradrenergic activation enhances the detailedness of object memory in a newly designed object discrimination task. We found that control mice trained for 3 min were only able to discriminate a dissimilar, but not similar, object to the one encoded during training during the 1-day retention test. Most importantly, both more extensive training and yohimbine treatment enhanced the detailedness of this memory, allowing the mice to also discriminate the similar object from the familiar one. This yohimbine effect on memory detailedness was associated with an enhanced retention-induced neuronal activity within the aIC and PRh. Finally, this yohimbine effect on enhancing memory detailedness and retention-induced neuronal activity became progressively lost during later retention intervals.

Extensive evidence indicates that noradrenergic activation is crucially involved in strengthening the consolidation of long-term memory (McGaugh, 2000; McGaugh & Roozendaal, 2002; McGaugh, 2004). Norepinephrine or a β-adrenoceptor agonist administered systemically or directly infused into relevant brain regions, such as the BLA, immediately posttraining enhances the retention of many different types of training experiences (Introini-Collison *et al.*, 1991; Ferry *et al.*, 1999; Hatfield & McGaugh, 1999; LaLumiere *et al.*, 2003; Huff *et al.*, 2006; Roozendaal *et al.*, 2006; Roozendaal *et al.*, 2008; Song *et al.*, 2020). Conversely, the administration of a β-adrenoceptor antagonist post-training impairs the consolidation of memory for these training experiences (Hatfield & McGaugh, 1999; Miranda & McGaugh, 2004). However, these learning and memory tasks typically did not allow any conclusion of whether the noradrenergic activation also enhanced the accuracy or detailedness of the memory. This question is important as evidence from particularly the human stress and memory field suggests that although stress and emotional arousal might enhance the strength of memory, these memories might become less accurate and are subject to de-contextualization, over-confidence and incorporation of misinformation (Payne *et al.*, 2002; Loftus, 2005; Payne *et al.*, 2006; Rimmele *et al.*, 2011; Qin *et al.*, 2012). Therefore, in recent years, we initiated a new line of research that started

to investigate whether and how noradrenergic activation could also influence the quality of memory. In some of these experiments we showed that noradrenergic activation, either by systemic yohimbine administration or norepinephrine infusion into the BLA, actually enhances the episodic-like accuracy on an inhibitory avoidance discrimination task, allowing the animals to differentiate a dangerous from a safe training context (Atucha *et al.*, 2017; Roozendaal & Mirone, 2020). In the present study, we sought to examine whether noradrenergic activation could also enhance the accuracy of memory for objects (i.e., non-hippocampal dependent memory). For this, we developed a new behavioral task that allowed us to assess whether animals were able to discriminate the training object from either a dissimilar or similar novel object. We found that posttraining yohimbine administration enhanced the detailedness of the memory, in an almost similar manner as more extensive training did.

Interestingly, these findings also require us to reformulate some older findings acquired on the classical object recognition task. Previous findings in both mice and rats indicated that 3 min of object training was insufficient to induce 24-h memory for the training object (Dornelles *et al.*, 2007; Roozendaal *et al.*, 2010; Nirogi *et al.*, 2012; Chen *et al.*, 2018; Song *et al.*, 2020). Noteworthy, in our own studies involving this classical object recognition (involving two objects only), we assessed memory for the familiar object as compared to a second object that was similar to the training object. In the present study, we found that although the animals after 3 min of training were not able to discriminate the familiar object from this similar object, it is incorrect to conclude that they did not acquire any memory of the training object, as they were well able to discriminate the training object from a dissimilar novel object.

We further found that the yohimbine effect on enhancing memory detailedness was associated with an increased retention-induced c-Fos expression within the aIC and the PRh. These findings most likely represent increased excitatory activity as we found that c-Fos expression was predominantly found in non-GABAergic cells. Extensive studies indicate that cortical regions such as the aIC and PRh are involved in recognition memory (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 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). In contrast, we found no changes in c-Fos activity within the pIC. The IC is a large and heterogeneous brain region which can be divided into the aIC and pIC by the middle cerebral artery (Gogolla, 2017). Studies from both animals and humans have indicated that the aIC and pIC might be involved in regulating different memory

functions (Nerad, 1997; Craig & Craig, 2009; Kurth *et al.*, 2010). Our findings are in line with the view that the aIC is involved in recognition memory and the processing of information about items (Balderas *et al.*, 2008; Roozendaal *et al.*, 2010; Bermudez-Rattoni, 2014), whereas the pIC appears to be involved in the consolidation and extinction of learned fear responses (Casanova *et al.*, 2016; Zhu *et al.*, 2016). We further found that this increased retention induced activity within the aIC and PRh in both brain regions positively correlated with the mice' ability to discriminate the similar object, but not dissimilar object. It is however questionable whether the aIC and PRh contribute to detecting similar objects alike. Findings in both animals and humans suggest that novelty and familiarity information might be signaled through interacting but non-overlapping neural networks (Kafkas & Montaldi, 2014; Molas *et al.*, 2017). Neuroimaging study in humans indicated that the IC is one of the brain structures which activity is increased with familiarity strength, whereas the PRh is involved in novelty detection (Kafkas & Montaldi, 2018). A rat study indicated that the PRh is involved in the discrimination of objects with overlapping features via a cellular process that resembles pattern separation (Miranda *et al.*, 2017). Thus, it could be hypothesized that the recruitment of multiple memory systems, including the aIC and PRh is required during the retention test for the detection of familiarity and novelty, respectively, which together could increase the ability of animals to discriminate a similar stimulus. We further investigate this topic in Chapter 4.



A second objective of the present study was to examine the long-term fate of memory detailedness. According to the standard model of systems consolidation, episodic memories are initially dependent on the hippocampus and are progressively becoming more supported by on cortical areas (Zola-Morgan *et al.*, 1986; Squire & Alvarez, 1995; Frankland *et al.*, 2004; Wiltgen & Silva, 2007; Winocur & Moscovitch, 2011; Wiltgen & Tanaka, 2013). This neural reorganization of the memory trace is accompanied by a progressive loss of episodic-like detail. We previously found that norepinephrine administration into the BLA can prevent or slowdown this systems consolidation process in maintaining long-term accurate memory (Atucha *et al.*, 2017). However, little is known regarding whether originally cortical-dependent memories also undergo a similar type of time-dependent reorganization. In other words, is a decrease in memory detailedness for objects over time related to a neural reorganization of the memory trace such that this initially detailed memory of the object is slowly replaced by a more generalized representation of the memory; and would this process be accompanied by also a transfer of the memory trace to other brain regions? And, if this is indeed the case, is noradrenergic activation able to modify this process? To address this question, we tested animals at different time intervals after training. We found, both after

yohimbine treatment and more extensive training, that not only the animals' ability to discriminate the similar object, but also dissimilar object was gradually lost over time. This finding strongly suggest that the animals did not only show a reduction in memory detailedness over time, but seem to completely lose memory of the training object. This was supported by the finding that we found no yohimbine effect on retention-induced neuronal activity at these later retention intervals. Interestingly, retention-induced c-Fos activity in particularly the PRh of saline-treated groups was higher at later retention intervals. This finding might support the view that all experimental groups treated all three objects during the retention test as a novel object, and not as a familiar object. As such, were unable to find any evidence for a process of systems consolidation for this neutral, cortically-dependent, object memory. In the extended training experiment, we did not determine neuronal activity and thus we do not know whether the effects on memory detailedness over time in that experiment are mediated by the same neurobiological mechanism.

In summary, the present findings support the view that noradrenergic activation can increase the detailedness of object memory, which is associated with an enhanced activation of both the aIC and PRh. As such, they pave the way for a further investigation of the specific neural circuits and molecular mechanisms underpinnings of these effects. Particularly new technologies such as fiber photometry and chemogenetics could be optimally combined with the use of a variety of readily available transgenic mouse to further investigate the potential role of the aIC and PRh in establishing noradrenergic enhancement of object memory detailedness.

## Disclosure statement

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## References

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*(1), 115.

Atucha, E., Vukojevic, V., Fornari, R. V., Ronzoni, G., Demougin, P., Peter, F., Atsak, P., Coolen, M. W., Papassotiropoulos, A., & McGaugh, J. L. (2017). Noradrenergic activation of the basolateral amygdala maintains hippocampus-dependent accuracy of remote memory. *Proceedings of the National Academy of Sciences of the United States of America, 114*(34), 9176-9181.

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.

Banks, P., Warburton, E., Brown, M., & Bashir, Z. (2014). Mechanisms of synaptic plasticity and recognition memory in the perirhinal cortex *Progress in molecular biology and translational science* (Vol. 122, pp. 193-209): Elsevier.

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 and Memory, 12*(5), 447-449.

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

Casanova, J. P., Madrid, C., Contreras, M., Rodríguez, M., Vasquez, M., & Torrealba, F. (2016). A role for the interoceptive insular cortex in the consolidation of learned fear. *Behavioural Brain Research, 296*, 70-77.

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.

Craig, A. D., & Craig, A. (2009). How do you feel--now? The anterior insula and human awareness. *Nature reviews neuroscience, 10*(1).

Dornelles, A., de Lima, M. N., Grazziotin, M., Presti-Torres, J., Garcia, V. A., Scalco, F. S., Roesler, R., & Schroder, N. (2007). Adrenergic enhancement of consolidation of object recognition memory. *Neurobiology of Learning and Memory, 88*(1), 137-142.

Dunsmoor, J. E., & Paz, R. (2015). Fear generalization and anxiety: Behavioral and neural mechanisms. *Biological Psychiatry, 78*(5), 336-343.

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). Involvement of alpha1-adrenoceptors in the basolateral amygdala in modulation of memory storage. *European Journal of Pharmacology, 372*(1), 9-16.

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nature reviews neuroscience, 6*(2), 119-130.

Frankland, P. W., Bontempi, B., Talton, L. E., Kaczmarek, L., & Silva, A. J. (2004). The involvement of the anterior cingulate cortex in remote contextual fear memory. *Science, 304*(5672), 881-883.

Gogolla, N. (2017). The insular cortex. *Current Biology, 27*(12), R580-R586.

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.

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.

Huff, N. C., Frank, M., Wright-Hardesty, K., Sprunger, D., Matus-Amat, P., Higgins, E., & Rudy, J. W. (2006). Amygdala regulation of immediate-early gene expression in the hippocampus induced by contextual fear conditioning. *Journal of Neuroscience, 26*(5), 1616-1623.

Introini-Collison, I. B., Miyazaki, B., & McGaugh, J. L. (1991). Involvement of the amygdala in the memory-enhancing effects of clenbuterol. *Psychopharmacology, 104*(4), 541-544.

Ito, T., Inoue, K., & Takada, M. (2015). Distribution of glutamatergic, gabaergic, and glycinergic neurons in the auditory pathways of macaque monkeys. *Neuroscience, 310*, 128-151.

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.

Kafkas, A., & Montaldi, D. (2018). How do memory systems detect and respond to novelty? *Neuroscience Letters, 680*, 60-68.

Kurth, F., Eickhoff, S. B., Schleicher, A., Hoemke, L., Zilles, K., & Amunts, K. (2010). Cytoarchitecture and probabilistic maps of the human posterior insular cortex. *Cerebral Cortex, 20*(6), 1448-1461.

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.

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.

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.

Loftus, E. F. (2005). Planting misinformation in the human mind: A 30-year investigation of the malleability of memory. *Learning and Memory, 12*(4), 361-366.

Lopresto, D., Schipper, P., & Homberg, J. R. (2016). Neural circuits and mechanisms involved in fear generalization: Implications for the pathophysiology and treatment of posttraumatic stress disorder. *Neuroscience and Biobehavioral Reviews, 60*, 31-42.

Mason, J. W. (1968). A review of psychoendocrine research on the sympathetic-adrenal medullary system. *Psychosomatic Medicine, 30*(5), 631-653.

McGaugh, J. L. (2000). Memory--a century of consolidation. *Science, 287*(5451), 248-251.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience, 27*, 1-28.

McGaugh, J. L., & Roozendaal, B. (2002). Role of adrenal stress hormones in forming lasting memories in the brain. *Current Opinion in Neurobiology, 12*(2), 205-210.

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., 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).

Miranda, M. I., & McGaugh, J. L. (2004). Enhancement of inhibitory avoidance and conditioned taste aversion memory with insular cortex infusions of 8-br-camp: Involvement of the basolateral amygdala. *Learning and Memory, 11*(3), 312-317.

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.

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

Nerad, L. (1997). Lesions of the posterior insular cortex impair water maze performance in the rat. *International Journal of Neuroscience, 90*(1-2), 1-7.

Nirogi, R., Abraham, R., Jayarajan, P., Medapati, R. B., Shanmuganathan, D., Kandikere, V., Irappanavar, S., Saralaya, R., Benade, V., Bhyrapuneni, G., & Muddana, N. (2012). Difference in the norepinephrine levels of experimental and non-experimental rats with age in the object recognition task. *Brain Research, 1453*, 40-45.

Norman, G., & Eacott, M. (2005). Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. *Behavioral Neuroscience, 119*(2), 557.

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 of the United States of America, 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.

Payne, J., Jackson, E., Ryan, L., Hoscheidt, S., Jacobs, J., & Nadel, L. (2006). The impact of stress on neutral and emotional aspects of episodic memory. *Memory, 14*(1), 1-16.

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

Peng, T., Thorn, K., Schroeder, T., Wang, L., Theis, F. J., Marr, C., & Navab, N. (2017). A basic tool for background and shading correction of optical microscopy images. *Nature Communications, 8*(1), 14836.

Pezze, M. A., Marshall, H. J., Fone, K. C., & Cassaday, H. J. (2017). Role of the anterior cingulate cortex in the retrieval of novel object recognition memory after a long delay. *Learning and Memory, 24*(7), 310-317.

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.

Qin, S., Hermans, E. J., van Marle, H. J., & Fernández, G. (2012). Understanding low reliability of memories for neutral information encoded under stress: Alterations in memory-related activation in the hippocampus and midbrain. *Journal of Neuroscience, 32*(12), 4032-4041.

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

Roozendaal, B., Castello, N. A., Vedana, G., Barsegyan, A., & McGaugh, J. L. (2008). Noradrenergic activation of the basolateral amygdala modulates consolidation of object recognition memory. *Neurobiology of Learning and Memory, 90*(3), 576-579.

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. *Journal of Neuroscience, 30*(14), 5037-5046.

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

Roozendaal, B., & McGaugh, J. L. (2011). Memory modulation. *Behavioral Neuroscience, 125*(6), 797-824.

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

Roozendaal, B., Okuda, S., Van der Zee, E. A., & McGaugh, J. L. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America, 103*(17), 6741-6746.

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

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., & Schmid, B. (2012). Fiji: An open-source platform for biological-image analysis. *Nature methods, 9*(7), 676-682.

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.

Song, Q., Bolsius, Y., Ronzoni, G., Henckens, M. J. A. G., & Roozendaal, B. (2020). Noradrenergic enhancement of object recognition and object location memory in mice. *Stress*, 1-25.

Squire, L. R., & Alvarez, P. (1995). Retrograde amnesia and memory consolidation: A neurobiological perspective. *Current Opinion in Neurobiology, 5*(2), 169-177.

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 of the United States of America, 106*(23), 9447-9452.

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., Fernandez, G., Deisseroth, K., Greene, R. W., & Morris, R. G. (2016). Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature, 537*(7620), 357-362.

Vogel-Ciernia, A., & Wood, M. A. (2014). Examining object location and object recognition memory in mice. *Current Protocols in Neuroscience, 69*, 8.31.31-17.

Wiltgen, B. J., & Silva, A. J. (2007). Memory for context becomes less specific with time. *Learning and Memory, 14*(4), 313-317.

Wiltgen, B. J., & Tanaka, K. Z. (2013). Systems consolidation and the content of memory. *Neurobiology of Learning and Memory, 106*, 365-371.

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., & Moscovitch, M. (2011). Memory transformation and systems consolidation. *Journal of the International Neuropsychological Society, 17*(5), 766-780.

Zhu, Y., Du, R., Zhu, Y., Shen, Y., Zhang, K., Chen, Y., Song, F., Wu, S., Zhang, H., & Tian, M. (2016). Pet mapping of neurofunctional changes in a posttraumatic stress disorder model. *Journal of Nuclear Medicine, 57*(9), 1474-1477.

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1986). Human amnesia and the medial temporal region: Enduring memory impairment following a bilateral lesion limited to field ca1 of the hippocampus. *Journal of Neuroscience, 6*(10), 2950-2967.

3

## Supplementary Materials

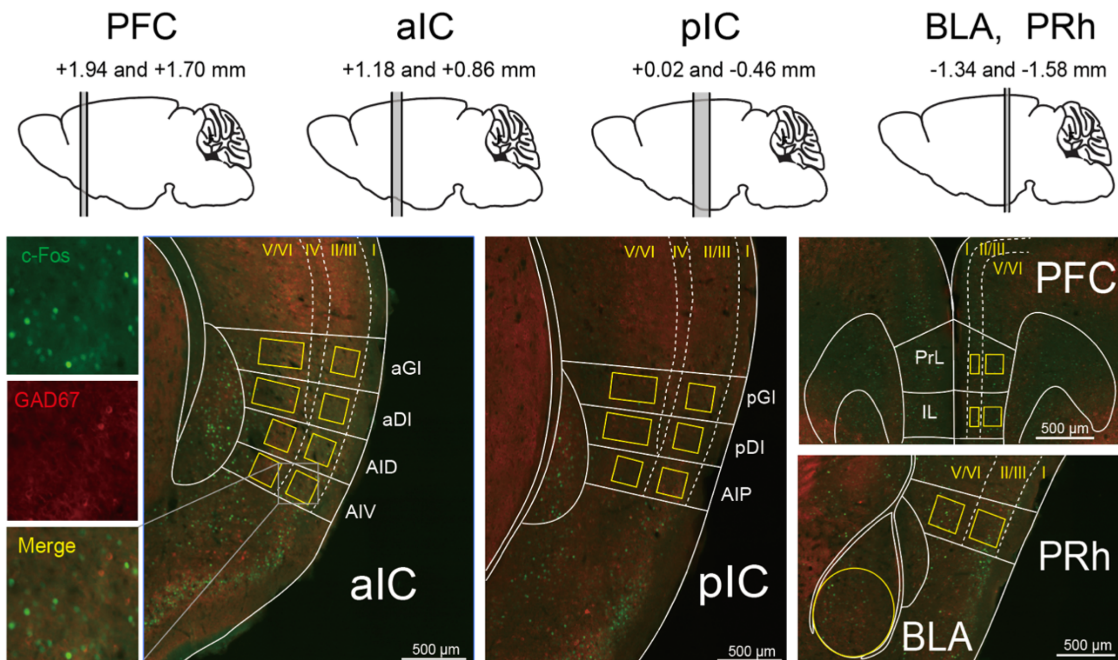



**Figure S1. Diagram illustrating the different regions of interest.** Anterior insular cortex (aIC): anterior granular insular cortex (aGI), anterior dysgranular insular cortex (aDI), agranular insular cortex dorsal part (AID) and agranular insular cortex ventral part (AIV); Posterior insular cortex (pIC): posterior granular insular cortex (pGI), posterior dysgranular insular cortex (pDI) and agranular insular cortex posterior part (AIP); Prefrontal cortex (PFC): prelimbic cortex (PrL) and infralimbic cortex (IL); Perirhinal cortex (PRh) and basolateral amygdala (BLA). The yellow areas show the exact locations in which the number of c-Fos-positive cells was counted within each region of interest. Scale bar = 500 µm.

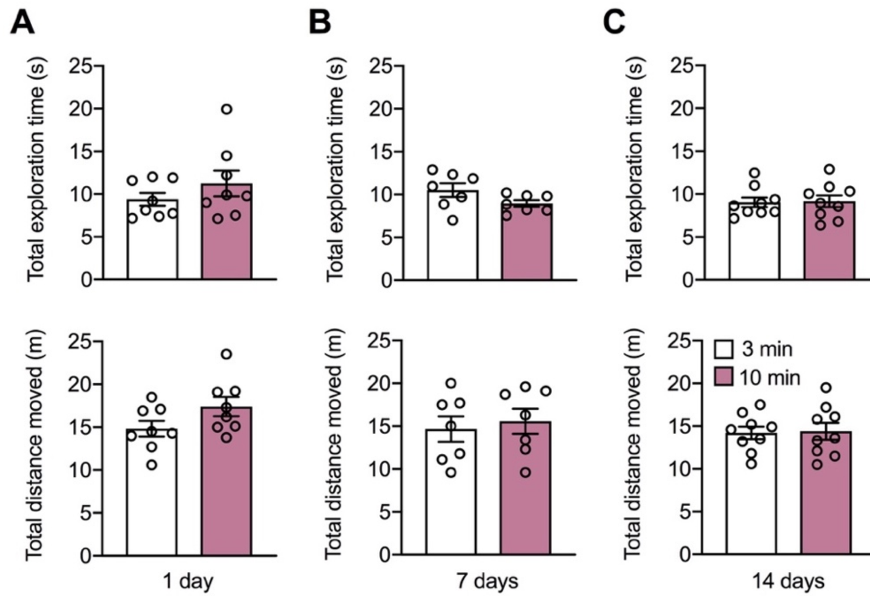



**Figure S2. Effect of training duration on the total exploration time and total distance moved during the retention test.** Mice trained for 3 min or 10 min did not differ in their total exploration time of the three objects or total distance moved at the 1-day (**A**; 3-min: n = 8, 10-min: n = 8), 7-day (**B**, 3-min: n = 7, 10-min: n = 7) or 14-day retention test (**C**, 3-min: n = 9, 10-min: n = 9). Data are shown as mean ± SEM, dots represent individual data points.

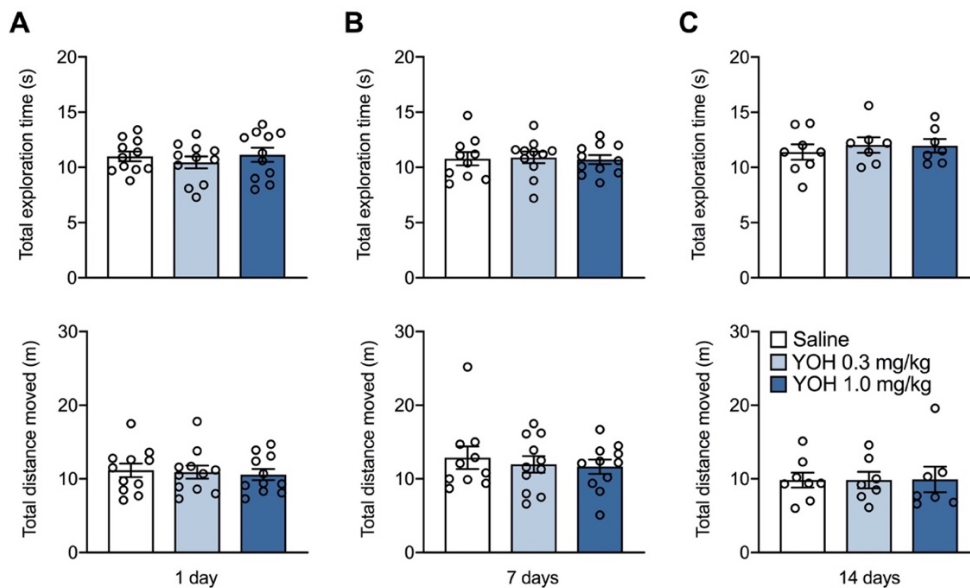
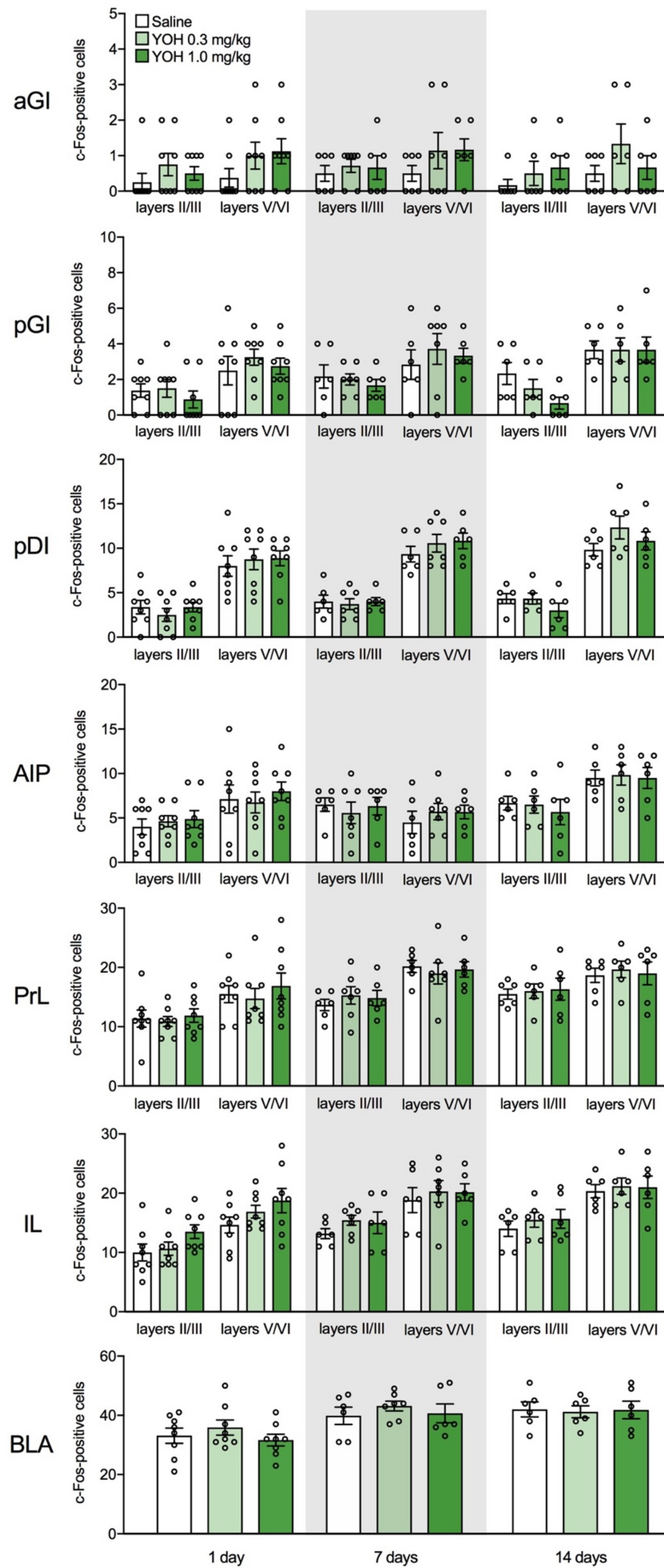



**Figure S3. Effect of yohimbine treatment on the total exploration time and total distance moved during the retention test.** Yohimbine treatment did not affect the total exploration time of the three objects or total distance moved by the mice at the 1-day (**A**, Saline: *n* = 11, YOH 0.3 mg/kg: *n* = 11, YOH 1.0 mg/kg: *n* = 11), 7-day (**B**, Saline: *n* = 10, YOH 0.3 mg/kg: *n* = 11, YOH 1.0 mg/kg: *n* = 11) or 14-day retention test (**C**, Saline: *n* = 8, YOH 0.3 mg/kg: *n* = 7, YOH 1.0 mg/kg: *n* = 7). Data are shown as mean ± SEM, dots represent individual data points.



