

DO YOU REMEMBER WHAT YOU DID LAST SUMMER?

On the formation and alteration of memories for stressful experiences

DONDERS
S E R I E S

Lycia Dienneke de Voogd

DO YOU REMEMBER WHAT YOU DID LAST SUMMER?

ON THE FORMATION AND ALTERATION OF MEMORIES FOR
STRESSFUL EXPERIENCES

Lycia Dieneke de Voogd

The work described in this thesis was carried out at the Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center Nijmegen.

ISBN 978-94-6284-107-9

Cover design

Linda de Voogd

Print

Uitgeverij

Paranimfen

Isabella Wagner

Tjerk Gutteling

Famke Ernst

Copyright © Lycia Dienneke de Voogd, 2017

ℒT_EX

DO YOU REMEMBER WHAT YOU DID LAST SUMMER?

ON THE FORMATION AND ALTERATION OF MEMORIES FOR
STRESSFUL EXPERIENCES

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,
volgens besluit van het college van decanen
in het openbaar te verdedigen op donderdag 11 mei 2017
om 12.30 uur precies

door

Lycia Dieneke de Voogd

geboren op 21 maart 1983
te Utrecht

Promotor

Prof. dr. G. S. E. Fernández

Copromotor

Dr. E. J. Hermans

Manuscriptcommissie

Prof. dr. A. H. Schene

Prof. dr. R. Cools

Dr. U. Rimmele (*Université de Genève, Zwitserland*)

“Everybody probably has isolated glimpses of certain hours of his nursery life, the position in which he stood or sat, the light of the room, what his father or mother said, etc. These moments so oddly selected for immunity from the tooth of time probably owe their good fortune to historical peculiarities which is now impossible to trace. Very likely we were reminded of them again soon after they occurred; that became a reason why we should again recollect them, etc., so that at last they became ingrained.
”

William James 1890 (page 670)

Contents

1 General introduction	3
2 Disentangling the roles of arousal and amygdala activation in emotional declarative memory	23
3 Amygdala-hippocampal connectivity during post-encoding rest as a trait predicts enhanced memory under stress	41
4 Awake reactivation of emotional memory traces through hippocampal-neocortical interactions	61
5 Goal-directed eye movements during extinction deactivate amygdala and reduce fear recovery	83
6 General discussion	101
Appendix	133
<i>Nederlandse samenvatting</i>	134
<i>Dankwoord</i>	137
<i>Curriculum Vitae</i>	143
<i>List of publications</i>	144
<i>Donders series</i>	145



Chapter 1

General introduction

Introduction

Memories may not always be as “oddly” selected as they appear. Our memory system is highly selective and not every piece of information we encounter will be stored into long-term memory. The experiences that have been emotional (positive and negative) or sometimes even stressful are “immune to the tooth of time” and preferentially stored in long-term memory. These experiences might have been a fight your father and mother had when you were a child. It might have been such a situation of which you vividly remember the position in which you stood or sat, the light of the room and so on. In a world full of dangerous encounters, we would benefit from remembering where and how these encounters occurred, in order to predict the occurrence of similar events in the future. The stress response to these stressors can have many negative consequences in daily life, but in the first place, it is useful. It helps us to escape from such dangerous situations and it facilitates the storage of these events. Influential work by James McGaugh, that started already in the 1960s, has shown that long-term memories are not created instantly. The effects of stress on memory might arise already at the time of the encounter (*i.e.*, encoding), but directly following the occurrence of an event memory storage is still susceptible to external influences. When time passes memories become more and more resistant to these external influences; a process that is referred to as consolidation. Thus, to understand why some memories are more persistent than others it is crucial to study the consolidation of memory.

Understanding consolidation is also of interest from a clinical perspective, because persistent memories (*e.g.*, traumatic memories) can severely affect functioning in daily life. It is not always possible to prevent traumatic events from happening, we can try to alter memory for these events after they occurred. Most of our understanding of the consolidation of memories for stressful experiences comes from animal studies, but it is unclear how the animal findings translate to humans. Studies in humans have mostly focused on memory encoding or retrieval. The reason for this is that with the available imaging methods it seemed only possible to study stimulus-evoked activity. Consolidation, however, occurs during undefined periods of rest and is unpredictable in time. This makes it impossible to detect with conventional neuroimaging techniques. Along with others in the field, I developed new multivariate analysis methods that make it possible to study this elusive phenomenon called consolidation.

Another reason why it is important to study consolidation of memory for stressful experiences in humans, is that in animals the effects of stress on memory consolidation have mostly been studied at the synaptic level. Yet we know that memories are (re)organized in the brain at a much larger scale. Specifically the latter can be investigated well in humans with imaging methods such as functional magnetic resonance imaging (fMRI). With this technique it is possible to investigate the entire

brain at the same time (including regions that are nested deep in the brain). Thus, in this thesis I will study the consolidation of memories for stressful experiences in humans by investigating periods of awake rest following memory encoding. Lastly, I will focus on manipulating these memories with a novel method that serves as a translation of a technique that is already in clinical use.

What is stress?

We first need to understand what “stress” is. First there is a stressor, which can be defined as an internal or external stimulus or event (physical or psychological) that threatens the homeostasis of an organism (Selye, 1936). The moment we encounter something threatening a set of defensive behaviors gets activated. For example, a robber with a gun, a wasp, the depth when looking down from a high building, or an audience when we have to give a presentation. This “stress response” can avoid possible harm from these stressors. Two stress-systems can be distinguished namely, (1) the sympatho-adrenomedullary system (Cannon, 1929), which is a fast system, and (2) the Hypothalamic–Pituitary–Adrenal (HPA) axis, which is a slower system (Selye & Fortier, 1950; Selye, 1936). Additional to these bodily changes we are often of course also able to verbalize that we are afraid or stressed (Schachter & Singer, 1962). The HPA-axis has historically been seen as the stress system (Selye & Fortier, 1950; Selye, 1936), but the two systems are dependent of each other (de Kloet et al., 2005). Specifically, these two systems were shown to interact, especially in the modulatory effects on memory (Roozendaal, Okuda, Van der Zee, and McGaugh, 2006). The sympatho-adrenomedullary system is also often studied in the context of fear learning (**Chapter 2, Chapter 4 and Chapter 5**) and the HPA-axis in the case of other stress-induction methods such as aversive movie clips (**Chapter 2**). Therefore, both systems should be considered when investigating the stress response.

The sympatho-adrenomedullary system

The fast system involves the adrenal medulla that activates the Autonomic Nervous System (ANS). The ANS has two complementary systems in itself, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The sympathetic branch can be seen as the “gas” pedal and is often referred to as the fight-or-flight system (Cannon, 1929). The parasympathetic branch can be seen as the “brake” and is therefore classically been referred to as the rest-or-digest system (Berntson et al., 1991), but the PNS also plays a role in the stress response, for example by promoting freezing responses (Fanselow, 1994). Threatening encounters activate the SNS and adrenal medulla which leads to release of epinephrine (or adrenaline) in the body and nor-epinephrine in the brain and rest of the body

(Aston-Jones & Cohen, 2005; Sara, 2009). SNS activation further leads to for example, increased heart rate, blood flow to the muscles, and dilation of pupils (de Kloet et al., 2005). The principal site for the release of nor-epinephrine in the brain is the locus coeruleus (LC), a region in the brainstem. The LC is a small region, and is for this reason difficult to investigate (especially with non-invasive imaging methods used in humans). Together the LC and nor-epinephrine release has been referred to as the LC-NE system. This system is relevant immediately at the time of the encounter because it increases vigilance (Aston-Jones & Bloom, 1981), attention (Nieuwenhuis et al., 2005), and perception (Foote et al., 1975).

The Hypothalamic–Pituitary–Adrenal-axis

Stressful experiences also activate a slower system, the Hypothalamic-Pituitary-Adrenal axis (HPA-axis de Kloet et al., 2005). This system starts by activation of the paraventricular nucleus of the hypothalamus which in turn secretes vasopressin and the corticotropin-releasing hormone (CRH). These together then stimulate the secretion of the adrenocorticotrophic hormone (ACTH). ACTH ultimately acts on the adrenal cortex that produces glucocorticoid (e.g., cortisol in humans). In turn, each of these nodes has feedback loops, making this altogether a complicated system. Cortisol reaches a peak around 20-30 minutes after stress onset and can bind to two different receptor types, namely the mineralcorticoid receptor (MR) as well as the glucocorticoid receptor (Joels et al., 2012). The non-genomic effects of the HPA-axis are fast and overlap in time with the sympathetic-adrenal-medullary system, but the genomic effects of the HPA-axis last till hours after stress onset (Henckens et al., 2011, 2010; Hermans et al., 2014; Joels et al., 2012; Karst et al., 2005). Thus, one potential function of the HPA-axis could be to restore homeostasis in the aftermath of stress (de Kloet et al., 2005).

Other factors that influence the two stress systems

The two stress systems are not only influenced by stressors, but also by other factors such as physical activity or the circadian rhythm. Cortisol levels peak after waking up (Kirschbaum et al., 1999). To be able to measure the relatively small effect (compared to the circadian rhythm) of stress on cortisol levels, the vast majority of the human stress studies are performed in the afternoon. Cortisol levels are furthermore considered to differ between men and women. The menstrual cycle was shown to influence cortisol levels (Kirschbaum et al., 1999) and for this reason typically only male participants are included. The analyses from **Chapter 3** were performed on existing data that only included men for these reasons. (**Chapter 2, Chapter 4 and Chapter 5**), the sample included an equal number of males and females. However, it is not only important that a sample is representative of the population (note that this is not per se the case for other variables such as age,

socio-economic status or race). The stress response might also be gender specific, because it is known that the HPA-axis interacts with the gonadal axis (Viau, 2002). Moreover, fear-related and anxiety disorders are much more prevalent in women than men (McLean et al., 2011). For this reason it is important to include both women and men in stress research.

Stress and memory

It may not come as a surprise that exactly these stress systems that help us respond adaptively to stressors, are also involved in the formation of memories for those stressful experiences. Stress and memory are therefore inextricably linked. These stress systems are not only activated by unconditioned stressors, but also by learned stressors (LeDoux, 2012). In **(Chapter 2, Chapter 4 and Chapter 5)** the stress response is therefore also used as a memory measure of learned fear, and is measured via skin conductance (an index of sympathetic activation; Lang et al., 1993) and pupil dilation (an indirect index of locus coeruleus-noradrenergic activity Aston-Jones & Cohen, 2005; Bradley et al., 2008; Gilzenrat et al., 2010). Arousing stimuli (e.g., a picture of a mutilated body) do not only elicit a stress response, but are also better remembered compared to items that do not (e.g., a picture of a chair Bradley et al., 1992; Buchanan et al., 2006; Kleinsmith & Kaplan, 1963). Memories for stressful experiences contain multiple dimensions and it is shown that stress does not enhance memory for all aspects per se. Central details related to the stressor are better remembered (Wiemers, Sauvage, Schoofs, Hamacher-Dang, and Wolf, 2013), while peripheral non-stressful aspects are remembered worse (Kensinger, Garoff-Eaton, and Schacter, 2007). Despite this inaccurate memory, the subjective feeling of remembering remains high (Rimmele et al., 2011). On the other hand it, items that are non-arousing by themselves but that are encoded in a stressful context where found to be better remembered compared to items encoded in a neutral context (Henckens et al., 2009). Interestingly, when stress is induced after learning, memory for the learned material is enhanced as well (Smeets et al., 2008). Specifically the processes that take place after such learning events are poorly understood in humans.

The role of the amygdala in stress

In addition to the primary structures involved in the stress response mentioned above, the amygdala is one of the most important sites implicated in the stress response. The amygdala is a structure in the medial temporal lobe (MTL). It has the name “amygdala” because of its almond-like shape (“amygdala” is Greek for “almond”). The exact function of the amygdala has been a topic of debate. On the one hand, the amygdala is involved in the initiation of the acute stress re-

sponse. This has led to the claim that the amygdala is the “fear-system” of the brain (LeDoux, 2003; Öhman & Mineka, 2001, although see (LeDoux, 2014)). On the other hand, others have stressed the role of the amygdala in enhancing mnemonic processes (McGaugh, 2002). The two roles of the amygdala in the initiation of the stress response and memory enhancement are difficult to dissociate, especially in humans.

The first evidence that the amygdala is involved in the initiation of the stress response comes from lesion studies performed by Brown and Shafer in 1888. They removed parts of the temporal lobe in rhesus monkeys and investigated the behavioral changes as a consequence of this (Brown & Shafer, 1888). The authors described that the rhesus monkeys became indifferent to the people handling them and showed no signs of fear. These findings were later confirmed by more well-known studies performed by Klüver and Bucy in 1937. They also induced lesions (although for a different purposes) in the MTL, including the amygdala in rhesus monkeys which resulted in a loss in emotional responses and loss of fear (Klüver & Bucy, 1937). Although these studies have formed the basis for the notion that the amygdala is involved in initiating stress responses, it is important to note that the lesions in these monkeys were not specific to the amygdala. Additional to the loss of fear, memory deficits were observed (Brown & Shafer, 1888). More evidence indicates that the amygdala plays a role in the initiation of the stress response. For instance, the LC receives projections from the central nucleus of the amygdala (Van Bockstaele et al., 2001). Furthermore, electrical stimulation of the amygdala leads to changes in autonomic responses in both humans and animals (Chapman et al., 1954; Kaada et al., 1954; Reis & LeDoux, 1987). A more recent study stimulated the basolateral amygdala (BLA) terminals in the central nucleus of the amygdala (CeN) using optogenetics and found an increase in anxiety-related behavior (Tye et al., 2011). This did not occur when the BLA was stimulated, suggesting that these effects are thus not uniform across the whole amygdala.

Furthermore, evidence for the role of the amygdala in the stress response in humans comes from patients or from correlational imaging work in humans. For instance, human patients with Urbach-Wiethe disease (UWD) which leads to selective bilateral amygdala damage (Terburg et al., 2012). They typically show deficits in conditioned fear responses (Bechara et al., 1995; Klumpers et al., 2015a). Functional neuroimaging work on the amygdala revealed activation of this structure related to the processing of arousing material, such as threatening or salient stimuli and faces (Hariri et al., 2002; Morris et al., 1997; Vuilleumier et al., 2001; Whalen et al., 1998). The vast majority of these studies have used negatively arousing stimuli, but it was also shown that amygdala responsivity was associated with subjective arousal and skin conductance responses of positive pictures (Bonnet et al., 2015). However, these studies do not provide causal evidence for a role of

the amygdala in the expression of (conditioned) arousal/fear in humans. Interestingly, responses to unconditioned stimuli are usually intact in these patients (Bechara et al., 1995; Klumpers et al., 2015a; LaBar et al., 1995) which means that noradrenergic-sympathetic stress responses can be present in the absence of a functional amygdala. In nonhuman primates it was shown that the amygdala is not necessary for the expression of conditioned fear (Antoniadis et al., 2009). Recent data on one patient with amygdala damage, furthermore, indicated that this patient is able to experience subjective feelings of fear and panic after carbon dioxide (CO₂) inhalation (Feinstein, 2013, but see (Feinstein et al., 2011)). These findings indicate that the amygdala is not the main initiator of the stress response in the case of unconditioned stressors. With respect to learned stressors, the amygdala might be critical for learning, but does not initiate a stress response to these learned stressors.

The role of the amygdala in memory encoding

It could be that the amygdala elicits a stress response and this subsequently alters mnemonic processes. A large body of animal studies have shown that the amygdala modulates mnemonic processing that are dependent on other brain regions (Ferry & McGaugh, 1999; McGaugh, 2004; McIntyre et al., 2005; Roozendaal et al., 2008; Roozendaal & McGaugh, 2011). For example, injections of d-amphetamine in the amygdala were shown to enhance memory that is dependent on regions such as the caudate nucleus and the hippocampus (Packard et al., 1994). However, there is also data demonstrating that noradrenergic manipulations are ineffective in modulating memory in the absence of a functioning amygdala (Cahill & McGaugh, 1991; Liang et al., 1982). Thus, this data suggests that the role of the amygdala in memory might arise after the initiation of the stress response.

Early studies investigating declarative memory in humans have indeed shown that arousal at the time of encoding is associated with enhanced memory. For example, stimuli that are perceived as more arousing (Bradley et al., 1992) or stimuli that elicit a stress response (Buchanan et al., 2006; Kleinsmith & Kaplan, 1963), are typically well remembered. Indeed, activation of the amygdala was related to the processing of arousing material, such as threatening or salient stimuli and faces (Hariri et al., 2002; Morris et al., 1997; Vuilleumier et al., 2001; Whalen et al., 1998). Amygdala activity during encoding furthermore predicts later memory for such stimuli (Dolcos et al., 2004; Erk et al., 2003; LaBar & Cabeza, 2006; Murty et al., 2011; Richardson et al., 2004). Furthermore, this subsequent memory-related amygdala activation at the time of encoding seems to correspond with subjective arousal (Canli et al., 2000).

These findings in humans are in line with a role for the amygdala in activating autonomic responses to threat (Chapman et al., 1954; Gläscher & Adolphs, 2003;

Kaada et al., 1954; Reis & LeDoux, 1987; Reyes et al., 2011). However, these data are also in line with a role for the amygdala in mediating the effects of the stress response on memory (Roozendaal et al., 2009; Roozendaal & McGaugh, 2011). This means that the existing human neuroimaging studies on emotional declarative memory are inconclusive about these two interpretations. In these paradigms, amygdala activity and the noradrenergic-sympathetic stress response cannot be disentangled because the to-be-remembered stimuli are arousing by themselves (*i.e.*, the arousing items later remembered might be more arousing than arousing items later forgotten). It is therefore unclear what roles arousal and activation of the amygdala play in enhancing memory. Thus, the first question I would like to answer in this thesis is; what are the roles of arousal and amygdala activation during encoding in enhancing declarative memory for stressful experiences?

Q1: What are the roles of arousal and amygdala activation during encoding in enhancing declarative memory for stressful experiences?

Synaptic and systems consolidation

Long-term and permanent memories are not created instantly (McGaugh, 1966). Following encoding, memory storage is still susceptible to outside influences and as time passes memories become resistant to these outside influences. This process has been referred to as consolidation. The term consolidation was introduced by Müller and Pilzecker (1900), who first discovered that memories are fragile after encoding (Lechner et al., 1999). For example, they showed that learning new information can interfere with information that was previously learned, and suggested that memory formation continues after learning has taken place (Lechner et al., 1999). Also drug manipulation or brain lesions following encoding were shown to interfere with memory formation (Dudai, 2004). One famous case study provided influential evidence that memories continue to be formed after encoding. Patient H.M. underwent bilateral medial temporal lobe resection, including the removal of the hippocampus with the purpose of reducing epileptic seizures (Schmolck et al., 2002; Scoville & Milner, 1957). After surgery he was not able to form new memories. Moreover, he also lost recent memories, while remote memories remained intact. This does not only suggest that memories are consolidated, it also suggests that memories are reorganized in the brain and after consolidation the hippocampus is no longer necessary for memory retrieval.

Two consolidation processes have been proposed (Dudai, 2004; Frankland & Bontempi, 2005). The first takes place at the cellular level via strengthening of synaptic connections between neurons. It is therefore referred to as “synaptic consolidation”. Synaptic consolidation lasts up to several hours (Davis & Squire, 1984). The second type of consolidation takes place at a larger scale between distant brain regions and lasts up to days, months or some have suggested years (Frankland & Bontempi, 2005). This involves a reorganization of brain circuits and is referred to as “systems consolidation”. These two processes are assumed to be related, but it is unclear how they influence each other exactly.

It was shown that memory for stressful experiences is strengthened due to preferential consolidation (McGaugh, 2000, 2013), additional to the immediate effects of the stress response on attentional, sensory, and mnemonic (Dolcos et al., 2004; LaBar & Cabeza, 2006). After learning has occurred, neuromodulatory systems continue playing a role in long-term memory formation (Roozendaal et al., 2009). Most evidence for the role of the stress response in memory consolidation is based on findings at a synaptic level and it is so far unknown whether the stress response affects systems consolidation as well.

Synaptic consolidation of stressful experiences and the modulatory role of the amygdala

Synaptic modifications form the neural basis of learning and memory (Hebb, 1949). These connections are plastic and can change rapidly. Long-term potentiation (LTP) is a strengthening of synapses (Lømo, 1966) via an increase of AMPA receptors at the postsynaptic membrane and long-term depression (LTD) is a weakening of connections and a decrease of these AMPA receptors. LTP can be induced via high-frequency stimulation of neurons. Often hippocampal neurons are used to study LTP. These changes at the synapse start at the time of encoding. They continue thereafter, which raises the question if there is a difference between synaptic encoding and synaptic consolidation. This is a difficult question to answer, but it seems unlikely that there is a clear cut-off where encoding stops and consolidation starts.

If stress enhances memory, then these basic processes that are thought to underlie learning and memory should be affected by stress as well. Indeed, a large body of studies have shown that stress hormones such as norepinephrine and glucocorticoids can facilitate synaptic plasticity (Krugers et al., 2012). Ex-vivo studies have shown that β -adrenergic receptor activation facilitates LTP of hippocampal neurons (Thomas et al., 1996) and increases AMPA receptors at postsynaptic synapses (Hu et al., 2007). Also, glucocorticoids were shown to increase synaptic transmission in the hippocampus (Karst et al., 2005). Norepinephrine and glucocorticoids do not only affect synaptic plasticity in isolation, but both in synergy enhance synaptic

strength even further (Pu et al., 2007). Moreover, glucocorticoids are insufficient to enhance memory consolidation without norepinephrine (Roozendaal et al., 2006). Importantly, the amygdala mediates these neuromodulatory systems by lowering the threshold for synaptic modification or LTP in other regions (Roozendaal et al., 2009). Electrophysiological experiments using anesthetized rats have shown that electrical stimulation of the (basolateral) amygdala modulates synaptic plasticity in the hippocampus (Abe, 2001) and noradrenergic activation of the BLA, in rats, increases the expression of activity-regulated cytoskeletal (Arc) protein, which is involved in regulating synaptic plasticity and memory consolidation (Guzowski et al., 2000), in the hippocampus. Also, behavioral experiments in rodents have shown that when norepinephrine is infused into the amygdala following learning, memory is enhanced, and when these β -adrenergic receptors are blocked, memory is impaired (Gallagher & Kapp, 1981). Another important finding that indicates the modulatory role of the amygdala in memory is that injections of d-amphetamine in the caudate nucleus or hippocampus improves memory that is dependent on those regions, but when d-amphetamine is infused in the amygdala it enhances both types of memory (Packard et al., 1994).

Systems consolidation of stressful experiences and the modulatory role of the amygdala

All these synaptic changes are very local and do not necessarily explain the reorganization that takes place at a large scale. The standard model of systems consolidation (Marr, 1970; McClelland et al., 1995; Squire & Alvarez, 1995) states that initially memories are dependent on the hippocampus, but over time memory retrieval is no longer dependent on the hippocampus. The hippocampus serves as a “binding” function between neocortical regions which over time is no longer necessary (Marr, 1970). It was proposed that other regions take over this binding function (Frankland & Bontempi, 2005). As mentioned above, evidence for this notion comes from patient studies showing that recent memories are disrupted when the hippocampus is lesioned, but remote memories are intact. More recent theories on systems consolidation have stated that not all memories may become independent of the hippocampus (Nadel et al., 2007; Yassa & Reagh, 2013). Semantic memories (*i.e.*, general knowledge; *e.g.*, “the King of the Netherlands is Willem Alexander”) might become hippocampal independent, but not episodic memories (*i.e.*, memory for specific events; *e.g.*, “I remember I once met Willem Alexander on Kingsday”). A clear line between semantic and episodic is, however, difficult to draw. One hypothesis is, that especially memories for stressful experiences, are episodic by nature and may therefore never become independent of the hippocampus (Nadel & Moscovitch, 1997; Nadel et al., 2007).

Stressful experiences where indeed found to prompt interactions between dis-

tant regions (Hermans et al., 2011, 2014; Paz et al., 2006). Electrophysiology studies in rodents have demonstrated that amygdala-hippocampal theta coherence increases during the expression of conditioned fear in mice (Seidenbecher et al., 2003). Furthermore, after chronic immobilization stress in rats, beta and gamma synchrony was enhanced between the lateral amygdala and the CA1 region of the hippocampus, which lasted up to 10 days (Ghosh et al., 2013). Indeed, it was shown that increases in theta coherence between amygdala and hippocampus during sleep after fear learning was predictive for later fear retention (Popa et al., 2010). A recent study showed that electrical stimulation of the basolateral complex of the amygdala (BLA) after rodents had seen novel objects leads to enhanced memory for those objects, as well as enhanced synchrony in the gamma frequency range in the hippocampus (Bass & Manns, 2015). Human studies did indicate that during encoding amygdala-hippocampal interactions predict enhanced memory retrieval (Dolcos et al., 2005), but it is unknown whether these systems-level interactions continue to play a role after learning. The second question I would like to answer in this thesis is therefore: which system-level interactions continue to play a role in enhancing declarative memory for stressful experiences?

Q2: Which systems-level interactions continue to play a role in enhancing declarative memory for stressful experiences?

Memory replay

It is not clear what William James meant exactly with “Very likely we were reminded of them again soon after they occurred; that became a reason why we should again recollect them, etc., so that at last they became ingrained.”. Maybe he meant that we are reminded of previous experiences via external cues which initiates a process of recollection. While this might be possible, it is known that that internal processes play a role in strengthening memory after learning. As should be clear by now, memories are not instantly stored, but continue to be formed after events occurred (McGaugh, 2000). The brain might keep memories active by replaying experiences after they occurred. The story of memory replay starts with the understanding that the hippocampus is not only involved in memory, but is actually a critical site for spatial representations of the environment (O’Keefe & Nadel, 1978). From rodent studies it is known that the hippocampus contains cells that fire in certain patterns depending on the location of the rat in a particular environment or space (O’Keefe

& Dostrovsky, 1971). This means that when a rat walks through a maze recordings of those place cells would reveal a pattern of activation in space and time.

The first discovery of replay occurred when these hippocampal place cells were also recorded during sleep after rats had walked through a maze. It was shown that the temporal and spatial firing patterns that were measured during behavior reoccurred during sleep in a similar fashion (Wilson & McNaughton, 1994). Replay was also recorded in the neocortex which was coordinated with replay in the hippocampus (Ji & Wilson, 2007; Sirota et al., 2003). Moreover, neocortical replay was found with increased probability during the occurrence of hippocampal sharp-wave/ripples (Logothetis et al., 2012; Peyrache et al., 2009). It is thought that replay is the underlying mechanism of systems consolidation and evidence for the systems consolidation theories explained in the previous paragraph (McKenzie & Eichenbaum, 2011). Replay occurs during both sleep and awake states (Carr et al., 2011).