Saline
YOH 0.3 mg/kg
YOH 1.0 mg/kg
aGI
pGI
pDI
AIP
PrL
IL
BLA
c-Fos-positive cells
layers II/III
layers V/VI
1 day
7 days
14 days

**Figure S4. Effect of posttraining noradrenergic activation on retention test-induced neuronal activity over time**. The number of retention-induced c-Fos-positive cells within each region of interest at the different retention intervals (1-day retention test: Saline: $n$ = 8, YOH 0.3 mg/kg: $n$ = 8, YOH 1.0 mg/kg: $n$ = 8; 7-day retention test: Saline: $n$ = 6, YOH 0.3 mg/kg: $n$ = 7, YOH 1.0 mg/kg: $n$ = 6; 14-day retention test: Saline: $n$ = 6, YOH 0.3 mg/kg: $n$ = 6, YOH 1.0 mg/kg: $n$ = 6.) Data are shown as mean ± SEM, dots represent individual data points. Anterior granular insular cortex (aGI), posterior granular insular cortex (pGI), posterior dysgranular insular cortex (pDI), agranular insular cortex posterior part (AIP), prelimbic cortex (PrL), infralimbic cortex (IL) and basolateral amygdala (BLA).

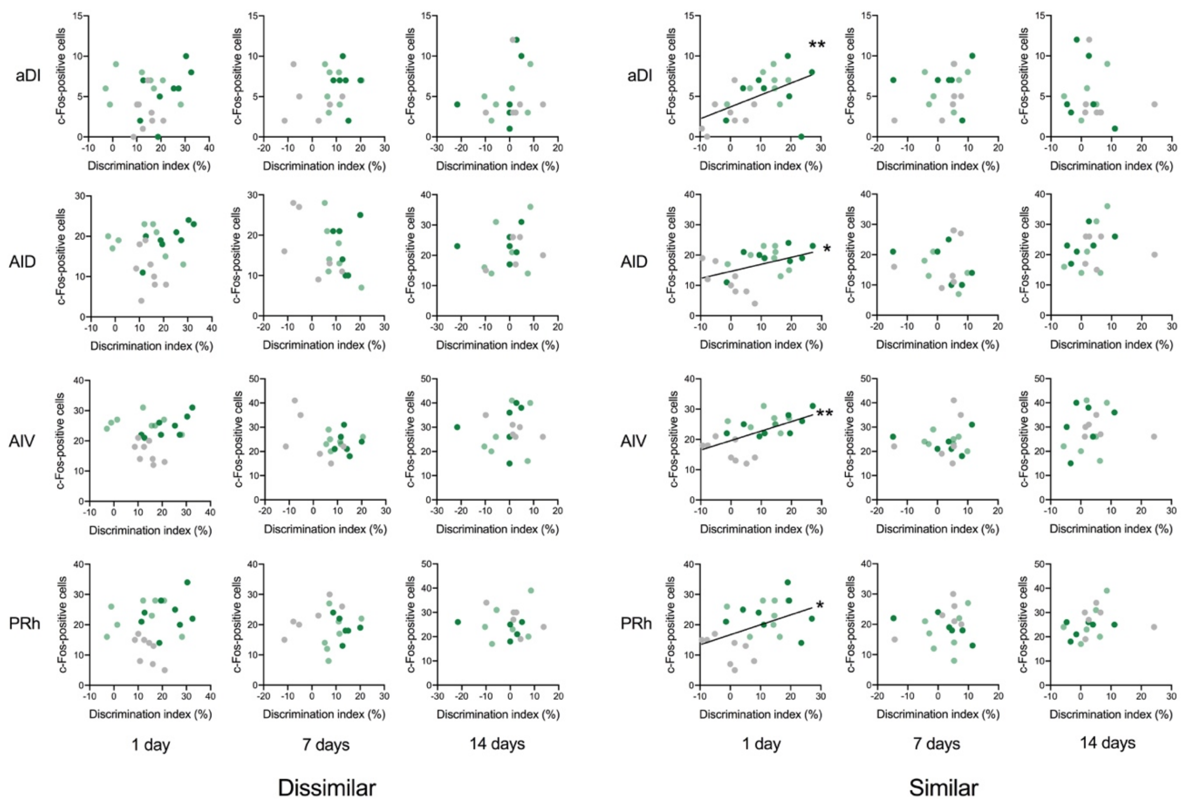



**Figure S5. Correlation between discrimination index and c-Fos activity in the different brain regions of interest**. The number of retention-induced c-Fos-positive cells within the anterior granular insular cortex (aDI), agranular insular cortex dorsal part (AID), agranular insular cortex ventral part (AIV) and perirhinal cortex (PRh) positively correlated with the $DI_{similar}$ at the 1-day retention test. No significant correlations were found with the $DI_{dissimilar}$ or at the 7-day or 14-day retention intervals. Data are shown as mean ± SEM, dots represent individual data points. * $p < 0.05$, ** $p < 0.01$.

**Table S1. Object exploration time and total distance moved during the training session**

| Training data items | 3 min | 10 min |
|---|---|---|
| Object exploration time (s) | 10.0 ± 0.4 | 18.0 ± 0.6 *** |
| Total distance moved (m) | 13.0 ± 0.6 | 35.4 ± 1.3 *** |

Data are shown as mean ± SEM. *** $p < 0.001$ vs 3-min group

**Table S2. Training data of the object discrimination memory task**

| Training data items | Saline | YOH 0.3 mg/kg | YOH 1.0 mg/kg |
|---|---|---|---|
| Object exploration time (s) | 10.6 ± 0.2 | 10.6 ± 0.3 | 10.1 ± 0.2 |
| Total distance moved (m) | 8.4 ± 0.5 | 8.1 ± 0.5 | 7.9 ± 0.6 |

Data are shown as mean ± SEM.

# CHAPTER

# 4

## Noradrenergic enhancement of memory detailedness - a role for the anterior insular cortex and perirhinal cortex

## Abstract

Noradrenergic activation has been implicated in enhancing the detailedness of object recognition memory. However, little is known concerning the neuronal mechanisms mediating this effect. In a first experiment, we used TRAP2 mice to investigate the effects of noradrenergic enhancement on neuronal activity both during memory consolidation and later memory recall, as well as the overlap between these two traces. We first validated that the noradrenergic stimulant yohimbine (0.3 mg/kg) administered systemically immediately after training to these mice enhanced memory detailedness on the object discrimination task. During the training session of this task, the mice could freely explore three copies of a single object, and on the later retention test they were exposed to one copy of the same familiar object and two objects looking either very similar or dissimilar to the familiar one. As expected, yohimbine treatment enhanced the mice' capability to discriminate the similar from the familiar object at a 3-day retention test, indicative of an enhanced memory detailedness for the familiar object, while such yohimbine effect was lost at the 7-day retention interval. Next, we examined the neuronal activity changes underlying this behavior by systemically injecting yohimbine as well as 4-hydroxytamoxifen posttraining, to induce fluorescent labeling of activated neurons. After 3 days, mice were exposed to three familiar objects, three similar or three dissimilar objects to trigger memory recall, and neuronal responses were analyzed using immunohistochemistry. Whereas noradrenergic activation had no major effects on neuronal activity during consolidation, it significantly increased (re-)exposure-induced neuronal activity within the anterior agranular insular cortex in response to the familiar or similar object, likely mediating familiarity detection, whereas it increased activity within the perirhinal cortex in response to the dissimilar or similar object, likely mediating novelty detection. To next investigate whether the norepinephrine-induced recruitment of basolateral amygdala (BLA) projections to the anterior insular cortex (aIC) are involved in mediating the yohimbine effect on memory detailedness, in a second experiment we inactivated the BLA-aIC pathway during memory consolidation by an inhibitory chemogenetic manipulation, and revealed that this manipulation selectively blocked the yohimbine effect to discriminate the similar object from the familiar one. This indicates that noradrenergic enhancement of the detailedness of object memory requires an intact BLA-aIC circuit. Moreover, these findings propose the intriguing existence of two parallel memory systems, both being modified by noradrenergic activation, with the aIC being crucially involved in detecting familiarity, and the perirhinal cortex coding novelty.

**Keywords:** norepinephrine; object recognition; memory detailedness; neuronal activity; chemogenetics

## Introduction

Extensive evidence indicates that norepinephrine is released in the brain and periphery during emotionally arousing conditions (Mason, 1968; Aston-Jones *et al.*, 1996; McIntyre *et al.*, 2002), and that this noradrenergic activation plays a crucial role in strengthening the consolidation of long-term memory (McGaugh, 2004; Sara, 2009; Roozendaal & McGaugh, 2011; Takeuchi *et al.*, 2016). Previous work has shown a particularly important role for the basolateral amygdala (BLA) in orchestrating the memory-enhancing effects of norepinephrine by regulating neural plasticity and information storage processes in other brain regions (McGaugh, 2004; Roozendaal & McGaugh, 2011; McIntyre *et al.*, 2012; McGaugh, 2013). Noradrenergic activation has also been found to enhance the consolidation of object recognition memory (Roozendaal *et al.*, 2008; Song *et al.*, 2020).

In recent experiments, we showed that noradrenergic activation not only enhances the strength, but also the accuracy, of memory (Atucha & Roozendaal, 2015; Atucha *et al.*, 2017; Bahtiyar *et al.*, 2020; Roozendaal & Mirone, 2020). In Chapter 3, we found that the noradrenergic stimulant yohimbine administered systemically after an object training experience enhanced the detailedness, and thus improved accuracy, of object recognition memory. To examine the detailedness of object memory, we used a novel object discrimination task in which mice during the training session could explore three identical objects. During the retention test trial, three different objects were used: 1) the previously explored training object (i.e., familiar object), 2) a novel object that was highly similar to the training object (i.e., similar object), and 3) a novel object that had a completely different shape and texture than the training object (i.e., dissimilar object). We found that mice given a saline control injection after a brief training session discriminated only the dissimilar, but not similar, object from the familiar object on the retention test. However, mice that had received yohimbine posttraining had created a more detailed memory of the training object and were also able to successfully discriminate the similar object from the familiar one.

We further found that this yohimbine effect on enhancing memory detailedness was associated with an increased retention-induced neuronal activity within the anterior insular cortex (aIC) and perirhinal cortex (PRh), which correlated positively with the mice' ability to discriminate the similar object, but not dissimilar object. Many previous findings have shown that both cortical regions are importantly involved in object recognition memory (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 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 suggested that their function is modulated by

noradrenergic activity in the BLA (Perugini *et al.*, 2012; Laing & Bashir, 2014; Beldjoud *et al.*, 2015; Chen *et al.*, 2018). However, their exact contribution to coding memory detailedness is rather unclear. Recent findings suggested that memory detailedness might require the detection of both novelty and familiarity, and that novelty and familiarity information might be signaled through separate, but interacting, neural networks (Kafkas & Montaldi, 2014; Molas *et al.*, 2017). Therefore, it could be hypothesized that the recruitment of multiple memory systems is required during the retention test for the detection of familiarity and novelty, respectively, which together could mediate the effect of yohimbine treatment on the animal's ability to discriminate a similar stimulus. However, in our previous study we were not able to determine whether the increased neuronal activation within the aIC and PRh was related to successful memory recall of the familiar object or the detection of novelty. Furthermore, we could not investigate whether this yohimbine effect on enhancing memory detailedness was also dependent on functional interactions with the BLA.

In the present study, we therefore performed two experiments to examine the neural mechanisms related to the yohimbine effect on enhancing memory detailedness. In a first experiment, we used TRAP2 × tdTomato double transgenic mice in which the systemic administration of 4-hydroxytamoxifen (4-OHT) induces the permanent fluorescent labeling of activated neurons (Guenthner *et al.*, 2013; DeNardo *et al.*, 2019). This enabled us to investigate the effects of noradrenergic stimulation on neuronal activity during memory consolidation and object (re-)exposure, as well as the overlap of these two traces. We first validated whether yohimbine administration to these mice enhanced memory detailedness on the object discrimination task. Next, to examine the neuronal activity changes underlying this behavior, mice were trained on the object discrimination task and given a systemic injection of yohimbine as well as 4-OHT posttraining to label those neurons that were activated during the training and consolidation phase. After 3 days, mice were either exposed to three familiar objects, three similar or three dissimilar objects to trigger memory recall, and neuronal responses to (re-)exposure of each of these objects were analyzed within the aIC, PRh and BLA using immunohistochemistry. To further determine whether noradrenergic activation affected the recruitment of the same neurons during memory consolidation and recall, we assessed the reactivation rate between training-induced and (re-)exposure-induced neuronal activity. In a second experiment, we investigated whether the norepinephrine-induced recruitment of BLA projections to the aIC is responsible for the enhanced memory detailedness. For this, we inactivated the BLA-aIC pathway during memory consolidation by an inhibitory chemogenetic manipulation (Sternson & Roth, 2014), and determined whether this manipulation selectively impaired the yohimbine effect on the

mice' ability to discriminate the similar, but not the dissimilar, object from the familiar one.

## Material and methods

### Experiment 1

#### Animals

A total of 118 male TRAP2 Fos$^{2A\text{-}iCreER}$ × tdTomato transgenic mice (further referred to as TRAP2 mice) were used for this experiment, which were 10 weeks old at the time of the behavioral experiments. Mice were bred in house by crossing two founder mouse lines: Female homozygous Fos$^{2A\text{-}iCreER}$ mice (*Fos*$^{tm2.1(icre/ERT2)Luo}$/J, 030323) with male homozygous conditional tdTomato mice (B6.Cg-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$/J, 007909) that were both purchased from The Jackson Laboratory (Bar Harbor, ME, USA). In these mice, the injection of 4-OHT induces the fluorescent labeling of neurons that express the immediate-early gene c-Fos over a labeling window of ~12 h (DeNardo *et al.*, 2019). The mice were kept in a temperature-controlled (22 °C) vivarium room with a regular 12-h/12-h light/dark cycle (lights on between 7:00 and 19:00 h). The vivarium room had a light intensity of 47 lux and humidity of 72%. Mice had *ad libitum* access to food and water. Animals were initially group housed (2-3 mice per cage), but were single housed during the course of behavioral training and testing. Behavioral training and testing was performed during the light phase of the diurnal cycle, between 10:00 and 15:00 h. 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 discrimination task

The experimental apparatus used for the object discrimination task was a gray, round plastic box (40 cm diameter, 40 cm height) with the floor covered with sawdust. During both the training and retention test sessions, three objects were placed equally spaced along the perimeter of the apparatus, 5 cm away from the wall. The objects were secured to the floor of the box with Velcro tape. A camera was mounted above the box to videotape the behavior of the animals during the training and test sessions.

Mice were first handled for 1 min each on four consecutive days to become accustomed to the experimenter. Subsequently, the animals received three 5-min habituation sessions to the experimental box during which they could freely explore the experimental apparatus without the objects. This habituation procedure is required to guarantee sufficient exploration of the objects during the training session (Stefanko *et al.*, 2009). On the training trial, the

mice were placed into the apparatus and allowed to explore three identical copies of either a glass light bulb (6 cm diameter, 11 cm length) or the similar looking glass vial (5.5 cm diameter, 5 cm height), randomized across animals. All animals were allowed to explore the objects for 3 min, followed by immediate posttraining drug administration. To avoid the presence of olfactory trails, feces were removed, sawdust was stirred, and the objects were thoroughly cleaned with 70% ethanol in between animals.

Memory was tested, in separate groups of animals, either 3 days (the minimal period required for the expression of tdTomato in activated cells) (Guenthner *et al.*, 2013) or 7 days after the training trial. During the retention test, three different objects were placed at the same locations as during training: the previously explored training object (familiar object), a novel object that was highly similar to the training object (glass jar or light bulb, similar object), and a novel object that had a completely different shape and texture than the training object (wooden pyramid, 7 cm × 7 cm × 7 cm, dissimilar object). All combinations and locations of objects were used in a balanced manner across animals to reduce potential confounding influences due to preference for a particular object or location. Pilot experiments had indicated that the animals do not display an innate preference for any of the three objects or locations used (See Chapter 3, Box I). For testing, the mice were placed in the apparatus and allowed to explore the objects for 5 min.

To assess the neural activity patterns during memory encoding and consolidation as well as those induced by memory recall of the distinct objects, other groups of trained mice were subjected to a (re-)exposure session instead of a retention test 3 days after the training trial. During the (re-)exposure session, the animals were allowed to explore either three familiar objects, three similar objects, or three dissimilar objects which were placed at the same locations as during training for 5 min. After the re-exposure session, the mice were left undisturbed in their home cage until sacrifice for immunohistochemistry 1 h later.

Videos of the training and test sessions were analyzed offline by a trained observer blind 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 are not actively engaged in exploring the object, but rather exhibit grooming behavior or are using the object 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). Re-scoring of a subset of the behavioral videos by an independent second rater confirmed the reliability of scoring ($r_{(40)} = 0.935$, $p < 0.001$, n = 20 videos per rater). Video analysis software (EthoVision XT, Noldus Information Technology, Wageningen, The Netherlands) was used to measure the total distance moved by the mice in the experimental apparatus during the training, retention testing and (re-)exposure sessions, which serves as a measure of exploration of the experimental apparatus.

To determine both the robustness and detailedness of the memory, two different discrimination indexes (DI) were calculated, one depicting the difference in time exploring the dissimilar and familiar object, divided by the total time exploring these two objects ($DI_{dissimilar}$), and the other depicting the difference in time exploring the similar and familiar object, divided by the total time exploring these two objects ($DI_{similar}$).

4

$$DI_{dissimilar} = \frac{(time\ dissimilar\ -\ time\ familiar)}{(time\ dissimilar\ +\ time\ familiar)} \times 100\%$$

$$DI_{similar} = \frac{(time\ similar\ -\ time\ familiar)}{(time\ similar\ +\ time\ familiar)} \times 100\%$$

The $DI_{dissimilar}$ is indicative of whether the mice discriminated the familiar object as compared to the dissimilar novel object and thus reflects having memory for the training object during the retention test. The $DI_{similar}$ is indicative of whether the mice successfully discriminated the familiar object as compared to the highly similar novel object and thus reflects having a detailed memory for the training object.

### Systemic drug administration

Yohimbine (17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride; 0.3 mg/kg; Sigma-Aldrich), an $\alpha_2$-adrenoceptor antagonist which increases noradrenergic activity (Szemeredi *et al.*, 1991), was dissolved in saline and administered intraperitoneally, in a volume of 0.01 mL/g of body weight, immediately after the training trial. This yohimbine dose was selected based on its memory-enhancing effect in Chapter 3. Drug solutions were freshly prepared before each experiment.

4-OHT (50 mg/kg; Sigma-Aldrich) was injected intraperitoneally, in a volume of 0.005 mL/g of body weight, immediately after the yohimbine injection in order to permanently label the activated neurons during training and consolidation. 4-OHT was first dissolved in absolute ethanol by ultra-sonication at 37 °C overnight, then corn oil (Sigma-Aldrich) was added to

generate a final concentration of 10 mg/mL (DeNardo *et al.*, 2019). The final solution contained 10% ethanol and 90% corn oil. The final 4-OHT solution was freshly prepared before each experiment.

### Immunohistochemistry

The mice were anesthetized with an overdose of sodium pentobarbital and perfused transcardially with ice-cold 0.1 M phosphate-buffered saline (PBS), pH 7.4, followed by ice-cold 4% paraformaldehyde (PFA). Brains were post-fixed overnight in 4% PFA and then cryoprotected in 30% sucrose in PBS for 72 h at 4 °C. Coronal slices of 35-µm thickness were cut on a cryostat, and collected in 0.1 M PBS with 0.1% sodium azide, and stored at 4 °C. For immunohistochemistry procedures, two sections of each of the brain regions investigated were selected according to the Franklin and Paxinos mouse brain atlas (Franklin and Paxinos, 3rd edition, 2007): aIC (anteroposterior (AP), +1.18 to +0.86 mm relative to Bregma), PRh (AP, -1.34 to -1.58 mm) and BLA (AP, -1.34 to -1.58 mm). Sections were rinsed in 0.5% Triton X-100 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) in PBS for 1 h at RT. Sections were then incubated with primary antibodies (c-Fos (guinea pig anti-c-Fos; 1:500, Synaptic Systems) and glutamic acid decarboxylase 67 (GAD67) (mouse anti-GAD67; 1:500, Merck)) overnight at RT. The incubation buffer contained 5% NDS and 0.1% acetylated bovine serum albumin (BSA-c; Aurion). 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, 1:500, Jackson ImmunoResearch; donkey anti-mouse Alexa Fluor 488, 1:500, Thermo Fisher) for 3 h at RT. All procedures starting from the secondary antibody incubation onwards were performed in the dark. Then, sections were briefly rinsed and incubated with 4’,6-diamidine-2’-phenylindole dihydrochloride (DAPI, 1:5,000) in PBS for 15 min at RT, washed three times in PBS for 10 min per wash, mounted on gelatin-coated slides, air-dried and coverslipped with FluoroSave mounting medium (Sigma-Aldrich).

### Imaging and quantification

Fluorescent images of the regions of interest (ROIs) were taken at 20× magnification using an automated high-content fluorescence microscope (Leica DMI 6000B, Germany) and image processing was performed in FIJI (NIH, version 1.0) (Schindelin *et al.*, 2012). First, tiles of images were corrected for background and signal bleaching by the BaSiC plugin (Peng *et al.*, 2017), and were stitched to a single image by the Grid/StitchCollection plugin in FIJI. Then, a set of ROIs for each brain region was created based on the Allen Mouse

Brain Atlas (https://portal.brain-map.org/) (Figure S1). For the agranular subdivisions of the aIC (agranular insular cortex dorsal part (AID) and agranular insular cortex ventral part (AIV)) and the PRh, two squared areas (200 × 200 μm) were selected to cover layers II/III and layers V/VI, respectively. For the BLA, a circular area (600 μm in diameter) was selected. Within each ROI, the number of cells showing expression of tdTomato, c-Fos or GAD67 was counted manually. To analyze the relatively low co-expression of markers, double-stained neurons were counted within the whole areas of layers II/III and layers V/VI of the AID, AIV and PRh as well as within the BLA. The areas were drawn along the border of each layer using the freehand selection tool in FIJI, and the number of cells showing expression of each of the markers was converted to number of cells per $mm^2$.