If replay is the mechanism through which consolidation occurs, and if stress enhances consolidation then it could be the case that stress hormones increase or enhance these replay events. This is so far unknown, but there are findings that are in line with this hypothesis. For example, awake replay was shown to be enhanced for novel (Foster & Wilson, 2006) and reward-related (Singer & Frank, 2009) experiences. This suggests that awake replay could be boosted by catecholaminergic, neuromodulatory changes during salient events (Carr et al., 2011). Indeed, a recent study showed that optogenetic stimulation of hippocampal dopaminergic midbrain neurons in mice exploring novel environments enhances reactivation of pyramidal cell assemblies during subsequent sleep (McNamara et al., 2014). As mentioned above, norepinephrine strengthens memory consolidation (McGaugh, 2002, 2000), and lowers the threshold for synaptic modification (Roozendaal et al., 2009). Spontaneous replay of information occurs specifically during sharp-wave/ripples (Karlsson & Frank, 2009; Skaggs & McNaughton, 1996). When synaptic plasticity is experimentally induced, sharp-wave/ripples are increased (Behrens et al., 2005; Buzsaki, 1984). Thus, these periods of synaptic plasticity have indirectly been linked to replay events through the occurrence of sharp-wave/ripples (Carr et al., 2011). That these replay events are relevant for memory was demonstrated by studies showing that hippocampal reactivation predicts subsequent spatial memory performance (Dupret et al., 2010).

Based on these findings, it could be the case that when stress hormones and neurotransmitters induce synaptic plasticity, this also affects memory replay. There is a relatively small number of human functional neuroimaging studies that investigated replay because of methodological challenges. Conventional neuroimaging techniques cannot be used to detect these replay events that occur during undefined and unpredictable periods of rest. Initial studies using novel analyses

techniques have produced findings which are in agreement with replay studies in animals. For example, enhanced post-learning functional connectivity between hippocampus and neocortical regions, measured Blood-Oxygen-Level Dependent (BOLD) fMRI, predicted memory retention (Tambini et al., 2010). Furthermore, multivariate analyses have revealed that encoding-related activity patterns reactivated spontaneously during post-learning rest (Deuker et al., 2013; Gruber et al., 2016; Staresina et al., 2013; Tambini & Davachi, 2013). In conclusion, it is possible to measure reactivation of memory traces in humans. Thus, we tested the hypothesis that memory traces of stressful experiences would be reactivated stronger than memory traces for non-stressful experiences. The third question I would like to answer in this thesis is: do stressful experiences alter awake memory reactivation in humans?

Q3: Do stressful experiences alter awake memory reactivation in humans?

Maladaptive memory formation

So far, I have discussed how stress can enhance memory and how this can be an adaptive process. However, for some individuals an event can be so stressful that it leads to involuntary memories and intrusions, in some cases resulting in post traumatic stress syndrome (PTSD). Indeed, memory persistence has been indicated as one of “the seven sins of memory” (Schacter, 1999). To quote Daniel Schacter (1999): “*Studies of traumatic memories reveal that failures to forget can sometimes be even more disabling than forgetting itself*”. Those individuals who suffer from traumatic memories would benefit from therapy that is focused on attenuating these memories, which are typically remote and already underwent consolidation. The most widely used technique to alter memories in therapy is through repeated exposure to reminders to the traumatic experience. For example, through repeated exposure of the traumatic car accident by means of pictures, the memory of the car accident becomes less aversive over time. This is based on a fundamental mechanism known as extinction (Bouton, 1993). It is thought that the process of extinction creates a new safety memory (*i.e.*, new memories of cars not being a threat) rather than overwriting the old memory. Interestingly, recent studies have indicated that memories might undergo the same process as consolidation after reactivating them (Nader et al., 2000). This would make it potentially possible to overwrite them instead of merely creating a new safety memory.

Fear learning

The most widely used model for fear learning is Pavlovian fear conditioning. It is thought fear conditioning underlies fear-related disorders such as PTSD. Fear conditioning involves a single neutral cue (*e.g.*, a yellow square on a computer screen) called the conditioned stimulus (CS) and an aversive stimulus (*e.g.*, an electric shock to the fingers), called the unconditioned stimulus (UCS). In rodent studies, the CS is typically an auditory tone and the UCS an aversive foot shock (LeDoux, 2003). When the CS and UCS are coupled in time participants learn that the CS predicts the UCS, presumably via Hebbian learning (Hebb, 1949). Over time fear or stress responses to the UCS are carried over to the CS. Thus, when the yellow square (CS) is encountered again participants typically show increased skin conductance responses (an index of sympathetic activation; Lang et al., 1993) and increased pupil dilation (an indirect index of locus coeruleus-noradrenergic activity; Aston-Jones & Cohen, 2005; Bradley et al., 2008; Gilzenrat et al., 2010). This type of cue-conditioning is dependent on the amygdala, since lesions in the amygdala make it impossible for rats to learn that the CS predicts the UCS (LeDoux, 2003; Maren, 1999, 2001). In **(Chapter 2, Chapter 4 and Chapter 5)** we used an electric shock to the fingers as an UCS. In **Chapter 5** we used simple cues, namely colored squares, as CSs. In **Chapter 2 and Chapter 4** we used pictures of a conceptual category, namely animals and fruits/vegetables, (Dunsmoor et al., 2012) as CSs, because this allowed us to investigate reactivation of these categories during rest and additionally test for item recognition.

Extinction learning

Once the CS-UCS association is made, it can be very difficult to unlearn it. One way would be to extinguish conditioned responses through repeated presentations of the CS without the UCS (Bouton, 1993; Maren, 2011). Even though extinction is effective, with the passage of time responses to the CS can return. Also when the CS is presented in a different context, or after a reminder UCS (without the CS), responses can return. For this reason a large body of studies investigate how extinction learning can be improved in order to prevent the return of conditioned responses (Dunsmoor et al., 2015). Since extinction learning is considered a new form of learning rather than overwriting the old fear memory (*i.e.*, the CS-UCS association), it might be that new learning is not strong enough to permanently overwrite the conditioned response. In **Chapter 5** we investigated whether we could improve extinction learning.

Reconsolidation

Maybe the best way to improve therapy would be to erase the entire CS-UCS association. However, it was for a long time the belief that once memories underwent

consolidation, they would remain fixated (McGaugh et al., 1996). An influential paper from 2000 by Nader and colleagues showed that memories can actually become labile again after reactivation. After a brief reminder a memory would undergo a similar process as consolidation, which was therefore referred to as reconsolidation. When the amygdala was targeted via protein synthesis inhibitors, it was possible to disrupt memory (Nader et al., 2000). However, this pharmacological treatment cannot be used in humans, because it is lethal. A safe drug that can be used is propranolol, β -adrenergic receptor blocker. Human studies have investigated whether propranolol could alter memories have provided opposing results in experimental studies with healthy participants (Bos et al., 2012; Kindt et al., 2009), and yielded mixed results in PTSD patients (Brunet et al., 2008; Wood et al., 2015). For these reasons, non-invasive techniques that can potentially improve extinction retention have gained a lot of attention (Coelho et al., 2015; Dunsmoor et al., 2015; Schiller et al., 2010, 2013). It was shown that performing extinction following a brief reminder (presumably leading to reconsolidation) diminished fear recovery in both animals and humans (Monfils et al., 2009; Schiller et al., 2010), suggesting it would be possible to erase memories via non-invasive means.

The amygdala as a target region

Since fear conditioning is dependent on the amygdala (LeDoux, 2003; Maren, 1999, 2001), animal studies have targeted the amygdala in an attempt to disrupt the CS-UCS association (Nader et al., 2000). In humans, the role of the amygdala in fear expression is less clear (Bach et al., 2011; Fullana et al., 2016; Mechias et al., 2010), but studies on patients with amygdala lesions indicate that the amygdala plays a role in the initial formation of the CS-UCS association as well (Bechara et al., 1995; Klumpers et al., 2015a). Pharmacological interventions in humans aiming to disrupt memory implicitly assume the drug effects obtained, for example with propranolol, are exerted via targeting the amygdala (Kindt et al., 2009; Kroes et al., 2015). Indeed, amygdala reactivity measured with BOLD-fMRI in humans was shown to decrease after propranolol administration (Hurlemann et al., 2010). The effects of propranolol on extinction learning are not clear. Some studies found that propranolol reduced recovery of fear (Kindt et al., 2009; Kroes et al., 2015), but other studies found it impaired extinction learning (Bos et al., 2012). This might be because the amygdala is not only involved in fear acquisition, but is also critically involved in extinction learning (Fitzgerald, Seemann, and Maren, 2014; Maren, 2001). During memory reactivation, multiple processes can occur at the same time making it possible to impair extinction as well as enhance it (Eisenberg et al., 2003). Moreover, the effects of systemic propranolol administration are not limited to the amygdala (Hermans et al., 2011).

It might also be possible to manipulate activity in the amygdala via non-invasive

means. This is a new idea that is based on recent insights into resource competition between large-scale brain networks (Fox et al., 2005; Hermans et al., 2014). It was shown that large-scale brain networks act reciprocally (Fox et al., 2005), and compete for resources (Hermans et al., 2014). This means that when one network or a set of regions is activated, other regions or networks are deactivated. For example, tasks that require endogenous attention, such as working memory tasks, typically activate a dorsal fronto-parietal network (Corbetta & Shulman, 2002). When this network (often referred to as the executive control network) is activated, regions in a posterior-medial network are typically deactivated, including the amygdala (Cousijn et al., 2012; Qin et al., 2009). The reciprocal relationship between these networks is also reflected in behavior. For example, acute stress impairs performance on a two-back working memory task (Qin et al., 2009). In the reverse direction, there is also data indicating that high cognitive load reduces stress responses. It was found that startle responses are reduced during the performance of a working memory task (Vytal et al., 2012). Evidence for the reciprocal nature of working memory and amygdala function comes from patients with amygdala lesions showing enhanced working memory performance (Morgan et al., 2012). These data together do not only indicate that activation in one large-scale network suppresses activation in another large-scale network, but also that this goes along with impairment in functions that are supported by the suppressed network. Since tasks that require goal-directed attention suppress activity in regions that support memory formation, it might be possible to disrupt (re)consolidation by performing a working memory task during this crucial period.

Eye Movement Desensitization and Reprocessing

Another way to activate the dorsal-frontal parietal network next to working memory tasks is via goal-directed eye movements (Jamadar et al., 2013). This is relevant because eye movements are currently used in a therapy called Eye Movement Desensitization and Reprocessing (EMDR) that aims to reduce traumatic symptoms (Shapiro, 1989). It was shown before that antisaccades, another type of goal-directed eye movements, indeed suppress activity in the hippocampus and amygdala (Herweg et al., 2014). It is not clear whether the smooth pursuit like eye movements used during EMDR also deactivate the amygdala.

During EMDR treatment, patients divide their attention between recalling traumatic memories and making lateral eye movements directed by the therapist's hand. EMDR is an evidence-based therapy and part of mental health care guidelines in many countries (Bisson et al., 2013; Lee & Cuijpers, 2013). Although eye movements are central to the procedure, it is unclear if they play any role in the therapeutic outcome making this therapy controversial (Herbert et al., 2000; Muris & Merckelbach, 1999). So far, scientific evidence on the efficacy of EMDR have been mostly

comprising subjective reports (Andrade et al., 1997; van den Hout et al., 2001) and a mechanistic explanation for how eye movements could reduce traumatic symptoms does not exist. Moreover, since patients also recall their traumatic memories, the effects observed with EMDR could also be due to normal extinction learning (Rogers & Silver, 2002). Insight into the potential role of eye movements and the neurobiological mechanisms underlying this manipulation is not only crucial to further optimize this therapy, but would also importantly advance fundamental understanding of extinction learning. Therefore, the final question I would like to address in this thesis is: can memory be altered by disrupting amygdala activation via non-invasive means?

Q4: Can memory be altered by disrupting amygdala activation via non-invasive means?

Thesis outline

As explained above, stressful experiences are typically well remembered. While this can be partly explained by the immediate effects of stress at encoding, memory for such events are further strengthened by subsequent consolidation processes. Thus, to understand why some memories are more persistent than others it is crucial to study the consolidation of memory. So far such research in humans investigating the neural correlates of consolidation is scarce. Specifically the neural correlates that underlie the consolidation of memories for stressful experiences has not yet been studied. Moreover, animal research has shown how neuromodulatory systems that are involved in stress promote synaptic consolidation, but it is unclear how stress alters systems-level interactions known to underlie long-term memory formation.

In this thesis I addressed the question to what extent altered systems-level interactions play a role during consolidation of memories for stressful experiences and further investigate how such “offline” processes can be targeted to alter memory retention. To do so, I have combined functional MRI with physiological stress measures and studied task-free periods of rest using novel developed multivariate analyses techniques.

In **Chapter 2** we investigated the roles of arousal and the amygdala in the formation of declarative memory for stressful experiences. A large body of evidence in animals and humans implicates the amygdala in promoting memory for stressful experiences. Although the amygdala can trigger threat-related noradrenergic-sympathetic responses, amygdala activation and noradrenergic-sympathetic re-

sponses do not always concur in humans. We combined a subsequent-memory paradigm with a fear-conditioning paradigm in which we could disentangle amygdala activation and the noradrenergic-sympathetic response. The paradigm consisted of neutral items (*i.e.*, conditioned stimuli) which belonged to two conceptual categories. One category consisted of pictures of animals and the other of pictures of fruits. Functional MRI, skin conductance (index of sympathetic activity), and pupil dilation (indirect index of central noradrenergic activity) were acquired during acquisition and recognition memory for individual items was tested 24 hours later.

In **Chapter 3** we investigated whether amygdala-hippocampal interactions following learning continue to play a role in memory for stressful experiences. Animal models stated that the amygdala mediates the effects of stress on consolidation of hippocampal-dependent memories. To test this hypothesis, we used an existing data set consisting of a sample of 120 healthy male participants performing an incidental encoding task and subsequently underwent a resting-state functional MRI in a stressful and a neutral context. Stress responses were assessed by measures of salivary cortisol, blood pressure, heart rate, and subjective ratings of mood. Memory was tested afterwards outside of the scanner.

In **Chapter 4** we investigated whether spontaneous reactivations across hippocampal-neocortical circuits following learning are stronger for such stressful experiences using functional MRI. To test this, participants underwent a delay fear conditioning paradigm including pictures part of a conceptual category (*i.e.*, animals and fruits/vegetables). Task blocks were interleaved with blocks of awake rest. Counterbalanced across participants, exemplars of one category (CS+), but not the other (CS-), were paired with mild electrical shocks. To individually estimate category specific patterns of BOLD, an independent localizer paradigm preceded the conditioning paradigm. This localizer paradigm involved exposure to unique exemplars from the two categories which did not reoccur during conditioning. Fear recall (differential conditioned pupil dilation) was tested 24 hours later.

And finally, in **Chapter 5** we investigated whether disrupting amygdala activation with a behavioral manipulation, namely eye movements, following reactivation of such stressful experiences could disrupt memory retention. Additionally, this study was meant to establish an experimental model of EMDR that would allow us to investigate the underlying mechanisms. We proposed that EMDR might exerts its effect through amygdala deactivation, which occurs as a consequence of the goal-directed eye movements that are being made. We therefore developed a paradigm which integrates goal-directed eye movements into an established Pavlovian fear conditioning paradigm. Similar to EMDR treatment, goal-directed eye movements were performed following memory reactivation during extinction learning. Memory was assessed by testing for spontaneous recovery and recovery following reinstatement of skin conductance responses.



Chapter 2

Disentangling the roles of arousal and amygdala activation in emotional declarative memory

Lycia D. de Voogd, Guillén Fernández and Erno J. Hermans

Abstract

A large body of evidence in animals and humans implicates the amygdala in promoting memory for arousing experiences. Although the amygdala can trigger threat-related noradrenergic-sympathetic arousal, in humans amygdala activation and noradrenergic-sympathetic arousal do not always concur. This raises the question how these two processes play a role in enhancing emotional declarative memory. The present study was designed to disentangle these processes in a combined subsequent-memory/fear-conditioning paradigm with neutral items belonging to two conceptual categories as conditioned stimuli. Functional MRI, skin conductance (index of sympathetic activity), and pupil dilation (indirect index of central noradrenergic activity) were acquired throughout procedures. Recognition memory for individual items was tested 24h later. We found that pupil dilation and skin conductance responses were higher on CS+ (associated with a shock) compared to CS- trials, irrespective of later memory for those items. By contrast, amygdala activity was only higher for CS+ items that were later confidently remembered compared to CS+ items that were later forgotten. Thus, amygdala activity and not noradrenergic-sympathetic arousal, predicted enhanced declarative item memory. This dissociation is in line with animal models stating that the amygdala integrates arousal-related neuromodulatory changes to alter mnemonic processes elsewhere in the brain.

This chapter has been published as: de Voogd LD, Fernández G, Hermans EJ (2016) Disentangling the roles of arousal and amygdala activation in emotional declarative memory. *Social Cognitive and Affective Neuroscience* 11(9):1471–1480 (de Voogd et al., 2016b)

Introduction

The amygdala has been shown to be critically involved in promoting memory in both animals (McGaugh, 2004; Roozendaal & McGaugh, 2011) and humans (LaBar & Cabeza, 2006; Murty et al., 2011). The increased retention found for emotional declarative memory is thought to be driven by arousal (Cahill & McGaugh, 1995). Indeed, efferent pathways from the (central nucleus of the) amygdala are involved in regulating arousal-related autonomic, endocrine, neuromodulatory, and behavioral responses to threat (LeDoux et al., 1988; Reyes et al., 2011). In human fear-conditioning experiments, however, amygdala activity is often absent even though a sympathetic arousal response (*e.g.*, skin conductance) is robustly measured (see Bach et al., 2011; Fullana et al., 2016; Mechias et al., 2010). This indicates that amygdala activity and arousal-related sympathetic activity do not always coincide. Therefore, these findings raise the question what exact roles these two processes play in enhancing emotional declarative memory.

Early studies investigating declarative memory have shown that arousal at the time of encoding is associated with enhanced memory. For example, stimuli that are perceived as more arousing (Bradley et al., 1992) or stimuli that elicit a sympathetic arousal response, as measured using skin conductance responses (SCRs; Buchanan et al., 2006; Kleinsmith & Kaplan, 1963), are typically well remembered. Functional neuroimaging work on the amygdala, which normally lacks the resolution to dissociate amygdala subregions, has revealed activation of this structure related to processing of arousing material, such as threatening or salient stimuli and faces (Hariri et al., 2002; Morris et al., 1997; Vuilleumier et al., 2001; Whalen et al., 1998). Amygdala activity during encoding furthermore predicts later memory for such stimuli (Dolcos et al., 2004; Erk et al., 2003; Hamann, 2001; LaBar & Cabeza, 2006; Murty et al., 2011; Richardson et al., 2004). Indeed, subsequent memory-related amygdala activation at the time of encoding seems to correspond with subjective arousal (Canli et al., 2000).

These findings are in line with a role for the amygdala in activating arousal-related autonomic responses to threat (Chapman et al., 1954; Gläscher & Adolphs, 2003; Kaada et al., 1954; Reis & LeDoux, 1987; Reyes et al., 2011). Even though this could be a potential pathway through which mnemonic processes are altered, there is also data demonstrating that noradrenergic manipulations are ineffective in modulating memory in the absence of a functional amygdala (Cahill & McGaugh, 1991; Liang et al., 1982). Such findings indicate that amygdala activation during encoding of arousing material observed in humans may alternatively be a consequence of arousal-related noradrenergic-sympathetic activation, and reflect a modulation of mnemonic processing of the arousing material elsewhere in the brain (Ferry & McGaugh, 1999; McGaugh, 2004; McIntyre et al., 2005; Roozendaal et al., 2008; Roozendaal & McGaugh, 2011; Strange & Dolan, 2004; Van Stegeren et al., 1998).

Existing human neuroimaging studies on emotional declarative memory, however, are inconclusive about these interpretations. In these paradigms, amygdala activity and noradrenergic-sympathetic arousal cannot be disentangled because the to-be-remembered stimuli are arousing by themselves (*i.e.*, the arousing items later remembered might be more arousing than arousing items later forgotten). It is therefore unclear whether the amygdala activity found for items later remembered reflects neural activity associated with the initiation of a noradrenergic-sympathetic arousal response or an enhancement of mnemonic processing induced by this response.

There are also human neuroimaging findings that challenge the view of a tight coupling between amygdala activity and noradrenergic-sympathetic arousal responses. Dissociations between these two responses are often seen in neuroimaging experiments using classical fear conditioning, a widely used model for fear learning in which a neutral stimulus is associated with an unconditioned stimulus (UCS) such as an electrical shock. After acquisition of the fear association, participants exhibit robust and persistent noradrenergic-sympathetic arousal responses to the previously neutral stimulus (LaBar et al., 1998; Maren, 2001). Although lesion studies in humans indicate that the amygdala is necessary to acquire conditioned fear (Bechara et al., 1995; Klumbers et al., 2015a; LaBar et al., 1995), a persistent amygdala response during the expression of conditioned fear is usually not observed (Bach et al., 2011; Fullana et al., 2016; Mechias et al., 2010). This latter finding is in line with data from nonhuman primates showing that the amygdala is not necessary for the expression of conditioned fear (Antoniadis et al., 2009). These studies show that a noradrenergic-sympathetic arousal response to conditioned stimuli does not require activation of the amygdala. Thus, existing data from human fear conditioning experiments reveal a clear dissociation between amygdala activation and noradrenergic-sympathetic arousal responses, but cannot establish what the roles of these two processes are in enhancing declarative memory.

We therefore designed a functional MRI study to disentangle the roles of these two processes by orthogonalizing arousal and item memory. Participants took part in a combined subsequent-memory/fear-conditioning paradigm with neutral items belonging to two conceptual categories as stimuli (Dunsmoor et al., 2012). During encoding, items of one of the two categories (CS+; counterbalanced across participants) were paired with an aversive electrical shock in 50% of the presentations, while items of the other category (CS-) were never reinforced. In contrast to typical emotional memory paradigms, the specific item itself therefore does not trigger noradrenergic-sympathetic arousal responses. Participants returned to the lab 24h later for a recognition test in which they were shown the items seen during encoding and new items they had not seen before. Subsequent memory effects during encoding were tested by separating confidently remembered items from misses

and unsure hits (*i.e.*, forgotten items). Physiological responses to CS+ and CS- items were measured using skin conductance (an index of sympathetic activation; Lang1993) and pupil dilation (an indirect index of locus coeruleus-noradrenergic activity; Aston-Jones & Cohen, 2005; Bradley et al., 2008; Gilzenrat et al., 2010). We reasoned that if the role of the amygdala in emotional enhancement of declarative memory is to modulate mnemonic processing of the to-be-remembered material rather than to generate the noradrenergic-sympathetic arousal response, then (1) amygdala activation should predict subsequent memory for items belonging to the CS+ category, but not show a differential conditioning effect (CS+>CS-); and (2) noradrenergic-sympathetic activation should show a robust differential conditioning effect, but should not be directly associated with subsequent item memory.

Methods

Participants

Twenty-four right-handed healthy volunteers (12 female, 12 male; 19-32 years [mean=23.25]) took part in the study. An additional seven participants did not complete the entire experiment due to apparatus failure or non-compliance with instructions. Exclusion criteria were: current or lifetime history of psychiatric, neurological, or endocrine illness, abnormal hearing or (uncorrected) vision, average use of more than 3 alcoholic beverages daily, current treatment with any medication that affects central nervous system or endocrine systems, average use of recreational drugs weekly or more, habitual smoking, predominant left-handedness, intense daily physical exercise, and any contraindications for MRI. All participants gave written informed consent and were paid for their participation. This study was approved by the local ethical review board (CMO region Arnhem-Nijmegen).

Design and procedure

Participants were tested in a subsequent-memory/fear-conditioning paradigm (see Figure 2.1) including neutral items belonging to two distinct conceptual categories. In 50% of the trials, one category was paired to an electrical shock (*i.e.* unconditioned stimulus; UCS). On day 1, first, the intensity of electrical shock was adjusted individually using a standardized procedure (see below). Following this procedure, participants underwent the subsequent-memory/fear-conditioning paradigm. Twenty-four hours later, recognition memory was tested for the individual items presented during encoding. This test also included the same amount of unseen lures. Which items served as targets and which ones as lures was randomized across subjects. Additionally, the experimental procedure included a category representation localizer paradigm and resting-state blocks. Analyses on these data

will be reported elsewhere. All experiments were programmed using Presentation® software (Version 0.70, www.neurobs.com).

Stimuli

Stimuli consisted of 128 items which were either animals or fruits/vegetables. We excluded items with a higher threat value (such as lions and snakes) to avoid additional arousal and facilitated conditioning (Öhman & Mineka, 2001). The pictures were selected from the Hemera Photo-Objects set (<http://hemera-technologies-inc.software.informer.com>) and publicly available resources on the internet. Luminance of all pictures, including the grey background, was equalized.

Subsequent-memory/fear-conditioning paradigm

The encoding paradigm included 32 CS+ items (50% reinforcement rate) and 32 CS- items (Dunsmoor et al., 2012). Which of the two categories (animals or fruit/vegetables) served as CS+ was randomly counterbalanced across participants. The paradigm included two acquisition blocks and each block comprised 16 CS+ and 16 CS- items presentations, each with a 5 s duration. The intertrial interval (ITI) varied randomly between 3.5 and 6.5 s. Items were presented in a pseudorandom order with no more than three repetitions of the same category. Participants were instructed to figure out the relationship between the categories and the UCS, but did not do any other task when viewing the items. Sympathetic arousal and amygdala activity was measured in response to the individual items.