### Statistics

Data are expressed as mean ± SEM. The $DI_{dissimilar}$ and $DI_{similar}$ were analyzed with a three-way mixed ANOVA with drug condition (saline and yohimbine) and retention interval (3 and 7 days) as between-subject variables, and type of object ($DI_{dissimilar}$ and $DI_{similar}$) as within-subject variable. Follow-up testing of the discrimination indexes at each retention interval separately was performed with a two-way mixed ANOVA with drug condition as between-subject variable and type of object as within-subject variable. Total exploration time of the objects and total distance moved were analyzed with two-way ANOVAs with drug condition and retention interval as between-subject variables. Tukey *post-hoc* *t*-tests (correcting for two comparisons for the DI) were used to determine the source of the significance in the ANOVAs. One-sample *t*-tests were used to determine whether the $DI_{dissimilar}$ or $DI_{similar}$ differed from zero (i.e. chance level).

Based on our behavioral observations, the re-exposure session was only performed at the 3-day retention interval, and immunohistochemistry data were analyzed with a mixed ANOVA with drug condition and (re-)exposed object (familiar, similar and dissimilar) as between-subject variables, and cortical layers (layers II/III and layers V/VI) (for the AID, AIV and PRh) as within-subject variables. When appropriate, Tukey *post-hoc* analyses (correcting for two comparisons for layer, and three comparisons for type of object) were used to determine the source of the significance in the ANOVAs. Main effects of layer were followed up by paired *t*-tests. For all statistical tests, $p < 0.05$ was accepted for statistical significance. The number of mice per group is indicated in the figure legends.

## Experiment 2

### Animals

A total of 53 male wild-type CB57BL/6J mice (11 weeks old at the time of the behavioral experiments) from Charles River Breeding Laboratories (Kisslegg, Germany) were used. Animals were single housed upon arrival in a temperature-controlled (22 °C) vivarium room with a regular 12-h/12-h light/dark cycle (lights on between 7:00 and 19:00 h). Mice had *ad libitum* access to food and water. Behavioral training and testing was performed during the light phase of the diurnal cycle, between 10:00 and 15:00 h. 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

All viral vectors were purchased from Addgene (Cambridge, MA, USA). For virus delivery, 8-week-old 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-hSyn-DIO-hM4D(Gi)-mCherry (600 nL, $2.3\times10^{13}$ GC/mL; Cat# 44362-AAV9) or its control virus AAV9-hSyn-DIO-mCherry (600 nL, $2.1\times10^{13}$ GC/mL; Cat# 50459-AAV9) was delivered bilaterally into the BLA (from Bregma: AP, -1.10 mm; mediolateral (ML), ±3.20 mm; dorsoventral (DV), –4.65 mm) using a 10-µL microsyringe with a 26 G needle (Nanofil; WPI, Sarasota, FL, USA). Additionally, the retrograde virus ENN.AAV.hSyn.HI.eGFP-Cre.WPRE.SV40 (600 nL, $1.17\times10^{13}$ GC/mL; Cat# 105540-AAVrg) was injected bilaterally into the aIC (from Bregma: AP, +1.40 mm; ML, ±3.20 mm; DV, –3.60 mm) to ensure selective hM4D(Gi) expression in aIC-projecting BLA neurons. After the surgery, mice could recover for 14 days to allow sufficient expression of the hM4D(Gi) DREADD receptors (Tervo *et al.*, 2016).

### Object discrimination task

See Experiment 1, except that retention was tested 1 day after the training trial, and no (re-)exposure session was performed.

### Systemic drug administration

Yohimbine administration occurred similar to Experiment 1.

The potent DREADD activator clozapine (0.03 mg/kg; Sigma-Aldrich) was injected to all animals, in a volume of 0.005 mL/g of body weight, intraperitoneally immediately after the training session (Gomez *et al.*, 2017; Zerbi *et al.*, 2019). This low dose of clozapine was selected based on pilot data that it effectively inhibited the BLA-aIC pathway (see: Figure 3C). Clozapine was first dissolved in 1 M hydrochloric acid, and 0.1 M PBS was added to generate a final concentration of 0.006 mg/mL. Drug solutions were freshly prepared before each experiment.

### Verification of viral transfection

The mice were anesthetized with an overdose of sodium pentobarbital and perfused transcardially with ice-cold 0.1 M PBS, pH 7.4, followed by ice-cold 4% PFA. Brains were post-fixed overnight in 4% PFA and then cryoprotected in 30% sucrose for 72 h at 4 °C. Coronal slices of 35-μm thickness were cut at the viral injection sites (aIC; AP, +1.50 to +1.30 mm, and BLA; AP, -1.00 to -1.20 mm), collected in 0.1 M PBS with 0.1% sodium azide, and mounted on gelatin-coated slides, air-dried and coverslipped with FluoroSave mounting medium (Sigma-Aldrich). Fluorescent images were taken at 10× magnification using an automated high-content fluorescence microscope (Leica DMI 6000B, Germany). Successful viral transfection was confirmed by abundant mCherry expression within the BLA in both hemispheres (>50% of the area size).

### Statistics

Data are expressed as mean ± SEM. To verify yohimbine effects in the mice injected with the control virus, the $DI_{dissimilar}$ and $DI_{similar}$ were analyzed using two-way mixed ANOVA with drug condition (saline and yohimbine) as between-subject variable, and type of object as within-subject variable. Next, we tested for the presence of a similar effect of yohimbine in the mice treated with the hM4D(Gi) virus. Critical differences between virus groups were tested using independent samples *t*-tests. Total exploration time of the objects and total distance moved were analyzed with two-way ANOVAs with drug condition and type of DREADD virus as between-subject variables. Tukey *post-hoc t*-tests (correcting for two comparisons for the DI) were used to determine the source of the significance in the ANOVAs. One-sample *t*-tests were used to determine whether the $DI_{dissimilar}$ or $DI_{similar}$ differed from zero (i.e. chance level). For all statistical tests, $p < 0.05$ was accepted for statistical significance. The number of mice per group is indicated in the figure legends.

## Results

## Experiment 1

### Effect of posttraining noradrenergic stimulation in TRAP2 mice on the detailedness of object memory over time

In this first experiment, we aimed at verifying that systemic yohimbine (0.3 mg/kg) administration immediately after object training enhances both the detailedness and longevity of the memory in TRAP2 × tdTomato double transgenic mice, as well as determining the neural correlates of this effect. As a minimum of 72 h after the 4-OHT injection is required to induce sufficient expression of tdTomato in activated cells (Guenthner *et al.*, 2013), retention was tested, in separate groups of animals, either at 3 days or 7 days after the training session (Figure 1A). Posttraining drug treatment groups did not differ in total object exploration time or total distance moved during the training session (both $p$'s > 0.20, Table S1).

A three-way mixed ANOVA for the two discrimination indexes at retention testing indicated significant main effects of drug condition ($F_{(1,56)} = 7.80$, $p = 0.007$) and type of object ($F_{(1,56)} = 4.46$, $p = 0.04$), as well as a significant drug condition × type of object × retention interval interaction effect ($F_{(1,56)} = 6.02$, $p = 0.02$). All other effects were not significant (all $p$'s > 0.10). Two-way ANOVAs for the total object exploration time and total distance moved during the retention test revealed no significant effects of drug condition, retention interval, or drug condition × retention interval interaction (all $p$'s > 0.06, Figure S2). To follow up on the three-way interaction effect, we investigated the effect of yohimbine treatment on the two discrimination indexes at the 3-day and 7-day retention intervals separately.

At the 3-day retention test, a two-way mixed ANOVA for the two discrimination indexes indicated a significant main effect of drug condition ($F_{(1,27)} = 4.41$, $p = 0.045$) and a significant drug condition × type of object interaction effect ($F_{(1,27)} = 4.60$, $p = 0.04$). There was no significant main effect of type of object ($F_{(1,27)} = 2.10$, $p = 0.16$). The $DI_{dissimilar}$ of mice treated with yohimbine did not differ significantly from that of saline-treated animals (independent samples *t*-test: $t_{(27)} = 0.92$, $p_{corr} = 0.37$, Figure 1B), with the $DI_{dissimilar}$ of both drug condition groups being significantly greater than zero (i.e., chance level) (saline: $t_{(14)} = 3.31$, $p = 0.005$; yohimbine: $t_{(13)} = 3.68$, $p = 0.003$). These findings indicate that both saline and yohimbine-treated mice identified the dissimilar object as a novel object. However, we found a significant effect of yohimbine treatment on the mice' ability to identify the similar object as a novel object (independent samples *t*-test: $t_{(27)} = 2.83$, $p_{corr} = 0.009$, Figure 1B). The $DI_{similar}$ of mice treated with saline did not differ from chance level ($t_{(14)} = 0.69$, $p = 0.50$), whereas

the $DI_{similar}$ of mice treated with yohimbine was significantly higher than chance ($t_{(13)} = 3.98$, $p = 0.002$). These findings thus indicate that mice treated with saline were able to discriminate the dissimilar, but not similar, object from the familiar object at the 3-day retention test, whereas mice treated with yohimbine were able to also discriminate the similar object from the familiar object, reflecting a more detailed memory of the training object.

At the 7-day retention test, a two-way mixed ANOVA for the two discrimination indexes indicated no significant main effects of drug condition ($F_{(1,27)} = 3.39$, $p = 0.08$) or type of object ($F_{(1,27)} = 2.37$, $p = 0.14$), and no drug condition × type of object interaction effect ($F_{(1,27)} = 1.73$, $p = 0.20$). The $DI_{dissimilar}$ of mice treated with saline was no longer different from zero ($t_{(14)} = 1.01$, $p = 0.33$, Figure 1B), whereas that of mice treated with yohimbine was still significantly greater than chance level ($t_{(13)} = 3.22$, $p = 0.007$). At this time point, the $DI_{similar}$ of none of the two groups differed from zero (saline: $t_{(14)} = 1.89$, $p = 0.36$; yohimbine: $t_{(13)} = 2.03$, $p = 0.11$), indicating that yohimbine was not able to maintain the detailedness of object memory after 7 days.



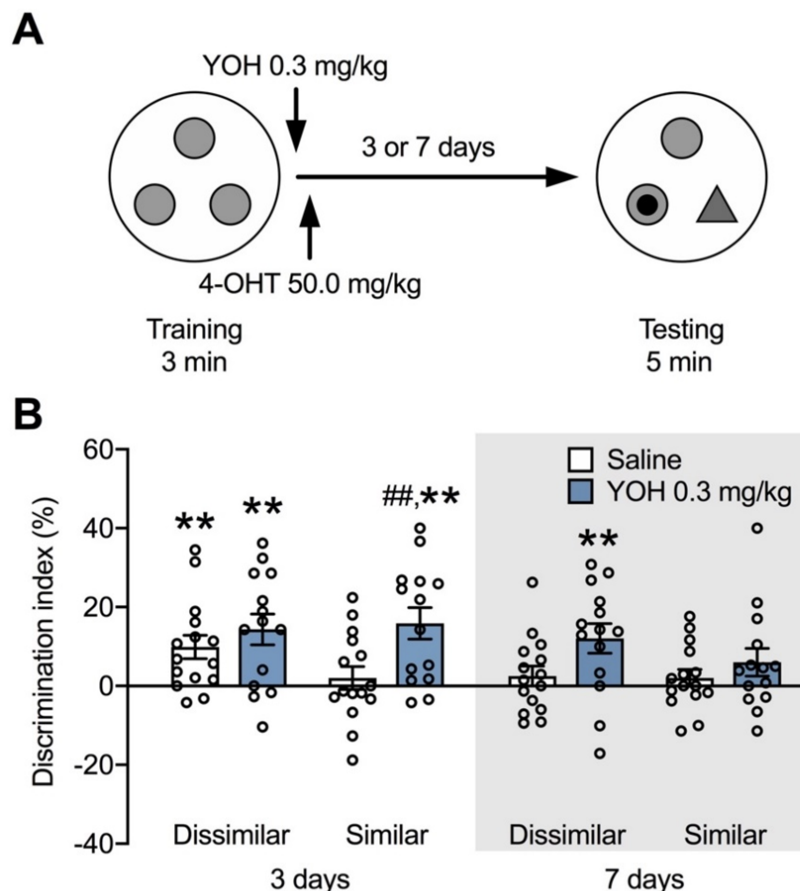


**Figure 1. Effect of posttraining noradrenergic activation in TRAP2 mice on the detailedness of object memory over time**. **A,** Experimental design of the object discrimination memory task. Mice could freely explore three identical objects for 3 min. An immediate posttraining injection of yohimbine (YOH, 0.3 mg/kg) or saline was given intraperitoneally, followed by an intraperitoneal injection of 4-OHT (50.0 mg/kg). Retention was tested, in separate groups of animals, 3 days or 7 days later. On the retention test, one object was identical to the training object (i.e., familiar object), one novel object was highly similar to the training object and another novel object was dissimilar to the training object. **B,** At the 3-day retention test, saline-treated mice discriminated the dissimilar, but not similar, object from the familiar one, whereas mice treated with yohimbine were able to discriminate both the dissimilar and similar object from the familiar object (Saline: $n = 15$, YOH: $n = 14$). At 7 days, mice treated with saline no longer discriminated the dissimilar or similar object from the familiar object, whereas mice treated with yohimbine continued to display discrimination of the dissimilar, but not similar, object (Saline: $n = 15$, YOH: $n = 14$). Data are shown as mean ± SEM, dots represent individual data points. ** $p < 0.01$ vs. chance level; ## $p_{corr} < 0.01$ vs. saline group.

## Effect of posttraining noradrenergic stimulation on training-induced and (re-)exposure-induced neuronal activity

In Chapter 3, we found that posttraining yohimbine administration increased retention-induced c-Fos expression within both the aIC (AID and AIV) and PRh. However, we could not determine by the detection of which object (i.e., the familiar, similar or dissimilar object) this activation was induced. As such, we were unable to determine whether it was related to successful detection of familiar features (i.e., memory recall of the familiar object) or the detection of novelty in case of the similar or dissimilar objects. Therefore, in this experiment we examined retention-induced neuronal activity within these ROIs, as well as the BLA, after (re-)exposure to only a single type of object (Figure 2A). Moreover, we included an additional labeling of neurons activated during the training and (early) consolidation period (by tdTomato) next to the labeling of neurons activated by the (re-)exposure session (marked by c-Fos) to examine whether yohimbine treatment affected the reactivation rate of the neurons involved in storing the memory (Figure 2B). As our behavioral findings indicated that the effect of yohimbine treatment on memory detailedness was limited to the 3-day retention test, we only performed the object (re-)exposure session at the 3-day interval.

During the (re-)exposure session, animals were allowed to explore either three familiar objects, three similar objects, or three dissimilar objects for 5 min. Two-way ANOVAs for total object exploration time and total distance moved revealed no significant effects of drug condition, type of (re-)exposed object, or drug condition × type of (re-)exposed object interaction effect during the training (all $p$'s > 0.14, Table S2) or (re-)exposure session (all $p$'s > 0.22, Figure S3). These data indicate that during the (re-)exposure session, all groups spent a comparable time exploring the objects, regardless of their drug administration or the

type of object they were (re-)exposed to, thus excluding the possibility that differences in neuronal activity were caused by the amount of object exploration as such.

### *Training-induced neuronal activity*

To determine training/early consolidation-induced neuronal activity, we assessed the number of tdTomato-positive cells in the aIC (AID and AIV), PRh and BLA (Figure S1). For the cortical (sub)regions (AID, AIV and PRh), we assessed the number of tdTomato-positive cells separately within the input (layers II/III) and output (layers V/VI) areas.

For the AID, a mixed ANOVA for the number of tdTomato-positive cells revealed a significant drug condition × layer interaction effect ($F_{(1,58)} = 8.71$, $p = 0.005$), but no main effect of drug condition or layer (both *p*'s > 0.08). The interaction effect indicated that the yohimbine affected tdTomato expression in layers II/III and layers V/VI in a different manner. However, Tukey's *post-hoc* tests did not reveal any significant drug condition effect within each separate layer (both *p*'s > 0.13, Figure 2C).



For the AIV, we found a significant main effect of layer ($F_{(1,58)} = 12.66$, $p < 0.001$), but no main effect of drug condition or drug condition × layer interaction effect (both *p*'s > 0.12, Figure 2C).
Overall, higher tdTomato expression was observed in layers II/III compared to layers V/VI (paired *t*-test: $t_{(59)} = 3.53$, $p < 0.001$).

For the PRh, we found no significant main effect of drug condition or layer, and no drug condition × layer interaction effect (all *p*'s > 0.13, Figure 2C).

For the BLA, there was no main effect of drug condition either ($p = 0.89$, Figure 2C).

### *(Re-)exposure-induced neuronal activity*

Next, we examined the (re-)exposure-induced c-Fos expression within these different brain regions.

For the AID, we found significant main effects of drug condition ($F_{(1,54)} = 40.62$, $p < 0.001$), type of (re-)exposed object ($F_{(2,54)} = 7.14$, $p = 0.002$) and layer ($F_{(1,54)} = 9.37$, $p = 0.003$). All other effects were not significant (all *p*'s > 0.10). Overall, higher c-Fos expression was observed in layers II/III compared to layers V/VI (paired *t*-test: $t_{(59)} = 3.15$, $p = 0.003$). Exploratory analyses revealed that yohimbine-treated animals had significantly more c-Fos-

expressing cells in response to the familiar (layers II/III: $p_{corr} = 0.007$, layers V/VI: $p_{corr} = 0.02$), and similar object (layers II/III: $p_{corr} < 0.001$, layers V/VI: $p_{corr} < 0.001$), whereas no significant effect of yohimbine treatment was observed in response to the dissimilar object (layers II/III: $p_{corr} = 0.10$, layers V/VI: $p_{corr} = 0.09$). This made that in the saline condition there was no significant main effect of type of (re-)exposed object (layers II/III: $F_{(2,27)} = 0.66$, $p = 0.53$; layers V/VI: $F_{(2,27)} = 1.03$, $p = 0.37$), which was present in the yohimbine condition (layers II/III: $F_{(2,27)} = 3.90$, $p = 0.03$; layers V/VI: $F_{(2,27)} = 3.40$, $p = 0.048$). Tukey's *post-hoc* tests revealed that yohimbine-treated animals that were exposed to the familiar object had significantly more c-Fos expression compared to yohimbine-treated animals that were exposed to the dissimilar object (layers II/III: $p_{corr} = 0.05$; layers V/VI: $p_{corr} = 0.045$, Figure 2D).

For the AIV, we found significant main effects of drug condition ($F_{(1,54)} = 89.01$, $p < 0.001$), type of (re-)exposed object ($F_{(2,54)} = 23.40$, $p < 0.001$) and layer ($F_{(1,54)} = 99.40$, $p < 0.001$), as well as interaction effects for drug condition × layer ($F_{(1,54)} = 12.87$, $p = 0.001$), drug condition × type of (re-)exposed object ($F_{(2,54)} = 15.59$, $p = 0.001$), type of (re-)exposed object × layer ($F_{(2,54)} = 12.76$, $p < 0.001$), and drug condition × type of (re-)exposed object × layer ($F_{(2,54)} = 6.89$, $p = 0.002$). Similar to the AID, yohimbine-treated animals that were exposed to either the familiar or similar object had significantly more c-Fos-expressing cells in the AIV than saline-treated animals that were exposed to these objects (familiar: layers II/III: $p_{corr} < 0.001$, layers V/VI: $p_{corr} = 0.002$; similar: layers II/III: $p_{corr} < 0.001$; layers V/VI: $p_{corr} < 0.001$). In contrast, yohimbine treatment did not increase the number of c-Fos-expressing cells in animals that were exposed to the dissimilar object compared to corresponding saline-treated animals (layers II/III: $p_{corr} = 0.23$, layers V/VI: $p_{corr} = 0.16$). In the saline condition, there was no significant main effect of type of (re-)exposed object (layers II/III: $F_{(2,27)} = 3.30$, $p = 0.05$; layers V/VI: $F_{(2,27)} = 0.54$, $p = 0.59$), which was observed in the yohimbine condition (layers II/III: $F_{(2,27)} = 27.15$, $p < 0.001$; layers V/VI: $F_{(2,27)} = 4.28$, $p = 0.02$). Tukey's *post-hoc* tests revealed that yohimbine-treated mice that were re-exposed to the familiar object had significantly more c-Fos expressing cells than yohimbine-treated mice that were exposed to the similar (layers II/II: $p_{corr} = 0.01$; layers V/VI: $p_{corr} = 0.98$, Figure 2D) or dissimilar object (layers II/III: $p_{corr} = 0.001$; layers V/VI: $p_{corr} = 0.04$). Moreover, yohimbine-treated mice that were exposed to the similar object had significantly more c-Fos-expressing cells than yohimbine-treated mice that were exposed to the dissimilar object (layers II/III: $p_{corr} < 0.001$; layers V/VI: $p_{corr} = 0.06$). Significant interactions with layer seemed to be caused by most prominent effects of yohimbine on neuronal responses to the familiar and similar object in layers II/III.

For the PRh, we found significant main effects of drug condition ($F_{(1,54)} = 87.27$, $p < 0.001$) and type of (re-)exposed object ($F_{(2,54)} = 40.97$, $p < 0.001$), as well as interaction effects for drug condition × type of (re-)exposed object ($F_{(2,54)} = 23.36$, $p < 0.001$), and drug condition × type of (re-)exposed object × layer ($F_{(2,54)} = 3.62$, $p = 0.03$). All other effects were not significant (all $p$'s > 0.05). Comparison between drug conditions indicated that yohimbine-treated animals that were exposed to either the dissimilar or similar object had significantly more c-Fos-expressing cells than saline-treated animals that were exposed to these objects (all $p_{corr}$'s < 0.001). In contrast, yohimbine treatment did not increase the number of c-Fos-expressing cells in animals that were re-exposed to the familiar object (layers II/III: $p_{corr}$ = 0.53, layers V/VI: $p_{corr}$ = 0.24). Tukey's *post-hoc* tests revealed that saline-treated animals that were exposed to the dissimilar object had significantly more c-Fos expression than saline-treated animals that were exposed to either the familiar (layers II/III: $p_{corr}$ = 0.01; layers V/VI: $p_{corr}$ = 0.04; Figure 2D) or similar object (layers II/III: $p_{corr}$ = 0.01; layers V/VI: $p_{corr}$ = 0.09), whereas yohimbine-treated animals that were exposed to either the dissimilar or similar object had significantly more c-Fos expression than yohimbine-treated animals that were exposed to the familiar object (all $p_{corr}$'s < 0.001). Comparisons between layers revealed that yohimbine-treated animals that were exposed to the similar object had significantly more c-Fos expression in layers V/VI than in layers II/III ($p_{corr}$ = 0.03). No significant differences between layers were found in yohimbine-treated animals that were exposed to either the familiar or dissimilar object, nor in saline-treated animals after exposure to any of the three objects (all $p$'s > 0.34).

For the BLA, we found no effect of either drug condition or type of (re-)exposed object on the number of c-Fos-positive cells (all $p$'s > 0.10, Figure 2D).

### *Reactivation rate between training-induced and (re-)exposure-induced neuronal activity*

To further examine whether yohimbine treatment affected the recruitment of the same neurons during memory consolidation and recall, we investigated the reactivation rate of tdTomato- and c-Fos-positive cells, which was defined as the number of neurons double positive for tdTomato and c-Fos, divided by the total number of tdTomato-positive cells (Kitamura *et al.*, 2017; DeNardo *et al.*, 2019).

For the AID, we found a significant main effect of drug condition ($F_{(1,54)} = 7.55$, $p = 0.008$). All other effects were not significant (all $p$'s > 0.13). However, exploratory Tukey's *post-hoc*

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tests did not reveal any significant drug condition effect within each separate layer for each of the objects (all $p$'s > 0.07, Figure 2E).

For the AIV, we found a significant main effect of drug condition ($F_{(1,54)} = 19.77$, $p < 0.001$) and type of (re-)exposed object ($F_{(2,54)} = 3.87$, $p = 0.03$), as well as interaction effects for drug condition × layer ($F_{(2,54)} = 4.77$, $p = 0.03$), and drug condition × type of (re-)exposed object ($F_{(2,54)} = 3.21$, $p = 0.048$). All other effects were not significant (all $p$'s > 0.16). Tukey's *post-hoc* tests revealed that the yohimbine treatment significantly increased the reactivation rate in animals that were exposed to either the familiar (layers II/III: $p_{corr} = 0.007$; layers V/VI: $p_{corr} = 0.03$) or similar object (layers II/III: $p_{corr} = 0.02$; layers V/VI: $p_{corr} = 0.08$), but not to the dissimilar object (layers II/III: $p_{corr} = 0.13$; layers V/VI: $p_{corr} = 0.48$) compared to that of corresponding saline-treated animals (Figure 2E). In the saline condition, there was no significant main effect of type of (re-)exposed object (layers II/III: $F_{(2,27)} = 0.18$, $p = 0.83$; layers V/VI: $F_{(2,27)} = 0.04$, $p = 0.96$), which was observed in the yohimbine condition (layers II/III: $F_{(2,27)} = 2,43$, $p = 0.10$; layers V/VI: $F_{(2,27)} = 4.22$, $p = 0.03$). Tukey's *post-hoc* tests revealed that yohimbine-treated mice that were exposed to the familiar object had a significantly higher reactivation rate than yohimbine-treated mice that were exposed to the dissimilar object (layers II/II: $p_{corr} = 0.72$; layers V/VI: $p_{corr} = 0.03$), but not similar object (layers II/III: $p_{corr} = 0.35$; layers V/VI: $p_{corr} = 0.89$). There was no difference in the reactivation rate between yohimbine-treated mice that were exposed to the similar object and those exposed to the dissimilar object (layers II/III: $p_{corr} =0.09$; layers V/VI: $p_{corr} = 0.08$). The significant drug condition × layer interaction seemed to be caused by most prominent effects of yohimbine on layers II/III.

For the PRh, we found significant main effects of drug condition ($F_{(1,54)} = 5.53$, $p = 0.02$) and layer ($F_{(1,54)} = 7.19$, $p = 0.01$). All other effects were not significant (all $p$'s > 0.13). Overall, a higher reactivation rate was observed in layers II/III compared to layers V/VI (paired $t$-test: $t_{(59)} = 2.77$, $p = 0.008$). Exploratory Tukey's *post-hoc* tests revealed that yohimbine significantly increased the reactivation rate in animals that were exposed to the dissimilar object (layers II/III: $p_{corr} = 0.24$; layers V/VI: $p_{corr} = 0.03$), whereas effects in response to the familiar (layers II/III: $p_{corr} = 0.62$; layers V/VI: $p_{corr} = 0.51$) or similar (layers II/III: $p_{corr} = 0.24$; layers V/VI: $p_{corr} = 0.64$) objects were non-significant (Figure 2E).

For the BLA, we found a significant interaction effect for drug condition × type of (re-)exposed object ($F_{(2,54)} = 5.51$, $p = 0.048$). All other effects were not significant (all $p$'s > 0.08). Following up on the interaction effect, Tukey's *post-hoc* tests revealed that this was

driven by a significant effect of yohimbine treatment on the reactivation rate in response to the similar object ($p_{corr}$ = 0.003), but not familiar ($p_{corr}$ = 0.99) or dissimilar object ($p_{corr}$ = 0.78) compared to that of corresponding saline-treated animals (Figure 2E). The yohimbine-treated group displayed a significant effect of type of object ($F_{(2,27)}$ = 4.24, $p$ = 0.03), with the reactivation rate in animals that were exposed to the similar object being significantly higher than that in response to the familiar object ($p_{corr}$ = 0.04), and a near to significant difference to the response to the dissimilar object ($p_{corr}$ = 0.06). Reactivation rates in animals that were exposed to the familiar object and dissimilar object did not differ from each other ($p_{corr}$ = 0.97). Saline-treated mice did not display any differences in reactivation rate to the different objects ($F_{(2,27)}$ = 0.43, $p$ = 0.65).

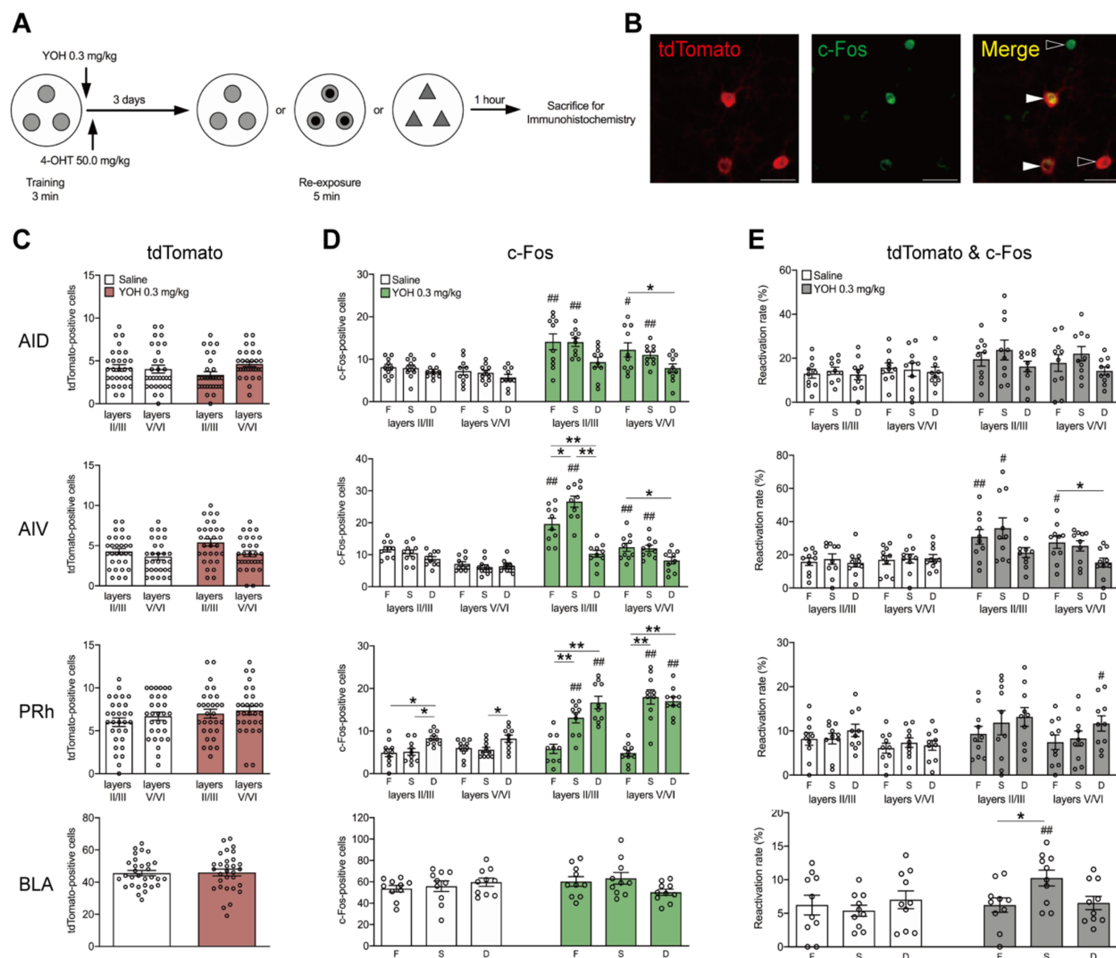


**Figure 2. Effect of posttraining noradrenergic activation in TRAP2 mice on training-induced and (re-)exposure-induced neuronal activity**. **A,** Experimental design of the object (re-)exposure task. Mice could freely explore three identical objects for 3 min. An immediate posttraining injection of yohimbine (YOH, 0.3 mg/kg) or saline was given intraperitoneally, followed by an intraperitoneal injection of 4-OHT (50.0 mg/kg). (Re-)exposure occurred 3 days later, during which mice were (re-)exposed to three objects that were either

identical to the training object (i.e., familiar object), three novel objects that were highly similar to the training object or three novel objects that were dissimilar to the training object. **B,** Representative images for double staining of tdTomato (red) and c-Fos (green) labeled neurons activated during training/consolidation and memory recall, respectively. Scale bar = 20 μm. White arrows point to double-labeled neurons, whereas open arrows point to single-labeled neurons. **C,** The number of tdTomato-positive cells within the agranular insular cortex dorsal part (AID), agranular insular cortex ventral part (AIV), perirhinal cortex (PRh) and basolateral amygdala (BLA) was not affected by yohimbine administration (Saline: $n$ = 30, YOH: $n$ = 30). **D,** Yohimbine administration increased the number of c-Fos-positive cells within layers II/III and layers V/VI of the AID and the AIV when animals were exposed to the familiar and similar, but not dissimilar, objects. Yohimbine administration increased the number of c-Fos-positive cells within layers II/III and layers V/VI of the PRh when animals were exposed to the similar and dissimilar, but not familiar, objects. Yohimbine administration did not affect the number of c-Fos-positive cells in the BLA (familiar object (F), similar object (S), dissimilar object (D); Saline-F: $n$ = 10, Saline-S: $n$ = 10, Saline-D: $n$ = 10, YOH-F: $n$ = 10, YOH-S: $n$ = 10, YOH-D: $n$ = 10). **E,** Yohimbine administration increased the reactivation rate (the number of neurons double positive for tdTomato and c-Fos, divided by the total number of tdTomato-positive cells) within layers II/III and layers V/VI of the AIV when animals were exposed to the familiar or similar, but not dissimilar, objects. Yohimbine administration increased the reactivation rate within layers V/VI of the PRh when animals were exposed to the dissimilar, but not similar or familiar, objects. Yohimbine administration increased the reactivation rate within the BLA when animals were exposed to similar object only. (Saline-F: $n$ = 10, Saline-S: $n$ = 10, Saline-D: $n$ = 10, YOH-F: $n$ = 10, YOH-S: $n$ = 10, YOH-D: $n$ = 10). Data are shown as mean ± SEM, dots represent individual data points. * $p_{corr} < 0.05$, ** $p_{corr} < 0.01$ between (re-)exposed objects; # $p_{corr} < 0.05$, ## $p_{corr} < 0.01$ vs. corresponding saline group. N.B. Significant differences between layers are not indicated, but only mentioned in the text.

### *Low expression levels of tdTomato and c-Fos in GABAergic neurons*

Lastly, we investigated whether changes in the number of tdTomato- or c-Fos-positive cells reflected a change in excitatory or inhibitory neuronal activity. For this, we counted the number of cells that showed co-expression of tdTomato or c-Fos with GAD67, a marker for GABAergic inhibitory neurons (Ito *et al.*, 2015) (Figure S1). Neuronal activation appeared to be almost exclusively restricted to excitatory, i.e., GAD67-negative, cells, with GAD67 co-expression being extremely sparse for both of the activity markers (average of 1% of the activated cells). Moreover, for none of the brain regions, significant changes in the number of cells that showed tdTomato-GAD67, c-Fos-GAD67 or tdTomato-c-Fos-GAD67 co-expression were observed (all $p$'s > 0.66; data not shown). Additionally, relative inhibitory tone, calculated as the number of cells showing co-expression of either tdTomato with GAD67 or of c-Fos with GAD67, divided by the total number of cells expression either tdTomato or c-Fos, was overall very low (<1%) and not affected by yohimbine administration or type of (re-)exposed object (all $p$'s > 0.34; data not shown). Thus, these findings suggest that the changes in the number of cells expressing tdTomato and/or c-Fos described above likely reflect an increase in excitatory activity.

## Experiment 2

### Effect of posttraining silencing of the BLA-aIC pathway on noradrenergic activation induced enhanced detailedness of object memory

Given the evidence that noradrenergic effects on object recognition memory critically depend on BLA interactions with the aIC (Chen *et al.*, 2018), we next examined whether selectively silencing the BLA-aIC pathway (Figure 3A) during the memory consolidation period by means of DREADD would block the effect of yohimbine administration on enhanced memory detailedness. The low dose of clozapine (0.03 mg/kg) we used in our experiment effectively inhibited activity of the BLA-aIC pathway in animals expressing hM4D(Gi), reported by a significant lower c-Fos expression in aIC-projecting BLA (mCherry-expressing) neurons 1 h after object discrimination training and posttraining yohimbine administration (independent samples *t*-test: $t_{(5)} = 7.68$, $p < 0.001$ vs control virus; Figure 3C).

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Two-way ANOVAs for total object exploration time and total distance moved revealed no significant effects of drug condition, type of DREADD virus, or drug condition × type of DREADD virus interaction effect during the training (all $p$'s > 0.86, Table S3) or retention test (all $p$'s > 0.80, Figure S4).

In mice treated with the control virus, a two-way mixed ANOVA for the two discrimination indexes verified a significant main effect of type of object ($F_{(1,21)} = 9.70$, $p < 0.001$), as well as a significant drug condition × type of object interaction effect ($F_{(1,21)} = 5.66$, $p = 0.03$), without a main effect of drug condition ($F_{(1,21)} = 2.06$, $p = 0.17$, Figure 3E). Similar to what was observed in Experiment 1, the $DI_{dissimilar}$ of mice treated with yohimbine did not differ significantly from that of saline-treated animals (independent samples *t*-test: $t_{(21)} = 0.14$, $p$ = 0.89), with both drug condition groups displaying a $DI_{dissimilar}$ that was significantly greater than zero (saline: $t_{(11)} = 3.91$, $p = 0.003$; yohimbine: $t_{(10)} = 3.22$, $p = 0.009$), indicating that mice of both treatment groups identified the dissimilar object as a novel object. However, again, a significant effect of yohimbine treatment on the mice' ability to identify the similar object as novel object was observed (independent samples *t*-test: $t_{(21)} = 2.43$, $p = 0.02$,). Whereas the $DI_{similar}$ of mice treated with saline did not differ from chance level ($t_{(11)} = 0.31$, $p = 0.76$), the $DI_{similar}$ of mice treated with yohimbine was significantly greater than zero ($t_{(10)}$ = 3.16, $p = 0.01$). These findings thus verified that the yohimbine treatment induced a more detailed memory of the training object in mice treated with the control virus.

Next, we examined whether we could block this yohimbine effect on memory detailedness by silencing the BLA-aIC pathway posttraining. A two-way mixed ANOVA for the two discrimination indexes indicated a significant main effect of type of object ($F_{(1,21)}$ = 10.00, $p$ = 0.005), but no significant main effects of drug condition ($F_{(1,21)}$ = 0.14, $p$ = 0.72) or significant drug condition × type of object interaction effect ($F_{(1,21)} < 0.001$, $p$ = 1.00, Figure 3E). In this group, neither the $DI_{dissimilar}$ (independent samples *t*-test: $t_{(21)}$ = 0.38, $p$ = 0.71) nor $DI_{similar}$ (independent samples *t*-test: $t_{(21)}$ = 0.26, $p$ = 0.80) of mice treated with yohimbine differed significantly from that of saline-treated mice. Further, one-sample *t*-tests indicated that the $DI_{dissimilar}$ of mice of both drug condition groups was significantly greater than zero (saline: $t_{(11)}$ = 2.64, $p$ = 0.02; yohimibine: $t_{(10)}$ = 3.00, $p$ = 0.01), whereas the $DI_{similar}$ of both mice treated with saline or yohimbine did not differ from chance (saline: $t_{(11)}$ = 0.17, $p$ = 0.87; yohimbine: $t_{(10)}$ = 0.48, $p$ = 0.64). Comparison between viruses indicated a significant effect of DREADD inhibition on the $DI_{similar}$ of mice treated with yohimbine ($t_{(21)}$ = 2.13, $p$ = 0.04), with no other significant differences between both virus conditions (all *p*'s > 0.34). These findings thus revealed that the DREADD inhibition of the BLA-aIC pathway selectively impaired the mice' ability to discriminate the similar, but not the dissimilar, object from the familiar one, under yohimbine treatment.

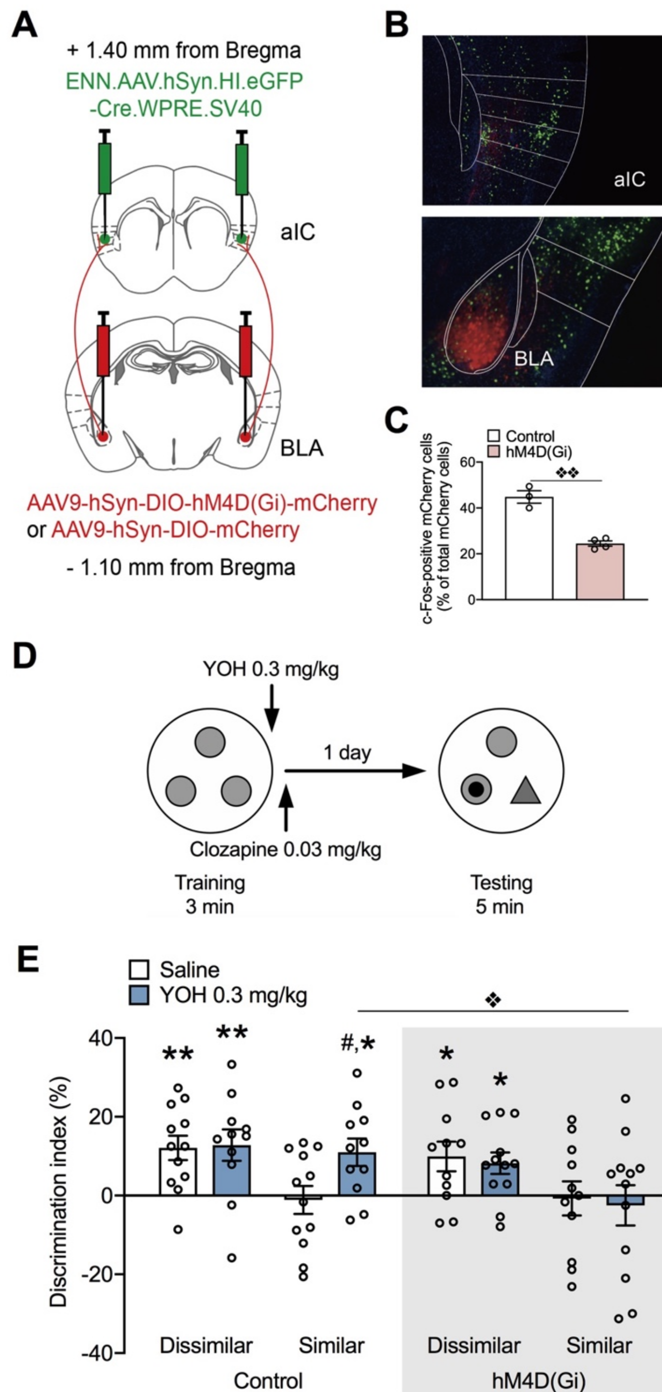


**Figure 3. Silencing of the basolateral amygdala – anterior insular cortex pathway blocks the effect of posttraining noradrenergic activation on the detailedness of object memory**. **A,** To ensure selective hM4D(Gi) expression in basolateral amygdala (BLA) neurons projecting to the anterior insular cortex (aIC), AAVrg.hSyn.HI.eGFP-Cre.WPRE.SV40 Cre-expressing retrograde virus was injected bilaterally into the aIC, and Cre-dependent AAV9-hSyn-DIO-hM4D(Gi)-mCherry or its control virus AAV9-hSyn-DIO-mCherry was delivered bilaterally into the BLA. **B,** Example images demonstrating aIC-projecting BLA neurons expressing Cre-dependent hM4D(Gi) containing mCherry (red), and neurons expressing the Cre-expressing retrograde virus

containing EGFP that was injected in the aIC (green). **C,** The low dose of clozapine (0.03 mg/kg) effectively inhibited activity of the BLA-aIC pathway in animals expressing hM4D(Gi) reported by a significant lower c-Fos expression in aIC-projecting BLA (mCherry-expressing) neurons 1 h after object discrimination training and posttraining yohimbine administration (Control: $n$ = 3, hM4D(Gi): $n$ = 4). **D,** Experimental design of the object discrimination task. Mice could freely explore three identical objects for 3 min. An immediate posttraining injection of yohimbine (YOH, 0.3 mg/kg) or saline was given intraperitoneally, followed by an intraperitoneal injection of clozapine (0.03 mg/kg). Retention was tested 1 day later. On the retention test, one object was identical to the training object (i.e., familiar object), one novel object was highly similar to the training object and another novel object was dissimilar to the training object. **E,** In mice expressing the control virus, yohimbine treatment induced accurate discrimination of the similar object relative to the familiar one. However, in mice expressing the hM4D(Gi) virus, this yohimbine-induced discrimination of the similar object was not found. All groups discriminated the dissimilar object relative to the familiar one, which was not affected by yohimbine treatment (Saline-Control: $n$ = 12, YOH-Control: $n$ = 11, Saline-hM4D(Gi): $n$ = 11, YOH-hM4D(Gi): $n$ = 12,). Data are shown as mean ± SEM, dots represent individual data points. * $p < 0.05$, ** $p < 0.01$ vs. chance level; # $p < 0.05$ vs. saline group; ❖ $p < 0.05$, ❖❖ $p < 0.01$ between DREADD virus.

## Discussion

This study was aimed at investigating the neural mechanisms underlying the effect of posttraining systemic yohimbine administration on the enhancement of memory detailedness in an object discrimination task. We first validated that posttraining noradrenergic activation in TRAP2 mice was able to enhance memory detailedness. In an object re-exposure task, we found that the yohimbine treatment generally increased neuronal activation within the aIC after exposure to the familiar object, whereas it increased overall neuronal activation within the PRh after exposure to the dissimilar object. Besides this general effect on retention-induced neuronal activity, a similar effect was observed in terms of reactivation of the neurons originally activated during the learning trial, with yohimbine increasing reactivation in the aIC and PRh in response to the familiar and dissimilar objects, respectively. These findings support the idea that these two brain regions might be involved in the detection of familiarity and novelty information, respectively. Most interestingly, yohimbine increased neuronal activation in both of these brain regions after exposure to the similar-looking novel object, suggesting that the display of memory detailedness requires a coordinated recruitment of the neural circuits implicated in both familiarity and novelty detection. Whereas yohimbine treatment did not affect overall activity in the BLA during either training or (re-)exposure, it increased the reactivation of neurons involved in object learning when exposed to the similar object, indicating that noradrenergic activation increases the activation of a subset of BLA neurons involved in modulating memory consolidation upon detection of a similar object. Finally, we found that inactivation

the BLA-aIC pathway with an inhibitory DREADD manipulation after the training session selectively blocked the enhancing effect of noradrenergic activation on memory detailedness, but that this inactivation did not affect recognition memory *per se*.

Extensive evidence indicates that noradrenergic activation, as induced by emotional arousal, enhances the consolidation of memory processing, including object recognition memory (Ferry *et al.*, 1999; McGaugh, 2004; Roozendaal *et al.*, 2008; Roozendaal & McGaugh, 2011; Song *et al.*, 2020). However, whether such memory enhancement also influences the quality and longevity of such memories remained largely elusive. In Chapter 3, we first examined this question by training and testing mice on an object discrimination task. Our findings indicated that posttraining administration of the noradrenergic stimulant yohimbine enhanced the detailedness of object recognition memory at a 1-day retention test, but that this memory detailedness was progressively lost when animals were tested at 7 and 14 days after the training session. Comparable to those findings, here we show, in TRAP2 mice, that yohimbine administration enhanced memory detailedness at a 3-day retention test. We used TRAP2 mice (Guenthner *et al.*, 2013; DeNardo *et al.*, 2019) in the current study as this enabled us to investigate the effects of noradrenergic enhancement on the neuronal activity during both the consolidation phase and (re-)exposure to a specific object, as well as the overlap between these two neuronal activity patterns. We selected a 3-day retention interval for this study as it takes a minimum of 72 h to induce sufficient expression of tdTomato in activated cells after the 4-OHT injection (Guenthner *et al.*, 2013).

Previous findings in Chapter 3 indicated that posttraining yohimbine administration increased retention-induced c-Fos expression within both the aIC (AID and AIV) and PRh, and that this increased activity positively correlated with the mice' ability to discriminate the similar object, but not dissimilar object. However, in that experiment we were unable to determine whether this increased activation was induced as a result of exploring the familiar, similar, or dissimilar object, and thus the detection of familiarity vs. novelty. Therefore, in this experiment, we examined retention-induced neuronal activity after (re-)exposure to only a single type of object. We found here that tdTomato expression, reflecting neuronal activity during the memory encoding and consolidation phase, was generally very low (i.e., much lower than the number of cFos-expressing cells in response to object (re-)exposure), suggesting a lower sensitivity of this labeling system compared to regular immunohistochemistry, with sub-threshold expression of c-Fos not inducing fluorescent labeling. Moreover, the yohimbine administration did not induce any marked changes in the number of tdTomato-expressing neurons within the three brain regions we investigated.

These findings were rather unexpected as we previously found that a memory-enhancing dose of norepinephrine administered into the BLA after a standard object recognition training session induced a large reduction in neuronal activity within the aIC 1 h later, determined by a decreased number of neurons expressing either the phosphorylated form of the transcription factor cAMP response element-binding (pCREB) protein or the neuronal activity marker c-Fos (Chen *et al*., unpublished findings). In addition, previous rat studies indicated that neuronal activity within the PRh is increased in response to the presentation of novel visual stimuli (Wan *et al.*, 1999) or an episodic-like object recognition task (VanElzakker *et al.*, 2008; Barbosa *et al.*, 2013). Moreover, human neuroimaging studies have reported on higher PRh activity during the encoding of items that were subsequently remembered (Bisby *et al.*, 2016). As previous findings have suggested a dynamic regulation of neuronal activity following stress hormone administration, with activity in the aIC initially being increased in response to salience and emotional arousal, but suppressed in its later aftermath (Hermans *et al.*, 2014), the time window during which neuronal activity is labeled in these transgenic mice (up to 5-6 h post 4-OHT injection), might be too long to detect such transient changes.