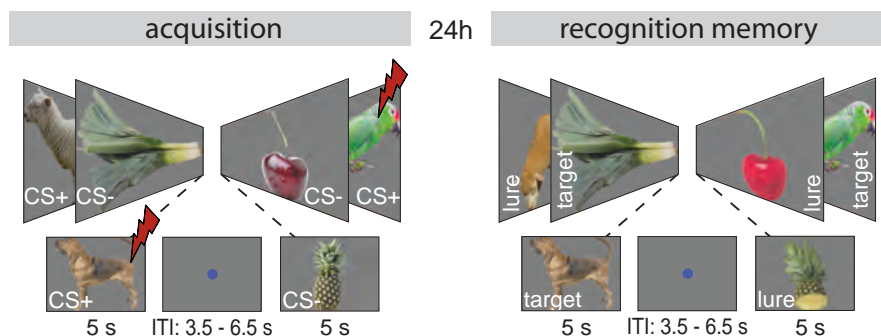


Figure 2.1: Overview of the experimental design

The experiment took place on two consecutive days. During acquisition, items from one of the two categories (CS+) were associated with an electrical shock. CS+ and CS- items were shown in pseudorandom order during the conditioning blocks (32 CSs per block). CS+s co-terminated with shock on 50% of the acquisition trials. During recognition, pictures from acquisition (64) were mixed with lures (64). Participants had to indicate whether it was an old or a new picture. Responses included three confidence bins (very sure, sure, unsure). ITI, inter trial interval.

Item recognition memory test

The recognition test contained all 64 items presented during encoding (targets) with an additional 64 new items (lures), each with a 5 s duration. The intertrial interval (ITI) varied randomly between 3.5 and 6.5 s. The lures were similar to the targets to prevent ceiling effects, which would make it impossible to test for subsequent memory effects. For example, if during encoding a dog was presented, then one of the lures was also a dog, but a different one. Participants were instructed to indicate whether they had seen the picture before, or whether it was a new picture. Response options consisted of three confidence bins (very sure, sure, unsure). Items were presented in a consecutive order. The presentation order of targets and lures was random.

For the subsequent memory analyses, we only included the very sure and sure hits in the remembered category to restrict this category to confident memory and not guesses (see Murray & Ranganath, 2007; Takashima et al., 2006; Turk-Browne et al., 2006; Wagner et al., 1998). Instead of omitting the unsure hits, we collapsed these with the misses to accommodate the low number of misses (*i.e.*, too low to reliably estimate subsequent memory effects). Memory accuracy increased with confidence [$F(2,46) = 78.85$, $p=1.37\text{E-}15$, $\eta_p^2 = .77$] and was higher for the very sure [$F(1,23)=179.216$, $p=2.42\text{E-}12$, $\eta_p^2=.89$] as well as the sure [$F(1,23)=17.951$, $p=3.12\text{E-}4$, $\eta_p^2=.44$] bins compared to unsure bins. Although at the group level, there was still above-chance level performance in the unsure bin [$F(1,21)=15.721$, $p=.001$, $\eta_p^2=.43$], at the individual level, there were on average only 1.6 unsure hit trials more than unsure false alarm trials per participant. The vast majority of the unsure hit trials is therefore likely to reflect forgotten items that were correctly guessed. We therefore define forgotten items as a combination of unsure hits and all misses.

Measurements of sympathetic arousal

Electrodermal activity was assessed using two Ag/AgCl electrodes attached to the distal phalanges of the first and second finger of the left hand using a BrainAmp MR system and recorded using BrainVision Recorder software (Brain Products GmbH, Munich, Germany). Skin conductance responses (SCR) were analyzed using in-house software implemented in Matlab 7.14 (MathWorks). SCR amplitudes were determined for each trial within a latency window from 1 to 5 seconds after stimulus onset, where the peak could only occur 500 ms after baseline. Responses were square root-transformed prior to statistical analysis. Pupil dilation was measured using an MR-compatible eye tracking system (MEye Track-LR camera unit, SMI, SensoMotoric Instruments). Data were analyzed using in-house software (Hermans et al., 2013) implemented in Matlab 7.14 (MathWorks), which was based on methods described previously by others (Siegle et al., 2003). Eyeblick artifacts were identified by differentiating the signal to detect eye pupil changes occurring too rapidly (<60

ms) to represent actual dilation. Blinks were removed from the signal using linear interpolation. Pupil diameter for each trial was normalized by dividing the signal with the average of 1 s pre-stimulus onset baseline. The averaged baseline-corrected pupil diameter within a 1 to 5 s window during picture presentation was used as response measure. Statistical analyses on SCR and pupil dilation were done by comparing later remembered (confident hits) and later forgotten (misses and unsure hits) items for both CS types.

Physiological noise correction

Finger pulse was recorded using a pulse oximeter affixed to the third finger of the left hand. Respiration was measured using a respiration belt placed around the participant's abdomen. Pulse and respiration measures were used for retrospective image-based correction (RETROICOR) of physiological noise artifacts in BOLD-fMRI data (Glover et al., 2000). Raw pulse and respiratory data were processed offline using in-house software for interactive visual artifact correction and peak detection, and were used to specify fifth-order Fourier models of the cardiac and respiratory phase-related modulation of the BOLD signal (Van Buuren et al., 2009), yielding 10 nuisance regressors for cardiac noise and 10 for respiratory noise. Additional regressors were calculated for heart rate frequency, heart rate variability, (raw) abdominal circumference, respiratory frequency, respiratory amplitude, and respiration volume per unit time (Birn et al., 2006), yielding a total of 26 RETROICOR regressors.

Peripheral stimulation

Electrical shocks were delivered via two Ag/AgCl electrodes attached to the distal phalanges of the second and third finger of the right hand using a MAXTENS 2000 (Bio-Protech) device. Shock duration was 200 ms, and intensity varied in 10 intensity steps between 0V-40V/0mA-80mA. During the standardized shock intensity adjustment procedure, each participant received and subjectively rated five shocks, allowing shock intensity to converge to a level experienced as uncomfortable, but not painful. The resulting average intensity step was 5.5 (SD: 2.0) on a scale from 1 to 10 intensity steps.

MRI data acquisition and multi-echo weighting

MRI scans were acquired using a Siemens (Erlangen, Germany) MAGNETOM Skyra 3.0T MR scanner. T2*-weighted blood oxygenation level-dependent (BOLD) images were recorded using a customized multi-echo EPI sequence with ascending slice acquisition (37 axial slices; TR, 2.38 s; TE, 15 ms and 36 ms; Generalized Autocalibrating Partially Parallel Acquisitions (GRAPPA; Griswold et al., 2002) acceleration factor 4; flip angle, 90°; slice matrix size, 106x106; slice thickness, 2.0 mm; slice gap,

0.26 mm; field of view (FOV), 212 x 212 mm; bandwidth: 1748 Hz/px; echo spacing: 0.7 ms). The functional scans only had partial brain coverage which was aligned to the temporal pole and included the amygdala and (partially) the dorsal anterior cingulate cortex. To allow for correction of distortions due to magnetic field inhomogeneity, we acquired field maps using a dual echo 2D gradient-echo sequence (64 axial slices; TR, 1020 ms; TE, 10 ms and 12.46 ms; flip angle, 90°; slice matrix size, 64x64, slice thickness, 2 mm; FOV, 224 x 224 mm). A high-resolution structural image (1 mm isotropic) was acquired using a T1-weighted 3D magnetization-prepared rapid gradient-echo sequence (MP-RAGE; TR, 2.3 s; TE, 3.03 ms; flip angle, 8°; FOV, 256 x 256 x 192 mm).

To correct EPI images for head motion, geometric distortions due to magnetic field inhomogeneity, and interactions between these, we used an integrated fieldmap-based unwarp/realign method (Hutton et al., 2002). Unwarping and realignment parameters were estimated from the first echo and applied to both echoes. Next, to account for regional variation in susceptibility-induced signal dropout, voxel-wise weighted sums of both echoes were calculated based on local contrast-to-noise ratio (Poser et al., 2006).

MRI data preprocessing and analyses

MRI data for the subsequent-memory/fear-conditioning paradigm were pre-processed in standard stereotactic (MNI152) space (using SPM8; <http://www.fil.ion.ucl.ac.uk/spm>; Wellcome Department of Imaging Neuroscience, London, UK). Mutual information maximization based rigid body registration was used to register structural and (motion and geometric distortion-corrected) functional images. Structural images were segmented into grey matter, white matter, and cerebrospinal fluid (CSF) images using a unified probabilistic template registration and tissue classification method (Ashburner and Friston, 2005). Tissue images were then registered with site-specific tissue templates (created from 384 T1-weighted scans) using DARTEL (Ashburner, 2007), and registered (using an affine transformation) with the MNI152 template included in SPM8. Identical transformations were applied to all functional images, which were resliced into 2 mm isotropic voxels and smoothed with a 6 mm FWHM Gaussian kernel.

For statistical analyses, responses to CS+ remembered items (confident hits), CS+ forgotten items (misses and unsure hits), CS- remembered items (confident hits), CS- forgotten items (misses and unsure hits), and shocks were estimated using a finite impulse response (FIR) model which included the two runs. This first-level model makes no assumptions regarding the haemodynamic response function (HRF) shape, and yields independent response estimates for all 6 TR bins within the peri-stimulus time histogram. The last bin before CS offset, but still before the shock onset was used to provide the best possible estimate of the peak

of the BOLD response to CSs. With this procedure, responses to CS+ trials can be fully separated from those to shocks. To verify this, we performed a separate FIR model in which everything was the same except for the regressors of interest. We included CS+ reinforced, CS+ unreinforced and CS- trial as regressors and compared the CS+ reinforced and the CS+ unreinforced trials in a direct contrast for the same bin. This did not yield any whole brain differences in CS response estimates (FWE $p < .05$, whole-brain cluster corrected, or within the amygdala after small volume correction). The first-level models additionally included six movement parameter regressors (3 translations, 3 rotations) derived from rigid body motion correction, 26 RETROICOR physiological noise regressors (see above), high pass filtering (1/128 Hz cut-off), and AR(1) serial correlations correction. Single-subject contrast maps obtained from first-level analyses for the 4 conditions were entered into a second-level factorial ANOVA to test for the interaction and a second-level random effects analyses (one sample t-test) for additional simple effect analyses. We used a cluster-forming voxel-level threshold of $p < .005$ (uncorrected). Alpha was set at .05, whole-brain family-wise error (FWE) corrected at the cluster level using Gaussian Random Field Theory based methods (Friston et al., 1996). Based on a priori hypotheses, results for amygdala were corrected for a reduced search volume using small volume corrections (SVC) based on an anatomical mask of the amygdala (Automated Anatomical Labeling atlas; Tzourio-Mazoyer et al., 2002).

Results

Item recognition memory test

Memory accuracy in the item recognition test was assessed by comparing the hit rates and false alarm rates for the CS+ and CS- items. Overall performance was above chance level [overall hit rate > false alarm rate; $F(1,22)=153.65$, $p=2.13E-11$, $\eta_p^2=.88$]. There was no accuracy difference between the CS+ and CS- items [$F(1,22)=.07$, $p=.80$, $\eta_p^2=.003$]. We found a non-significant trend towards a more liberal response bias (*i.e.*, tendency to say “old”) for the CS+ items [$F(1,22)=2.857$, $p=.11$, $\eta_p^2=.12$]. See Table 2.1 and Table 2.2 for descriptive statistics.

Table 2.1: Grouping of number of trials based on subsequent memory performance

	Misses	Unsure	Hits Sure	Very sure
CS+r	4.83(2.76)	2.25(1.65)	3.63(2.28)	4.96(2.44)
CS+ur	5.42(2.83)	2.58(2.24)	3.46(1.67)	4.29(2.87)
Total	10.25(4.95)	4.83(3.24)	7.07(3.32)	9.25(4.95)
CS-	12.08(4.28)	4.50(3.08)	6.88(3.29)	8.08(5.29)

Notes: Very sure and sure were grouped as later remembered and misses and unsure hits were grouped as later forgotten. r, reinforced; ur, unreinforced

Table 2.2: *Proportion of memory performance based on confidence interval*

	Unsure	Hit rate Sure	Very sure	Unsure	False Alarm rate Sure	Very sure
CS+r	0.56(0.33)	0.63(0.27)	0.82(0.19)	0.41(0.32)	0.25(0.23)	0.23(0.30)
CS+ur	0.52(0.27)	0.62(0.22)	0.81(0.30)	0.46(0.27)	0.23(0.22)	0.16(0.24)
Total	0.56(0.28)	0.62(0.19)	0.80(0.21)	0.45(0.24)	0.25(0.18)	0.18(0.23)
CS-	0.53(0.25)	0.56(0.20)	0.77(0.19)	0.36(0.20)	0.25(0.17)	0.12(0.11)

Physiological measures

First, our sympathetic arousal measures revealed robust differential conditioning effects. SCRs [$F(1,23)=19.975$, $p=1.75\text{E-}4$, $\eta_p^2=.47$] as well as pupil dilation responses [$F(1,23)=27.58$, $p=2.50\text{E-}5$, $\eta_p^2=.55$] were higher for CS+ items compared to CS- items. There was no difference in SCRs between items later remembered (confident hits) and items later forgotten (misses and unsure hits) $F(1,23)=1.681$, $p=.21$, $\eta_p^2=.07$], and no interaction between CS type (CS+, CS-) and memory (Remembered, Forgotten) [$F(1,23)=.562$, $p=.46$, $\eta_p^2=.02$] in SCRs. For pupil dilation responses, we did find an interaction between CS type (CS+, CS-) and later memory (Remembered, Forgotten) [$F(1,23)=5.49$, $p=.03$, $\eta_p^2=.21$]. An unexpected finding, however, was that this interaction was driven by an increased pupil dilation for CS- items that were later forgotten compared to CS- items that were later remembered [$t(23)=3.098$, $p=.005$, $D=1.29$]. Pupil dilation was similar for CS+ items that were later remembered versus later forgotten [$t(23)=.82$, $p=.94$, $D=0.34$]. In conclusion, we found a differential conditioning effect in the sympathetic arousal measures, however, sympathetic arousal to CS+ items did not predict item memory. See Figure 2.2.

Functional MRI

We then tested whether there was a differential conditioning response in the amygdala, however this was not the case (no voxels exceeding the clustering threshold of $p<.005$, uncorrected). Whole-brain analyses showed activation in the anterior insula, left [cluster size=3376mm³, cluster $p=.002$, whole-brain corrected] and right [cluster size=9480mm³, cluster $p=4.843\text{E-}08$, whole-brain corrected] in response to the CS+ pictures versus CS- pictures. Deactivations were found in the ventral medial prefrontal cortex (vmPFC) [cluster size=3784mm³, cluster $p=.001$, whole-brain corrected]. See Table 2.3 and Figure 2.3A.

Next, we tested whether there was an interaction between CS type (CS+, CS-) and subsequent memory (Remembered, Forgotten). We found a significant cluster in the right amygdala (cluster $p=.048$, SVC). As expected, amygdala activity was higher for CS+ pictures later remembered than for CS+ pictures later forgotten (cluster $p=.044$, SVC). There was no difference between the CS- pictures later remembered and CS- pictures later forgotten (no voxels exceeding the clustering threshold of $p<.005$, uncorrected). As can be seen from Figure 2.3C, the interac-

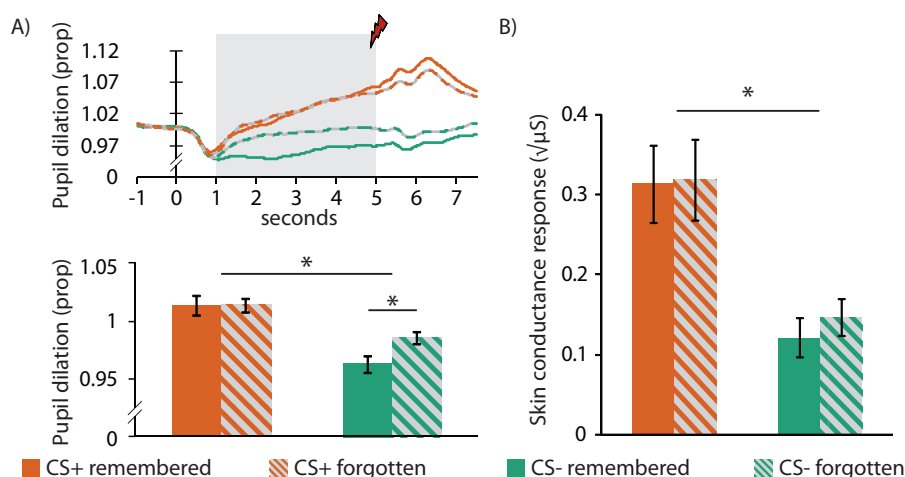


Figure 2.2: Behavioral and physiological results

A) Pupil dilation responses related to memory formation for both CS types and averaged across encoding trials that were later forgotten and those that were later remembered. B) Skin conductance responses related to memory formation for both CS types and encoding trials that were later forgotten and those that were later remembered. Rem, trials later remembered; For, trials later forgotten.

tion cluster seems to lie toward the edge of the dorsal part of the AAL amygdala mask. We therefore performed additional analyses to ensure we can attribute the activation cluster to the amygdala. These analyses show that 64% of all voxels of that cluster are within the mask, including the peak voxel ($t=3.55$, $p=.021$ FWE-SVC voxel level). The activation cluster is not part of another, bigger cluster, and the remaining voxels outside of the mask fall within white matter and not within another structure. Furthermore, when we increase the whole-brain cluster-defining threshold from $p<.005$ to $p<.001$ we see that the percentage of voxels that fall within the mask increases to 82% ($p=.017$, FWE-SVC cluster level). Thus, the central part of the cluster (including the peak voxel) is within the AAL amygdala mask and we therefore attribute the cluster to the amygdala.

Lastly, for the main effect of subsequent memory (Remembered, Forgotten) we found activations in the hippocampus extending into the parahippocampal gyrus, left [cluster size=2256mm³, cluster $p=.018$, whole-brain corrected] and right [cluster size=2400mm³, cluster $p=.013$, whole-brain corrected], and fusiform gyrus, left [cluster size=26360mm³, cluster $p=1.11E-16$, whole-brain corrected] and right [cluster size=8736mm³, cluster $p=1.46E-07$, whole-brain corrected], among others (see Table 2.3). There were no significant deactivations. In conclusion, amygdala activity is not enhanced for CS+ items overall, but does predict memory for CS+ items. See Figure 2.3B.

Table 2.3: Peak voxel coordinates and cluster statistics and size for the subsequent-memory/fear-conditioning paradigm

Region		Side	x(mm)	y(mm)	z(mm)	Cluster p	Size mm3
CS+ > CS-							
	Anterior insula	R	36	26	2	4.843E-08	9480
	Anterior insula	L	-30	24	-4	.002	3376
	Supramarginal gyrus	R	60	-46	26	.007	2696
CS- > CS+							
	Ventromedial prefrontal cortex	R/L	0	58	-6	.001	3784
<i>Remembered > forgotten</i>							
	Superior occipital gyrus / Cuneus / Precuneus	R	26	-68	42	9.281E-08	9040
	Fusiform gyrus / Inferior occipital gyrus	L	-30	-60	-14	1.110E-16	26360
	Inferior frontal gyrus	R	48	34	12	8.064E-06	6216
	Parahippocampal gyrus / hippocampus	L	-16	-12	-22	.018	2256
	Inferior orbital frontal cortex	L	-36	32	-14	.001	3776
	Fusiform gyrus / Inferior occipital gyrus	R	52	-62	-12	1.464E-07	8736
	Parahippocampal gyrus / hippocampus	R	26	-12	-28	.013	2400
	Supramarginal gyrus	L	-58	-22	36	.038	1944
<i>Interaction</i>							
	Amygdala	R	30	-6	-14	.048 (SVC)	168
CS+ remembered > CS- forgotten							
	Amygdala	R	28	-8	-14	.044 (SVC)	184

Notes: All coordinates are defined in MNI152 space. All reported statistics are significant at $p < .05$, cluster corrected.

Discussion

The aim of the current study was to disentangle the roles of noradrenergic-sympathetic arousal and the amygdala in emotional declarative memory by using an experimental design in which we were able to orthogonalize arousal (CS+ vs. CS-) and subsequent item memory (remembered vs. forgotten items). We found that skin conductance and pupil dilation showed a robust differential conditioning effect, but did not predict subsequent item memory. In contrast, amygdala activity did not show a differential conditioning effect, but predicted subsequent item memory specifically for CS+ trials. Thus, we demonstrate a dissociation between the roles of amygdala activation and noradrenergic-sympathetic arousal in emotional declarative memory.

We found robust differential conditioning effects in our noradrenergic-sympathetic arousal measures, but not in the amygdala. This finding seems to contradict findings from the rodent literature showing that the (central nucleus of the) amygdala is involved in regulating autonomic (LeDoux et al., 1988) and noradrenergic responses (Reyes et al., 2011). Indeed, stimulation of the amygdala leads to changes in autonomic responses in both humans and animals (Chapman et al., 1954; Kaada et al., 1954; Reis & LeDoux, 1987). Our null finding, however, is consistent with the human neuroimaging literature on fear conditioning (Bach et al., 2011; Fullana et al., 2016; Mechias et al., 2010). In humans, differential conditioning effects in the amygdala are often only seen during the first few trials, when fear learning takes place (Büchel & Dolan, 2000; LaBar et al., 1998). Furthermore, using a Pavlovian

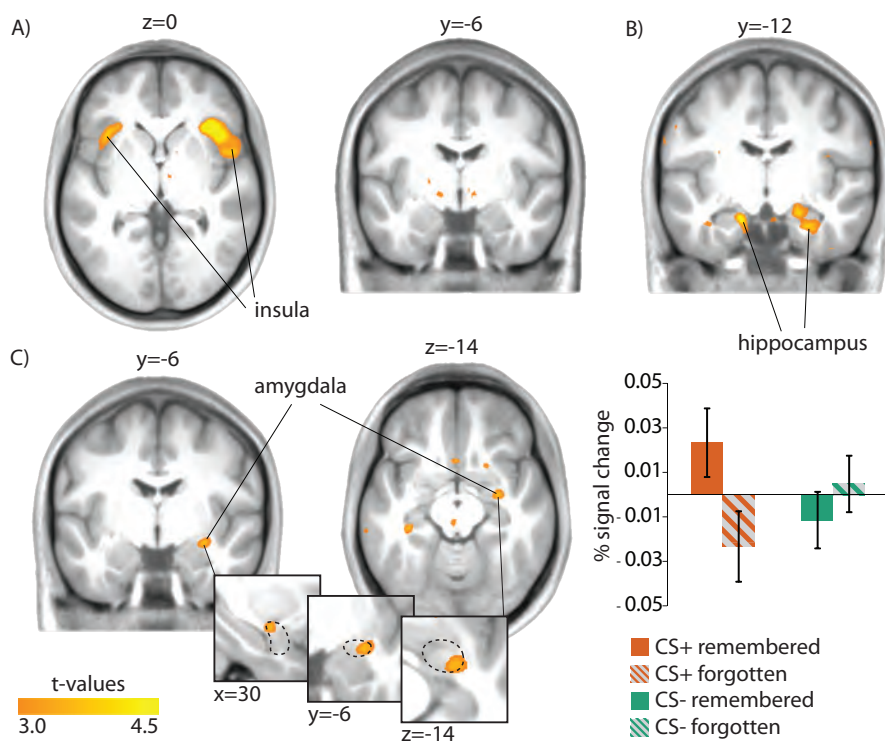


Figure 2.3: Subsequent memory analyses of the categorical fear conditioning paradigm

A) Brain activation for the contrast CS+ > CS- (bilateral insula) B) Brain activation for the contrast Remembered > Forgotten (bilateral hippocampus) C) Brain activation for the interaction CS type and later memory (right amygdala). Post hoc test shows increased amygdala activity for CS+ remembered versus CS+ forgotten ($p=.04$, Small Volume Corrected). For visualization purposes a threshold of $p<.005$ uncorrected was used. For corrected inferential statistics based on cluster size see Table 2.3. Extracted average from the contrast estimates from the significant cluster from the interaction contrast in the amygdala is plotted in a bar graph for illustration purposes.

reversal learning paradigm, it has been shown that BOLD signal in the amygdala tracks an associability signal rather than a reinforcement prediction error signal (Li et al., 2011), meaning that amygdala responsivity is related to the extent to which a cue has previously been accompanied by an unexpected event. Thus, our findings fit with the existing human neuroimaging literature and suggest that, rather than fear expression, activation of the amygdala primarily reflects enhanced encoding of relevant information in ambiguous situations or when the predictive value of information is uncertain (Davis & Whalen, 2001; Whalen et al., 1998).

Although functional neuroimaging data can only provide correlational evidence, this interpretation is also in line with data from studies on amygdala lesions. Although the rodent literature has indicated that the amygdala plays a crucial role

in both fear acquisition and expression (LeDoux, 2003), in primates, the amygdala does not seem to be crucially involved in the expression of conditioned fear (Antoniadis et al., 2009). Human patients with selective bilateral amygdala damage typically show deficits in conditioned fear responses (Bechara et al., 1995; Klumpers et al., 2015a). However, this is not causal evidence for a role of the amygdala in the expression of conditioned fear in humans, since the amygdala lesion is also present when the fear association is learned. Nevertheless, responses to unconditioned stimuli are usually intact (Bechara et al., 1995; Klumpers et al., 2015a; LaBar et al., 1995) meaning that noradrenergic-sympathetic arousal responses can be present in the absence of a functional amygdala. Recent data on one patient with amygdala damage, furthermore, indicated that this patient is able to experience subjective feelings of fear and panic after CO₂ inhalation (Feinstein, 2013, but see (Feinstein et al., 2011)). Finally, humans with amygdala damage typically do not show an emotional enhancement effect of episodic memory (Cahill & McGaugh, 1995; LaBar et al., 1998). This indeed suggest that noradrenergic-sympathetic arousal is ineffective in modulating memory in the absence of a functional amygdala indicated by rodent data (Cahill & McGaugh, 1991; Liang et al., 1982). Thus, our findings are in line with amygdala lesion data showing that the amygdala is crucially involved in fear acquisition and modulating memory processes rather than expressing fear.

Second, we found that amygdala activity was increased on CS+ items that were later remembered compared to CS+ items that were later forgotten, even though both evoked noradrenergic-sympathetic arousal responses. This subsequent memory effect in the amygdala is in line with previous literature (Dolcos et al., 2004; Erk et al., 2003; Hamann, 2001; LaBar & Cabeza, 2006; Murty et al., 2011; Richardson et al., 2004). However, these previous studies could not disentangle the separate roles of noradrenergic-sympathetic arousal and amygdala activation in enhancing declarative memory. Amygdala responses found in these paradigms could therefore reflect a response to arousing material (Hariri et al., 2002; Morris et al., 1997; Vuilleumier et al., 2001; Whalen et al., 1998) as well as perceptual-mnemonic processes. We therefore extend these findings by showing that noradrenergic-sympathetic arousal only predicts declarative memory for arousing stimuli when coinciding with amygdala activation.

Our data are furthermore in line with findings in rodents showing that the amygdala, in particular the basolateral amygdala (BLA), is necessary for arousal-related neuromodulators to have an effect on memory processes elsewhere in the brain (McGaugh, 2004; Roozendaal & McGaugh, 2011). Indeed, direct infusion of -neuromodulatory agents affecting the noradrenergic system into the BLA after learning, have been shown to enhance memory (Ferry & McGaugh, 1999; McIntyre et al., 2005). This is even the case for learning events that are low in arousal (Roozendaal et al., 2008), meaning that in absence of noradrenergic-sympathetic

arousal induced by the stimulus, noradrenergic activation in the amygdala can influence memory. Moreover, the effects of these post-training manipulations of noradrenergic activity in the BLA influence memory types that are dependent on other brain regions such as the hippocampus, caudate nucleus, and insular cortex (Beldjoud et al., 2015; Hatfield & McGaugh, 1999; Packard et al., 1994). Additionally, these effects are blocked when the amygdala is lesioned (Cahill & McGaugh, 1991; Liang et al., 1982). In humans it was found that β -adrenergic antagonist (*i.e.*, propranolol) administration blocks the emotional enhancement effect for arousing material (Cahill et al., 1994; Van Stegeren et al., 1998) and abolishes the subsequent memory effect in the amygdala (Strange & Dolan, 2004). Importantly, the emotional enhancement effects are driven by central and not per se peripheral noradrenergic activation (Van Stegeren et al., 1998). These findings align closely with the present study in showing the importance of noradrenergic activation of the amygdala, but do not directly demonstrate a dissociation between the noradrenergic-sympathetic response and amygdala activation.

Rodent data showing functional specificity within amygdala subregions raise the question whether we can attribute the BOLD activation found in the present study to any subregion of the amygdala. Although we observed that the activation lies more toward the central nucleus of the amygdala rather than the BLA, it is questionable whether we can draw inferences at this level of spatial specificity with BOLD-fMRI at this resolution. A comparison between subregions of the amygdala using BOLD-fMRI is inherently difficult because signal loss and distortion due to magnetic field inhomogeneity increases towards the ventral part of the brain, where the BLA is located (Merboldt et al., 2001; Sladky et al., 2013). Moreover, we applied spatial smoothing to improve signal-to-noise ratio and accommodate the anatomical and functional variability between subjects, but this further reduces the spatial accuracy. Thus, whether the effect we observed can be attributed to a specific subregion of the amygdala remains an open question.

Our behavioral data did not show enhanced item memory recognition for CS+ items compared to CS- items, even though previous studies using a similar paradigm did find a memory enhancement for CS+ items (Dunsmoor et al., 2012, 2015). A plausible explanation for this null finding is that the lures in our paradigm were more similar to the targets (*i.e.*, if the target was a dog, the lure was a different dog). We included a relatively small number of trials (*i.e.*, 64 encoding trials) due to the fear conditioning procedure. This made the task more difficult in order to prevent ceiling effects and to be able to reliably investigate subsequent memory effects. Another crucial difference is that our task did not include expectancy ratings for the UCS (Dunsmoor et al., 2012, 2015). These expectancy ratings might have had similar effects on encoding as do judgments tasks (*e.g.*, living/non-living judgments in response to objects or animals) in memory

paradigms, which are used to ensure more elaborate encoding (Gabrieli et al., 1997; Takashima et al., 2006; Turk-Browne et al., 2006). A lack of an overall emotional enhancement effect is not uncommon, nevertheless, in studies using recognition memory tests (Richardson et al., 2004; Windmann & Kutas, 2001), even when amygdala activity predicts recognition of individual emotional items (Richardson et al., 2004). Indeed, the effect of emotion on memory is thought to be reduced (or not present) when assessing memory via recognition instead of recollection (Yonelinas & Ritchey, 2015). When we compared all items later remembered versus all items later forgotten we did find a strong subsequent memory effect in the hippocampus and parahippocampal gyrus. These findings are consistent with a crucial role for these regions in non-emotional declarative memory (Wagner et al., 1998). Thus, hippocampal and parahippocampal activations predict overall memory, while the amygdala specifically predicts memory for CS+ items.

To summarize, we demonstrate that noradrenergic-sympathetic activation is not sufficient to enhance emotional declarative memory, but requires additional activation of the amygdala. Our data show that these two processes do not play a uniform role in memory. These findings support animal models stating that the amygdala integrates arousal-related neuromodulatory changes to modulate mnemonic processes elsewhere in the brain and thereby strengthens declarative memory.



Chapter 3

Amygdala-hippocampal connectivity during post-encoding rest as a trait predicts enhanced memory under stress

Lycia D. de Voogd, Floris Klumpers, Guillén Fernández and Erno J. Hermans

Abstract

Declarative memories of stressful events are less prone to forgetting than mundane events. Animal research has demonstrated that such stress effects on consolidation of hippocampal-dependent memories require the amygdala. In humans, it has been shown that during learning, increased amygdala-hippocampal interactions are related to more efficient memory encoding. Animal models predict that following learning, amygdala-hippocampal interactions are instrumental to strengthening the consolidation of such declarative memories, yet this remains to be empirically verified. To test this, we analyzed data from a sample of 120 healthy male participants who performed an incidental encoding task and subsequently underwent resting-state functional MRI in a stressful and a neutral context. Stress was assessed by measures of salivary cortisol, blood pressure, heart rate, and subjective ratings. Memory was tested afterwards outside of the scanner. Our data show that memory was stronger in the stress context compared to the neutral context and that the stress-induced cortisol responses predicted this memory enhancement. Interestingly, amygdala-hippocampal connectivity during post-encoding awake rest regardless of context (stress or neutral) predicted the enhanced memory performance under stress. Thus, our findings are in line with a role for intrinsic functional connectivity during rest between the amygdala and the hippocampus in the state effects of stress on strengthening memory.

This chapter has been published as: de Voogd LD, Klumpers, F, Fernández G, Hermans EJ (2017) Intrinsic functional connectivity between amygdala and hippocampus during rest predicts enhanced memory under stress. *Psychoneuroendocrinology* 75:192–202 (de Voogd et al., 2017)

Introduction

Stressful events are typically well remembered (Gold et al., 1975; McGaugh, 2002). Due to consolidation processes, the enhanced memory retention found for these events increases over time (LaBar et al., 1998). Animal data indicate that during consolidation, the amygdala can modulate hippocampal-dependent memories (McGaugh, 2002) which presumably underlies the increased retention for stressful events. Evidence for the involvement of the amygdala and hippocampus in memory for emotional material in humans is, however, limited to the time of encoding (Dolcos et al., 2005; Fastenrath et al., 2014; Richardson et al., 2004). Whether amygdala-hippocampal interactions continue playing a role in the effects of stress on declarative memory in humans after learning is unknown.

The amygdala and hippocampus are both anatomical and functionally connected. Anatomical studies in rodents have indicated there are reciprocal connections between the amygdala and hippocampus (Pitkänen et al., 2000). With BOLD-fMRI in humans, it has been shown that the amygdala and hippocampus are functionally connected during awake rest (Roy et al., 2009). Changes in these connectivity patterns due to state changes have also been observed. Electrophysiology studies in rodents, for instance, demonstrated that amygdala-hippocampal theta coherence increases during the expression of conditioned fear in mice (Seidenbecher et al., 2003). Furthermore, after chronic immobilization stress in rats, beta and gamma synchrony was enhanced between the lateral amygdala and the CA1 region of the hippocampus, which lasted up to 10 days (Ghosh et al., 2013). In humans, increased connectivity between the amygdala and hippocampus has been observed following fear learning (de Voogd et al., 2016a; Hermans et al., 2016). We therefore first hypothesized that functional connectivity between the hippocampus and amygdala would be elevated during post-encoding rest within a stressful context compared to a neutral context.

Amygdala-hippocampal interactions have furthermore been shown to be relevant for later memory in animals and humans. In rodents, it was shown that increases in theta coherence between amygdala and hippocampus during sleep after fear learning was predictive for later fear retention (Popa et al., 2010). Indeed, the amygdala is critically involved in the consolidation of long-term memories by regulating memory processes in regions elsewhere in the brain, such as the hippocampus (McGaugh, 2002; Roozendaal et al., 2009). For example, early studies have shown that stimulation of the amygdala after learning enhances retention for avoidance training (Gold et al., 1975), and injections of d-amphetamine in the amygdala enhances hippocampal-dependent memory in a spatial water-maze task (Packard et al., 1994). A recent study showed that electrical stimulation of the basolateral complex of the amygdala (BLA) after rodents have seen novel objects leads to enhanced memory for those objects, as well as enhanced synchrony in the

gamma frequency range in the hippocampus (Bass & Manns, 2015). Indeed, the amygdala can influence hippocampal neural plasticity (Abe, 2001) indicating that the amygdala might still be involved in memory formation after the learning event has taken place by strengthening synaptic consolidation in the hippocampus.

Evidence for the involvement of the amygdala and hippocampus in declarative memory in humans mostly comes from studies that have investigated encoding processes. With BOLD-fMRI it was shown that during encoding, amygdala-hippocampal connectivity is stronger for emotionally arousing stimuli that were successfully encoded compared to neutral stimuli (Dolcos et al., 2005), with a directionality from the amygdala to the hippocampus (Fastenrath et al., 2014). The critical importance of interactions between amygdala and hippocampus during encoding of emotional material has furthermore been shown in patients with damage to either of these two regions (Richardson et al., 2004).

Also, when β -adrenergic activation is blocked via systemic administration of propranolol, the emotional enhancement effect for arousing material (Van Stegeren et al., 1998) as well as the subsequent memory effect in the amygdala (Strange & Dolan, 2004) is diminished. Amygdala-hippocampal interactions might continue playing a role after learning. For instance, it was shown that when stress is induced after learning, memory for the learned material is enhanced as well (Smeets et al., 2008). Moreover, systemic administration of cortisol shortly before learning enhanced recall for emotional material not immediately but 24h later (Kuhlmann & Wolf, 2006). These data together indicate that amygdala-hippocampal interactions are crucial for the enhancing effects of stress on memory, but whether interactions between these regions continue to play a role after learning in humans is unknown. We therefore tested the hypothesis that the enhanced functional connectivity between the amygdala and hippocampus during post-encoding rest due to stress would predict enhanced long-term declarative memory under stress.

To test our hypotheses, we analyzed an existing data set from a functional MRI study investigating the effects of stress on cognition (Berkers et al., 2016; Everaerd et al., 2015; Henckens et al., 2016; Klumpers et al., 2015b). A large sample of 120 healthy men come to the lab twice and performed the same tasks once interleaved with aversive movie clips in the stressful context and once interleaved with neutral movie clips in the neutral context. The order of the sessions was counter-balanced. During each session, participants performed an incidental encoding task, which was followed by a final movie clip, and subsequently underwent a resting-state scan (6 min 30 s). Within this time frame it is possible to probe early consolidation processes as has been shown in previous studies (de Voogd et al., 2016a; Hermans et al., 2016; Tambini et al., 2010). The encoding task included 32 neutral faces which were paired with either a neutral (*e.g.*, driver) or a negative (*e.g.*, murderer) identity. Participants were instructed to judge whether the face

matched the identity. Memory for the association was tested at the end of the experiment outside of the scanner. Our first prediction was that amygdala-hippocampal connectivity would be increased in the stressful context compared to the neutral context and that this enhancement would be predicted by acute stress (assessed with stress-induced cortisol levels). Secondly and more importantly, we predicted that amygdala-hippocampal connectivity during post-encoding awake rest in a stressful context would predict enhanced memory performance as compared to the neutral context. Additionally, we explored the effect individual differences in trait anxiety and depression on memory performance and amygdala-hippocampal connectivity, because individual differences in these personality traits were shown to underlie memory dysfunction as well as functional alterations in regions such as the amygdala and hippocampus (Sandi & Richter-Levin, 2009).

Methods

Participants

One-hundred-twenty right-handed healthy male volunteers (range: 18-31 years [$M=21.9$ $SD=2.6$]) completed the study. Participants reported no regular use of psychoactive drugs or history of neurologic and psychiatric disorders. Exclusion criteria for participation were a current or past psychiatric or neurological disorder, history of somatic disease potentially affecting the brain, regular use of psychoactive drugs during the preceding 6 months, history of substance abuse, current or past alcohol dependence, or MRI contraindications. Only male participants were included in this study because of the difficulty in controlling for the effects of menstrual cycle on the stress response (Kirschbaum et al., 1999). We excluded 5 participants from the analyses due to movement during the anatomical scan leading to inaccurate segmentation ($n=1$), sleeping ($n=2$), and excessive movement (>4 SD above the mean displacement) during one of the resting-state sessions ($n=2$). Participants gave written informed consent and were paid for their participation. This study was approved by the local ethical review board (CMO region Arnhem-Nijmegen, The Netherlands).

Design and procedure

The data presented here were acquired as part of a large study which involved two lab visits (see Figure 3.1A). Participants underwent a neutral and a stress induction session in the afternoon of which the order was counterbalanced and separated by an average of 2 weeks (minimum of 5 days). All test sessions took place between noon and 6 pm to control for diurnal variation in cortisol levels. The stress and neutral sessions included three and four experimental tasks, respectively. The results from some of these tasks have been reported elsewhere (Berkers et al., 2016;

Everaerd et al., 2015; Henckens et al., 2016; Klumpers et al., 2015b). Here, we test an independent hypothesis on previously unreported data (resting-state functional MRI).

To induce a stressful state, highly aversive movie clips were shown in the MRI scanner during the stress session (Hermans et al., 2011). These clips consisted of scenes of a movie (*Irréversible*, 2002, by Gaspar Noé) containing extreme physical and sexual aggression and violence against men and women. As a control condition, neutral, non-arousing scenes of another movie (*Comment j'ai tué mon père*, 2001, by Anne Fontaine) were shown in the scanner during the neutral session. The stressful and the neutral movie clips were similar in the amount of speech, human (face) presence, luminance, environment, and language. The participants were asked to watch the movie clips from an eye-witness perspective.

The face-identity association encoding task (Berkers et al., 2016) was the 3rd task in both sessions (see Figure 3.1A). It was followed by a final movie clip (2 min 11 sec), after which the resting-state scan was conducted. Only the neutral session additionally included a fear conditioning task. Therefore, the memory tests in the two sessions, which were conducted at the end of each session outside of the scanner, were not performed at the exact same time point relative to the encoding task. The memory test in the stress session (range: 18 to 46 min after encoding) was performed on average 15 minutes earlier than the neutral session (range: 12 to 60 min after encoding). There was, however, substantial between-subjects variation in the time delay difference between sessions [neutral minus stress range: -24 min to +34 min]. This allowed us to use regression analyses (of memory performance onto time delay) to test whether the time delay may have affected our findings independent of the stress manipulation.

Finally, a structural scan was obtained at the end of the stressful session. The total duration of scanning was approximately 105 min per session.

Face-identity association task

The encoding task contained 32 neutral faces that were associated with an identity (see Figure 3.1B). There were 16 neutral (*e.g.*, driver) and 16 negative (*e.g.*, murder) identities which were presented in writing simultaneously with the face. The face-identity associations were presented in a block design. There were eight blocks (24 s per block) and each block contained 4 associations (6 s per association), which were either all neutral or all negative. Participants were instructed to indicate whether they thought the identity would fit the face via a button press. Additionally, the task contained three baseline blocks during which participants had to make a perceptual judgment (*i.e.*, indicating whether the left or right ear was higher). The association pairs as well as the block order were counterbalanced across participants.

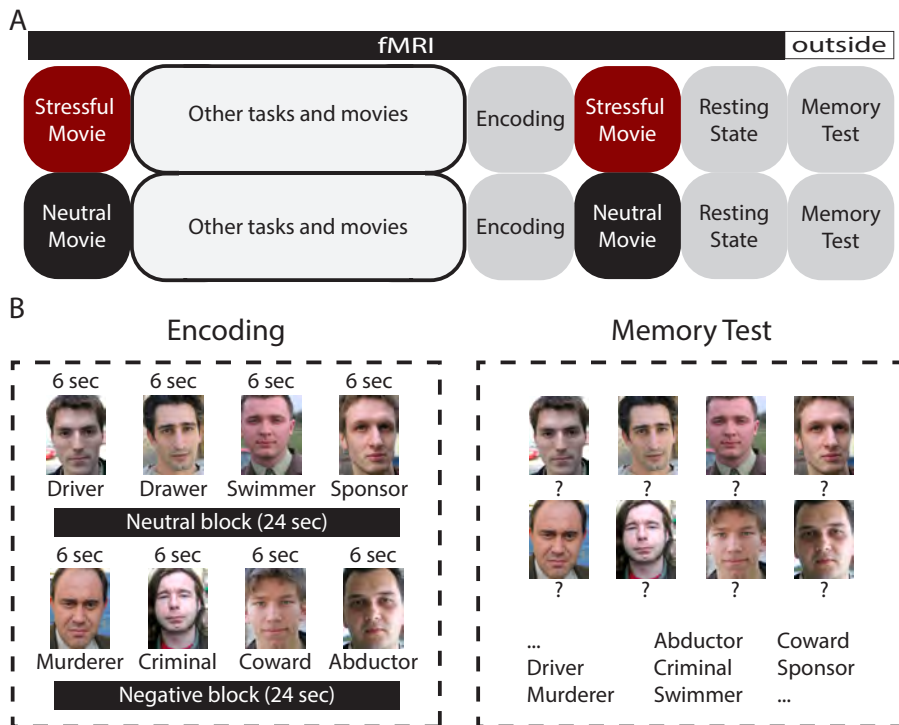


Figure 3.1: Overview of the experimental design

A) Overview of the experimental design. Within-subject design with a stress and neutral session. In the stressful context, participants performed tasks which were interleaved with aversive movie clips. In the neutral context, the tasks were interleaved by neutral movie clips that were matched to the stress movies. Participants performed a face-identity association paradigm and underwent a resting-state scan (6 min 30 s). During the resting-state scan participants were instructed to keep their eyes open. Saliva cortisol samples, blood pressure and mood state was assessed at three time points; before the start of the scanning procedure ($t = 45$ min), following the first task ($t = 95$ min) and at the end the scanning session ($t = 130$ min). Heart rate frequency and heart rate variability was assessed during the resting-state scan. B) Face-identity association paradigm. During encoding, participants were instructed to indicate whether they thought the identity would fit the face via a button press. During recall, participants were instructed to pair the identities with the faces, and to do so only in case they would remember the association with high confidence.

Resting-state scan

The resting-state scan (6 min 30 s) was performed after the encoding task and the final movie clip. During the resting-state scan participants were instructed to remain alert and awake, let their mind wander freely, avoid repetitive mental activity such as counting, and keep their eyes open (Van Dijk et al., 2010).

Memory recall test

Participants were tested for their memory of the associations after leaving the scanner room. The memory test consisted of a list of all faces and a list of all identities that were presented during encoding. Participants were instructed to pair the identities with the faces, and to do so only in case they would remember the association with high confidence. As a memory measure, we took the total percentage correct (see: Berkers et al., 2016). Theoretical chance level was 1/32 (3.125%), but participants were instructed to only fill in the associations of which they were certain.

Stress measurements

During the course of the experiment, saliva samples, blood pressure, and mood state was assessed at three time points. The first assessment was before the start of the scanning procedure ($t = 45$ min), a second following the first task ($t = 95$ min), and the final assessment at the end of the session ($t = 130$ min). Saliva samples were obtained using Salivette cotton swabs (Sarstedt, Rommelsdorf, Germany). Additionally, participants collected two extra samples at the same time of day (early afternoon and late afternoon) on the day before the visit for the second session. The average of the samples taken at home was used as baseline. Participants had to place a cotton swab in their mouth and chewed gently on it for 1 min to produce saliva. All samples were stored at -20°C until assaying. Laboratory analyses were performed at the Department of Biopsychology, Technical University of Dresden (Dresden, Germany). Biochemical analysis of free cortisol in saliva was performed using a commercially available chemiluminescence immunoassay (IBL Inc.). Mood state was assessed using the Positive and Negative Affect Schedule (PANAS) questionnaire (Watson, Clark, and Tellegen, 1988). Resting blood pressure measurements were obtained using a standard automatic blood pressure device, and during the experiment in the MRI scanner using a Ambulo™ 2400 device. The stress induction effects reported in this study have already been reported elsewhere (see: Everaerd et al., 2015; Henckens et al., 2016).

Finger pulse was recorded using a 50 Hz pulse oximeter and was continuously assessed during scanning. The average heart rate frequency (HRF) and heart rate variability (HRV) during the resting-state scan were used to test for difference between the stress and neutral session. Additionally, pulse measures were used for retrospective image-based correction (RETROICOR) of physiological noise artifacts in BOLD-fMRI data (Glover et al., 2000). Raw pulse was processed offline using in-house software for interactive visual artifact correction and peak detection, and were used to specify fifth-order Fourier models of the cardiac phase-related modulation of the BOLD signal (Van Buuren et al., 2009), yielding 10 nuisance regressors for cardiac noise. Additional regressors were calculated for HRF and HRV,

yielding a total of 12 regressors.

Personality questionnaires

Participants filled in the Dutch versions of the Beck Depression Inventory (BDI; Beck et al., 1996) and the State-Trait Anxiety Inventory (STAI-t; Van der Ploeg, 1980). The average BDI score was 4.3 (range: 0-18) and for the STAI-t it was 35.5 (range: 21-60).

MRI data acquisition

MRI scans were acquired using a Siemens (Erlangen, Germany) 1.5 T Avanto MR scanner. A series of 265 T2*-weighted blood oxygenation level-dependent (BOLD) images were recorded using gradient echo-planar imaging with ascending slice acquisition (27 axial slices; TR, 1.49 s; TE, 35 ms; flip angle, 80°; slice thickness, 3.5 mm; slice matrix size, 64x64; slice gap, 0.7 mm; FOV, 224 x 224 mm; bandwidth, 1906 Hz/px; echo spacing, 0.59 ms). We discarded the first five volumes to allow for T1 equilibration. A high-resolution structural image (1 mm isotropic) was acquired using a T1-weighted 3D magnetization-prepared rapid gradient-echo sequence (MP-RAGE; TR, 2730 ms; TE, 2.95 ms; flip angle, 7°; FOV, 256 x 256 x 176 mm).

MRI data preprocessing in native space and group analyses in standard stereotactic space

All resting-state EPI images were preprocessed in native space (*i.e.*, without stereotactic normalization) to optimally accommodate interindividual structural variability of the hippocampus. Mutual information maximization-based rigid body registration was used to register structural and (motion-corrected) functional images. The bilateral hippocampus was individually defined in native space using automated anatomical segmentation of T1-weighted images using FSL FIRST (see <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST>).

First-level models were applied to the realigned and co-registered functional images in native space and contained the mean (de-noised and de-trended) time course of the hippocampus (left, right) as a regressor of interest and 37 additional nuisance regressors. These included six motion parameter regressors (3 translations, 3 rotations), the zero-centered squares of the six motion parameters, the first derivatives of the six motion parameters, the zero-centered squares of the derivatives of the six motion parameters, 10 RETROICOR cardiac phase regressors, HRF and HRV (see section 2.6). High pass filtering (1/128 Hz cut-off) and AR(1) serial correlations correction was also included. Heart rate recording failed for ten participants. Therefore, the model did not include the 10 RETROICOR, HRF, and HRV regressors for these participants.

For the purpose of a whole-brain group analyses we first segmented the struc-

tural images into grey matter, white matter, and CSF images using a unified probabilistic template registration and tissue classification method (Ashburner & Friston, 2005). Tissue images were then registered with in-house site-specific tissue templates using DARTEL (Ashburner, 2007), and registered (using an affine transformation) with the MNI152 template included in SPM8. Next, the beta images, obtained at the first level analyses in native space, of the whole-brain connectivity maps with the hippocampus (left, right) were transformed into standard stereotactic (MNI152) space using DARTEL, resliced into 2 mm isotropic voxels, and smoothed with a 6 mm FWHM Gaussian kernel.

The second-level model included single-subject normalized beta maps for the regressor containing the hippocampal time course in the first-level analyses. These maps were entered into a hemisphere (left, right) by session (stress, neutral) repeated measures ANOVA. The model included the following covariates: (1) the difference in memory performance between stress and neutral conditions, (2) the interaction term of this difference and session (stress vs. neutral), and (3) session order. We used a cluster-forming voxel-level threshold of $p < .001$ (uncorrected). Alpha was set at .05, whole-brain family-wise error (FWE) corrected at the cluster level using Gaussian Random Field Theory based methods (Friston et al., 1996). Based on a priori hypotheses, results for amygdala were corrected for a reduced search volume using small volume corrections (SVC) based on an anatomical mask of the amygdala (Automated Anatomical Labeling atlas; Tzourio-Mazoyer et al., 2002).

Time course extraction and ROI segmentation

Additionally, we extracted the averaged (de-noised and de-trended) BOLD-fMRI voxel time courses for amygdala and hippocampus for both the left and right hemisphere, from the functional images in native space (described in section 2.9). This was done to investigate the correlation between the amygdala-hippocampal connectivity between sessions. Functional connectivity was calculated using (Fisher's z transformed) pairwise Pearson's correlations between the mean time course of the amygdala and hippocampus for both left and right hemisphere. A repeated-measures ANOVA was performed with hemisphere (left, right) and session (stress, neutral) as within-subject factors and session order as between-subject factor to compare the connectivity between the sessions. Additionally, across-subject partial (*i.e.*, controlling for the order of the session) Pearson's correlations were performed to investigate the association between these native-space functional connectivity measures and memory performance. The latter was merely done to confirm our whole-brain analyses. Similar to the hippocampus, the amygdala was individually defined in native space using automated anatomical segmentation of T1-weighted images using FSL FIRST (see <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST>). Statistical

testing. Partial eta squared (η_p^2) effect size estimates are reported for all relevant tests. Pearson's correlations were used for correlations across participants. Alpha was set at .05 throughout.