However, yohimbine administration induced some very interesting and novel findings with respect to the pattern of neuronal activity in response to the object (re-)exposure session. In saline-treated animals, we found no differences in neuronal activity within the aIC in response to exposure to the three different objects, whereas neuronal activity within the PRh was higher in response to the dissimilar object compared to the familiar or similar object. This finding is thus in concordance with our behavioral results indicating that saline-treated animals were able to successfully discriminate the dissimilar, but not similar, object from the familiar one. In yohimbine-treated animals we however found that neuronal activity was significantly higher within the aIC in response to the familiar object, whereas neuronal activity within the PRh was higher in response to the dissimilar object. These findings thus strongly suggest that the aIC and PRh might play a different role in the detection of familiarity and novelty information, respectively. Extensive evidence indicates that both the aIC and PRh are critically involved in object recognition memory (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 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), but only a few studies have investigated their role in specific aspects of recognition memory. One study revealed that the PRh might be involved in the discrimination of overlapping representations in object recognition memory via a cellular process that resembles pattern separation, and that such mechanisms are only recruited

when discrimination of similar objects is required (Miranda *et al.*, 2017). Moreover, highly consistent with our present observation, findings of a human neuroimaging study indicated that the aIC is one of the brain structures which activity is increased with familiarity strength, whereas the PRh is involved in novelty detection (Kafkas & Montaldi, 2014). In support for a role of the aIC in detecting familiarity, we previously found that enhancing gene expression within the aIC after an object training experience, by posttraining administration of the histone deacetylase inhibitor sodium butyrate, selectively reduced the exploration of the familiar object on the retention test (i.e., reflecting a better memory of the training object), but that this manipulation did not affect exploration of a novel object.

Interestingly, the yohimbine effect on enhancing memory detailedness was paralleled by an increased neuronal activity within both the aIC and PRh after exposure to the similar object. We found a similar, but much less pronounced, effect of yohimbine on the reactivation rate of neurons in the AIV and PRh after exposure to the similar object, possibly explained by the increased (re-)exposure-induced activity. An interesting observation was that this increased c-Fos expression in response to the similar object within the AIV was most pronounced in layers II/III, whereas in the PRh it was strongest in layers V/VI. Early tracing studies in rats have indicated that the cortical efferents arise in deep layers (Insausti *et al.*, 1997), whereas the superficial cortical layers (I-III) are regarded more as 'input layers' (Sewards & Sewards, 2003). Moreover, previous studies in rats revealed that BLA-projecting neurons in the PRh originate predominantly from layers V/VI (McIntyre *et al.*, 1996), whereas a more recent study showed that the BLA-projecting cells in the IC are largely confined to both layers II and V (Haaranen *et al.*, 2020). As such, these findings seem to suggest that noradrenergic activation modulates the input of the AIV and the output of the PRh. These findings suggest that memory detailedness requires a coordinated activation of the neural circuits involved in both familiarity and novelty. This would be consistent with the findings of a neuroimaging study that concluded that although the neural systems responsible for processing familiar and novel stimuli are non-overlapping, they are functionally connected (Kafkas & Montaldi, 2014; Molas *et al.*, 2017). Interestingly, whereas overall neuronal activity in the BLA - during both memory consolidation and recall - was not affected by posttraining noradrenergic stimulation, yohimbine treatment significantly increased the reactivation of memory encoding neurons upon exposure to the similar object. These findings indicating that, during the detection of the similar object, noradrenergic activation induces an increased recruitment of the BLA neurons originally activated during learning, suggesting an enhanced activation of a memory-modulating BLA-originating circuit, potentially activating the aIC and

PRh. Future studies should however investigate the projection sites of these reactivated cells.

Another objective of the present study was to examine the role of the BLA-aIC pathway in mediating the effect of yohimbine administration on the enhancement of memory detailedness. Many previous studies have shown that the BLA and aIC closely interact in mediating the effect of emotional arousal or specific pharmacological manipulations on different forms of recognition memory (Miranda & McGaugh, 2004; Miranda *et al.*, 2008; Beldjoud *et al.*, 2015; Rodríguez-Durán *et al.*, 2017; Chen *et al.*, 2018). Previous findings from our lab indicated a particularly important role for a functional interaction between the BLA and aIC in mediating the memory-enhancing effect of norepinephrine on object recognition memory (Beldjoud *et al.*, 2015; Chen *et al.*, 2018). In conditioned taste aversion, an electrophysiological study showed that long-term potentiation in the BLA-IC pathway strengthens long-term memory, whereas long-term depression in this pathway facilitates extinction of this memory (Rodríguez-Durán *et al.*, 2017). However, these studies did not provide evidence for the involvement of the direct anatomical pathway the IC and BLA (McDonald & Jackson, 1987; Shi & Cassell, 1998; Gehrlach *et al.*, 2020) between both brain regions in the modulation of object recognition memory, nor the modulation of its detailedness. In this study, we found that silencing of the BLA-aIC pathway with a posttraining DREADD manipulation selectively blocked the effect of systemic yohimbine administration on the enhanced memory detailedness and impaired the ability of mice to discriminate the similar object from the familiar one. Noteworthy, silencing of the BLA-aIC pathway did not impair the ability of mice to discriminate the dissimilar object. Further, clozapine administration did not appear to alter retention performance in control animals. These findings thus indicate that the inhibitory chemogenetic manipulation did not impair object recognition memory as such, but rather impaired the effect of noradrenergic activation, which is in line with many previous findings indicating that BLA is not the storage site of the enhanced memories (Packard *et al.*, 1994; Cahill & McGaugh, 1998), but a node of modulation such that stress hormones require an intact BLA in order to exert their actions on other brain regions (McGaugh *et al.*, 1996; Roozendaal *et al.*, 1996; Ikegaya *et al.*, 1997; Setlow *et al.*, 2000; Roozendaal *et al.*, 2001; McReynolds *et al.*, 2010; Roozendaal & McGaugh, 2011; Holloway-Erickson *et al.*, 2012). As the IC and BLA are reciprocally connected, future experiments should also investigate whether the pathway from the aIC to the BLA plays a role in mediating this effect.

In summary, the present findings indicate the intriguing possibility that the aIC and PRh might each play a very specific role in mediating the effect of noradrenergic activation on memory detailedness, and confirm the critical role of the BLA-aIC circuit in the effect of noradrenergic activation on the detailedness of object memory. As such, they pave the way for a further investigation of other related specific neural circuits, such as the BLA-PRh, aIC-BLA and PRh-BLA pathways, as well as the molecular underpinnings of these effects.

## Disclosure statement

No potential conflict of interest was reported by the author(s).


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## References


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*(1), 115.

Aston-Jones, G., Rajkowski, J., Kubiak, P., Valentino, R. J., & Shipley, M. T. (1996). Role of the locus coeruleus in emotional activation *Progress in brain research* (Vol. 107, pp. 379-402): Elsevier.

Atucha, E., & Roozendaal, B. (2015). The inhibitory avoidance discrimination task to investigate accuracy of memory. *Frontiers in Behavioral Neuroscience, 9*, 60.

Atucha, E., Vukojevic, V., Fornari, R. V., Ronzoni, G., Demougin, P., Peter, F., Atsak, P., Coolen, M. W., Papassotiropoulos, A., & McGaugh, J. L. (2017). Noradrenergic activation of the basolateral amygdala maintains hippocampus-dependent accuracy of remote memory. *Proceedings of the National Academy of Sciences of the United States of America, 114*(34), 9176-9181.

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

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.

Banks, P., Warburton, E., Brown, M., & Bashir, Z. (2014). Mechanisms of synaptic plasticity and recognition memory in the perirhinal cortex *Progress in molecular biology and translational science* (Vol. 122, pp. 193-209): Elsevier.

Barbosa, F., Santos, J. R., Meurer, Y., Macêdo, P., Ferreira, L., Pontes, I., Ribeiro, A., & Silva, R. (2013). Differential cortical c-fos and zif-268 expression after object and spatial memory processing in a standard or episodic-like object recognition task. *Frontiers in Behavioral Neuroscience, 7*(112).

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*(108), 108.

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 and Memory, 12*(5), 447-449.

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

Bisby, J. A., Horner, A. J., Hørlyck, L. D., & Burgess, N. (2016). Opposing effects of negative emotion on amygdalar and hippocampal memory for items and associations. *Social Cognitive and Affective Neuroscience, 11*(6), 981-990.

Cahill, L., & McGaugh, J. L. (1998). Mechanisms of emotional arousal and lasting declarative memory. *Trends in Neurosciences, 21*(7), 294-299.

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.

DeNardo, L. A., Liu, C. D., Allen, W. E., Adams, E. L., Friedmann, D., Fu, L., Guenthner, C. J., Tessier-Lavigne, M., & Luo, L. (2019). Temporal evolution of cortical ensembles promoting remote memory retrieval. *Nature Neuroscience, 22*(3), 460-469.

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). Involvement of alpha1-adrenoceptors in the basolateral amygdala in modulation of memory storage. *European Journal of Pharmacology, 372*(1), 9-16.

Gehrlach, D. A., Gaitanos, T. N., Klein, A. S., Weiand, C., Hennrich, A. A., Conzelmann, K.-K., & Gogolla, N. (2020). A whole-brain connectivity map of mouse insular cortex. *BioRxiv*.

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., Ellis, R. J., Richie, C. T., Harvey, B. K., & Dannals, R. F. (2017). Chemogenetics revealed: Dreadd occupancy and activation via converted clozapine. *Science, 357*(6350), 503-507.

Guenthner, C. J., Miyamichi, K., Yang, H. H., Heller, H. C., & Luo, L. (2013). Permanent genetic access to transiently active neurons via trap: Targeted recombination in active populations. *Neuron, 78*(5), 773-784.

Haaranen, M., Schäfer, A., Järvi, V., & Hyytiä, P. (2020). Chemogenetic stimulation and silencing of the insula, amygdala, nucleus accumbens, and their connections differentially modulate alcohol drinking in rats. *Frontiers in Behavioral Neuroscience, 14*.

Hermans, E. J., Henckens, M. J., Joels, M., & Fernandez, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in Neurosciences, 37*(6), 304-314.

Holloway-Erickson, C. M., McReynolds, J. R., & McIntyre, C. K. (2012). Memory-enhancing intra-basolateral amygdala infusions of clenbuterol increase arc and camkiiα protein expression in the rostral anterior cingulate cortex. *Frontiers in Behavioral Neuroscience, 6*, 17.

Ikegaya, Y., Nakanishi, K., Saito, H., & Abe, K. (1997). Amygdala β-noradrenergic influence on hippocampal longterm potentiation in vivo. *Neuroreport, 8*(14), 3143-3146.

Insausti, R., Herrero, M. T., & Witter, M. P. (1997). Entorhinal cortex of the rat: Cytoarchitectonic subdivisions and the origin and distribution of cortical efferents. *Hippocampus, 7*(2), 146-183.

Ito, T., Inoue, K., & Takada, M. (2015). Distribution of glutamatergic, gabaergic, and glycinergic neurons in the auditory pathways of macaque monkeys. *Neuroscience, 310*, 128-151.

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.

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

Laing, M., & Bashir, Z. (2014). Β-adrenoceptors and synaptic plasticity in the perirhinal cortex. *Neuroscience, 273*, 163-173.

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.

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.

Mason, J. W. (1968). A review of psychoendocrine research on the sympathetic-adrenal medullary system. *Psychosomatic Medicine, 30*(5), 631-653.

McDonald, A. J., & Jackson, T. R. (1987). Amygdaloid connections with posterior insular and temporal cortical areas in the rat. *Journal of Comparative Neurology, 262*(1), 59-77.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience, 27*, 1-28.

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., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: Interaction with other brain systems. *Proceedings of the National Academy of Sciences, 93*(24), 13508-13514.

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., McGaugh, J. L., & Williams, C. L. (2012). Interacting brain systems modulate memory consolidation. *Neuroscience and Biobehavioral Reviews, 36*(7), 1750-1762.

McIntyre, D. C., Kelly, M. E., & Staines, W. A. (1996). Efferent projections of the anterior perirhinal cortex in the rat. *Journal of Comparative Neurology, 369*(2), 302-318.

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.

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).

Miranda, M. I., & McGaugh, J. L. (2004). Enhancement of inhibitory avoidance and conditioned taste aversion memory with insular cortex infusions of 8-br-camp: Involvement of the basolateral amygdala. *Learning and Memory, 11*(3), 312-317.

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 and Memory, 15*(7), 468-476.

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.

Norman, G., & Eacott, M. (2005). Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. *Behavioral Neuroscience, 119*(2), 557.

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 of the United States of America, 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.

Packard, M. G., Cahill, L., & McGaugh, J. L. (1994). Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. *Proceedings of the National Academy of Sciences, 91*(18), 8477-8481.

Peng, T., Thorn, K., Schroeder, T., Wang, L., Theis, F. J., Marr, C., & Navab, N. (2017). A basic tool for background and shading correction of optical microscopy images. *Nature Communications, 8*(1), 14836.

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.

Pezze, M. A., Marshall, H. J., Fone, K. C., & Cassaday, H. J. (2017). Role of the anterior cingulate cortex in the retrieval of novel object recognition memory after a long delay. *Learning and Memory, 24*(7), 310-317.

Rodríguez-Durán, L. F., Martínez-Moreno, A., & Escobar, M. L. (2017). Bidirectional modulation of taste aversion extinction by insular cortex ltp and ltd. *Neurobiology of Learning and Memory, 142*, 85-90.

Roozendaal, B., Castello, N. A., Vedana, G., Barsegyan, A., & McGaugh, J. L. (2008). Noradrenergic activation of the basolateral amygdala modulates consolidation of object recognition memory. *Neurobiology of Learning and Memory, 90*(3), 576-579.

Roozendaal, B., Dominique, J.-F., Ferry, B., Setlow, B., & McGaugh, J. L. (2001). Basolateral amygdala–nucleus accumbens interactions in mediating glucocorticoid enhancement of memory consolidation. *Journal of Neuroscience, 21*(7), 2518-2525.

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. *Journal of Neuroscience, 30*(14), 5037-5046.

Roozendaal, B., & McGaugh, J. L. (2011). Memory modulation. *Behavioral Neuroscience, 125*(6), 797-824.

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

Roozendaal, B., Okuda, S., Van der Zee, E. A., & McGaugh, J. L. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America, 103*(17), 6741-6746.

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

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

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., & Schmid, B. (2012). Fiji: An open-source platform for biological-image analysis. *Nature methods, 9*(7), 676-682.

Setlow, B., Roozendaal, B., & McGaugh, J. L. (2000). Involvement of a basolateral amygdala complex–nucleus accumbens pathway in glucocorticoid-induced modulation of memory consolidation. *European Journal of Neuroscience, 12*(1), 367-375.

Sewards, T. V., & Sewards, M. A. (2003). Input and output stations of the entorhinal cortex: Superficial vs. Deep layers or lateral vs. Medial divisions? *Brain research reviews, 42*(3), 243-251.

Shi, C. J., & Cassell, M. (1998). Cascade projections from somatosensory cortex to the rat basolateral amygdala via the parietal insular cortex. *Journal of Comparative Neurology, 399*(4), 469-491.

Song, Q., Bolsius, Y., Ronzoni, G., Henckens, M. J. A. G., & Roozendaal, B. (2020). Noradrenergic enhancement of object recognition and object location memory in mice. *Stress*, 1-25.

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 of the United States of America, 106*(23), 9447-9452.

Sternson, S. M., & Roth, B. L. (2014). Chemogenetic tools to interrogate brain functions. *Annual Review of Neuroscience, 37*, 387-407.

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., Fernandez, G., Deisseroth, K., Greene, R. W., & Morris, R. G. (2016). Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature, 537*(7620), 357-362.

Tervo, D. G. R., Hwang, B.-Y., Viswanathan, S., Gaj, T., Lavzin, M., Ritola, K. D., Lindo, S., Michael, S., Kuleshova, E., & Ojala, D. (2016). A designer aav variant permits efficient retrograde access to projection neurons. *Neuron, 92*(2), 372-382.

VanElzakker, M., Fevurly, R. D., Breindel, T., & Spencer, R. L. (2008). Environmental novelty is associated with a selective increase in fos expression in the output elements of the hippocampal formation and the perirhinal cortex. *Learning and Memory, 15*(12), 899-908.

Vogel-Ciernia, A., & Wood, M. A. (2014). Examining object location and object recognition memory in mice. *Current Protocols in Neuroscience, 69*, 8.31.31-17.

Wan, H., Aggleton, J. P., & Brown, M. W. (1999). Different contributions of the hippocampus and perirhinal cortex to recognition memory. *Journal of Neuroscience, 19*(3), 1142-1148.

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.

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 e705.

## Supplementary Materials

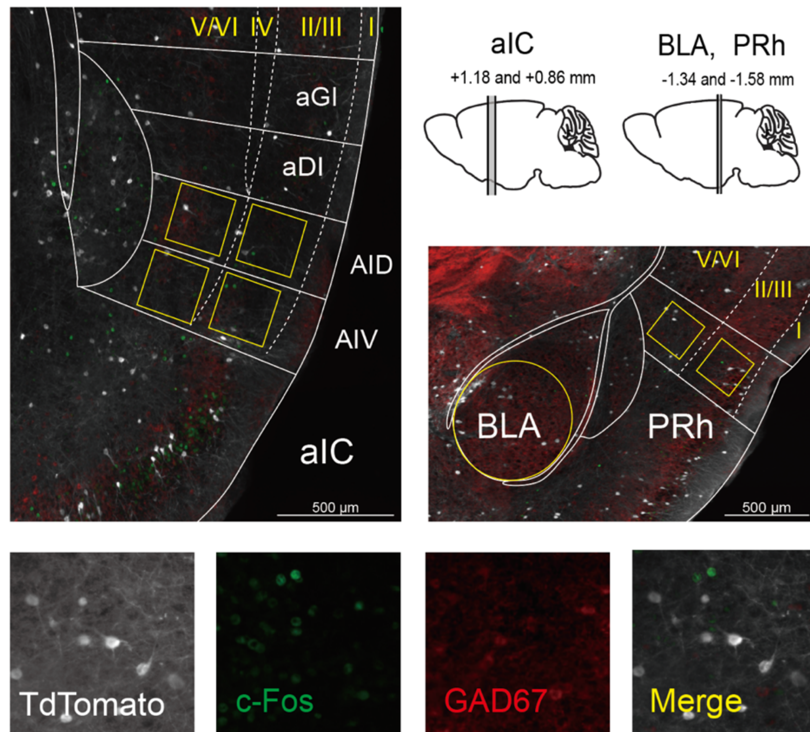


**Figure S1. Diagram illustrating the different regions of interest.** Agranular insular cortex dorsal part (AID) and agranular insular cortex ventral part (AIV); perirhinal cortex (PRh) and basolateral amygdala (BLA). The yellow squares and circle show the exact locations in which the number of neurons was counted within each region of interest. Scale bar = 500 µm. Other abbrevations: aIC, anterior insular cortex; aGI, anterior granular insular; aDI, anterior dysgranular insular.

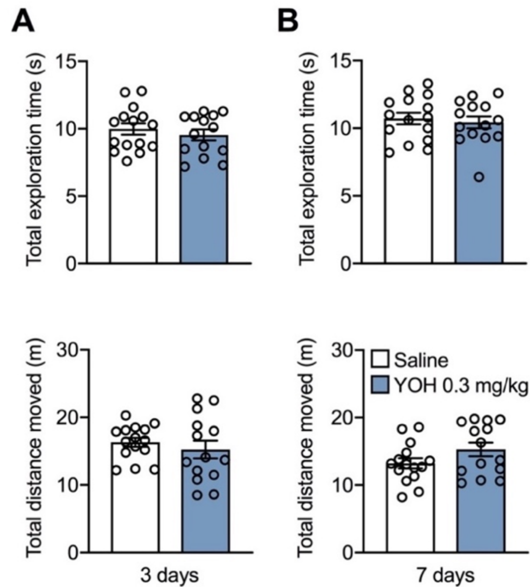


**Figure S2. Effect of yohimbine treatment on the total exploration time and the total distance moved during the retention test.** Yohimbine treatment did not affect the total exploration time of the three objects or the total distance moved by the mice at the 3-day (**A**, Saline: $n$ = 15, YOH: $n$ = 14) or 7-day (**B**, Saline: $n$ = 15, YOH: $n$ = 14) retention test. Data are shown as mean ± SEM, dots represent individual data points.

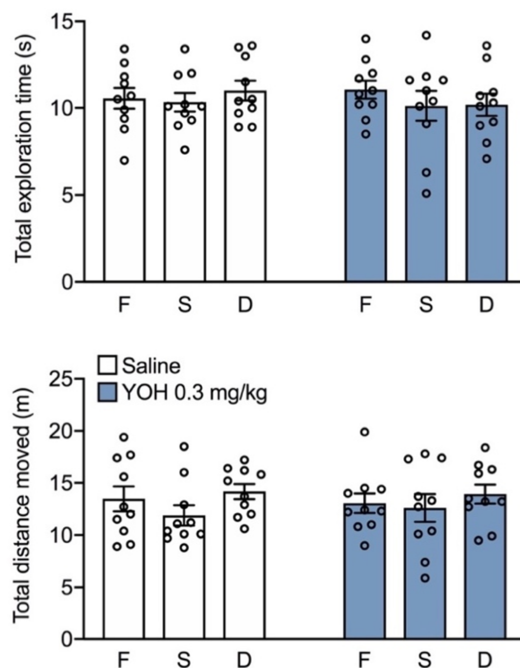


**Figure S3. Effect of yohimbine treatment on the total exploration time and the total distance moved during the (re-)exposure session.** Yohimbine treatment did not affect the total exploration time of the three objects or the total distance moved by the mice at the 3-day (re-)exposure session (familiar object (F), similar object (S), dissimilar object (D); Saline-F: $n$ = 10, Saline-S: $n$ = 10, Saline-D: $n$ = 10, YOH-F: $n$ = 10, YOH-S: $n$ = 10, YOH-D: $n$ = 10). Data are shown as mean ± SEM, dots represent individual data points.

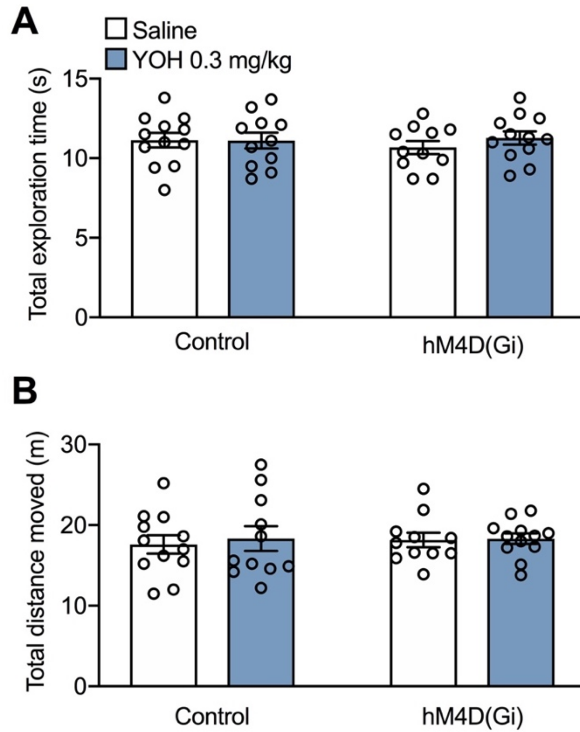


**Figure S4. Effect of yohimbine treatment and DREADD manipulation on the total exploration time and the total distance moved during the retention test.** Yohimbine treatment or DREADD manipulation did not affect the total exploration time of the three objects **(A)** or the total distance moved **(B)** by the mice during the retention test (Saline-Control: $n$ = 12, YOH-Control: $n$ = 11, Saline-hM4D(Gi): $n$ = 11, YOH-hM4D(Gi): $n$ = 12). Data are shown as mean ± SEM, dots represent individual data points.

**Table S1. Training data of the object discrimination memory task – Experiment 1**

| Training data items | Saline | YOH 0.3 mg/kg |
|---|---|---|
| Object exploration time (s) | 8.6 ± 0.2 | 8.6 ± 0.2 |
| Total distance moved (m) | 12.2 ± 0.4 | 13.1 ± 0.5 |

Data are shown as mean ± SEM.

**Table S2. Training data of the object (re-)exposure task– Experiment 1**

| Training data items | Saline | YOH 0.3 mg/kg |
|---|---|---|
| Object exploration time (s) | 9.9 ± 0.4 | 9.9 ± 0.3 |
| Total distance moved (m) | 12.8 ± 0.5 | 13.0 ± 0.7 |

Data are shown as mean ± SEM.