Results

Stress measures

Successful stress induction in this dataset has also been reported elsewhere (see: Everaerd et al., 2015; Henckens et al., 2016). To confirm successful stress induction in the current sample we tested the same salivary hormone measures, physiological measures, and self-reports. Baseline-corrected salivary cortisol levels [$F(1,112)=10.526$, $p=.002$, $\eta_p^2=.09$], baseline-corrected systolic blood pressure [$F(1,113)=8.020$, $p=.005$, $\eta_p^2=.07$], as well as baseline-corrected self-report of negative affect (PANAS questionnaire) [$F(1,112)=35.535$, $p=2.97\text{E-}8$, $\eta_p^2=.24$] were higher for the stress session compared to the neutral session.

Furthermore, during the resting-state session heart rate frequency (expressed in beats per minutes) was higher for the stress session compared to neutral [$F(103)=17.439$, $p=6.22\text{E-}5$, $\eta_p^2=.15$], and heart rate variability was decreased [$F(103)=9.539$, $p=.003$, $\eta_p^2=.09$]. In conclusion, these data show that the stress manipulation was successful: cortisol, blood pressure, heart rate frequency, and heart rate variability were altered as intended during the stress context as compared to the neutral context.

Behavioral data

On average, 27.28% (SD=16.56) of the 32 associations were remembered which was above what could be expected based on chance (1/32) [$t(114)=15.642$, $p=3.79\text{E-}30$, $D=2.93$]. We next compared memory performance between the stress and neutral context. The memory tests in the two sessions, however, were not performed at the exact same time point relative to the encoding task (see section 2.2.). Therefore, we investigated first the effect of the time delay on memory performance. There was no indication that within each session, the between participants' time delays predicted memory performance in either the neutral [$r(112)=.08$, $p=.38$] or the stress [$r(112)=.05$, $p=.63$] condition. The difference in time delay between the sessions was also not associated with a difference in memory performance between the sessions [$F(1,112)=.122$, $p=.73$, $\eta_p^2=.001$]. Thus, despite this difference in time of testing, there was no indication this influenced performance across or within subjects.

Next, we therefore investigated the effect of session (stress versus neutral) on memory performance. We found a main effect of session [$F(1,113)=4903$, $p=.029$, $\eta_p^2=.042$], meaning that the associations in the stress session ($M=28.53$, $SD=17.13$) were better remembered than the associations in the neutral session ($M=26.03$,

SD=18.41). When we controlled for the time delay, the difference between the sessions remained significant [$F(1,112)=4.869$, $p=.029$, $\eta_p^2=.042$]. The order of the sessions interacted with the effect of stress [$F(1,113)=13.57$, $p=3.54\text{E-}4$, $\eta_p^2=.11$]. Only when the stress session was first, memory was enhanced for the this session ($M=28.83\%$, $SD=15.35$) compared to the neutral ($M=22.07\%$, $SD=17.58$) session [$t(56)=4.431$, $p=4.41\text{E-}5$, $D=1.18$]. When the neutral session was first, memory in the stress ($M=28.23\%$, $SD=18.84$) session did not differ from the neutral ($M=29.92\%$, $SD=18.54$) session [$t(57)=.987$, $p=.33$, $D=0.26$]. For the functional MRI analyses, the order of the sessions was therefore added as a covariate.

Finally, we tested whether individual differences in cortisol responses to stress would predict memory enhancement under stress. Indeed, we found that the stronger the stress-induced cortisol response was, the greater the memory was enhanced due to stress [$r(112)=.24$, $p=.01$]. See Figure 3.2. In summary, stress enhanced memory performance and the stress-induced cortisol response predicted this memory enhancement due to stress.

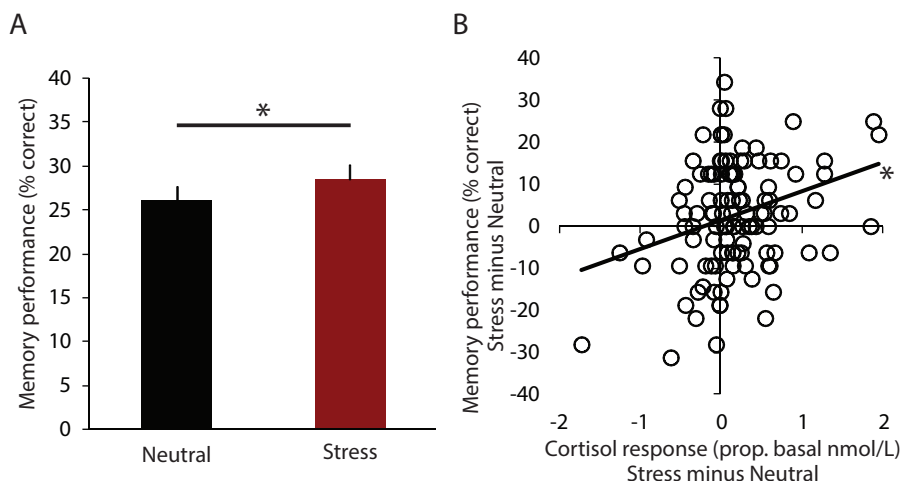


Figure 3.2: Memory performance

A) Average memory performance (percentage correct) for both sessions. B) Across-subject correlation between baseline-corrected, stress induced cortisol responses (nmol/L) and difference in memory performance (percentage correct) under stress. * $p<.05$

Whole-brain functional connectivity with the hippocampus and memory performance during post-encoding rest

We then tested the hypothesis that amygdala-hippocampal connectivity as a function of stress would predict the enhanced memory under stress. We performed a whole-brain connectivity analysis with the time course of the hippocampus as a seed for each participant, and subsequently performed a second-level ANOVA

with hemisphere (left, right) and session (stress, neutral) as within-subject factors, the difference in memory performance between stress and neutral conditions, the interaction term of this difference and session (stress vs. neutral), and session order as covariates. There were no significant differences in amygdala-hippocampal connectivity between the sessions (no voxels exceeded the clustering threshold, even at a more liberal threshold of $p < .005$, uncorrected). For whole-brain connectivity results see Table 3.1. Then, we did not find any significant connectivity increases across the brain or within the amygdala (no voxels exceeded the clustering threshold, even at a more liberal threshold of $p < .005$, uncorrected) predicting enhanced memory performance. Interestingly, we did find that the average connectivity (*i.e.*, the averaged connectivity across both sessions) between the hippocampal seed (left and right combined) and a cluster in the left amygdala predicted memory enhancement under stress (cluster size=256 mm³, cluster $p = .004$, FWE-SVC). See Figure 3.3 and Table 3.1. Thus, our data indicate that hippocampal connectivity with the (left) amygdala predicted memory enhancement under stress and this was regardless of state (*i.e.*, acquired in stress or neutral sessions).

Table 3.1: Peak voxel coordinates and cluster statistics and size for post-encoding resting state with hippocampus (left, right) as seeds

Region	Side	x(mm)	y(mm)	z(mm)	Z-score	Cluster p	Size mm ³
<i>Neutral > Stress</i>							
Precuneus / Post central gyrus / Superior parietal lobule	L/R	-6	-48	58	5.54	$p < .001$	90904
Lingual / Calcarine sulcus / Cuneus	L/R	-20	-82	-12	5.60	$p < .001$	72048
Inferior occipital gyrus	R	36	-92	-6	4.67	$p < .001$	4176
<i>Main effect memory [stress - neutral]</i>							
Amygdala	L	-20	-6	-16	4.14	$p = .004^*$	256
<i>Main effect memory [stress - neutral] - Left hippocampus</i>							
Amygdala	L	-20	-6	-16	3.72	$p = .009^*$	152
Amygdala	L	-30	-6	-14	4.43	$p = .016^*$	72
<i>Main effect memory [stress - neutral] - Right hippocampus</i>							
Amygdala	L	-20	-6	-18	3.78	$p = .008^*$	176

Notes: All coordinates are defined in MNI152 space. All reported statistics are significant at $p < .05$, cluster-level corrected for the whole brain unless indicated otherwise. *Small volume corrected for region of interest.

Region of interest analyses

We then reasoned that amygdala-hippocampal connectivity, in relation to memory enhancement under stress, may constitute a trait rather than a state factor. If this is the case, then the connectivity measures between sessions would be correlated with each other. We therefore extracted the average time courses from the anatomically defined amygdala and hippocampus in a native-space analysis. This

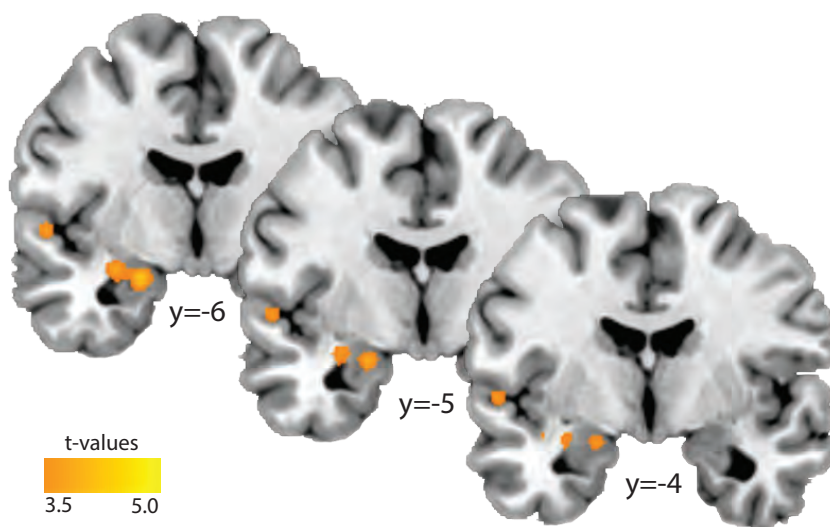


Figure 3.3: Functional connectivity during post-encoding rest

Whole brain connectivity with hippocampal seeds (left, right) during post-encoding rest predicting memory enhancement under stress. A significant cluster was found in the amygdala (FWE-SVC). Statistical parametric maps are thresholded at $p < .001$, uncorrected, for visualization purposes. Whole-brain cluster-level corrected inferential statistics are reported in Table 3.1.

allowed us to compute amygdala-hippocampal functional connectivity measures for each session, which we could then correlate between the two sessions. Indeed, the average time-course connectivity between the amygdala and hippocampus was correlated between the two sessions across subjects [$r(112) = .35$, $p = 1.41\text{E-}4$]. See Figure 3.3. Lastly, confirming our results obtained using the whole-brain voxel-wise approach described above, the mean amygdala-hippocampal connectivity did not differ significantly between the sessions [$F(1,113) = .21$, $p = .65$, $\eta_p^2 = .002$]. Furthermore, we found a significant correlation between the mean amygdala-hippocampal connectivity and memory enhancement under stress [left: $r(112) = .19$, $p = .02$; right $r(112) = .11$, $p = .26$; see Figure 3.4].

We next tested whether individual differences in cortisol responses to stress would predict functional connectivity between the amygdala and hippocampus. Cortisol responses did not predict the average functional connectivity [$r(112) = .07$, $p = .48$]. There was a non-significant trend towards a positive correlation between cortisol responses to stress and a difference in functional connectivity between the amygdala and hippocampus [$r(112) = .16$, $p = .09$]. Our ROI analyses confirmed the whole-brain analyses by showing that amygdala-hippocampal connectivity during rest regardless of state predicted a state effect on memory (*i.e.*, enhancement due to stress).

Correlations between psychological traits and functional connectivity / memory performance

Finally, because the functional connectivity between the amygdala and hippocampus did not differ between sessions, we investigated whether individual differences amygdala-hippocampal connectivity would be predicted by individual differences in measures of depression and anxiety. We therefore correlated the average amygdala-hippocampal connectivity with the outcome of the STAI-t (range: 21 to 60) and BDI (range: 0 to 18) questionnaires. However, this did not yield any significant correlations [STAI-t: $r(112)=.07$, $p=.46$ and BDI: $r(112)=.08$, $p=.40$]. There was also no correlation between the questionnaires and the enhanced memory under stress [STAI-t: $r(112)=-.08$, $p=.43$ and BDI: $r(112)=.07$, $p=.48$]. It is important to note that there is little variance in BDI questionnaire scores within a healthy population, potentially reducing the possibility to find a correlation. In conclusion, individual differences in amygdala-hippocampal connectivity and enhanced memory under stress were not explained by individual differences in trait characteristics of depression and anxiety.

Discussion

The aim of this study was to investigate the role of post-encoding amygdala-hippocampal connectivity in the consolidation of memories encoded under stress. For this, we used an existing dataset from a large study ($n=115$) investigating the influence of stress on cognition. We found that memory performance was enhanced under stress, and that stress-induced cortisol responses predicted this memory enhancement. Critically, amygdala-hippocampal connectivity also predicted stress-induced memory enhancement, but did so regardless of context (stress, neutral). Amygdala-hippocampal connectivity during post-encoding awake rest did not differ between the sessions, and positively correlated across participants. Thus, our data indicate that amygdala-hippocampal connectivity during rest facilitates memory enhancement under stress as trait rather than a state factor.

We found that memory performance was increased under stress. It is important to note that this finding is possibly confounded by a difference in time delay between the encoding and retention test between sessions. There was, however, no indication that individual differences in time delay accounted for the difference in memory performance, either within or between the sessions. Despite this potential confound, this finding is in line with many previous studies in both animals (McGaugh, 2002; Roozendaal et al., 2009) and humans (Henckens et al., 2009; Smeets et al., 2008; Wiemers et al., 2013). Moreover, the stress-induced cortisol responses correlated positively with the enhancement under stress, indicating further that the difference in memory retention is more likely to be induced by stress.

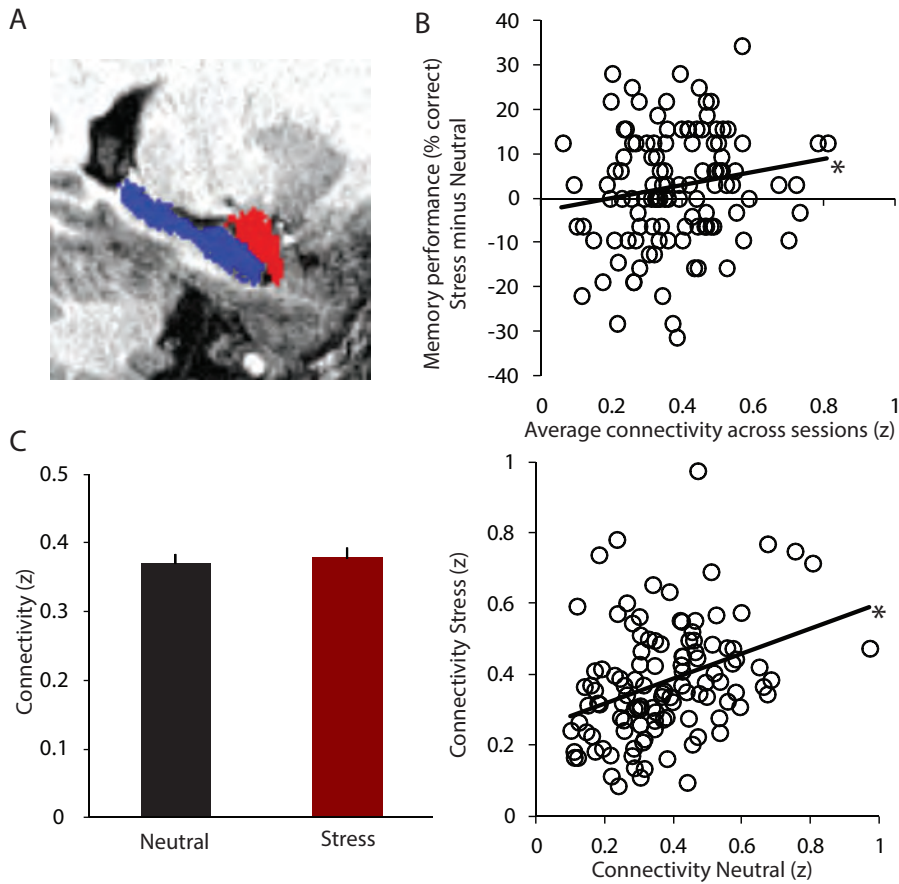


Figure 3.4: Region of interest analysis

A) Single subject example of amygdala and hippocampus segmentation. B) Across-subject correlation between average left amygdala-hippocampal connectivity across sessions (Fisher z-transformed) and enhanced memory performance under stress. C) Bar graph represents the average amygdala-hippocampal connectivity (Fisher z-transformed) for stress and neutral. Across subject correlation of the amygdala-hippocampal connectivity (Fisher z-transformed) between the sessions. *, $p < .05$.

Differences in memory retention between stressful and mundane events are partly due to immediate effects of stress on attentional, sensory, and mnemonic processes (de Voogd et al., 2016b; Dolcos et al., 2005). Indeed, human studies have shown that memory is enhanced for material that is encoded under stress compared to when it is encoded in a non-stressful context (Henckens et al., 2009; Wiemers et al., 2013). Critically, however, this difference in memory retention is further increased via preferential consolidation (LaBar et al., 1998; McGaugh, 2002). Memory for the learned material was also shown to be enhanced when stress is induced after learning (Smeets et al., 2008). Additional evidence for the importance of post-

encoding processes for later memory comes from pharmacological administration studies. Post-encoding intravenous infusion of epinephrine in humans was shown to enhance memory performance (Cahill et al., 2003). Administration of cortisol before learning, furthermore, did not affect immediate recall but did enhance recall for emotional material tested 24h later (Kuhlmann & Wolf, 2006). Thus, our data are in line with a large body of literature showing that stress and stress-sensitive hormones can improve memory performance by enhancing consolidation.

We found that post-encoding amygdala-hippocampal connectivity during rest predicted memory enhancement under stress regardless of context (stress or neutral). Animal studies have implicated a role for the amygdala in modulating memory after learning (McGaugh, 2002; Roozendaal et al., 2009). Specifically, it was shown that the amygdala mediates arousal-related neuromodulators' effects on memory processes elsewhere in the brain (Roozendaal et al., 2009), such as in the hippocampus (Packard et al., 1994). Additional studies have shown that when the amygdala is lesioned, the effect of arousal on memory is diminished (Cahill & McGaugh, 1991; Liang et al., 1982). Human patient studies have also found support for the involvement of the amygdala in mediating stress effects on hippocampal-dependent memory. For example, patients with lesions to the amygdala, resulting from Urbach-Wiethe disease, do not show an emotional enhancement of episodic memory consolidation (LaBar and Phelps, 1998). Furthermore, data from patients with damage to either the amygdala or hippocampus indicated that there are reciprocal interactions between these regions during encoding of emotional material (Richardson et al., 2004). Based on these findings, we expected that stress would increase the connectivity between amygdala and hippocampus, which would subsequently predict enhanced memory under stress. However, we did not find an increase in amygdala-hippocampal connectivity between the two sessions and found that amygdala-hippocampal connectivity as a trait predicted memory enhancement under stress.

What could be a possible explanation for this finding? First, amygdala-hippocampal connectivity between resting-state sessions did not differ. Previous human imaging studies using BOLD-fMRI have shown that the amygdala and hippocampus are indeed functionally connected during awake rest (Roy et al., 2009), but our findings are not in line with previous studies showing this connectivity is increased following fear learning (de Voogd et al., 2016a; Hermans et al., 2016). Although there was a trend towards a correlation between stress-induced cortisol responses and increased amygdala-hippocampal connectivity under stress, our data did not indicate that stress influenced connectivity between these regions. Second, across participants the functional connectivity between the sessions was highly correlated, even though the sessions were on different days and on average two weeks apart. Our data therefore indicate that functional connectivity between

the amygdala and hippocampus during rest is, at least, also a trait characteristic.

This is in line with previous findings on resting-state connectivity that have indicated that functional connectivity is a stable and strong trait characteristic despite additional influences of mental states (Geerligs et al., 2015). Furthermore, individual differences in intrinsic functional connectivity have been shown to be relevant for cognitive processes because these were shown to predict state-independent individual differences in intellectual performance (van den Heuvel et al., 2009) and learning (Gerraty et al., 2014). Importantly, there is a close link between functional connectivity and structural connectivity (van den Heuvel et al., 2009) suggesting that the relationship between trait differences in functional connectivity could be related to underlying anatomical connections. With regards to the amygdala and hippocampus, studies in animals have indeed indicated these regions are structurally connected (Pitkänen et al., 2000). Thus, our data extend these previous studies by showing that resting-state functional connectivity between the amygdala and the hippocampus as a trait factor can account for a state effects of stress on memory performance. A previous finding indeed showed that the amygdala to hippocampal volume ratio was predictive of interindividual differences in negative memory bias (Gerritsen et al., 2012). Thus, although stress might trigger state-dependent amygdala-hippocampal interactions during post-encoding rest, as has been shown in animals (McGaugh, 2002; Roozendaal et al., 2009) and humans (de Voogd et al., 2016a; Hermans et al., 2016), there are additional trait characteristics that play an important role in the effects of stress on memory. In particular, trait factors such as amygdala-hippocampal volume ratio (Gerritsen et al., 2012) or intrinsic connectivity between these regions (the present study), but also genetics (Li et al., 2013) may determine the degree to which hormones and neurotransmitters released during stress are able to engage the amygdala to modulate mnemonic processes in hippocampus. Finally, we asked whether this trait connectivity between the amygdala and hippocampus was related to individual differences in anxiety and depression. However, we did not find a correlation between amygdala-hippocampal connectivity and individual differences in our measures for trait anxiety (STAI-t) and depression (BDI). Previous studies have indicated that structural characteristics of the amygdala and hippocampus, such as volume ratio, are related to pathological anxiety and depression (MacMillan et al., 2003). Furthermore, depressed (Irwin et al., 2004) and social anxiety disorder patients (Liao et al., 2010) were found to have a different functional connectivity pattern between the amygdala and other parts of the brain compared to healthy controls. Since we did not find a relationship between amygdala-hippocampal connectivity and trait anxiety or depression in healthy volunteers, our results cannot be explained by underlying differences in anxiety and depression predispositions. This is, however, a healthy population with limited variance in these measures.

In conclusion, our data show that amygdala-hippocampal connectivity predicts the strengthening of memory under stress, but constitutes a trait rather than a state characteristic. This finding implicates a role for intrinsic functional connectivity between these regions in determining the degree to which stress-sensitive hormones and neurotransmitters are able to modulate memory formation.



Chapter 4

Awake reactivation of emotional memory traces through hippocampal-neocortical interactions

Lycia D. de Voogd, Guillén Fernández and Erno J. Hermans

Abstract

Emotionally arousing experiences are typically well remembered not only due to immediate effects at encoding, but also through further strengthening of subsequent consolidation processes. A large body of research shows how neuromodulatory systems promote synaptic consolidation. However, how emotionally arousing experiences alter systems-level interactions, presumably a consequence of modifications at a synaptic level, remains unclear. Animal models predict that memory traces are maintained by spontaneous reactivations across hippocampal-neocortical circuits during “offline” periods such as post-learning rest, and suggest this might be stronger for emotional memories. The present study was designed to test this hypothesis in humans using functional Magnetic Resonance Imaging. Participants underwent a two-category localizer paradigm followed by a categorical differential delay fear conditioning paradigm interleaved with blocks of awake rest. Counterbalanced across participants, exemplars of one category (CS+), but not the other (CS-), were paired with mild electrical shocks. Fear recall (differential conditioned pupil dilation) was tested 24h later. Analyses of the localizer paradigm replicate earlier work showing category-specific response patterns in neocortical higher-order visual regions. Critically, we show that during post-learning rest, spontaneous reactivation of these neocortical patterns was stronger for the CS+ than the CS- category. Furthermore, hippocampal connectivity with the regions exhibiting these reactivations predicted strength of fear recall 24h later. We conclude that emotional arousal during learning promotes spontaneous post-learning reactivation of neocortical representations of recent experiences, which leads to better memory when coinciding with hippocampal connectivity. Our findings reveal a systems-level mechanism that may explain the persistence of long-term memory for emotional experiences.

This chapter has been published as: de Voogd LD, Fernández G, Hermans EJ (2016) Awake reactivation of emotional memory traces through hippocampal–neocortical interactions. *NeuroImage*, 134, 563–572. (de Voogd et al., 2016a)

Introduction

Stressful and emotionally arousing experiences produce strong and lasting memories (Cahill et al., 1996; Gold et al., 1975; McGaugh, 2013; Schacter, 1999). This selectivity of memory is partly due to immediate effects of emotional arousal on attentional, sensory, and mnemonic processes (Davis & Whalen, 2001; Dolcos et al., 2004; LaBar & Cabeza, 2006; Visser et al., 2013), but is further strengthened by preferential consolidation (McGaugh, 2000). One account of this enhanced consolidation states that the amygdala engages neuromodulatory systems to alter thresholds for synaptic modification, a process that continues well beyond the duration of the learning experience (Roozendaal et al., 2009). Presumably as a consequence of synaptic modifications, emotionally arousing experiences may also prompt interactions between distant regions (Hermans et al., 2014; Pape & Pare, 2010; Paz et al., 2006). Such systems-consolidation processes are thought to engage hippocampal-neocortical circuits and take place during “off-line” periods such as awake rest or sleep (Frankland & Bontempi, 2005; Marr, 1970; McClelland et al., 1995; Rasch & Born, 2007). If such interactions play a role in selective consolidation of emotional memories, however, remains unknown.

Theories of systems-consolidation state that interactions within hippocampal-neocortical networks serve to reactivate recently acquired memory traces and gradually integrate them into existing neocortical networks (Frankland & Bontempi, 2005; Rasch & Born, 2007; Squire, 1992). Indeed, electrophysiological studies in rodents show that firing patterns of hippocampal place cells present during encoding reoccur during sleep (Wilson & McNaughton, 1994) and awake states (Carr et al., 2011). Furthermore, replay in the neocortex has been found with increased probability during the occurrence of hippocampal sharp waves (Logothetis et al., 2012; Peyrache et al., 2009). There is a relative paucity in human functional neuroimaging studies into these phenomena, but initial studies have produced findings which are in agreement with replay studies in animals. For example, enhanced post-learning interactions between hippocampus and neocortical regions, as measured using Blood Oxygenation Level-Dependent functional MRI (BOLD-fMRI), predicted memory retention (Tambini et al., 2010; Van Kesteren et al., 2010). Furthermore, pattern-recognition techniques applied to resting-state fMRI data revealed that encoding-related activity patterns reactivated spontaneously during post-learning rest (Deuker et al., 2013; Staeresina et al., 2013; Tambini & Davachi, 2013). We therefore hypothesized that emotionally arousing experiences may potentiate such reactivations of experience-specific activity patterns.

The amygdala plays a particularly important role in strengthening memory for emotionally arousing experiences (LaBar & Cabeza, 2006; McGaugh, 2000). It is critically involved in neuromodulatory influences that promote storage processes elsewhere in the brain (McGaugh, 2002). BOLD-fMRI studies in humans have

shown stronger amygdala activity and amygdala-hippocampal connectivity (Dolcos et al., 2005) during successful encoding of emotionally arousing compared to neutral stimuli. Electrophysiological recordings in rodents revealed that, during acquisition and expression of conditioned fear, theta coherence between amygdala and hippocampus is increased (Seidenbecher et al., 2003). This increase was also observed during sleep after fear learning, and was predictive for later expression of the fear memory (Popa et al., 2010). Furthermore, functional connectivity from the amygdala to the hippocampus increases after stress during awake rest (Ghosh et al., 2013). We therefore hypothesized that functional connectivity between the amygdala and hippocampus would be elevated during rest following an emotionally arousing experience.

To test our hypotheses, we conducted a categorical differential delay fear conditioning experiment in which exemplars belonging to two distinct categories served as conditioned stimuli (Dunsmoor et al., 2012). This type of delay conditioning has shown to elicit learning-related activity in the hippocampus (Dunsmoor et al., 2014), which fits with a role of the hippocampus in spatiotemporal binding of conceptual information (Brown & Aggleton, 2001; Gluck & Myers, 1993; Henke, 2010). Blocks of conditioning were interleaved with resting-state blocks (Figure 4.1). Exemplars of one of the two categories (CS+; counterbalanced across participants) paired with an aversive electrical shock on 50% of presentations, while exemplars of the other category (CS-) were never reinforced. We chose (1) animals and (2) fruits/vegetables as categories because these are known to elicit differential patterns of BOLD in neocortical representational regions (Haxby et al., 2001; Kriegeskorte et al., 2008). To individually estimate category specific patterns of BOLD, an independent localizer paradigm preceded the conditioning paradigm. This localizer paradigm involved exposure to unique exemplars from the two categories which did not reoccur during conditioning. Critically, this allowed us to examine category specific reactivations of the CS+ category versus the CS- category during awake rest following fear learning. Participants returned to the lab 24h later for a fear recall test in which they were shown a new unseen exemplar of both categories, none of which were reinforced. The differential pupil dilation response to the CS+ and CS- exemplar served as a measure of fear recall.

Our primary prediction was that spontaneous reactivation of category-specific patterns of BOLD signal during post-learning awake rest would be stronger for the CS+ compared to the CS- category. Second, we predicted that functional connectivity during awake rest between hippocampus and category-specific representational regions would increase for the CS+ compared to the CS- category. Third, we predicted that functional connectivity between amygdala and hippocampus would increase during post-learning awake rest compared to baseline. Fourth and finally, we predicted that individual differences in the strength of such reactivation effects

and functional coupling might predict differences in long-term expression of fear, as measured in differential conditioned pupil dilation 24h after acquisition.

Methods

Participants

Twenty-four right-handed healthy volunteers (12 female, 12 male; 19-32 years [mean=23.25]) completed the study. An additional seven participants did not complete the entire experiment due to apparatus failure or non-compliance with instructions. Participants gave written informed consent and were paid for their participation. Exclusion criteria were: current or lifetime history of psychiatric, neurological, or endocrine illness, abnormal hearing or (uncorrected) vision, average use of more than 3 alcoholic beverages daily, current treatment with any medication that affects central nervous system or endocrine systems, average use of recreational drugs weekly or more, habitual smoking, predominant left-handedness, intense daily physical exercise, and any contraindications for MRI. This study was approved by the local ethical review board (CMO region Arnhem-Nijmegen).

Design and procedure

Participants were tested in a categorical differential delay fear conditioning paradigm with a fear recall session 24h after learning (Figure 4.1). On day 1, procedures started with a category representation localizer paradigm. Next, intensity of electrical shock (unconditioned stimulus) was adjusted individually using a standardized procedure (see 2.7). Participants then underwent the categorical differential delay fear conditioning paradigm. Localizer and conditioning blocks were preceded and followed by resting-state blocks (5.4 min duration). Twenty-four hours later, we tested fear recall by presenting additional unreinforced trials of both the CS+ and the CS- categories. All experiments were programmed using Presentation® software (Version 0.70, www.neurobs.com).

Stimuli

The stimuli set consisted of 144 pictures of animals (N=72) and fruits/vegetables (N=72). Of the 144 pictures, 64 were used during the localizer, 64 during the categorical differential delay fear conditioning paradigm, and 8 were presented on the second day. The pictures were selected from the Hemera Photo-Objects set (<http://hemera-technologies-inc.software.informer.com>) and publicly available resources on the internet. Luminance of all pictures, including the grey background, was equalized. We excluded pictures with a higher threat value (such as lions and snakes) to avoid additional arousal and facilitated conditioning (Öhman & Mineka, 2001). Additionally, we created scrambled pictures by randomly mixing the phase

information of pictures from both categories (Reinders et al., 2005).

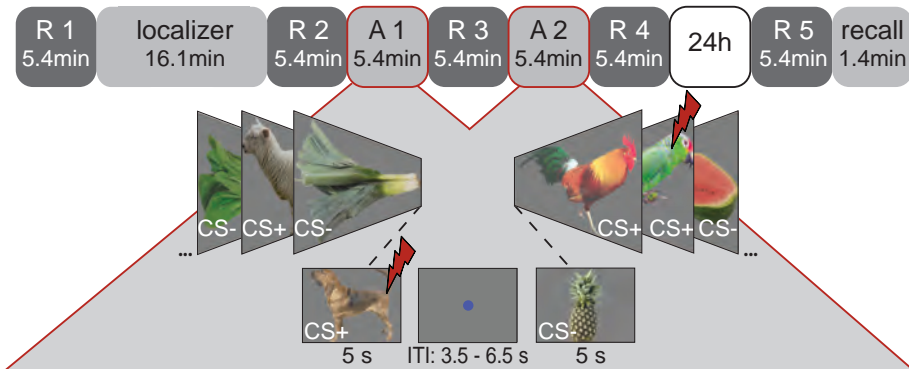


Figure 4.1: Overview of the experimental design

Acquisition blocks were intermixed with resting-state blocks (5.4 min each) on two consecutive days. During the localizer paradigm, exemplars of two categories and additional scrambled pictures were shown in a block design (20 s per block). During acquisition, one of the two categories was associated with an electrical shock. CS+ and CS- pictures were shown in pseudo-random order during conditioning blocks (32 CSs per block). CS+s co-terminated with shock on 50% of the acquisition trials. R1-R5, resting-state blocks; A1-2, acquisition blocks; ITI, inter trial interval.

Category representation localizer paradigm

The localizer paradigm consisted of 36 short blocks with a duration of 20 s each. Blocks contained either pictures of animals, pictures of fruits/vegetables, or scrambled pictures. During every block, 32 pictures were presented with a duration of 625 ms each. One picture in half of all blocks was overlaid with a red dot. To control for attention, participants were instructed to press a button upon detecting this stimulus. There were 12 additional rest blocks which consisted of a gray screen with a fixation dot. To preclude covariation of task regressors with linear drift, blocks were presented in a mirrored order. The order of the first half of all blocks was randomized.

Categorical differential delay fear conditioning paradigm

The categorical differential delay fear conditioning paradigm consisted of two acquisition blocks on day one and one fear recall block on day two. Exemplars of one of the two picture categories (animals or fruits/vegetables) served as CS+ (reinforced) stimuli, while exemplars of the other category served as CS- (unreinforced) stimuli (Dunsmoor et al., 2012). Which of the two picture categories served as CS+ was counterbalanced across participants. Each acquisition block comprised 16 CS+ and 16 CS- exemplar presentations, each with a 5 s duration. The intertrial interval (ITI) varied randomly between 3.5 and 6.5 s. Pictures were presented in

a pseudorandom order with no more than three repetitions of the same category. Half of all CS+ pictures was followed by shock (*i.e.*, the reinforcement rate was 50%). The following day, 4 CS+ pictures and 4 CS- pictures were presented with the same duration and ITI, and none of these CS+ stimuli was reinforced. Order of the first stimulus on day two (*i.e.*, CS+ first versus CS- first) was counterbalanced across participants.

Resting-state blocks

There were four resting-state blocks on day one and one on day two. Each had a 5.4 min duration (Figure 4.1). A black screen with green fixation dot was presented throughout these blocks. Participants were instructed to remain awake and alert, keep their eyes open, let their mind wander freely, and avoid repetitive mental activity such as counting (see Van Dijk et al., 2010). We verified compliance with the instruction to remain awake using eye-tracking.

Peripheral stimulation and measurements

Electrical shocks were delivered via two Ag/AgCl electrodes attached to the distal phalanges of the second and third fingers of the right hand using a MAXTENS 2000 (Bio-Protech) device. Shock duration was 200 ms, and intensity varied in 10 intensity steps between 0V-40V/0mA-80mA. During the standardized shock intensity adjustment procedure, each participant received and subjectively rated five shocks, allowing shock intensity to converge to a level experienced as uncomfortable, but not painful. The resulting average intensity step was 5.5 (SD: 2.0) on a scale from 1 to 10 intensity steps.

Pupil dilation was measured using an MR-compatible eye tracking system (MEye Track-LR camera unit, SMI, SensoMotoric Instruments). Pupil data was analyzed using in-house software (Hermans et al., 2013) implemented in Matlab 7.14 (MathWorks), which was based on methods described previously by others (Siegle et al., 2003). Eyeblink artifacts were identified by differentiating the signal to detect eye pupil changes occurring too rapidly (<60 ms) to represent actual dilation. Blinks were removed from the signal using linear interpolation. Pupil diameter for each trial was normalized by dividing the signal with the average of 1 s pre-stimulus onset baseline. The averaged baseline-corrected pupil diameter within a 1 to 5 s window during picture presentation was used as response measure. This time window precedes the shock and therefore, all reinforced and unreinforced trials are included in the analysis.

Finger pulse was recorded using a pulse oximeter affixed to the ring finger of the left hand. Respiration was measured using a respiration belt placed around the participant's abdomen. Pulse and respiration measures were used for retrospective image-based correction (RETROICOR) of physiological noise artifacts in

BOLD-fMRI data (Glover et al., 2000). Raw pulse and respiratory data were processed offline using in-house software for interactive visual artifact correction and peak detection, and were used to specify fifth-order Fourier models of the cardiac and respiratory phase-related modulation of the BOLD signal (Van Buuren et al., 2009), yielding 10 nuisance regressors for cardiac noise and 10 for respiratory noise. Additional regressors were calculated for heart rate frequency, heart rate variability, (raw) abdominal circumference, respiratory frequency, respiratory amplitude, and respiration volume per unit time (Birn et al., 2006), yielding a total of 26 RETROICOR regressors.

MRI data acquisition and multi-echo weighting

MRI scans were acquired using a Siemens (Erlangen, Germany) MAGNETOM Skyra 3.0T MR scanner. T2*-weighted blood oxygenation level-dependent (BOLD) images were recorded using a customized multi-echo EPI sequence with ascending slice acquisition (37 axial slices; TR, 2.38 s; TE, 15 ms and 36 ms; Generalized Autocalibrating Partially Parallel Acquisitions (GRAPPA; Griswold et al., 2002) acceleration factor 4; flip angle, 90°; slice matrix size, 106x106; slice thickness, 2.0 mm; slice gap, 0.26 mm; FOV, 212 x 212 mm; bandwidth: 1748 Hz/px; echo spacing: 0.7 ms). To allow for correction of distortions due to magnetic field inhomogeneity, we acquired field maps using a dual echo 2D gradient-echo sequence (64 axial slices; TR, 1020 ms; TE, 10 ms and 12.46 ms; flip angle, 90°; slice matrix size, 64x64, slice thickness, 2 mm; FOV, 224 x 224 mm). A high-resolution structural image (1 mm isotropic) was acquired using a T1-weighted 3D magnetization-prepared rapid gradient-echo sequence (MP-RAGE; TR, 2.3 s; TE, 3.03 ms; flip angle, 8°; FOV, 256 x 256 x 192 mm).

To correct EPI images for head motion, geometric distortions due to magnetic field inhomogeneity, and interactions between these, we used an integrated fieldmap-based unwarp/realign method (Hutton et al., 2002). Unwarping and realignment parameters were estimated from the first echo and applied to both echoes. Next, to account for regional variation in susceptibility-induced signal dropout, voxel-wise weighted sums of both echoes were calculated based on local contrast-to-noise ratio (Poser et al., 2006).

MRI data preprocessing in standard stereotactic space and group analyses

To replicate earlier findings and validate consistency of neural response patterns across participants for the localizer paradigm and conditioning blocks, MRI data for these tasks were pre-processed in standard stereotactic (MNI152) space (using SPM8; <http://www.fil.ion.ucl.ac.uk/spm>; Wellcome Department of Imaging Neuroscience, London, UK). Mutual information maximization based rigid body registration was used to register structural and (motion and geometric distortion-corrected) functional images. Structural images were segmented into grey matter,

white matter, and CSF images using a unified probabilistic template registration and tissue classification method (Ashburner & Friston, 2005). Tissue images were then registered with site-specific tissue templates (created from 384 T1-weighted scans) using DARTEL (Ashburner, 2007), and registered (using an affine transformation) with the MNI152 template included in SPM8. Identical transformations were applied to all functional images, which were resliced into 2 mm isotropic voxels and smoothed with a 6 mm FWHM Gaussian kernel.

For statistical analysis of the localizer paradigm, neural responses to the animal blocks, the fruits/vegetable blocks and scrambled blocks were modeled in three separate regressors using 20 s box car functions. For the conditioning blocks, responses to reinforced CS+s, unreinforced CS+s, and CS-s were modeled in three separate regressors using 5 s box car functions. Responses to shocks were modeled in an additional regressor using a delta function. All task regressors were temporally convolved with the canonical SPM8 hemodynamic response function. Both models additionally included six movement parameter regressors (3 translations, 3 rotations) derived from rigid body motion correction, 26 RETROICOR physiological noise regressors (see Section 2.7), high pass filtering (1/128 Hz cut-off), and AR(1) serial correlations correction. Single subject contrast maps obtained from first-level analyses (for the localizer: animals vs. fruits/vegetables; for conditioning blocks: unreinforced CS+ vs. CS-) were entered into second-level random effects analyses (one sample t-test). We used a cluster-forming voxel-level threshold of $p < .005$ (uncorrected). Alpha was set at .05, whole-brain family-wise error (FWE) corrected at the cluster level using Gaussian Random Field Theory based methods (Friston et al., 1996).

MRI data preprocessing of localizer and resting-state blocks in native space

For our main analyses of interest (reactivation of category representations and functional connectivity), we preprocessed all MRI data in native space (*i.e.*, without stereotactic normalization) to optimally accommodate interindividual structural variability and variability in the topography of higher-order category representations in inferotemporal cortex (Haxby et al., 2001). EPI volumes (corrected for motion and geometric distortions) recorded during the localizer paradigm and during resting-state blocks were co-registered with structural scans using mutual information maximization. Smoothing with a 6 mm FWHM Gaussian kernel was applied to localizer images. For resting-state blocks, we used a multiple regression model to remove nuisance signals from each voxel's time course through residualization. This model included a high pass filter (using a 1/128 Hz cut-off discrete cosine transform; Fox et al., 2005), six movement parameter regressors (3 translations, 3 rotations), and 26 RETROICOR physiological noise regressors (see Section 2.7). Regions of interest (ROIs) for amygdala and hippocampus were individually

defined in native space using automated anatomical segmentation of T1-weighted images using FSL FIRST (see <http://www.fmrib.ox.ac.uk/fsl/first/index.html>). See Figure 4.6B for a single-subject example.

Representational similarity analyses between localizer and resting-state blocks

The purpose of representational similarity analyses was to detect and quantify spontaneous reactivations of category representations in inferior/ventral temporal cortex during resting-state blocks following fear learning. To do so in an unbiased manner, we regressed multi-voxel patterns of activity sampled during the category representation localizer paradigm (*i.e.*, data acquired prior to and thus independently of fear learning) on data acquired in the same voxels during resting-state blocks (cf. Staresina et al., 2013). This procedure was performed independently for the category associated with shock (CS+) and the category not associated with shock (CS-). Native space data from the localizer paradigm were analyzed using the same GLM as in the group analyses described above. To confine our analyses to the anterior part of the ventral visual stream or inferior/ventral temporal cortex (Haxby et al., 2001; Kriegeskorte et al., 2008; Wimber et al., 2015), we used an anatomical mask comprising inferior temporal gyrus, fusiform gyrus, and parahippocampal gyrus as defined in the Automated Anatomical Labeling atlas (Tzourio-Mazoyer et al., 2002). The DARTEL-generated spatial normalization parameters were applied in a reverse fashion to transfer this MNI152 space mask into native space for each participant. Voxels overlapping with the anatomically defined amygdala and hippocampus masks were excluded. We then generated a contrast map for animals versus fruits/vegetables and a contrast map for fruits/vegetables versus animals. Next, we selected a subset of voxels, since we needed a unique set of voxels for both categories. Therefore, for both contrast maps and in each hemisphere, we identified the 500 voxels, as is commonly done in multivariate analyses (Deuker et al., 2013; Eger et al., 2009; Ethofer et al., 2009), with the highest t-values and extracted these t-statistics to form individual category-specific response vectors. See Figure 4.4B and C for two single-subject examples. To quantify spontaneous reactivation of these category-specific response patterns, we calculated Pearson's correlations of these vectors with (de-noised and de-trended, see Section 2.10) data acquired within corresponding voxels during resting-state blocks. This procedure yielded 2 (categories) * 2 (hemispheres) * 135 (TRs) * 5 (resting-state blocks) correlation coefficients, which were Fisher's z transformed ($z = 0.5[\log_e((1+r)/(1-r))]$) to normalize distributions. Resulting z values were averaged across all 135 TRs for each resting-state block, and entered into a repeated measures ANOVA including CS type (CS+, CS-), hemisphere (left, right), and block as within-subject factors, and CS+ type (which stimulus category representation was associated with shock) as between-subjects factor. Differential pupil dilation on the first trial on day two was

added to the model as a covariate to test for an interaction with fear recall.

Functional connectivity analyses of resting-state blocks

Functional connectivity analyses were performed to investigate learning-related changes in (1) hippocampal-neocortical connectivity and (2) amygdala-hippocampal connectivity (Tambini et al., 2010; Van Kesteren et al., 2010). We averaged (de-noised and de-trended, see Section 2.10) BOLD-fMRI voxel time courses for four regions of interest in each hemisphere: amygdala and hippocampus (both anatomically defined), and the two sets of 500 category-specific responsive voxels in inferotemporal cortex (as explained in 2.11). Functional connectivity between regions was calculated using pairwise Pearson's correlation coefficients, which were Fisher's z transformed. Resulting z values were analyzed using repeated measures ANOVAs. For hippocampal-neocortical connectivity, the ANOVA included CS type (CS+, CS-), hemisphere (left, right), and block as within-subject factors, and Group (which stimulus category representation was associated with shock) as between-subjects factor. For amygdala-hippocampal connectivity, this ANOVA included block (1-4) as within-subject factor. Differential pupil dilation on the first trial on day two was added to the model as a covariate to test for an interaction with fear recall. The analysis of amygdala-hippocampal connectivity involves a comparison between blocks instead of a comparison between spatially distinct regions (representing CS+ and CS-; which were counterbalanced between subjects), and could therefore be confounded by differences in subject motion. To mitigate this concern, we reran this analysis with additional motion artifact corrections, namely global signal regression and spike regression (Power, Schlaggar, and Petersen, 2015). This approach did not change the results and conclusions. We therefore report all results with the original de-trending, motion correction, and physiological noise corrections as described in section 2.10.

Statistical testing

Partial eta squared (η_p^2) effect size estimates are reported for all relevant tests. Spearman rank order correlations were used for correlations across participants. Alpha was set at .05 throughout.

Results

Functional localizer

First, we tested whether the exemplars belonging to the two categories would elicit consistent localized patterns of BOLD signal across participants (Haxby et al., 2001; Kriegeskorte et al., 2008) by using conventional group analyses in standard stereotactic (MNI152) space. As expected, we found significant category-selective

clusters consistent with prior research (Dunsmoor et al., 2014). We found clusters responsive to animal exemplars in the lateral fusiform gyrus extending into the inferior temporal gyrus, left (cluster size=4416 mm³, cluster $p<.001$, corrected) and right (cluster size=2224 mm³, cluster $p=.009$, corrected) and clusters selective to fruits/vegetables exemplars in the medial fusiform gyrus extending into the parahippocampal gyrus, left (cluster size=3344 mm³, cluster $p=.001$, corrected) and right (cluster size=1752 mm³, cluster $p=.03$, corrected). For full whole-brain results see Figure 4.2 and Table 4.1. To accommodate known between-subject functional-anatomical variability (Kanwisher et al., 1997; Wohlschläger et al., 2005) and increase detection power, we used non-stereotactically normalized data and individual estimation of category-specific BOLD patterns for representational similarity analyses between the localizer paradigm and the resting-state blocks, which are described below.

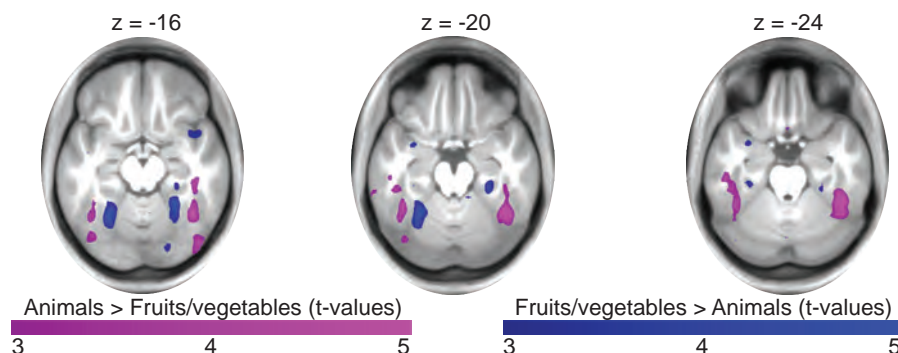


Figure 4.2: Localizer paradigm

Significant clusters in the inferior/ventral temporal cortex responding to blocks with animals versus fruits/vegetables exemplars (violet) and significant clusters responding to blocks with fruits/vegetables versus animal exemplars (blue). Statistical parametric maps are thresholded at $p=.005$, uncorrected, for visualization purposes. Whole-brain cluster-level corrected inferential statistics are reported in Table 4.1. Maps are overlaid onto the averaged normalized T1-weighted image of all participants.

Physiological measures

To confirm the effectiveness of our fear conditioning paradigm, we analyzed pupil dilation in response to the CS+ and CS- category. The repeated-measures ANOVA with CS type (CS+ and CS-) as a within-subjects factor and Group (animal CS+ and fruits/vegetables CS- or fruits/vegetables CS+ and animal CS-) as a between-subjects factor revealed a stronger pupil dilation for the pictures of the CS+ compared to the CS- category [$F(1,22)=21.31$, $p=1.34\text{E-}4$, $\eta_p^2=.49$]. Differential pupil dilation did not differ between participants that were fear conditioned to animals and participants that were fear conditioned to fruits/vegetables [$F(1,22)=.14$, $p=.71$, $\eta_p^2=.006$]. During fear recall on day 2, pupil dilation to CS+ was still higher than to

Table 4.1: Peak voxel coordinates and cluster statistics and size for the localizer paradigm

Region	Side	x(mm)	y(mm)	z(mm)	Cluster p	Size mm3
<i>Animals > Fruits/Vegetables</i>						
Middle occipital / middle temporal gyrus	R	50	-76	2	5.218E-15	19080
Middle occipital / middle temporal gyrus	L	-48	-82	6	3.143E-10	11248
Lateral fusiform gyrus	R	42	-52	-20	5.528E-05	4416
Cuneus / calcarine / superior occipital gyrus	L	-14	-94	18	1.915E-06	6104
Lingual gyrus / calcarine	R	16	-78	2	1.711E-07	7424
Lateral fusiform gyrus	L	-42	-48	-22	9.243E-03	2224
<i>Fruits/Vegetables > Animals</i>						
Lingual gyrus / calcarine	R	4	-84	-4	2.994E-03	2664
Medial fusiform gyrus	R	28	-50	-10	3.348E-02	1752
Medial fusiform gyrus	L	-28	-54	-18	5.867E-04	3344
Superior parietal gyrus	L	-18	-62	40	1.878E-02	1960

Notes: All coordinates are defined in MNI152 space. All reported statistics are significant at $p < .05$, cluster corrected.

CS- category [$F(1,20)=6.04$, $p=.02$, $\eta_p^2=.23$]. Recall of differential fear, however, exhibited substantial individual differences ($M=0.06$, $SD=0.12$), but was not explained by the presentation order (CS+ first or CS- first) [$F(1,20)=.08$, $p=.78$, $\eta_p^2=.004$]. See Figure 4.3.

Categorical differential delay fear conditioning paradigm

We verified whether the fear conditioning paradigm exhibited the expected task-related activity during CS presentation using conventional group analyses in standard stereotactic (MNI152) space. With a whole-brain analysis we first identified regions that were more responsive to pictures of the CS+ category versus pictures of the CS- category. In line with results commonly seen in fear conditioning paradigms, we observed robust differential BOLD responses in the left (cluster size=14104 mm³, cluster $p<.001$, corrected) and right anterior insula (cluster size=15312 mm³, cluster $p<.001$, corrected), dorsal anterior cingulate cortex (cluster size=5856 mm³, cluster $p<.001$, corrected), and thalamus (cluster size=2336 mm³, cluster $p=.007$, corrected) among others. For the reversed contrast (CS- > CS+) we found differential BOLD responses in the ventral medial prefrontal cortex (cluster size=11344 mm³, cluster $p<.001$, corrected), precuneus (cluster size=6472 mm³, cluster $p<.001$, corrected) and angular gyrus (cluster size= 6336mm³, cluster $p<.001$, corrected), among others. See Figure 4.3 and Table 4.2.