**Table S3. Training data of the object discrimination memory task – Experiment 2**

| Drug condition | Saline | | YOH 0.3 mg/kg | |
|---|---|---|---|---|
| Virus | Control | hM4D(Gi) | Control | hM4D(Gi) |
| Object exploration time (s) | 9.3 ± 0.3 | 9.1 ± 0.3 | 9.5 ± 0.4 | 9.4 ± 0.3 |
| Total distance moved (m) | 15.4 ± 1.1 | 15.4 ± 1.3 | 15.3 ± 1.1 | 15.3 ± 1.1 |

Data are shown as mean ± SEM.

*CHAPTER*

# 5

# Summary and general discussion

In this thesis, I investigated whether noradrenergic activation influences the detailedness of object recognition memory and its underlying neural mechanisms. This question was raised based on extensive evidence indicating that stress and emotional arousal have a very powerful impact on many aspects of learning and memory, ranging from the initial encoding to the storage and retrieval of information processing (McGaugh, 2003, 2013). Prior experiments have extensively investigated how stress and emotional arousal can enhance the strength of these memories. Such better memory for emotionally arousing events is typically a highly adaptive survival mechanism which helps us to remember significant life experiences (McGaugh, 2003). In fact, this phenomenon was already known in medieval times: To induce a lasting memory for a specific occasion, children were thrown into a river (to induce a stress response) after they witnessed an important event like a wedding or granting of land to a township (McGaugh, 2003). However, to what extent stress and emotional arousal also influence the quality of such memories, in terms of their accuracy and detailedness, had been much less examined.

Human behavioral studies have indicated that emotional memories could be altered in their quality as well, though the findings are conflicting: Some studies showed that emotional arousal enhances the accuracy of memory (Porter *et al.*, 2008; Hoscheidt *et al.*, 2014), whereas other studies reported that emotional memories are remembered in a more generalized manner (Morgan III *et al.*, 2004), recalled with overconfidence (Talarico & Rubin, 2003) or subject to incorporation of misinformation (Payne *et al.*, 2002; Sharot *et al.*, 2004; Rimmele *et al.*, 2011). It is obvious that an impaired memory accuracy can have severe and maladaptive consequences. For example, aberrant memory processing of stressful events, inducing strong, but less specific, fear memories, is thought to be a major risk factor for the development of post-traumatic stress disorder and phobias (Lissek *et al.*, 2005; de Quervain *et al.*, 2017; Lis *et al.*, 2020). Besides such clinical implications, disturbances in memory accuracy are also relevant to certain societal issues, e.g. accuracy of retrieved memories of eyewitness testimony in a courtroom or the emotional enhancement theories in didactics and education (Friedlander *et al.*, 2011; Lacy & Stark, 2013). Therefore, it is important to gain more understanding of the effects of stress and emotional arousal on the brain in regulating not only the strength of memory, but also its accuracy or detailedness.

Extensive evidence indicates that the strengthening of memory involves synergistic actions of both norepinephrine and glucocorticoid hormones (corticosterone in rodents, cortisol in humans) (McGaugh & Roozendaal, 2002; Joëls *et al.*, 2011; Roozendaal & McGaugh, 2011; Schwabe *et al.*, 2012; de Quervain *et al.*, 2017). Additionally, many other hormones,

neurotransmitters and peptides were found to have modulatory effects on memory consolidation (Izquierdo *et al.*, 2004; Roozendaal & McGaugh, 2011). However, little is known concerning the specific modulators that regulate the effect of emotional arousal on memory accuracy. Interestingly, recent studies from our laboratory indicated that norepinephrine and corticosterone exert opposite effects on memory accuracy (Bahtiyar *et al.*, 2020). In studies in rats, we showed that posttraining noradrenergic activation enhanced both the strength and accuracy of memory on an episodic-like inhibitory avoidance discrimination task (Atucha & Roozendaal, 2015; Atucha *et al.*, 2017; Roozendaal & Mirone, 2020), whereas corticosterone administration induced a strengthened, but generalized, memory for that training (Roozendaal & Mirone, 2020). These findings on the opposite effect of the two stress hormones on memory accuracy could possibly explain, at least in part, the contradictory results in humans.

Here, I will further focus on the effect of norepinephrine on the accuracy of memory. As I described above, previous rodent studies have provided strong evidence that noradrenergic activation is crucially involved in strengthening the consolidation of long-term memory of emotional experiences (McGaugh, 2004; Aston-Jones & Cohen, 2005; Sara, 2009; Roozendaal & McGaugh, 2011; Takeuchi *et al.*, 2016). Further, in recent studies, we have shown that such noradrenergic activation not only increases the strength, but also the accuracy, of hippocampus-dependent episodic-like memories (Atucha & Roozendaal, 2015; Atucha *et al.*, 2017; Roozendaal & Mirone, 2020). Moreover, we have found that noradrenergic activation during the consolidation period keeps the memories accurate over time by maintaining long-term hippocampal involvement in the memory (Atucha *et al.*, 2017). However, it is currently unknown whether noradrenergic activation also enhances accuracy or detailedness of other forms of memories, such as recognition memory, that depend on cortical brain regions (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 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). In this thesis, I therefore performed a series of experiments in mice to investigate whether noradrenergic activation enhances the detailedness of object recognition memory and its underlying cellular mechanisms.



## Summary of main findings

In **Chapter 2,** I first validated the effect of noradrenergic activation on the consolidation of object recognition memory in mice. Although many previous studies have examined the effect of noradrenergic activation on memory, including object recognition memory, these studies were all performed in rats (Dornelles *et al.*, 2007; Jurado-Berbel *et al.*, 2010; Nirogi *et al.*, 2012). However, due to the wide variety of available transgenic lines, in recent years mice became preferred as experimental animal model for neural circuitry-based investigations that could be optimally combined with new technologies such as optogenetics (Deisseroth, 2011) or DREADD (Sternson & Roth, 2014). It had not been examined whether pharmacological augmentation of noradrenergic signaling, thus on top of endogenous noradrenergic activity, can enhance memory consolidation in mice, which exhibit different cognitive abilities and show higher endogenous arousal levels compared to rats (Hok *et al.*, 2016; Stepanichev *et al.*, 2016).

Therefore, in this Chapter, I performed the object recognition memory and object location memory tasks in mice. In both tasks, the animals were given a short 3-min training trial during which they could explore two identical objects. For object recognition, memory for the object encountered during the training was determined by a preferred exploration of a novel object over the familiar object on the retention trial. Memory for the location of an object was determined by a preferred exploration of an object that had been moved to a new location on the retention trial. I could show that a systemic posttraining injection of yohimbine, a noradrenergic stimulant which increases noradrenergic signaling in the brain (Szemeredi *et al.*, 1991), was able to enhance both the "what" (object identity) and "where" (object location) components of object recognition memory (Song *et al.*, 2020). These findings were highly similar to those of previous studies in rats (Dornelles *et al.*, 2007; Jurado-Berbel *et al.*, 2010; Nirogi *et al.*, 2012), and made it possible for me to subsequently investigate the effects of noradrenergic activation on the detailedness of object recognition memory in mice.

In **Chapter 3,** I therefore examined whether noradrenergic activation and training duration influences the detailedness of object recognition memory, and how this is altered over time. As the standard object recognition paradigm (which is based on discrimination of a familiar object vs a novel object) does not allow for the investigation of memory detailedness, I first had to setup a novel memory task, termed the object discrimination task, in which I could assess memory detailedness. In this task, I use three different test objects (familiar object, similar object and dissimilar object) that vary in their resemblance with the training object. I found that when mice were trained for a brief 3-min period, they were able to discriminate the dissimilar object, but not the similar object, from the familiar one 1 day later. However, if

the animals had received more extensive training for 10 min, they were also able to discriminate the similar object. Thus, these findings indicate that this more extensive training created a more detailed memory of the training object. Noradrenergic activation with yohimbine given after a 3-min training trial was found to have a very similar enhancing effect on the detailedness of this memory when the animals were tested at 1 day after the training session. However, this yohimbine effect on enhanced detailedness became gradually lost when I tested the animals at 7 and 14 days after the training session, indicating that noradrenergic activation did not have any major effect on the maintenance of the memory over time.

To understand how yohimbine can enhance detailedness of object memory, I assessed retention-induced neuronal activity within several brain regions that are known to be involved in recognition memory (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 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). I found that retention-induced neuronal activity in yohimbine-treated animals was increased in the anterior insular cortex (aIC) and perirhinal cortex (PRh), but not in the posterior insular cortex (pIC) or basolateral amygdala (BLA). I further found that this increased retention-induced neuronal activity within both the aIC and PRh was positively correlated with the mice' ability to discriminate the similar object, but not dissimilar object.



Furthermore, consistent with the behavioral finding that the yohimbine effect on memory detailedness was gradually lost over time, I found no yohimbine effect on retention-induced neuronal activity at such later retention intervals. These findings strongly suggest that the animals do not only show a reduction in memory detailedness over time, but that there appears to be a complete loss of memory for the training object. Moreover, these findings also indicate that, unlike episodic-like memories, such cortex-dependent object memories seemingly do not follow a systems consolidation process (Zola-Morgan *et al.*, 1986; Squire & Alvarez, 1995; Frankland *et al.*, 2004; Wiltgen & Silva, 2007; Winocur & Moscovitch, 2011; Wiltgen & Tanaka, 2013).

In **Chapter 4**, I further investigated the role of the aIC and PRh in regulating the effect of noradrenergic activation on memory detailedness. In **Chapter 3**, I showed that noradrenergic activation increased retention-induced neuronal activity within both the aIC and PRh. However, that experimental design did not allow me to draw any conclusions as to whether this activity was induced by exploration of the familiar or similar object, and

whether these two brain regions have a similar or different role in this process. Therefore, in this Chapter I assessed retention-induced neuronal activity within these two brain regions after exposure to only a single object. Further, I aimed to determine to what extent those neurons were also active during the training and consolidation period by comparing this retention-induced neuronal activity with the pattern of neuronal activity during the training/consolidation period (i.e., reactivation rate). For this, I made use of a newly developed transgenic mouse line, termed the FosTRAP2 × tdTomato mice, in which an injection of the estrogen receptor agonist 4-hydroxytamoxifen (4-OHT) induces the permanent labeling of active (i.e., immediate-early gene c-Fos-expressing) neurons in a specific time window (Guenthner *et al.*, 2013; DeNardo *et al.*, 2019). This transgenic line is able to tag the neuronal representations activated during training and consolidation, which can be compared to later (re-)exposure-induced neuronal activity.

I first showed that posttraining yohimbine administration to these FosTRAP2 × tdTomato mice, highly comparable to wild-type mice, enhanced the detailedness of object memory. Next, in the object (re-)exposure task, I found that yohimbine treatment increased neuronal activity within the aIC after exposure to the familiar object, whereas it increased neuronal activity within the PRh after exposure to the dissimilar object. These findings thus support the idea that the aIC and PRh might be involved in the detection of familiarity and novelty information, respectively (Kafkas & Montaldi, 2014; Chen *et al.*, 2018). Most importantly, yohimbine increased neuronal activity within both the aIC and PRh after exposure to the similar object, suggesting that the display of memory detailedness requires a coordinated recruitment of the neural circuits implicated in both familiarity and novelty detection. Further, I found that the neuronal activity changes within the aIC, particularly the agranular insular cortex ventral part (AIV), were most pronounced within layers II/III, whereas the effects in the PRh were strongest in layers V/VI, which might reflect specific alterations in incoming vs outgoing information processing, respectively (Sewards & Sewards, 2003).

Unfortunately, tdTomato expression, reflecting neuronal activity during the memory encoding and consolidation phase, was generally very low, suggesting a lower sensitivity of this labeling system compared to regular immunohistochemistry. Moreover, yohimbine administration did not induce any marked changes in the number of tdTomato-expressing neurons within the brain regions we investigated. As will be discussed below, these findings were rather unexpected, but it is likely that the time window during which neuronal activity is labeled in these transgenic mice (up to 5-6 hours post 4-OHT injection) might be too long to detect any transient changes (Guenthner *et al.*, 2013). Further, I found that posttraining

yohimbine treatment did not affect the reactivation rate of neurons within the aIC or PRh. However, an interesting observation was that yohimbine significantly increased the reactivation rate of neurons within the BLA upon exposure to the similar object, thus indicating that noradrenergic activation increases the reactivation of a subset of BLA neurons involved in modulating memory consolidation upon detection of a similar object.

In the second experiment, I investigated whether the effect of yohimbine on memory detailedness is dependent on BLA projections to the aIC. An inhibitory DREADD manipulation (Sternson & Roth, 2014) was applied in order to inactivate the BLA-aIC pathway during the post-learning consolidation period. Many previous studies have shown that the BLA and IC (mostly not differentiating between the aIC and pIC) closely interact in mediating the effect of noradrenergic activity and emotional arousal on different forms of recognition memory (Miranda & McGaugh, 2004; Miranda *et al.*, 2008; Beldjoud *et al.*, 2015; Rodríguez-Durán *et al.*, 2017; Chen *et al.*, 2018). In this Chapter, I found that selective silencing of the BLA-aIC pathway blocked the yohimbine effect on memory detailedness, but that it spared the ability of mice to discriminate the dissimilar object. These findings are thus consistent with previous evidence indicating that the BLA does not appear to have a direct participation in recognition memory but that it mediates the modulatory effects of stress hormones by regulating information storage processes in other brain regions (McGaugh *et al.*, 1996; Roozendaal *et al.*, 1996; Ikegaya *et al.*, 1997; Setlow *et al.*, 2000; Roozendaal *et al.*, 2001; McReynolds *et al.*, 2010; Roozendaal & McGaugh, 2011; Holloway-Erickson *et al.*, 2012).

Collectively, these findings indicate that noradrenergic activation enhances the detailedness of object memory and that the aIC and PRh might each have a very specific role in this behavioral effect. In the following sections, I will further discuss the most interesting findings in more detail.

## Time-dependent effect of posttraining noradrenergic activation on the detailedness of object memory

The first major aim of this research was to determine whether posttraining noradrenergic activation enhances the detailedness of object memory. Extensive evidence indicates that noradrenergic activation is crucially involved in strengthening the consolidation of long-term memory (McGaugh, 2004; Aston-Jones & Cohen, 2005; Sara, 2009; Roozendaal &

McGaugh, 2011; Takeuchi *et al.*, 2016). These studies typically ascribed a ubiquitous role to the BLA (McGaugh, 2004; Roozendaal & McGaugh, 2011) and the locus coeruleus-norepinephrine system (Aston-Jones & Cohen, 2005; Sara, 2009; Takeuchi *et al.*, 2016) in facilitating the consolidation of memory of many different kinds of training experiences, though no conclusion was drawn as to whether the noradrenergic activation also enhanced the accuracy or detailedness of the memory.

In several recent studies, we have shown that noradrenergic activation not only increases the strength, but also the accuracy, of episodic-like memories in an inhibitory avoidance discrimination task *(*Atucha *et al*., 2017; Roozendaal & Mirone, 2020). In this inhibitory avoidance task, rats were trained in two different inhibitory avoidance apparatuses with a short interval in between, but footshock was given only in the latter apparatus. The findings indicated that whereas control rats were unable to accurately discriminate the context in which they had received footshock, rats that had received a systemic injection of yohimbine after the training trial showed a highly accurate memory of the training experience on the retention test 2 days later as indicated by long retention latencies only in the shock box (Roozendaal & Mirone, 2020). Interestingly, the BLA might have a unique role in mediating the effect of norepinephrine on enhancing this episodic-like accuracy as norepinephrine administration directly into the BLA induced a very similar enhancement of both the strength and accuracy of memory on the inhibitory avoidance discrimination task (Atucha *et al.*, 2017), whereas norepinephrine administration into the hippocampus only enhanced the strength, but not the accuracy, of that memory (Atucha *et al*. unpublished findings). These two studies show that noradrenergic activation, either by systemic administration of the noradrenergic stimulant yohimbine or by norepinephrine infusion into the BLA, enhances the episodic-like accuracy of memory in a hippocampus-dependent inhibitory avoidance discrimination task.

Noradrenergic activation is also known to enhance the consolidation of object recognition memory (Dornelles *et al.*, 2007; Roozendaal *et al.*, 2008; Roozendaal *et al.*, 2009; Barsegyan *et al.*, 2014; Beldjoud *et al.*, 2015; Song *et al.*, 2020); yet, the effect of noradrenergic activation on the detailedness of object recognition memory had not been investigated. In order to assess whether noradrenergic activation could enhance the accuracy/detailedness of memory for objects, I developed a new behavioral task. First of all, I found that animals after 3 min of object training were not able to discriminate the familiar object from a similar-looking object, but were still able to discriminate the training object from a dissimilar object, which is highly different from the training object, indicative of having a memory for the training object per se. Interestingly, the interpretation of these findings is

inconsistent with that of previous findings on the standard object recognition task in both mice and rats that indicated that 3 min of object training was insufficient to induce any 24-h memory for the training object (Dornelles *et al.*, 2007; Roozendaal *et al.*, 2010; Nirogi *et al.*, 2012; Chen *et al.*, 2018; Song *et al.*, 2020). However, critically, the two objects used in the standard object recognition task are the ones we used as similar objects (i.e., the jar and bulb). This suggests that one should reinterpret the lack of memory as reported in these original works rather as reflecting a lack of detailed memory.

Further, I found that posttraining systemic yohimbine administration enhanced the detailedness of the memory in a highly similar manner as more extensive training did. This memory detailedness enabled animals to discriminate the similar object from the training object during the 1-day retention test (and 3-day retention test in TRAP2 mice). As such, these findings are in line with the memory-enhancing effect of yohimbine as previously reported on the standard object recognition task, which in retrospect should be interpreted as an increase in memory accuracy for the training object (Roozendaal *et al.*, 2006; Nirogi *et al.*, 2012). Notably, in all experiments described in this thesis, noradrenergic activity was enhanced by a systemic injection of yohimbine, a centrally acting noradrenergic stimulant which induces a brain-wide increase in noradrenergic activity (Szemeredi *et al.*, 1991). Previous studies in rats showed that the memory-enhancing effects of systemic yohimbine administration on recognition memory are mimicked by the selective administration of norepinephrine into the BLA (Roozendaal *et al.*, 2008; Beldjoud *et al.*, 2015). My finding that silencing of the BLA-aIC pathway blocked the effect of yohimbine on memory detailedness clearly supports the idea that the BLA might also play a critical role in regulating norepinephrine effects on the detailedness of object memory. However, future studies should confirm this by examining whether norepinephrine administration directly into the BLA induces a similar enhancement of memory detailedness in the object discrimination task.

A second aim of my experiments was to examine whether such detailed memories induced by noradrenergic activation also remain accurate over time. In the study of Atucha *et al.* (2017), noradrenergic activation during memory consolidation was found to keep the memories accurate even 28 days later by maintaining long-term involvement of the hippocampus in the memory. In contrast, long-term memory of rats given a saline control infusion had become generalized at this remote retention interval. These findings in the control animals are in line with the hypothesis that episodic memories normally undergo a systems consolidation process, such that they are initially dependent on the hippocampus

and progressively become more supported by cortical areas (Zola-Morgan *et al.*, 1986; Squire & Alvarez, 1995; Frankland *et al.*, 2004; Wiltgen & Silva, 2007; Winocur & Moscovitch, 2011; Wiltgen & Tanaka, 2013), and suggest that norepinephrine administration into the BLA can prevent or slowdown this systems consolidation process and thereby maintain long-term episodic-like accuracy (Atucha *et al.*, 2017). At apparent contrast to these findings, in **Chapter 3** and **Chapter 4**, I found that the mice gradually lost the ability to discriminate both the similar and dissimilar objects at the later retention intervals (7 days and 14 days after training), with no evidence for yohimbine substantially preventing or slowing down this process. These findings are in line with those of a previous mouse study indicating that a long-term memory for objects generated by histone deacetylase inhibition (which increases gene transcription) was lost 7 days after training (Stefanko *et al.*, 2009). Moreover, the (re-)exposure experiment revealed that c-Fos activity in particularly the PRh after exposure to the familiar object was stronger at later retention intervals (7 days and 14 days after training). As will be further discussed below, such an enhanced neural activity in the PRh would be consistent with the hypothesis that the mice processed the objects as being novel during the later retention test, which would be indicative of forgetting rather than generalization. These findings thus indicate that cortex-dependent object memories seemingly do not follow a systems consolidation process.

It should be noted that mouse studies implementing an object-place learning task did present evidence for a systems consolidation process by showing an electrophysiological correlation between anterior cingulate cortex neuronal activity and object-place memory both at a 6 days retention test (Weible *et al.*, 2009), and at 30 days after the last training session, suggesting that anterior cingulate cortex neurons are involved in long-term object-place recognition memory (Weible *et al.*, 2012). Another study using an object-space task where animal were exposed to multiple pairs of different objects in different places, also confirmed a role for the prefrontal cortex in creating a stable long-term representation of the overlapping object location in space (Genzel *et al.*, 2019). However, critically, object recognition memory in these tasks involves the integration of memory for the object itself with its context and/or place. Although recognition of the object *per se* has been shown to depend on neuronal plasticity in the PRh and IC (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 2005; Balderas *et al.*, 2008; Albasser *et al.*, 2009; Roozendaal *et al.*, 2010; Banks *et al.*, 2014; Olarte-Sánchez *et al.*, 2015), integrating this with its context and/or place involves a network of interacting brain regions (Bussey *et al.*, 1999; Wan *et al.*, 1999) and is heavily relying on the hippocampus (Mumby *et al.*, 2002; Balderas *et al.*, 2008). Therefore, object memory that is incorporated with other components,

like place or context, seems to be subject to systems consolidation with the passage of time, but my findings did not provide evidence that memory for an object itself undergoes a systems consolidation process that could be potentially influenced by noradrenergic activity.

## Distinct role of the PRh and aIC in novelty and familiarity detection and their modulation by noradrenergic activation

Many previous studies have shown that both the aIC and PRh are involved in mediating object recognition memory (Ennaceur & Aggleton, 1997; Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 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). Findings from human neuroimaging studies have indicated that the role of the PRh and aIC in memory is that of detecting novelty and familiarity, respectively (Ranganath *et al.*, 2004; Kafkas & Montaldi, 2014). Kafkas & Montaldi (2014) used familiar, novel, or relatively familiar images that contained different levels of similarity to the familiar or novel images, and found that the brain regions responsive to novelty involved a large-scale network of regions along the ventral visual stream, including the PRh, hippocampus and parahippocampal cortex. Further studies have indicates that the PRh is particularly important for signaling novelty of an object itself or of object-object associations, while other regions such as the hippocampus, parahippocampal cortex and retrosplenial cortex are more important for signaling novel spatial context and object-context configurations (Wan *et al.*, 1999; Malkova & Mishkin, 2003; Norman & Eacott, 2005).

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The PRh has been suggested to detect novelty by processes resembling pattern separation (Miranda et al., 2017). It facilitates the discrimination of stimuli that display overlapping features by coordinating the recollection of the details of a specific item in a multidimensional manner; e.g. two items that are similar in only one dimension (like visual, auditory, olfactory, or semantic features) could still be represented quite differently by the PRh (Bussey *et al.*, 2005; Eichenbaum *et al.*, 2007; Graham *et al.*, 2010). A study in monkeys indicated that PRh lesions impaired the animals' ability to discriminate objects that shared many features (Bussey *et al.*, 2002). In addition, human studies showed that damage to the PRh was associated with an impaired capacity to perceptually discriminate objects with highly overlapping features (Barense *et al.*, 2005; Lee *et al.*, 2005; Lee, Buckley, *et al.*, 2006). Human imaging studies further provided evidence that PRh activity is increased during complex visual discrimination tasks (Lee, Bandelow, *et al.*, 2006), and that this activity is

closely related to the accuracy with which this discrimination occurs (O'Neil *et al.*, 2009). Interestingly, another study suggested that when highly familiar stimuli are used, no novelty signal is triggered within the PRh, as there is simply no novelty to detect (Eldridge *et al.*, 2005; Yonelinas *et al.*, 2005). These findings are thus in line with my findings of **Chapter 4** indicating that the PRh showed increased activity after (re-)exposure to novel and similar objects, but not to the familiar object. Moreover, these studies indicate a crucial role for the PRh in discriminating similar, yet distinct, representations, which is in line with our current findings of increased PRh activity in response to the similar object after posttraining yohimbine treatment being related to enhanced memory detailedness, as observed in **Chapter 4**.