Category reactivation during rest

We then tested our first primary hypothesis, namely that category-specific patterns of BOLD signal in the inferior/ventral temporal cortex, obtained during the localizer paradigm, would spontaneously reactivate more strongly for the CS+ compared to the CS- category during post-learning rest. To test this we used a representational similarity analysis approach (Kriegeskorte et al., 2008; Staeresina et al., 2013). We correlated the individually selected voxel activation patterns for both the animal

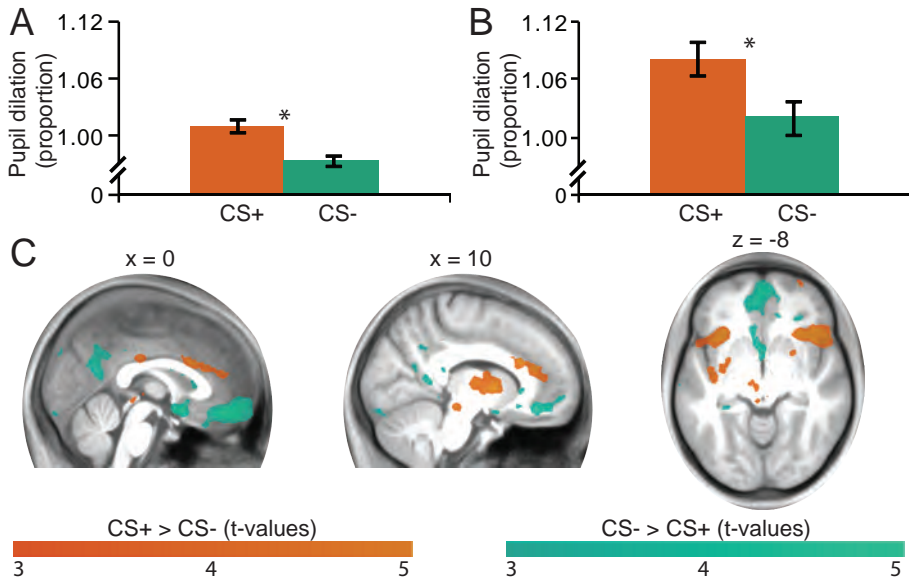


Figure 4.3: Categorical differential delay fear conditioning paradigm

(A) Average pupil dilation in response to CS+ and CS- category during the acquisition blocks on day 1. *, $p < .05$. (B) Pupil dilation in response to a single CS+ and CS- picture during fear recall on day 2. *, $p < .05$. (C) Regions responding more to pictures of the CS+ versus the CS- category (orange) and regions responding more to pictures of the CS- versus the CS+ category (green) during the acquisition blocks. Statistical parametric maps are thresholded at $p = .005$, uncorrected, for visualization purposes. Whole-brain cluster-level corrected inferential statistics are reported in Table 4.2. Maps are overlaid onto the averaged normalized T1-weighted image of all participants.

Table 4.2: Peak voxel coordinates and cluster statistics and size for categorical differential delay fear conditioning paradigm

Region	Side	x(mm)	y(mm)	z(mm)	Cluster p	Size mm ³
CS+ > CS-						
Anterior insula	R	32	24	2	7.732E-13	15312
Anterior insula	L	-40	18	-2	4.270E-12	14104
Supramarginal / Superior temporal gyrus	R	54	-44	32	6.014E-08	8000
Dorsal anterior cingulate cortex	L / R	8	26	26	2.985E-06	5856
Supramarginal / Superior temporal gyrus	L	-58	-26	20	2.572E-03	2720
Thalamus / Caudate	R	10	4	2	6.820E-03	2336
CS- > CS+						
Ventral medial prefrontal cortex	L / R	-2	46	-14	2.579E-10	11344
Precuneus	L / R	-4	-58	16	9.315E-07	6472
Angular gyrus	L	-50	-72	34	1.201E-06	6336
Olfactory / Caudate / Medial Orbital	L	-2	10	-12	1.207E-02	2120
Hippocampus	L	-26	-18	-20	1.373E-02	2072
Middle temporal gyrus	L	-64	-18	-14	3.558E-02	1728

Notes: All coordinates are defined in MNI152 space. All reported statistics are significant at $p < .05$, cluster corrected.

(either CS+ or CS-) and fruits/vegetable (either CS+ or CS-) category obtained during the localizer paradigm (*i.e.*, before one of these categories was associated with shock) with each volume during rest. This resulted in two average category specific

correlations (Fisher z-transformed) for every CS type (CS+, CS-) for all five rest blocks. There was an interaction of CS type (CS+, CS-) by Block (linear contrast 4 time points on day 1) [$F(1,22)=4.57$, $p=.04$, $\eta_p^2=.17$]. This interaction did not differ between participants that were conditioned to animals, versus participants that were conditioned to fruits/vegetables [$F(1,22)=3.03$, $p=.10$, $\eta_p^2=.12$]. Investigating both CS types (CS+,CS-) separately, we found a linear increase over time for the CS+ category specifically [$F(1,23)=5.22$, $p=.03$, $\eta_p^2=.19$]. Indeed, pattern reactivation of the CS+ category was higher post-learning compared to pre-learning [$F(1,23)=7.88$, $p=.01$, $\eta_p^2=.26$]. Reactivation for the CS+ category post-learning was higher compared to CS- category post-learning [$F(1,23)=6.641$, $p=.02$, $\eta_p^2=.22$] and was higher when tested against zero [$t(23)=3.442$, $p=.002$, $D=1.44$]. There was no linear change over time for the CS- category [$F(1,22)=1.34$, $p=.26$, $\eta_p^2=.06$]. The strength of the differential reactivation was not associated with interindividual differences in fear recall 24h later [$F(1,21)=.58$, $p=.46$, $\eta_p^2=.03$]. In conclusion, CS+ category specific voxels patterns obtained during the localizer paradigm reactivated more strongly during post-learning rest relative to baseline compared to the voxels patterns of the CS- category. See Figure 4.4A.

Hippocampus-Category specific regions

Next, we reasoned that if the hippocampus drives spontaneous reactivations of emotional memory traces, one would expect enhanced connectivity of the hippocampus with those regions that exhibited these reactivations. We tested the (Fisher z-transformed) correlation between the mean time course of the hippocampus and the category specific voxels identified with the localizer paradigm within each resting-state block. Although we did not find a CS type (CS+,CS-) by Block (linear contrast 4 time points on day 1) interaction [$F(1,22)=.04$, $p=.84$, $\eta_p^2=.002$], the CS type by Block interaction predicted fear recall [$F(1,21)=7.38$, $p=.01$, $\eta_p^2=.26$]. Further testing showed that this interaction was specific to the CS+ [$F(1,22)=8.22$, $p=.009$, $\eta_p^2=.27$] and not the CS- category [$F(1,22)<.001$, $p=.99$, $\eta_p^2<.001$]. For visualization purposes, we plotted the correlations of the post-learning connectivity minus the pre-learning connectivity with fear recall for the CS+ category [$\rho(22)=.41$, $p=.045$] and the CS- category [$\rho(22)=.05$, $p=.81$; see Figure 4.5). Thus, functional connectivity between the hippocampus and the CS+ category specific voxels patterns during post-learning rest relative to pre-learning rest was associated with interindividual differences in fear recall 24h later.

Amygdala-hippocampal connectivity

Finally, if the amygdala would boost these hippocampal-neocortical interactions, then amygdala-hippocampal connectivity should be increased during rest following an emotionally arousing experience. Because the present design does not allow us

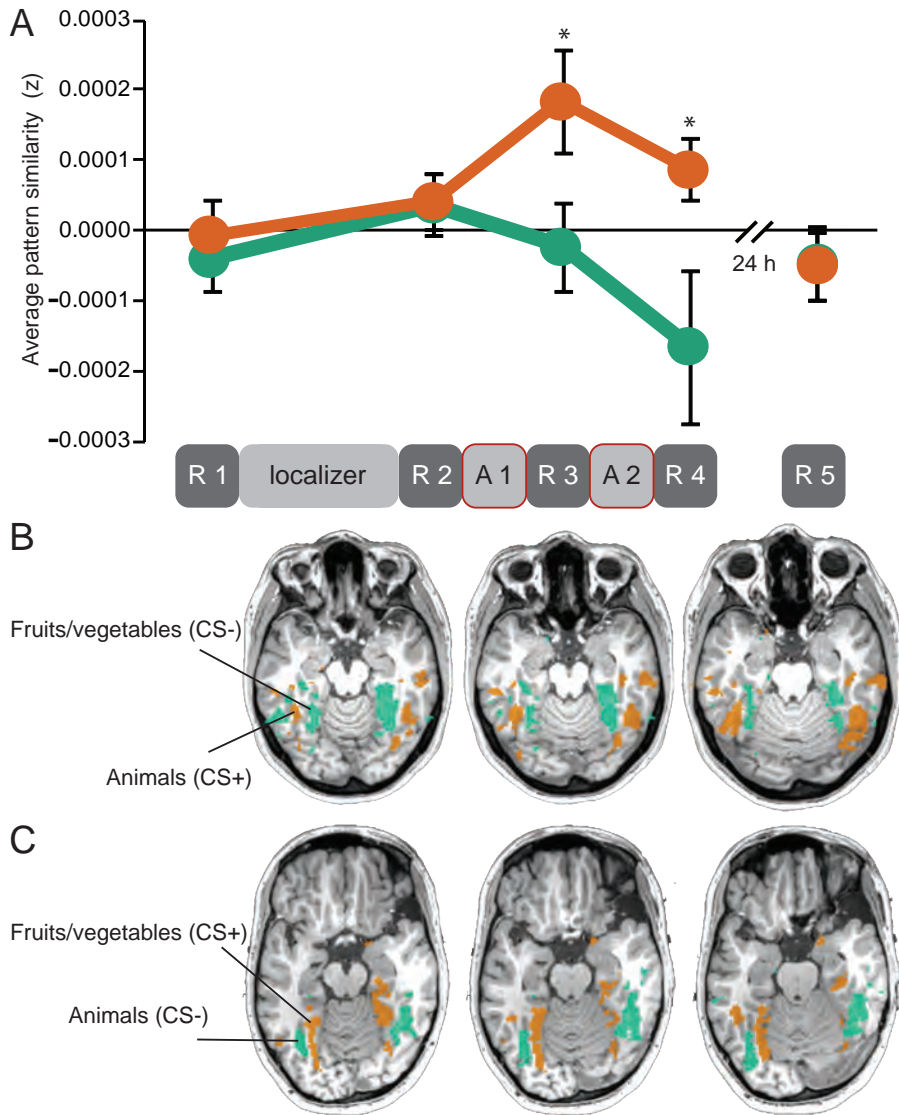


Figure 4.4: Category reactivation during rest

(A) Increase in pattern similarity between patterns of BOLD obtained during the localizer paradigm (i.e., before one of the two categories was associated with shock) and each resting-state block. Fisher Z-transformed correlations are plotted for the CS+ and the CS- category. *, $p < .05$. (B) Single subject example of the CS+ (fruits/vegetables) and CS- (animals) response patterns. (C) Single subject example of the CS+ (animals) and CS- (fruits/vegetables) response patterns.

to investigate amygdala-hippocampal connectivity patterns for the two CS types (CS+,CS-) separately, we expected to find an increase in connectivity during post-learning rest compared to baseline. We tested the (Fisher z-transformed) correlation between the mean time course of the hippocampus and the amygdala and found

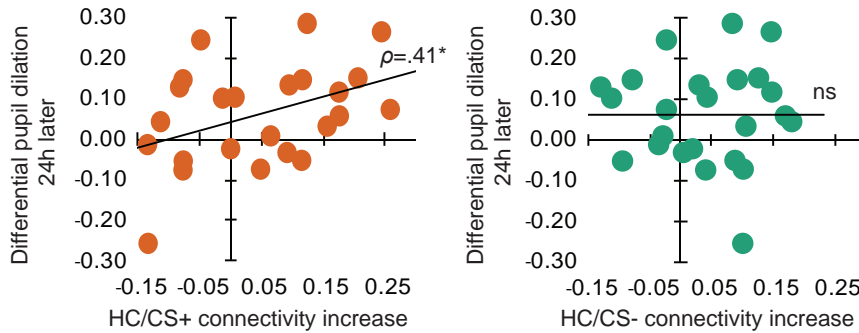


Figure 4.5: Functional coupling between the hippocampus and the category specific voxels patterns related to fear recall

Across-subject correlation between fear recall and the increase in within-subject correlation (Fisher z -transformed) of the time course of the hippocampus (HC) and the CS+ specific voxel patterns (left) and CS- specific voxel patterns (right) during the resting-state blocks. Fear recall was defined as differential pupil dilation 24h later. *, $p < .05$.

a linear increase over the four resting state blocks [$F(1,22)=7.39$, $p=.01$, $\eta_p^2=.25$] indicating that amygdala-hippocampal connectivity was increased during post-learning rest. See Figure 4.6A.

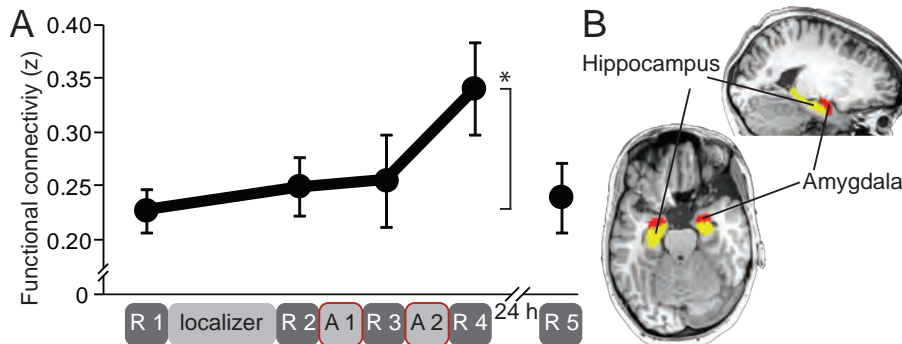


Figure 4.6: Functional coupling between the amygdala and the hippocampus

(A) Linear increase in the within-subject correlation (Fisher z -transformed) between the mean time course of the hippocampus and the amygdala during the resting-state blocks. *, $p < .05$. (B) Single subject example of amygdala and hippocampus segmentation.

Discussion

This study aimed to investigate systems-level interactions underlying consolidation of emotional memories by investigating spontaneous reactivation of memory traces with an emotional connotation and tracking changes in functional connectivity. We hypothesized that emotional memories are preferentially consolidated by increased

spontaneous reactivation of memory traces during post-learning awake rest. Firstly, we found increased spontaneous reactivation of category-specific patterns of BOLD signal during post-learning awake rest for a conceptual category that was coupled to an emotionally arousing event. Secondly, hippocampal connectivity with the regions exhibiting these reactivations and the hippocampus predicted interindividual differences in fear recall 24h later. Lastly, we found that amygdala-hippocampal connectivity was increased during post-learning awake rest compared to baseline.

We found a reactivation of neocortical patterns of BOLD responses within the inferior/ventral temporal cortex, which were obtained from a localizer paradigm (*i.e.*, before one of the two conceptual categories was associated with shock), during post-learning resting-state blocks. This finding extends earlier studies that found reactivations in other parts of the brain (Deuker et al., 2013; Staresina et al., 2013; Tambini & Davachi, 2013) by showing that reactivation during post-learning rest was specific for the category that was coupled to an emotional event. Our findings cannot be explained by other processes that can take place during learning, such as attentional, motivational, and arousal-related processes, because we sampled patterns of BOLD responses to exemplars of both stimulus categories before fear learning took place. Therefore, our effect can also not be explained by temporal autocorrelation of the BOLD signal or by the temporal structure of the experiment. Moreover, our voxel selection was orthogonal to our emotional manipulation by counterbalancing the shock association to one of the categories and controlling for reactivation effects in a spatially distinct region. Along the hierarchically organized ventral occipitotemporal cortex, these neocortical regions are thought to represent various features of an experience (Frankland & Bontempi, 2005; Osada et al., 2008). Low-level visual features are integrated into more complex, high-level feature sets along the anterior-posterior axis (Tyler et al., 2013). Our experiment contained exemplars from a conceptual category (Tranel et al., 1997) with which we aimed to capture these high-level feature representations. These categories elicit differential and localized patterns within the inferior/ventral temporal cortex (Haxby et al., 2001; Kriegeskorte et al., 2008), which we have replicated within our own dataset. We therefore interpret the observed correlations between task-evoked activity patterns in the localizer paradigm with patterns of spontaneous activity during post-learning awake rest as a reactivation of higher-order conceptual category representations.

The critical difference between the CS+ and the CS- category is that one of the two was associated with an emotionally arousing event. Our data show that only the category associated with an emotionally arousing event was reactivated. Emotional arousal was indicated by an increased pupil dilation, an indirect index of norepinephrine release (Sara, 2009), during fear learning in response to exemplars of the category linked to a shock. Although the reactivation of patterns of neocor-

tical BOLD responses is a process that takes place at a different spatiotemporal scale, our finding is in line with replay studies showing that awake replay is enhanced for novel (Foster & Wilson, 2006) and reward-related (Singer & Frank, 2009) experiences. This suggests that awake replay could be boosted by catecholaminergic neuromodulatory changes during salient events (Carr et al., 2011). Indeed, a recent study showed that optogenetic stimulation of hippocampal dopaminergic fibers from midbrain neurons in mice exploring novel environments enhances reactivation of pyramidal cell assemblies during subsequent sleep/rest (McNamara et al., 2014). Furthermore, it is well established that norepinephrine strengthens memory consolidation (McGaugh, 2002, 2000) and alters thresholds for synaptic modification (Roozendaal et al., 2009). Periods of synaptic plasticity have been indirectly linked to replay events through the occurrence of sharp-wave/ripples (Carr et al., 2011). Moreover, spontaneous replay of information occurs specifically during sharp-wave/ripples (Karlsson & Frank, 2009; Skaggs & McNaughton, 1996), and when synaptic plasticity is experimentally induced, sharp-wave/ripples are increased (Behrens et al., 2005; Buzsaki, 1984). Although direct evidence showing that enhanced catecholaminergic activity during emotionally arousing events induces awake replay is missing, our study is the first linking replay-like processes in humans to emotional arousal.

Next, we found that the connectivity between the hippocampus and the regions showing this enhanced reactivation was predictive of fear recall tested 24 hours later. Stabilization of memory traces is thought to depend on the hippocampally driven reactivation of encoded features (Carr et al., 2011), resulting in a gradual integration into existing neocortical networks (Frankland & Bontempi, 2005; Rasch & Born, 2007; Squire, 1992). Electrophysiological studies in rodents have indeed shown replay events within the hippocampus and neocortex (Ji & Wilson, 2007; Kudrimoti et al., 1999; Wilson & McNaughton, 1994), and reactivation occurs in a coordinated fashion between hippocampal and neocortical networks (Ji & Wilson, 2007; Qin et al., 1997). Moreover, silencing hippocampal cells prevents reactivation of neurons during retrieval that were active during fear learning (Tanaka et al., 2014). In addition, in humans, enhanced functional interactions between hippocampus and neocortical regions involved during item encoding were predictive of memory recall (Tambini et al., 2010). Our findings indicate that hippocampal-neocortical coupling during consolidation is not only involved in declarative associative memory tested immediately, but also in non-declarative memory such as fear conditioning tested at a later time point. We therefore show that the processes we captured are relevant for consolidation.

Lastly, we found an increased connectivity between the amygdala and the hippocampus during post-learning rest compared to baseline. The hippocampus and amygdala do not exhibit readily detectable category-selective BOLD response pat-

terns and we could therefore not distinguish between the CS+ versus CS- category representations. Thus, our finding may reflect a general increase in connectivity following emotional arousal. One limitation of this finding is that it is based only on a comparison between different resting-state blocks. Even though the increase in functional connectivity remained significant when taking additional measures to reduce motion artifact (Power et al., 2015), we cannot fully rule out the influence of subject motion. An interpretation of this finding in terms of increased connectivity is, however, in line with anatomical studies in rodents, which have indicated there are reciprocal connections between the amygdala and the hippocampus (Pitkänen et al., 2000), making it possible for these regions to functionally influence each other. Indeed, increased amygdala-hippocampal theta coherence during the acquisition and expression of conditioned fear (Seidenbecher et al., 2003) as well as during periods of paradoxical sleep after fear learning (Popa et al., 2010) have been demonstrated. Moreover stress has been shown to increase the influence of the amygdala on the hippocampus during awake rest (Ghosh et al., 2013). Our findings extend previous findings demonstrating amygdala-hippocampal connectivity in humans during rest using BOLD-fMRI (Roy et al., 2009) by showing that amygdala-hippocampal connectivity is increased after fear learning. We did not find a relationship between this functional coupling and individual differences in fear recall, which raises the question what the role of the amygdala in memory facilitation and regulating memory trace reactivation might be. The amygdala could act as a gating system by facilitating transmission of information from the hippocampal complex to neocortical storage sites (Bauer et al., 2007; Paz et al., 2006). On the other hand, the amygdala may be involved as a consequence, because activation of dentate gyrus cell assemblies related to a fear memory seem to be sufficient to cause amygdala activation (Ramirez et al., 2013). Our data cannot conclusively answer this question and therefore further research is needed. For example, invasive electrophysiology in rodents combined with catecholaminergic manipulations, or via specific interventions such as optogenetic techniques, could potentially shed light on the influence of the amygdala on awake memory trace reactivation (Hermans et al., 2014).

We did not find differential BOLD responses in the amygdala during fear acquisition blocks. Why would we then still expect the amygdala to play a role after learning? First of all, the absence of amygdala activity is consistent with the human neuroimaging literature on fear conditioning (Bach et al., 2011; Fullana et al., 2016; Mechias et al., 2010). Differential conditioning effects in the amygdala are often only seen during the first few trials, when fear learning takes place (Büchel & Dolan, 2000; LaBar et al., 1998). Furthermore, the amygdala has not only been implicated in fear conditioning. A large body of research in rodents shows how the amygdala is necessary for arousal-related neuromodulators to have an effect on memory

processes elsewhere in the brain (McGaugh, 2004; Roozendaal & McGaugh, 2011), for example the hippocampus (Beldjoud et al., 2015; Hatfield & McGaugh, 1999; Packard et al., 1994). These effects are blocked when the amygdala is lesioned (Cahill & McGaugh, 1991; Liang et al., 1982). Therefore, our findings are more in line with a role for the amygdala in modulating mnemonic processes elsewhere in the brain, rather than with a role for the amygdala in fear expression.

When enhanced memory trace reactivation is the mechanism through which emotionally arousing events get consolidated more strongly, one could speculate on how this mechanism might underlie extremely persistent and intrusive memories. For example, posttraumatic stress disorder (PTSD) is characterized by persistent vivid and mostly unwanted memories of traumatic events (Brewin et al., 2010; Ehlers, 2010). Stronger (re-)consolidation has been proposed as a mechanism by which these traumatic memories develop over time (Pitman, 1989) and our data might provide insight into how these memories get (re)consolidated more strongly. It has been suggested that memory retrieval or re-experiencing of the traumatic event might be caused by externally or internally driven reminders of that traumatic event (Ehlers et al., 2004). Awake replay has been proposed to be a potential mechanism through which retrieval is facilitated (Karlsson & Frank, 2009). Thus, the process we observe could first potentially be triggered by cues (Carr et al., 2011) and subsequently lower the threshold for spontaneous memory recall and start a vicious cycle of re-experiencing emotional memories. Future research, however, should focus on whether cues might trigger awake memory trace reactivation and secondly whether this reactivation can indeed facilitate retrieval.

Conclusions

In conclusion, our data support theories on systems consolidation (Frankland & Bontempi, 2005; Rasch & Born, 2007; Squire, 1992) stating that spontaneous interactions within hippocampal-neocortical networks is a potential mechanism through which information is bound together and subsequently retained. We demonstrate that emotional arousal during learning leads to stronger spontaneous reactivation of neocortical memory traces with an emotional connotation during post-learning awake rest. Thereby, this study reveals a potential mechanism by which emotional memories are selectively consolidated and may thereby explain why emotional memories are preferentially preserved in long-term memory.



Chapter 5

Goal-directed eye movements during extinction deactivate amygdala and reduce fear recovery

Lycia D. de Voogd, Jonathan W. Kanen, Karin Roelofs, Guillén Fernández and Erno J. Hermans

Abstract

Improving extinction learning is essential to optimize therapy for persistent fear-related disorders. We found that precisely timed eye movements during extinction transiently deactivate the amygdala and diminish spontaneous fear recovery. Amygdala deactivation furthermore predicted reduced fear recovery after reinstatement. Our findings show extinction learning can be improved with a behavioral manipulation and provide mechanistic understanding of a widely used treatment for traumatic symptoms which uses eye movements to enhance therapy.

Introduction

Introduction Extinction learning is core to most effective therapies for disorders of fear and anxiety (Bisson et al., 2013). Exposure therapy, for instance, results in the formation of an extinction memory that suppresses the expression of fear. Relapse of pathological fear is nevertheless common (Dunsmoor et al., 2015; Maren, 2011). Improving extinction learning is therefore an important goal of translational research into fear-related disorders (Dunsmoor et al., 2015). Pharmacological treatments have proven effective in preventing fear recovery in animal models (Nader et al., 2000), but these methods are often not applicable in humans (Nader et al., 2000) or have yielded inconsistent results in experimental models with humans (Bos et al., 2012; Kindt et al., 2009). Therefore, new non-invasive techniques have been developed that target reconsolidation of the original fear memory rather than enhance extinction learning (Schiller et al., 2010). Although these results are promising, their clinical application is so far unclear.

Clinically effective treatments, however, are not always derived from such experimental models. One example is Eye Movement Desensitization and Reprocessing (EMDR; Bisson et al., 2013), an evidence-based therapy and part of mental health care guidelines in many countries (Bisson et al., 2013; Lee & Cuijpers, 2013). Despite its wide use, a mechanistic, neurobiological understanding of EMDR is lacking. During treatment, patients divide their attention between recalling traumatic memories and making lateral eye movements directed by the therapist's hand. Eye movements are central to the procedure, but it is unclear if they play any role in the therapeutic outcome above normal extinction learning (Lee & Cuijpers, 2013). Insight into the potential role of eye movements and the neurobiological mechanisms underlying this manipulation is not only crucial to further optimize this therapy, but would also importantly advance fundamental understanding of extinction learning.