In contrast, human work has indicated that the neural network supporting familiarity detection includes the dorsomedial thalamic nucleus, medial prefrontal cortex and medial and lateral parietal cortex, including the IC (Kafkas & Montaldi, 2014). Other studies found that the aIC, rather than the IC as a whole, became robustly activated by the presentation of familiar words (Kikyo *et al.*, 2002; Craig & Craig, 2009) or music (Platel *et al.*, 1997), which supports a specific role of the aIC in familiarity detection. My findings of **Chapter 4** indicating that the aIC showed increased activity after (re-)exposure to familiar objects are in line with these human findings. It has been suggested that the dorsomedial thalamic nucleus in fact might orchestrate familiarity detection in this network by combining information from novelty-sensitive regions, such as the PRh and parahippocampal cortex, with the prefrontal cortex, which in turn performs familiarity computations (Kafkas & Montaldi, 2018). The PRh is closely connected with the brain systems involved in familiarity detection such as the aIC (Kealy & Commins, 2011), which supports such coordinated response to detect familiarity vs. novelty. To assess potential crosstalk between the aIC and PRh, we tested whether activity of these two regions during the processing of the similar item was correlated. Indeed, we observed a significant positive correlation between neural activity of the AIV and of the PRh ($r = 0.66$, $p = 0.04$). Interestingly, this association was only found in yohimbine-treated animals during the exploration of the similar object, and thus supports the idea that the display of memory detailedness requires functional interactions between the neural systems involved in novelty and familiarity detection. However, also other mechanisms underlying familiarity detection have been proposed, such that the anterior temporal system facilitates the detection of familiar features based on past experiences, encoded by the amygdala and orbitofrontal cortex which extract information about the salience and value of items to guide future evaluations (Ranganath & Ritchey, 2012). Since there are dense connections between the anterior temporal system and the aIC (Höistad & Barbas, 2008), it might be that

the familiarity-detection mechanism of the aIC shows a functional commonality with that of the anterior temporal system (Kafkas & Montaldi, 2014).

Importantly, aIC and PRh functioning is modulated by noradrenergic activity (in the BLA) (Perugini *et al.*, 2012; Laing & Bashir, 2014; Beldjoud *et al.*, 2015; Chen *et al.*, 2018). In **Chapter 3,** I observed that the yohimbine effect on enhancing memory detailedness was associated with an increased retention-induced c-Fos expression within both the aIC and PRh. In contrast, no changes in c-Fos activity were found within the pIC. This latter finding is in line with the more general view that the aIC, but not the pIC, is involved in recognition memory (Balderas *et al.*, 2008; Roozendaal *et al.*, 2010; Bermudez-Rattoni, 2014). One of our recent rat studies confirmed this by showing that norepinephrine administration into the aIC, but not into the pIC, after a 3-min training trial enhanced object recognition memory, whereas administration of the β-adrenoceptor antagonist propranolol into the aIC, but not the pIC, after a 10-min training trial impaired object recognition memory (Chen *et al*., unpublished findings). Interestingly, Western blot analyses showed that protein levels of both the $\beta_1$-adrenoceptor and $\beta_2$-adrenoceptor did not differ between the aIC and the pIC (Chen *et al*., unpublished findings), indicating that the selective involvement of the aIC in mediating noradrenergic effects on recognition memory was not caused by a higher sensitivity of the aIC to the effects of norepinephrine. It is therefore likely that this selective involvement of the aIC in object recognition is caused by its specific connectivity pattern with other brain regions. The aIC is extensively connected to the frontal lobe and cognitive-emotion-related areas among which the BLA (Shi & Cassell, 1998), whereas the pIC has dense connections with the central amygdala and parietal and temporal lobes (Augustine, 1996; Shura *et al*., 2014), but receives only sparse projections from the BLA (Chen et al., unpublished findings). In previous experiments, we found that functional interactions between the BLA and aIC are involved in regulating object recognition memory. For example, we found that an inhibition of noradrenergic activity in the BLA by the administration of propranolol blocked the effect of pharmacological manipulations of the aIC on object recognition memory (Chen *et al*., unpublished findings). These findings are consistent with my DREADD findings indicating that the BLA-aIC pathway plays an important role in regulating noradrenergic effects on the aIC underlying the object recognition memory.

Consistent with the finding that yohimbine administration enhanced memory detailedness that was associated with an increased neuronal activity within both the aIC and PRh, we previously found that norepinephrine administration into either the aIC or BLA reduced the exploration of the familiar object and increased the exploration of the novel object on the

retention test of a standard object recognition task (Chen *et al*., unpublished findings). Similarly, in the present study, I found a trend-level significant reduction in the exploration of the familiar object ($p$ = 0.07) and a tendency towards increased exploration of the similar object ($p$ = 0.09) after systemic yohimbine administration. These findings suggest that the enhancement of object recognition memory by noradrenergic activation, either induced systemically or locally in the aIC and/or BLA, is associated with an increased ability to detect both the familiar and novel object. These findings are thus in line with my neuronal activity findings. In the object (re-)exposure task, I found that yohimbine treatment increased the (re-)exposure-induced neuronal activity within the aIC after exposure to the familiar object, whereas it increased neuronal activity within the PRh after exposure to the dissimilar object. These findings are in line with the idea that the aIC and PRh might be involved in the detection of familiarity and novelty information, respectively (Kafkas & Montaldi, 2014; Chen *et al*., 2018), and that yohimbine enhanced the ability of the animals to detect both familiarity and novelty information. Most interestingly, I found that yohimbine increased neuronal activity within both the aIC and PRh after exposure to the similar object (similar in color, shape, and texture), indicating that the display of memory detailedness induces a coordinated recruitment of the neural circuits implicated in both familiarity and novelty detection.

## Effect of noradrenergic activity on memory consolidation within the aIC and PRh underlying memory detailedness

The findings as presented in this thesis show that posttraining yohimbine administration enhanced the detailedness of object memory and that this was associated with an altered retention-induced activation of the aIC and PRh. An important remaining question is: How can noradrenergic activity during the post-learning period alter information storage processes in these two brain regions to ensure such a detailed memory of the training object?

Extensive previous work has shown a particularly important role for the BLA in orchestrating the memory-enhancing effects of norepinephrine by regulating neural plasticity and information storage processes in other brain regions (McGaugh, 2004; Roozendaal & McGaugh, 2011; McIntyre *et al.*, 2012; McGaugh, 2013). Previous studies have also shown that noradrenergic activity in the BLA alters functioning of the aIC and PRh (Perugini *et al.*, 2012; Laing & Bashir, 2014; Beldjoud *et al.*, 2015; Chen *et al.*, 2018). In **Chapter 4**, I showed that inactivation of the BLA-aIC pathway by the posttraining DREADD manipulation

selectively blocked the effect of systemic yohimbine administration on the enhanced memory detailedness, thus confirming the interaction of the BLA with the aIC in mediating the memory-enhancing effects of norepinephrine in terms of memory detailedness. Future experiments should also examine the consequence of such inactivation of the BLA-PRh pathway on memory detailedness. Previous studies have already demonstrated that *in vitro* electrical stimulation of the BLA reduces the threshold for the induction of long-term potentiation in the PRh (Perugini *et al.*, 2012), and that administration of the β-adrenoceptor agonist isoprenaline combined with subthreshold electrical stimulation of the BLA-PRh pathway results in a long-lasting potentiation of synaptic plasticity within the PRh (Laing & Bashir, 2014). In the (re-)exposure task of **Chapter 4**, I found that noradrenergic activation induced an increased reactivation of the neurons in the BLA during the detection of the similar object, suggesting an enhanced activation of a memory-modulating BLA-originating circuit. These findings make it tempting to speculate that these re-activated BLA neurons are in fact reflecting the activation of efferent projections to the PRh and aIC, facilitating the detection of novelty and familiarity, respectively. Such an interpretation would be consistent with more general evidence that the BLA is also known to interact with efferent brain regions in regulating emotional arousal effects on memory recall (Roozendaal *et al.*, 2004).



There is extensive evidence that both the aIC and PRh are involved in memory storage processes underlying object recognition memory (Bermudez-Rattoni *et al.*, 2005; Norman & Eacott, 2005; Balderas *et al.*, 2008; Albasser *et al.*, 2009; Roozendaal *et al.*, 2010; Guzmán-Ramos & Bermúdez-Rattoni, 2012; Banks *et al.*, 2014; Bermudez-Rattoni, 2014; Olarte-Sánchez *et al.*, 2015). For example, it has been demonstrated that posttraining administration of the protein-synthesis blocker anisomycin into either the aIC or PRh impairs the consolidation of object recognition memory (Balderas *et al.,* 2008). An important question is whether the aIC and PRh might store similar or different features of an object and whether this consolidation process depends on functional interactions between these two brain regions. In **Chapter 4**, I used the TRAP2 mice in an attempt to specifically address this research question. However, no evidence was found for such hypothesized alterations in neuronal activity in the aIC and PRh during the memory consolidation phase. This is likely due to the insensitivity to transient neuronal activity changes in this transgenic model, which is characterized by a relatively long period during which neuronal activity is labeled (up to 5-6 hours) (Guenthner *et al.*, 2013). However, there is evidence from previous experiments in our laboratory and by others that do support the idea that consolidation processes in the aIC and PRh might be different. Our findings indicated that object recognition memory enhancement induced by locally increasing gene and protein expression in the aIC was

selectively associated with a reduced exploration of the familiar object on the retention test, but that this did not affect exploration of the novel object (Chen *et al.*, 2018), suggesting that aIC activation mainly modulates the processing on familiarity detection of previously encountered objects. On the other hand, another study revealed that the PRh is involved in the consolidation of overlapping representations in object recognition memory via a process that resembles pattern separation (Miranda *et al.*, 2017). Brain-derived neurotrophic factor (BDNF) and activity-regulated cytoskeletal-associated protein (Arc) were found to be required for the separated storage of overlapping object representations in the PRh, within this time-restricted window of the consolidation period (Miranda *et al.*, 2017). Therefore, it could be hypothesized that consolidation processes within the aIC are able to support familiarity detection which might be sufficient to discriminate the dissimilar object from the familiar one, but that consolidation processes within the PRh are required to also enhance memory detailedness by separating overlapping information, enabling the animals to also discriminate a similar object.

Interestingly, in a previous study we found that a memory-enhancing dose of norepinephrine administered into the BLA was found to reduce aIC activity during the consolidation period (Chen *et al*., unpublished findings). Both the number of neurons expressing the phosphorylated form of the transcription factor cAMP response element-binding (pCREB) protein and the neuronal activity marker c-Fos was decreased in this study (Chen *et al*., unpublished findings). Chen *et al*. further found that such reduced aIC activity was driven by an upregulation of GABAergic activity within the aIC, indicating an increased inhibitory tone (Chen *et al*., unpublished findings). The BLA innervates not only excitatory neurons within cortical areas, but has even stronger inputs directly onto GABAergic interneurons (McGarry & Carter, 2016). Based on the functional interactions between the aIC and PRh it is tempting to speculate that this reduction in aIC activity further enhances consolidation processes in the PRh, by boosting pattern separation, ultimately resulting in a more detailed memory. This idea is supported by human neuroimaging findings suggesting a model of transient changes in large-scale neural networks following stress, such that activity of the aIC is initially increased in response to stress and emotional arousal, but suppressed in its later aftermath (Hermans *et al.*, 2014). Specifically, exposure to emotional arousal first induces a rapid strengthening in connectivity within the salience network (and thus between the BLA and aIC) at the cost of the central executive network (Seeley *et al.*, 2007; Hermans *et al.*, 2011). Such rapid increase in salience network activity and connectivity is critically dependent on noradrenergic activity, and related to the increase in attention and detection of emotionally salient information (Hermans *et al.*, 2011; Hermans *et al.*, 2014). After a delay,

resource allocation to these two networks reverses: The salience network shuts off and the central executive network becomes active, which normalizes emotional reactivity and enhances higher-order cognitive processes (Hermans *et al.*, 2014; van Leeuwen *et al.*, 2018). Our rats study confirmed this idea by showing that administration of the GABAergic agonist muscimol into the aIC 1 hour after training enhanced object recognition memory, indicating that such inactivation of the salience network, including the aIC, might be necessary for mediating emotional arousal effects on the memory consolidation process in other brain regions (Chen *et al.*, unpublished findings). These findings suggest that an inactivation of the aIC activity during consolidation facilitates consolidation processes in other regions that are part of the executive control network. The aIC would then be activated again in a later phase during familiarity detection.

## Working model

Collectively, it could thus be hypothesized that noradrenergic activation after object training reduces aIC activity during the memory consolidation period, which is necessary for facilitating consolidation processing in other brain systems for novelty detection, especially the PRh. Thereby, the pattern separation mechanism of the PRh, and the memory processing supporting the detection of familiarity in the aIC, are both initiated. The BLA proposedly plays a critical role in both this suppression of aIC activity and activation of PRh activity during the consolidation phase via its dense connections with these two regions, modulating local synaptic plasticity. These norepinephrine-stimulated mechanisms during memory consolidation are hypothesized to result in an increased activity of the aIC and PRh during the retention test for familiarity and novelty detection, respectively. Together, based on a better storage of the detailed features of the object during consolidation, a coordinated activation of these two brain regions could thereby lead to a better evaluation of the object compared to the previously encountered one, thus discriminate the familiar, similar and novel stimuli during retention (Figure 1).

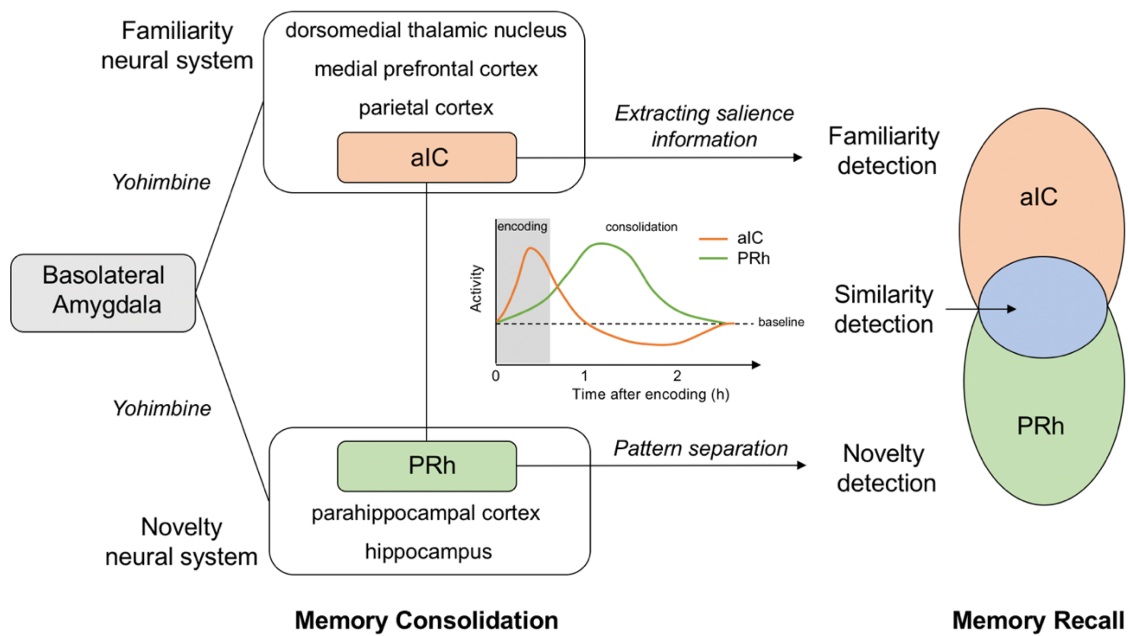


**Figure 1.** Schematic summarizing the role of BLA, aIC and PRh, and their neural activity changes in regulating the effect of noradrenergic activation on the detailedness of object recognition memory.

## Prospects for future work

Although based on data from prior literature and the findings in this thesis, several aspects of this model are still hypothetical and should be empirically tested. First of all, the current study is limited in showing the proposed real-time dynamic shifts in the activity of the neural networks. In the current study, neuronal activity was assessed by immediate-early gene expression, but this readout lacks the temporal specificity to dissociate between the initial encounter and exploration of certain objects (potentially triggering aIC activity) and the subsequent consolidation of this object into memory (potentially related to a suppression of aIC activity). Therefore, it would be important to also track neuronal activity changes within or between brain regions of interest across the distinct memory phases. For this, a newly developed technique, termed fiber photometry, could be used. Fiber photometry allows for the visualization of neuronal activity by means of calcium imaging with a fast temporal resolution through an optical fiber located at the target brain region (Girven & Sparta, 2017). This technique would enable us to directly assess neural activity changes within the aIC and PRh during the retention test while the animals are exploring the different test objects.

The aIC and the PRh are known to be reciprocally connected (Kealy & Commins, 2011), and the above-mentioned findings suggest that the discrimination of memory detailedness should depend on functional interactions between these two regions. In ongoing experiments, we are analyzing how the suppression of the BLA-aIC pathway as described in Chapter 4 modulates retention-induced activity in the aIC and PRh. It would also be interesting to assess how this suppression affects PRh activity during the memory consolidation phase itself. Moreover, it would be important to determine whether inactivation of the BLA-PRh pathway also blocks the yohimbine effect on memory detailedness and how this affects retention-induced neuronal activity patterns. Additionally, future experiments implementing neural circuitry manipulations using DREADD could be optimally used to manipulate direct crosstalk between the aIC and PRh in different memory phases. For instance, one could use an inhibitory DREADD to suppress the aIC input to the PRh to examine the hypothesis that such reduced aIC input would enhance the consolidation process in the PRh. Moreover, DREADD manipulations of the aIC-PRh circuit can be used to whether a crosstalk between these two brain regions is needed during the detection of selectively a similar object. Moreover, additional DREADD manipulations of specific cell types could inform us on the exact neuronal substrate of these effects. For example, one could selectively target GABAergic neurons within the aIC to test the hypothesis that an increase in their activity during memory consolidation would increase activity in the PRh related to the improved storage of detailed information, or during later novelty and familiarity detection.



## Conclusion

Overall, the research reported in this thesis revealed that noradrenergic activation enhances not only the strength, but also the detailedness of object memory. Increased memory detailedness was associated with the modulation of neuronal activity within the aIC and PRh, regions critically involved in the detection of familiarity and novelty, respectively. Moreover, I showed that the BLA is importantly involved in mediating the norepinephrine effect on increasing memory detailedness. As such, these findings provide novel insight into a hitherto unexplored fact of the manifold effects of emotional arousal on memory in guiding adaptive behaviors.

## References

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*(1), 115.

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*, 403-450.

Atucha, E., & Roozendaal, B. (2015). The inhibitory avoidance discrimination task to investigate accuracy of memory. *Frontiers in Behavioral Neuroscience, 9*, 60.

Atucha, E., Vukojevic, V., Fornari, R. V., Ronzoni, G., Demougin, P., Peter, F., Atsak, P., Coolen, M. W., Papassotiropoulos, A., & McGaugh, J. L. (2017). Noradrenergic activation of the basolateral amygdala maintains hippocampus-dependent accuracy of remote memory. *Proceedings of the National Academy of Sciences of the United States of America, 114*(34), 9176-9181.

Augustine, J. R. (1996). Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Research Reviews, 22*(3), 229-244.

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

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.

Banks, P., Warburton, E., Brown, M., & Bashir, Z. (2014). Mechanisms of synaptic plasticity and recognition memory in the perirhinal cortex *Progress in molecular biology and translational science* (Vol. 122, pp. 193-209): Elsevier.

Barense, M. D., Bussey, T. J., Lee, A. C., Rogers, T. T., Davies, R. R., Saksida, L. M., Murray, E. A., & Graham, K. S. (2005). Functional specialization in the human medial temporal lobe. *Journal of Neuroscience, 25*(44), 10239-10246.

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

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*(108), 108.

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 and Memory, 12*(5), 447-449.

Bussey, T. J., Muir, J. L., & Aggleton, J. P. (1999). Functionally dissociating aspects of event memory: The effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat. *Journal of Neuroscience, 19*(1), 495-502.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2002). Perirhinal cortex resolves feature ambiguity in complex visual discriminations. *European Journal of Neuroscience, 15*(2), 365-374.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2005). The perceptual-mnemonic/feature conjunction model of perirhinal cortex function. *The Quarterly Journal of Experimental Psychology Section B, 58*(3-4), 269-282.

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.

Craig, A. D., & Craig, A. (2009). How do you feel--now? The anterior insula and human awareness. *Nature reviews neuroscience, 10*(1).

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.

Deisseroth, K. (2011). Optogenetics. *Nature methods, 8*(1), 26-29.

DeNardo, L. A., Liu, C. D., Allen, W. E., Adams, E. L., Friedmann, D., Fu, L., Guenthner, C. J., Tessier-Lavigne, M., & Luo, L. (2019). Temporal evolution of cortical ensembles promoting remote memory retrieval. *Nature Neuroscience, 22*(3), 460-469.

Dornelles, A., de Lima, M. N., Grazziotin, M., Presti-Torres, J., Garcia, V. A., Scalco, F. S., Roesler, R., & Schroder, N. (2007). Adrenergic enhancement of consolidation of object recognition memory. *Neurobiology of Learning and Memory, 88*(1), 137-142.

Eichenbaum, H., Yonelinas, A. P., & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annual Review of Neuroscience, 30*, 123-152.

Eldridge, L. L., Engel, S. A., Zeineh, M. M., Bookheimer, S. Y., & Knowlton, B. J. (2005). A dissociation of encoding and retrieval processes in the human hippocampus. *Journal of Neuroscience, 25*(13), 3280-3286.

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.

Frankland, P. W., Bontempi, B., Talton, L. E., Kaczmarek, L., & Silva, A. J. (2004). The involvement of the anterior cingulate cortex in remote contextual fear memory. *Science, 304*(5672), 881-883.

Friedlander, M. J., Andrews, L., Armstrong, E. G., Aschenbrenner, C., Kass, J. S., Ogden, P., Schwartzstein, R., & Viggiano, T. R. (2011). What can medical education learn from the neurobiology of learning? *Academic Medicine, 86*(4), 415-420.

Genzel, L., Schut, E., Schröder, T., Eichler, R., Khamassi, M., Gomez, A., Navarro Lobato, I., & Battaglia, F. (2019). The object space task shows cumulative memory expression in both mice and rats. *PLoS Biology, 17*(6), e3000322.

Girven, K. S., & Sparta, D. R. (2017). Probing deep brain circuitry: New advances in in vivo calcium measurement strategies. *ACS Chemical Neuroscience, 8*(2), 243-251.

Graham, K. S., Barense, M. D., & Lee, A. C. (2010). Going beyond ltm in the mtl: A synthesis of neuropsychological and neuroimaging findings on the role of the medial temporal lobe in memory and perception. *Neuropsychologia, 48*(4), 831-853.

Guenthner, C. J., Miyamichi, K., Yang, H. H., Heller, H. C., & Luo, L. (2013). Permanent genetic access to transiently active neurons via trap: Targeted recombination in active populations. *Neuron, 78*(5), 773-784.

Guzmán-Ramos, K., & Bermúdez-Rattoni, F. (2012). Interplay of amygdala and insular cortex during and after associative taste aversion memory formation. *Reviews in the Neurosciences, 23*(5-6), 463-471.

Hermans, E. J., Henckens, M. J., Joels, M., & Fernandez, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in Neurosciences, 37*(6), 304-314.

Hermans, E. J., Van Marle, H. J., Ossewaarde, L., Henckens, M. J., Qin, S., Van Kesteren, M. T., Schoots, V. C., Cousijn, H., Rijpkema, M., & Oostenveld, R. (2011). Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. *Science, 334*(6059), 1151-1153.

Höistad, M., & Barbas, H. (2008). Sequence of information processing for emotions through pathways linking temporal and insular cortices with the amygdala. *Neuroimage, 40*(3), 1016-1033.

Hok, V., Poucet, B., Duvelle, E., Save, E., & Sargolini, F. (2016). Spatial cognition in mice and rats: Similarities and differences in brain and behavior. *Wiley Interdisciplinary Reviews: Cognitive Science, 7*(6), 406-421.

Holloway-Erickson, C. M., McReynolds, J. R., & McIntyre, C. K. (2012). Memory-enhancing intra-basolateral amygdala infusions of clenbuterol increase arc and camkiiα protein expression in the rostral anterior cingulate cortex. *Frontiers in Behavioral Neuroscience, 6*, 17.

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.

Ikegaya, Y., Nakanishi, K., Saito, H., & Abe, K. (1997). Amygdala β-noradrenergic influence on hippocampal longterm potentiation in vivo. *Neuroreport, 8*(14), 3143-3146.

Izquierdo, I., Cammarota, M., Medina, J. H., & Bevilaqua, L. R. (2004). Pharmacological findings on the biochemical bases of memory processes: A general view. *Neural Plasticity, 11*(3-4), 159-189.

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

Jurado-Berbel, P., Costa-Miserachs, D., Torras-Garcia, M., Coll-Andreu, M., & Portell-Cortes, 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.

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.

Kafkas, A., & Montaldi, D. (2018). How do memory systems detect and respond to novelty? *Neuroscience Letters, 680*, 60-68.

Kealy, J., & Commins, S. (2011). The rat perirhinal cortex: A review of anatomy, physiology, plasticity, and function. *Progress in Neurobiology, 93*(4), 522-548.

Kikyo, H., Ohki, K., & Miyashita, Y. (2002). Neural correlates for feeling-of-knowing: An fmri parametric analysis. *Neuron, 36*(1), 177-186.

Lacy, J. W., & Stark, C. E. (2013). The neuroscience of memory: Implications for the courtroom. *Nature reviews neuroscience, 14*(9), 649-658.

Laing, M., & Bashir, Z. (2014). B-adrenoceptors and synaptic plasticity in the perirhinal cortex. *Neuroscience, 273*, 163-173.

Lee, A. C., Bandelow, S., Schwarzbauer, C., Henson, R. N., & Graham, K. S. (2006). Perirhinal cortex activity during visual object discrimination: An event-related fmri study. *Neuroimage, 33*(1), 362-373.

Lee, A. C., Buckley, M. J., Gaffan, D., Emery, T., Hodges, J. R., & Graham, K. S. (2006). Differentiating the roles of the hippocampus and perirhinal cortex in processes beyond long-term declarative memory: A double dissociation in dementia. *Journal of Neuroscience, 26*(19), 5198-5203.

Lee, A. C., Bussey, T. J., Murray, E. A., Saksida, L. M., Epstein, R. A., Kapur, N., Hodges, J. R., & Graham, K. S. (2005). Perceptual deficits in amnesia: Challenging the medial temporal lobe 'mnemonic'view. *Neuropsychologia, 43*(1), 1-11.

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.

Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., & Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. *Behaviour Research and Therapy, 43*(11), 1391-1424.

Malkova, L., & Mishkin, M. (2003). One-trial memory for object-place associations after separate lesions of hippocampus and posterior parahippocampal region in the monkey. *Journal of Neuroscience, 23*(5), 1956-1965.

McGarry, L. M., & Carter, A. G. (2016). Inhibitory gating of basolateral amygdala inputs to the prefrontal cortex. *J Neurosci, 36*(36), 9391-9406.

McGaugh, J. L. (2003). *Memory and emotion: The making of lasting memories*: Columbia University Press.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience, 27*, 1-28.

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., Cahill, L., & Roozendaal, B. (1996). Involvement of the amygdala in memory storage: Interaction with other brain systems. *Proceedings of the National Academy of Sciences, 93*(24), 13508-13514.

McGaugh, J. L., & Roozendaal, B. (2002). Role of adrenal stress hormones in forming lasting memories in the brain. *Current Opinion in Neurobiology, 12*(2), 205-210.

McIntyre, C. K., McGaugh, J. L., & Williams, C. L. (2012). Interacting brain systems modulate memory consolidation. *Neuroscience and Biobehavioral Reviews, 36*(7), 1750-1762.

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.

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).

Miranda, M. I., & McGaugh, J. L. (2004). Enhancement of inhibitory avoidance and conditioned taste aversion memory with insular cortex infusions of 8-br-camp: Involvement of the basolateral amygdala. *Learning and Memory, 11*(3), 312-317.

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 and Memory, 15*(7), 468-476.

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

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.

Nirogi, R., Abraham, R., Jayarajan, P., Medapati, R. B., Shanmuganathan, D., Kandikere, V., Irappanavar, S., Saralaya, R., Benade, V., Bhyrapuneni, G., & Muddana, N. (2012). Difference in the norepinephrine levels of experimental and non-experimental rats with age in the object recognition task. *Brain Research, 1453*, 40-45.

Norman, G., & Eacott, M. (2005). Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. *Behavioral Neuroscience, 119*(2), 557.

O'Neil, E. B., Cate, A. D., & Köhler, S. (2009). Perirhinal cortex contributes to accuracy in recognition memory and perceptual discriminations. *Journal of Neuroscience, 29*(26), 8329-8334.

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.

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

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.

Platel, H., Price, C., Baron, J.-C., Wise, R., Lambert, J., Frackowiak, R. S., Lechevalier, B., & Eustache, F. (1997). The structural components of music perception. A functional anatomical study. *Brain: a journal of neurology, 120*(2), 229-243.

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.

Ranganath, C., & Ritchey, M. (2012). Two cortical systems for memory-guided behaviour. *Nature reviews neuroscience, 13*(10), 713-726.

Ranganath, C., Yonelinas, A. P., Cohen, M. X., Dy, C. J., Tom, S. M., & D'Esposito, M. (2004). Dissociable correlates of recollection and familiarity within the medial temporal lobes. *Neuropsychologia, 42*(1), 2-13.

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

Rodríguez-Durán, L. F., Martínez-Moreno, A., & Escobar, M. L. (2017). Bidirectional modulation of taste aversion extinction by insular cortex ltp and ltd. *Neurobiology of Learning and Memory, 142*, 85-90.

Roozendaal, B., Castello, N. A., Vedana, G., Barsegyan, A., & McGaugh, J. L. (2008). Noradrenergic activation of the basolateral amygdala modulates consolidation of object recognition memory. *Neurobiology of Learning and Memory, 90*(3), 576-579.

Roozendaal, B., Dominique, J.-F., Ferry, B., Setlow, B., & McGaugh, J. L. (2001). Basolateral amygdala–nucleus accumbens interactions in mediating glucocorticoid enhancement of memory consolidation. *Journal of Neuroscience, 21*(7), 2518-2525.

Roozendaal, B., Hahn, E. L., Nathan, S. V., de Quervain, D. J., & McGaugh, J. L. (2004). Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala. *Journal of Neuroscience, 24*(37), 8161-8169.

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. *Journal of Neuroscience, 30*(14), 5037-5046.

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

Roozendaal, B., & McGaugh, J. L. (2011). Memory modulation. *Behavioral Neuroscience, 125*(6), 797-824.

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

Roozendaal, B., Okuda, S., Van der Zee, E. A., & McGaugh, J. L. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America, 103*(17), 6741-6746.

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

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

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

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., Reiss, A. L., & Greicius, M. D. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *Journal of Neuroscience, 27*(9), 2349-2356.

Setlow, B., Roozendaal, B., & McGaugh, J. L. (2000). Involvement of a basolateral amygdala complex–nucleus accumbens pathway in glucocorticoid-induced modulation of memory consolidation. *European Journal of Neuroscience, 12*(1), 367-375.

Sewards, T. V., & Sewards, M. A. (2003). Input and output stations of the entorhinal cortex: Superficial vs. Deep layers or lateral vs. Medial divisions? *Brain research reviews, 42*(3), 243-251.

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

Shi, C. J., & Cassell, M. D. (1998). Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. *J Comp Neurol, 399*(4), 440-468.

Shura, R. D., Hurley, R. A., & Taber, K. H. (2014). Insular cortex: Structural and functional neuroanatomy. *J Neuropsychiatry Clin Neurosci, 26*(4), 276-282.

Song, Q., Bolsius, Y., Ronzoni, G., Henckens, M. J. A. G., & Roozendaal, B. (2020). Noradrenergic enhancement of object recognition and object location memory in mice. *Stress*, 1-25.

Squire, L. R., & Alvarez, P. (1995). Retrograde amnesia and memory consolidation: A neurobiological perspective. *Current Opinion in Neurobiology, 5*(2), 169-177.

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 of the United States of America, 106*(23), 9447-9452.

Stepanichev, M., Markov, D., Pasikova, N., & Gulyaeva, N. (2016). Behavior and the cholinergic parameters in olfactory bulbectomized female rodents: Difference between rats and mice. *Behavioural Brain Research, 297*, 5-14.

Sternson, S. M., & Roth, B. L. (2014). Chemogenetic tools to interrogate brain functions. *Annual Review of Neuroscience, 37*, 387-407.

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., Fernandez, G., Deisseroth, K., Greene, R. W., & Morris, R. G. (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*(5), 455-461.

van Leeuwen, J. M. C., Vink, M., Fernandez, G., Hermans, E. J., Joëls, M., Kahn, R. S., & Vinkers, C. H. (2018). At-risk individuals display altered brain activity following stress. *Neuropsychopharmacology, 43*(9), 1954-1960.

Wan, H., Aggleton, J. P., & Brown, M. W. (1999). Different contributions of the hippocampus and perirhinal cortex to recognition memory. *Journal of Neuroscience, 19*(3), 1142-1148.

Weible, A. P., Rowland, D. C., Monaghan, C. K., Wolfgang, N. T., & Kentros, C. G. (2012). Neural correlates of long-term object memory in the mouse anterior cingulate cortex. *Journal of Neuroscience, 32*(16), 5598-5608.

Weible, A. P., Rowland, D. C., Pang, R., & Kentros, C. (2009). Neural correlates of novel object and novel location recognition behavior in the mouse anterior cingulate cortex. *Journal of Neurophysiology, 102*(4), 2055-2068.

Wiltgen, B. J., & Silva, A. J. (2007). Memory for context becomes less specific with time. *Learning and Memory, 14*(4), 313-317.

Wiltgen, B. J., & Tanaka, K. Z. (2013). Systems consolidation and the content of memory. *Neurobiology of Learning and Memory, 106*, 365-371.

Winocur, G., & Moscovitch, M. (2011). Memory transformation and systems consolidation. *Journal of the International Neuropsychological Society, 17*(5), 766-780.

Yonelinas, A. P., Otten, L. J., Shaw, K. N., & Rugg, M. D. (2005). Separating the brain regions involved in recollection and familiarity in recognition memory. *Journal of Neuroscience, 25*(11), 3002-3008.

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1986). Human amnesia and the medial temporal region: Enduring memory impairment following a bilateral lesion limited to field ca1 of the hippocampus. *Journal of Neuroscience, 6*(10), 2950-2967.

# *Appendix*

**Samenvatting**

**Research data management**

**PhD portfolio**

**About the author**

**Acknowledgement**

**Donders Graduate School for Cognitive Neuroscience**

## Samenvatting

In dit proefschrift heb ik onderzocht of de neurotransmitter noradrenaline de gedetailleerdheid van onze herinneringen beïnvloedt en welke hersenprocessen daar een rol bij spelen. Deze onderzoeksvraag komt voort uit eerder opgedane kennis dat stressvolle en emotionele ervaringen een sterk effect hebben op veel aspecten van leren en geheugen, uiteenlopend van het aanleren tot de opslag en het ophalen van informatie. Eerdere studies hebben uitgebreid onderzocht hoe stress en emotie deze herinneringen versterken. Een beter geheugen voor emotionele gebeurtenissen is een adaptief verschijnsel dat helpt om vooral belangrijke gebeurtenissen te onthouden. Dit fenomeen was zelfs al bekend in de middeleeuwen: Om een blijvende herinnering te creëren aan een specifieke gebeurtenis werden kinderen ondergedompeld in een rivier (om stress te veroorzaken) nadat ze iets belangrijks hadden meegemaakt zoals een huwelijk of schenking van land. Echter, in welke mate stress en emotie ook de betrouwbaarheid van dergelijke herinneringen beïnvloeden, d.w.z. hun accuraatheid en gedetailleerdheid, is veel minder onderzocht.

Gedragsstudies bij de mens hebben aangetoond dat emotionele herinneringen anders kunnen zijn in kwaliteit, maar de bevindingen zijn tegenstrijdig. Sommige studies toonden aan dat emotie de accuraatheid van het geheugen verbetert, terwijl andere studies juist lieten zien dat emotionele herinneringen in een meer gegeneraliseerde, algemene wijze worden onthouden, en vaak verkeerde informatie bevatten. Het is duidelijk dat een minder betrouwbaar geheugen veel minder adaptieve waarde heeft. Zo is bijvoorbeeld een zeer sterke, maar weinig specifieke herinnering aan een stressvolle gebeurtenis een belangrijke risicofactor voor het ontwikkelen van posttraumatische stressstoornis en fobieën. Naast zulke klinische implicaties, zijn verstoringen in de betrouwbaarheid van het geheugen ook maatschappelijk relevant, zoals de accuraatheid van de herinneringen van ooggetuigen in de rechtbank of voor theorieën over emotionele versterking van leerprestaties in het onderwijs. Het is daarom belangrijk te begrijpen welke effecten stress en emoties hebben op de accuraatheid en gedetailleerdheid van het geheugen, en hoe deze effecten worden bewerkstelligd in het brein. In gedragsexperimenten bij proefdieren kan dit worden onderzocht.

In **Hoofdstuk 2** heb ik bij muizen onderzocht of noradrenaline na het kort exploreren van een object tijdens de leerfase ervoor zorgt dat dit object later beter herkend wordt. Hoewel vele studies al eerder de effecten van noradrenaline op het geheugen, inclusief dat voor objecten, hadden onderzocht, waren deze studies dusver alleen uitgevoerd bij ratten. Tegenwoordig worden echter vooral studies bij muizen gedaan omdat er veel transgene

muizenlijnen beschikbaar zijn die uitermate geschikt zijn om onderzoek te doen naar de betrokkenheid van specifieke hersencircuits bij het geheugen. In Hoofdstuk 2 heb ik daarom de effecten van noradrenaline op het geheugen van muizen onderzocht in twee verschillende leertaken: de objectherkenningstaak en de objectlocatieherkenningstaak. In de objectherkenningstaak kunnen muizen tijdens een trainingssessie twee dezelfde objecten voor een bepaalde tijd exploreren. Om vervolgens te testen of zij dit object daarna kunnen herkennen wordt een retentietest uitgevoerd. Tijdens deze testsessie is een van deze nu bekende objecten nog steeds aanwezig, maar is het andere object vervangen door een nieuw object. In de andere leertaak, de objectlocatieherkenningstaak, kunnen de muizen tijdens de trainingssessie ook twee dezelfde objecten exploreren, maar tijdens de testsessie is een van deze objecten verplaatst naar een nieuwe locatie. Aangezien muizen van nature een voorkeur hebben voor nieuwe objecten or locaties, kan uit de verhouding van exploratie van het nieuwe en bekende object of de nieuwe en bekende locatie worden bepaald in hoeverre zij het object en/of de locatie hiervan hebben onthouden. Ik kon aantonen dat het farmacologisch toedienen (in de buikholte) van yohimbine, een stof die de afgifte van noradrenaline in de hersenen vergroot, zowel het geheugen voor het object zelf als voor de locatie van dit object verbeterde (Song *et al.*, 2020). Deze bevindingen zijn zeer vergelijkbaar met die van voorgaande studies in ratten, en stelden mij in staat om vervolgens de effecten van noradrenaline op de gedetailleerdheid van het geheugen voor objecten te onderzoeken.

In **Hoofdstuk 3** heb ik onderzocht of noradrenaline en de duur van de trainingsfase de gedetailleerdheid van het geheugen voor objecten beïnvloeden, en hoe deze gedetailleerdheid verandert over de tijd. De standaard geheugentaak die gebruikt wordt voor het testen van het geheugen voor objecten was echter ongeschikt voor het onderzoeken van de gedetailleerdheid van het geheugen. Daarom heb ik een nieuwe geheugentaak ontwikkeld, genaamd de objectvergelijkingstaak. In deze taak gebruik ik drie verschillende objecten tijdens de retentietest; een exemplaar van het bekende object, een exemplaar van een object dat erg lijkt op het bekende object, en een exemplaar van een object dat totaal niet lijkt op het bekende object. Ik zag dat als de muizen tijdens de leerfase het object voor een periode van 3 min mochten verkennen, ze in staat waren tijdens de retentietest 1 dag later, het totaal verschillende object te herkennen als een nieuw object, maar ze maakten geen onderscheid tussen het bekende en het daarop lijkende object. Echter, als de leerfase verlengd werd tot 10 min, waren ze ook in staat het lijkende object te onderscheiden van het bekende object. Deze bevindingen toonden dus aan dat een langere leerperiode zorgt voor een gedetailleerder geheugen voor het object.

Wanneer ik yohimbine toediende na een leerfase van 3 min, zag ik een vergelijkbaar effect op de gedetailleerdheid van het geheugen voor het object als van een langere leerfase; muizen bleken dan ook in staat het lijkende object te onderscheiden van het bekende object 1 dag later. Echter, dit meer gedetailleerde geheugen door yohimbine raakte geleidelijk verloren met het verstrijken van de tijd. De muizen hadden geen gedetailleerd geheugen meer 7 en 14 dagen na de leerfase.

Om te begrijpen hoe yohimbine de aanvankelijke gedetailleerdheid van het geheugen voor het object kan verbeteren, heb ik gekeken naar de hersenactiviteit in reactie op de retentietest 1 dag na het leren. Ik heb daarbij specifiek gekeken naar hersengebieden waarvan we weten dat ze betrokken zijn bij het geheugen voor objecten; de anterieure (het voorste deel van de) insulaire cortex (aIC), de perirhinale cortex (PRh) en de basolaterale amygdala (BLA). Dit heb ik onderzocht met een immunofluorescentie experiment waarbij ik onder een microscoop het aantal cellen in deze hersengebieden telde dat immunoreactiviteit vertoonde voor c-Fos, een eiwit dat aangemaakt wordt in actieve hersencellen. Ik vond dat dieren die yohimbine toegediend hadden gekregen meer actieve hersencellen in reactie op de retentietest hadden in de aIC en in de PRh. Ik vond geen verschillen in de activiteit in de BLA. Deze toename in activiteit in de aIC en de PRh vertoonde een positieve correlatie met de mate waarin de muizen in staat waren het lijkende object van het bekende object te onderscheiden.

In **Hoofdstuk 4** heb ik verder onderzocht of deze toename in actieve hersencellen in de aIC en PRh werd veroorzaakt door het herkennen van het nieuwe, totaal verschillende object of juist door het bekende, lijkende object. Recente onderzoeken bij vooral de mens suggereren dat het verwerken van informatie voor nieuwe en bekende stimuli afhankelijk is van verschillende neurale systemen. Dit is een volledig nieuw idee en in hoeverre dit ook betrekking heeft op objectherkenning is niet eerder onderzocht. Daarom heb ik in dit hoofdstuk de activiteit van hersencellen in de aIC en PRh onderzocht in reactie op slechts een type object tijdens de retentietest. Dit experiment leverde een aantal interessante resultaten op. Ik vond namelijk dat het toedienen van yohimbine zorgde voor meer actieve cellen in de aIC na het verkennen van het bekende object, terwijl ik juist meer actieve cellen in de PRh vond na het verkennen van het totaal verschillende object. Deze resultaten laten dus zien dat de aIC vooral betrokken is bij het herkennen van bekende informatie en de PRh vooral bij het herkennen van nieuwe informatie. Mijn belangrijkste bevinding was dat het aantal actieve cellen in zowel de aIC als PRh omhoog ging na het verkennen van het lijkende

object. Deze bevinding suggereert dus dat om de details van een object goed te kunnen herkennen zowel hersengebieden die betrokken zijn bij het herkennen van bekende als nieuwe informatie gebruikt worden.
In een tweede experiment heb ik onderzocht of het effect van yohimbine op de gedetailleerdheid van het geheugen afhankelijk is van anatomische verbindingen tussen de BLA en de aIC. Met behulp van een specifieke techniek (DREADD) kon ik selectief de activiteit van deze verbinding onderdrukken na de leerfase. Ik kon hier aantonen dat het remmen van de activiteit van deze verbinding specifiek het effect van yohimbine op de gedetailleerdheid van het geheugen tijdens de testsessie blokkeerde terwijl het geen effect had op het herkennen van het nieuwe object.

In **Hoofdstuk 5** geef ik een samenvatting van mijn onderzoeksresultaten en heb getracht deze in een bredere context te plaatsen. Ik beschrijf drie belangrijke thema's. Ten eerste, wat zeggen mijn resultaten over de effecten van noradrenaline op de gedetailleerdheid van het geheugen en hoe dit verandert met de tijd? Ten tweede, welke rol spelen de aIC en PRh in de gedetailleerd van het geheugen en het herkennen van bekende en onbekende informatie? Ten derde, hoe kan noradrenaline na de leerfase in samenspraak met de aIC en PRh er uiteindelijk voor zorgen dat er een meer gedetailleerd geheugen ontstaat? Aan de hand van deze discussie presenteer ik tot slot een nieuw model hoe ik denk dat noradrenaline de gedetailleerdheid van het geheugen voor objectherkenning kan verbeteren. In dit model stel ik voor dat een verhoogde afgifte van noradrenaline in de hersenen in de periode na de leerfase zorgt voor een versterkte interactie tussen de BLA enerzijds en zowel de aIC als PRh anderzijds. Vorig onderzoek heeft aangetoond dat dit ervoor zorgt dat tijdens de opslag van de herinnering in het geheugen de activiteit in de aIC afneemt, en ik stel voor dat dit leidt tot een toename in de activiteit in de PRh. Dit zorgt er vervolgens voor dat het geheugen voor objecten niet alleen sterker wordt opgeslagen maar ook nauwkeuriger. Tijdens de herkenning van het object worden de aIC en PRh daardoor sterker geactiveerd en werken ze nauw samen om zo meer details van het object te kunnen ophalen en herkennen.

## 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:\ 4040000.02) |
| 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:\ 4040000.02) |
| 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:\ 4040000.02) |
| 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 | Qi Song |
|---|---|
| **Graduate school** | Donders Graduate School |
| **Department** | Cognitive Neuroscience |
| **(Co-)Promotor(s)** | Prof. Dr. Benno Roozendaal<br>Dr. Marloes Henckens |
| **Research period** | September 2016 – December 2020 |

## Professional courses and workshops

| Subject | Course provider | Year | EC |
|---|---|---|---|
| Anatomy & Physiology | Utrecht University | 2016 | 7.0 |
| Laboratory Animal Science | Radboudumc | 2017 | 3.0 |
| Advanced Conversation | Radboud University | 2017 | 1.5 |
| Scientific Integrity for PhD candidates | Radboudumc | 2017 | 1.0 |
| Presentation Skills | Radboudumc | 2017 | 1.5 |
| Perfecting Your Academic Writing Skills | Radboud University | 2017 | 1.5 |
| Image Analysis with FIJI | RIMLS | 2018 | 1.0 |
| Scientific Writing | Radboud University | 2018 | 3.0 |
| Lectures and others | | | 12 |
| | | **Total** | **31.5** |

## 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 |
| FENS Forum of Neuroscience | Online forum | 2020 |
| 3rd Swiss Stress Network Meeting | Online forum | 2021 |

## List of publications

**Song Q**., Bolsius, Y., Ronzoni, G., Henckens, M. J. A. G., & Roozendaal, B. (2020). Noradrenergic enhancement of object recognition and object location memory in mice. *Stress*, 1-25.

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 of the United States of America*, 116(14), 7077-7082. doi: 10.1073 /pnas. 1901513116.

**Song Q**, Wang Q, Wu W, Shao M, Zhang Y, Zhang Z, Wang Y, Tao L. (2016). Effects of new drug T-006 on learning and memory abilities in scopolamine-induced dementia Kunming mice. *Chinese Pharmacological Bulletin*, 32(6):812-817.

Zhang Y, Tao L, Fan L, Peng Y, Yang K, Zhao Y, **Song Q**, Wang Q. (2015). Different gap junction-propagated effects on cisplatin transfer result in opposite responses to cisplatin in normal cells versus tumor cells. *Scientific Reports*. 5:12563.

## About the author

Qi Song was born on 19th of March 1991 in Shucheng, Anhui province, China. In 2009, he started his bachelor study at Guangdong Pharmaceutical University in China, with a major in Pharmacy. In 2013, he was admitted to a 3-year Master research program at the National Platform for Pre-clinical Evaluation of New Drugs at Sun Yat-sen University, Guangzhou, China. He performed his research project under the supervision of Prof. Dr. Liang Tao on the effect of the novel drug T-006, an anti-Alzheimer's compound derived from Chinese medicinal component tetramethylpyrazine, on the learning and memory abilities in rodents. It was during this time that he opened the new door to neuroscience research and became fascinated by the mystery of learning and memory.

In 2016, he obtained his Master degree of Science and decided to apply for a PhD program. In the same year, he received a scholarship from the China Scholarship Council (CSC) to pursue his 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, he carried out his PhD project on the effect of noradrenergic activation in mice on memory detailedness and the underlying neural mechanisms. Most of the scientific studies he performed during his PhD are described in this thesis.

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## 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 Donders Graduate School for Cognitive Neuroscience (DGCN), which was officially recognised as a national graduate school in 2009. The Graduate School covers training at both Master's and PhD level and provides an excellent educational context fully aligned with the research programme of the Donders Institute.

The school successfully attracts highly talented national and international students in biology, physics, psycholinguistics, psychology, behavioral science, medicine and related disciplines. Selective admission and assessment centers guarantee the enrolment of the best and most motivated students.

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.

For more information on the DGCN as well as past and upcoming defenses please visit:
http://www.ru.nl/donders/graduate-school/phd/