One lead into a neural mechanistic account is that goal-directed eye movements are associated with activations in the dorsal fronto-parietal network, including frontal eye fields (Corbetta & Shulman, 2002), similar to working memory tasks that require goal-directed attention. Critically, working memory tasks are accompanied by robust deactivations in a posterior-medial network (Qin et al., 2009), including the amygdala. This is important because targeting the amygdala following memory reactivation, by blocking protein synthesis, prevents fear recovery in rodents (Nader et al., 2000). Similarly, systemic administration of propranolol, a β -adrenoceptor antagonist, presumably exerts its effects on fear recovery via inhibition of protein synthesis in the amygdala (Kindt et al., 2009). Amygdala reactivity measured with BOLD-fMRI in humans is furthermore decreased after propranolol administration (Hurlemann et al., 2010). We therefore hypothesized that (1) goal-directed eye movements could be used as a non-invasive tool to transiently suppress amygdala activity, comparable to working memory tasks, and (2) a well-timed application of

this deactivation following memory reactivation could prevent fear recovery.

Results

To test our first hypothesis, participants performed a two-back working memory task (Qin et al., 2009) and eye movements in separate blocks while undergoing functional MRI. As expected, both the working memory blocks [left: $p=.05$, right: $p=.035$; peak-voxel FWE-SVC] and eye-movement blocks [left: $p=.036$; right: $p=.05$, peak-voxel FWE-SVC] led to deactivations in the amygdala compared to fixation. Thus, comparable to a working memory task (Qin et al., 2009), goal-directed eye movements suppress amygdala activity (Table 5.1).

Table 5.1: Peak voxel coordinates and statistics of activations and deactivations of the two-back task and eye movements compared to fixation (Experiment 1)

Region	Side	x(mm)	y(mm)	z(mm)	Peak t	Size mm3
<i>Two-Back blocks > fixation</i>						
Dorsolateral prefrontal cortex (dlPFC)	L	-36	48	16	9.39	$p=.02^*$
Posterior parietal cortex	L	-26	-52	44	9.43	$p=.02^*$
Anterior insula	L	-24	20	0	18.87	$p=.007$
Anterior Insula	R	32	24	2	15.39	$p=.033$
<i>Two-Back blocks < fixation</i>						
Middle temporal gyrus	L	-60	-22	-12	22.72	$p=.002$
Superior frontal gyrus	L	-18	42	46	19.32	$p=.006$
Superior frontal gyrus (medial)	R	10	58	20	17.50	$p=.012$
Calcarine sulcus	R	4	-60	20	16.36	$p=.021$
Ventromedial prefrontal cortex (vmPFC)	R	6	38	-2	15.71	$p=.028$
Superior frontal gyrus	L	-16	60	26	15.51	$p=.031$
Amygdala	L	-26	4	-20	4.51	$p=.05^*$
Amygdala	R	26	-2	-18	4.88	$p=.035^*$
<i>Eye movements > fixation</i>						
Superior occipital gyrus	R	26	-74	18	19.88	$p=.005$
Precentral gyrus (frontal eye fields)	L	-40	-12	50	7.13	$p=.002^*$
Precentral gyrus (frontal eye fields)	R	32	-10	56	8.50	$p=.001^*$
Posterior parietal cortex	L	-20	-54	48	9.72	$p=.017^*$
<i>Eye movements < fixation</i>						
Amygdala	L	-26	-12	-14	4.87	$p=.036^*$
Amygdala	R	26	-8	-16	4.46	$p=.05^*$

Notes: all coordinates are defined in MNI152 space. All statistics listed are significant at $p<.05$, whole-brain family-wise error corrected unless indicated otherwise. *Small volume corrected for region of interest.

We then tested whether goal-directed eye movements can prevent fear recovery via this amygdala deactivation. We integrated eye movements into an established Pavlovian fear conditioning paradigm (Schiller et al., 2010, Figure 5.1A). Similar to EMDR, goal-directed eye movements were performed following unreinforced fear memory reactivation (*i.e.*, during extinction learning; Figure 5.1B). During acquisition (day one), two conditioned stimuli (CSs+) were associated with mild electrical shocks (unconditioned stimulus; US) and two stimuli (CSs-) were never reinforced. During extinction (day two), one CS+ and one CS- was followed by a laterally moving dot (duration: 10 s; speed: 1 Hz; visual angle: 11°). On day

three, we tested for spontaneous recovery (re-extinction1) and recovery following reinstatement (re-extinction2). We predicted that extinction learning with (versus without) eye movements on day two would lead to reduced recovery of differential (CS+ minus CS-; Schiller et al., 2010) skin conductance responses (SCR) on day three. We furthermore expected that eye movements would suppress amygdala activity (*i.e.*, replicating Experiment 1), and critically, that the strength of this deactivation would predict reduced fear recovery.

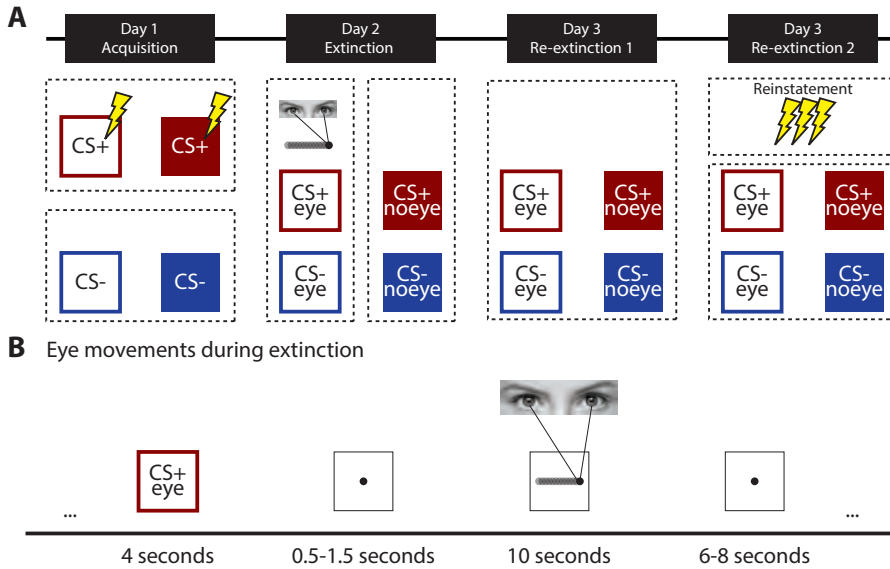


Figure 5.1: Overview of the experimental design

(A) The experiment took place on three consecutive days. During acquisition, two colored squares (CS+) were associated with an electrical shock (reinforcement rate was 37.5%) and two colored squares were never reinforced (CS-). CSs+ and CSs- were shown in pseudo-random order during conditioning. During extinction, one CS+ and one CS- was always followed by a laterally moving dot. A fixation dot (0.5-1.5 s) was presented between CSs and eye-movement blocks and following the eye-movement blocks (6-8 s). The interstimulus interval following the other CS+ and CS- consisted of a static fixation dot entirely. Day three consisted of a re-extinction (re-extinction1) phase and a re-extinction after reinstatement (re-extinction2) phase. (B) Time line of a single trial during extinction when participants made eye movements.

Replicating experiment 1, amygdala activity was suppressed during eye movements compared to fixation [$F(1,23)=4.576$, $p=.04$, $\eta_p^2=.17$]. There was no interaction with CS (CS+, CS-) [$F(1,23)=1.296$, $p=.27$, $\eta_p^2=.05$] (Figure 5.2). As anticipated, we found the opposite pattern in the frontal eye fields (Figure 5.3). SCR measures revealed successful acquisition (Figure 5.4A) and extinction (Figure 5.4B). Importantly, there was full extinction on the last trial [$F(1,23)=.260$, $p=.61$, $\eta_p^2=.01$] and no interaction with extinction manipulation (Eye, No-eye) [$F(1,23)=.991$, $p=.33$, $\eta_p^2=.04$].

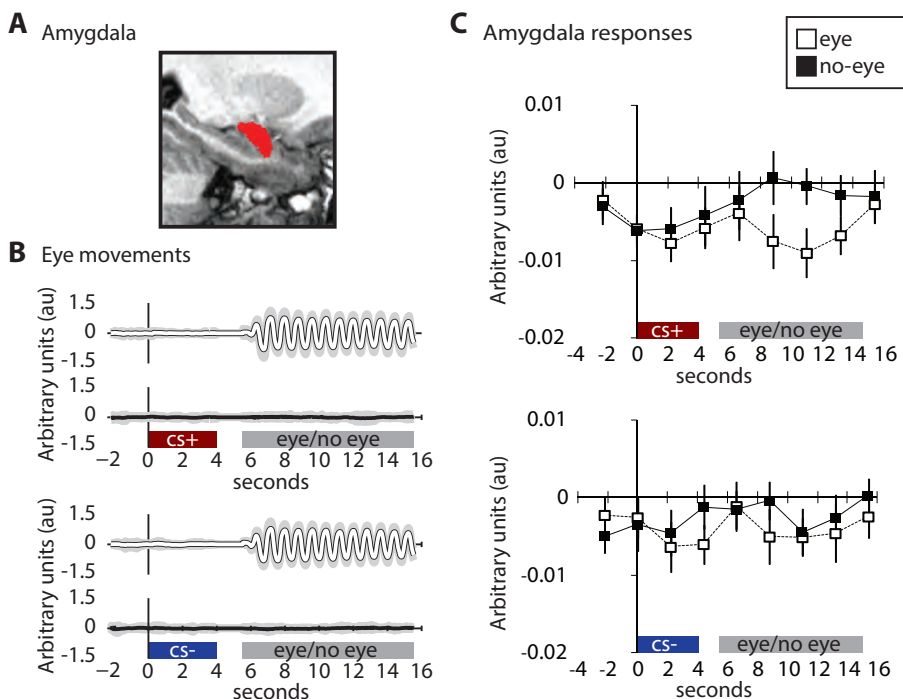


Figure 5.2: Eye movements suppress amygdala activity

(A) Single-subject example of the automated anatomical segmentation of the amygdala using FSL FIRST. (B) Eye-movement recordings during the eye-movement blocks and no-eye movement blocks within the extinction phase. The black and white lines reflect the mean across all participants and the gray shaded area the standard error of the mean (SEM). (C) Amygdala deactivation during the eye-movement blocks and no-eye movement blocks within the extinction phase, displayed separately for the CSs+ and CSs-. Error bars represent \pm standard error of the mean (SEM).

Spontaneous recovery of fear was defined as the increase in differential responding from the last trial of extinction (day two) to the first trial of re-extinction1 (day three) (Schiller et al., 2010). This spontaneous recovery index differed between the CSs extinguished with eye movements versus without [$F(1,22)=5.976$, $p=.02$, $\eta_p^2=.21$]. As expected, there was spontaneous recovery for extinction without [$t(22)=3.60$, $p=.002$], but not with eye movements [$t(22)=.694$, $p=.50$; Figure 5.4C]. Thus, eye movements during extinction learning indeed diminished spontaneous recovery. Analyses on the reinstatement recovery index, first trial of re-extinction2 minus last trial of re-extinction1, (Schiller et al., 2010) showed that differential responses returned on average [$F(1,22)=23.486$, $p=7.65E-5$, $\eta_p^2=.52$]. Notably, including strength of amygdala deactivation as a covariate revealed an interaction between this deactivation and extinction manipulation [$F(1,21)=7.252$, $p=.01$, $\eta_p^2=.26$]. Further testing showed that the stronger the amygdala deactivation, the less fear recovery occurred, when extinction had been accompanied by eye movements [$r(21)=.39$, $p=.028$, one-tailed; Figure 5.4E]. Amygdala deactivation also predicted the differ-

ence between the spontaneous recovery index and reinstatement recovery index [$r(20)=-.62$ $p=.002$]. Thus, although differential fear responses on average recovered after reinstatement, recovery was attenuated when participants had stronger amygdala deactivations during eye movements.

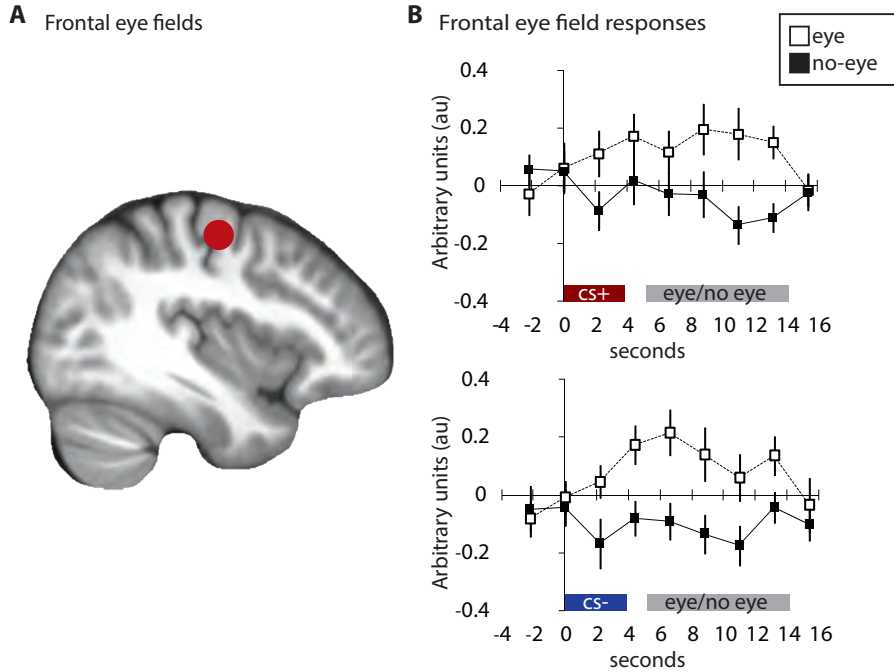


Figure 5.3: Activity in frontal eye fields associated with eye movements

(A) The frontal eye fields were defined using a bilateral sphere with 5 mm radius around the MNI peak coordinates reported in a meta-analysis of neuroimaging studies of eye movements (Jamadar et al., 2013). (B) Frontal eye field activation during the eye-movement blocks and no-eye movement blocks within the extinction phase, displayed separately for the CSs+ and CSs-. Activity during the eye movements was higher than during fixation [$F(1,23)=13.11$, $p=.001$, $\eta_p^2=.36$].

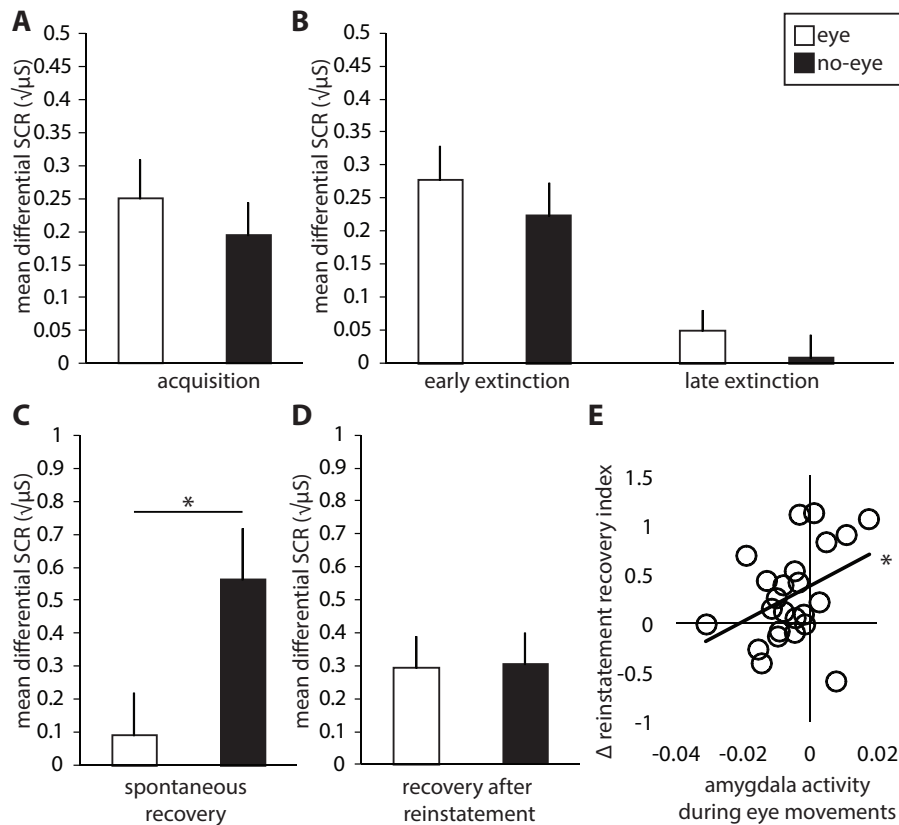


Figure 5.4: Conditioned skin conductance responses

(A) Skin conductance response (SCR) measures during acquisition revealed a robust differential conditioning effect (CS+ versus CS-) across all trials [$F(1,22)=18.54$, $p=2.86\text{E-}4$, $\eta_p^2=.46$]. Differential conditioning did not differ for the CSs which were later associated with eye movements versus no eye movements during extinction [$F(1,22)=1.945$, $p=.18$, $\eta_p^2=.08$]. (B) During early extinction, there was a differential conditioning effect [$F(1,23)=49.77$, $p=3.46\text{E-}7$, $\eta_p^2=.68$] which was non-significant during late extinction [$F(1,23)=.896$, $p=.35$, $\eta_p^2=.04$]. (C) Spontaneous recovery of differential fear. A repeated-measures ANOVA across all re-extinction1 trials revealed an interaction between extinction manipulation (eye, no-eye) and time [first versus second half of re-extinction1; $F(1,22)=6.723$, $p=.02$, $\eta_p^2=.23$]. Follow-up tests on the spontaneous recovery index (first trial of re-extinction1 minus last trial of extinction; shown here) revealed that spontaneous recovery differed between extinction manipulations [$F(1,22)=5.976$, $p=.02$, $\eta_p^2=.21$]. There was spontaneous recovery for extinction without [$t(22)=3.60$, $p=.002$], but not with eye movements [$t(22)=.694$, $p=.50$]. (D) Differential reinstatement recovery index (first trial re-extinction2 minus last trial re-extinction1). Differential responses returned after reinstatement [$F(1,22)=23.486$, $p=7.65\text{E-}5$, $\eta_p^2=.52$] but there was no interaction with extinction manipulation [$F(1,22)=.005$, $p=.94$, $\eta_p^2<.001$]. (E) For the reinstatement recovery index, amygdala deactivation strength (used as covariate of interest) interacted with extinction manipulation [$F(1,21)=7.252$, $p=.01$, $\eta_p^2=.26$]. Amygdala deactivation during the eye-movement blocks on day two correlated positively with the differential reinstatement recovery index for extinction learning with eye movements [displayed in graph: $r(21)=.39$, $p=.028$, one-tailed]. For the spontaneous recovery index, this was not the case [$F(1,21)=1.592$, $p=.22$, $\eta_p^2=.07$]. Error bars represent \pm standard error of the mean (SEM). * = $p < .05$.

Discussion

A potential explanation for why amygdala deactivation occurs, is that large-scale brain networks act reciprocally (Fox et al., 2005) and compete for resources (Hermans et al., 2014). Acute stress engages the amygdala but impairs dorsal fronto-parietal network functioning (Hermans et al., 2014). Our data confirm that engaging the dorsal fronto-parietal network has the opposite effect of deactivating the amygdala. A consequence of this might be that fear expression is attenuated. Startle responses, for instance, are reduced when performing a working memory task (Vytal et al., 2012). More evidence for the reciprocal nature of working memory and amygdala function comes from patients with amygdala lesions showing enhanced working memory performance (Morgan et al., 2012). The amygdala has been the main target site for pharmacological manipulations aiming to update the CS-US association following memory reactivation (Nader et al., 2000). If this mechanism underlies the role of eye movements in reducing traumatic symptoms, then any working memory task would have similar effects. Indeed, emotionality and vividness of autobiographical memories is reduced when memory reactivation is paired with working memory tasks (Engelhard et al., 2010). Our data therefore provide a parsimonious explanation for how both eye movements and working memory tasks could affect the emotionality of memories.

Spontaneous recovery was diminished after extinction with eye movements. The dominant view on post-extinction recovery (Dunsmoor et al., 2015; Maren, 2011) holds that this can be due to updating the original CS-US association or to the formation of a stronger new extinction memory. In line with the latter account, differential fear responses recovered after reinstatement, indicating the CS-US association was not fully eliminated. A similar reduction in spontaneous recovery was observed in a study in which the US was replaced by a non-aversive tone during extinction (Dunsmoor et al., 2015). One possibility, therefore, is that eye movements following CS presentation, similar to a tone, reduce the ambiguity of the CS created by US omission, and thereby strengthen extinction. However, unlike a tone, eye movements suppress amygdala activity and possibly attenuate fear responses (Vytal et al., 2012). This may allow for additional learned controllability over conditioned responses via subsequent suppression. This interpretation aligns with findings of reduced spontaneous recovery in rats when trained to actively avoid the US during extinction learning (Moscarello & LeDoux, 2013).

However, the amygdala is also crucially involved in encoding the CS-US association (Maren, 2011). Amygdala suppression following reactivation could therefore also lead to updating of the CS-US association (*e.g.*, as less aversive) rather than only facilitating new learning. Indeed, we found that stronger amygdala deactivation was predictive of reduced fear recovery following a reminder of the original memory (*i.e.*, the US). This possibility is in line with active forgetting studies, which show

that hippocampal deactivation due to top-down control (*i.e.*, trying not to think about a memory) following reactivation predicts later forgetting (Anderson & Oates, 2004). Our findings may therefore be due to a combination of new learning and unlearning the CS-US association. This interpretation aligns with recent views on safety learning that challenge the strict dichotomy between unlearning and new learning (Clem & Schiller, 2016). We thus propose that eye movements during extinction learning may update the CS-US association (Clem & Schiller, 2016) via newly learned instrumental control over CS-evoked fear responses following memory reactivation (Moscarello & LeDoux, 2013).

Our findings show that eye movements have added value in safety learning above reactivation alone. This effect, while likely not specific to eye movements, is associated with transient amygdala deactivation as a consequence of reciprocally coupled activation of the dorsal fronto-parietal network. Our findings align closely with pharmacological manipulations (Nader et al., 2000) which also target the amygdala. However, transiently suppressing amygdala activity with behavioral manipulations has clear advantages, because these are non-invasive, precise in time and duration, and shown to be clinically effective. Besides the theoretical value, our findings thus provide a parsimonious account of both a technique already in clinical use, and of experimental findings showing that eye movements and working memory tasks following reactivation alter retention of emotional memories.

Methods experiment 1

Participants

Nine right-handed healthy volunteers (5 female, 4 male; 24-30 years [$M=26.8$, $SD=2.0$]) completed the study. Exclusion criteria were any contraindications for MRI. All gave written informed consent and were paid for their participation. This study was approved by the local ethical review board (CMO region Arnhem-Nijmegen).

Experimental task

The tasks consisted of 6 blocks of a two-back working memory task (Qin et al., 2009), 6 blocks of smooth-pursuit lateral eye movement task, and an additional 8 blocks of low-level fixation baseline. The duration of each block was 27 seconds. Within each two-back block, participants saw a random sequence consisting of 15 single digits. Each digit was presented for 400 ms, followed by an interstimulus interval (ISI) of 1400 ms. Participants were asked to detect whether the current item had appeared two positions back in the sequence, and were instructed to make a button press when detecting a target. For the eye-movement blocks, participants were instructed to follow a laterally moving dot with their eyes. The speed of the eye movements was 1 Hz, based on previous laboratory models of EMDR (van den

Hout et al., 2013).

MRI data acquisition

MRI scans were acquired using a Siemens (Erlangen, Germany) MAGNETOM Skyra 3T MR scanner. T2*-weighted blood oxygenation level-dependent (BOLD) images were recorded using a customized EPI sequence with ascending slice acquisition (37 axial slices; TR, 1.89 s; TE, 25 ms; Generalized Autocalibrating Partially Parallel Acquisitions (GRAPPA; Griswold et al., 2002) acceleration factor 2; flip angle, 90°; slice matrix size, 64x64; slice thickness, 3.3 mm; slice gap, 0.3 mm; FOV, 212 x 212 mm; bandwidth: 1776 Hz/px; echo spacing: 0.65 ms). A structural image (1 mm isotropic) was acquired using a T1-weighted 3D magnetization-prepared rapid gradient-echo sequence (MP-RAGE; TR, 2.73 s; TE, 2.95 ms; flip angle, 7°; FOV, 256 x 256 x 176 mm).

MRI data preprocessing and statistical analyses

MRI data were pre-processed in standard stereotactic (MNI152) space for the purpose of whole-brain group analyses. Mutual information maximization-based rigid-body registration was used to register structural and functional images. Functional images were motion corrected using rigid-body transformations. Structural images were segmented into gray matter, white matter, and CSF images using a unified probabilistic template registration and tissue classification method (Ashburner & Friston, 2005). Tissue images were then registered with site-specific tissue templates (created from 384 T1-weighted scans) using DARTEL (Ashburner, 2007), and registered (using an affine transformation) with the MNI152 template included in SPM8. Identical transformations were applied to all functional images, which were resliced into 2 mm isotropic voxels and smoothed with a 6 mm FWHM Gaussian kernel.

Responses to the two-back task and lateral eye movements were modeled using box-car regressors (duration of 27 s). These two regressors were temporally convolved with the canonical hemodynamic response function (HRF) included in SPM8. Additionally, six movement parameter regressors (3 translations, 3 rotations) derived from rigid-body motion correction, high-pass filtering (1/128 Hz cut-off), and AR(1) serial correlation corrections were included in the model. Single-subject contrast maps of the two-back and eye-movement blocks against fixation were entered into second-level one-sample t-tests. Based on our priori hypotheses, results for the amygdala, dorsolateral prefrontal cortex (dlPFC), posterior parietal cortex (PPC), and frontal eye fields (FEF) were corrected for reduced search volumes using small volume corrections (SVC) and were family-wise error (FWE) corrected using voxel-level statistics. SVC of the amygdala was based on a group mask that was created by averaging individual amygdala segmentations (n=24) of T1-weighted im-

ages (using FSL FIRST, see <http://www.fmrib.ox.ac.uk/fsl/first/index.html>), which were warped into MNI space using DARTEL. SVC for dlPFC was based on Brodmann Areas 9, 10, and 46, and the PPC was based on Brodmann Areas 7 and 40 (Qin et al., 2009; Wager & Smith, 2003). The FEF were defined using a bilateral sphere with 5 mm radius around the MNI peak coordinates reported in a meta-analysis of neuroimaging studies of eye movements (Jamadar et al., 2013).

Methods experiment 2

Participants

Twenty-four right-handed healthy volunteers (12 female, 12 male; 20-34 years [$M=24.8$, $SD=3.6$]) completed the study. An additional 5 participants did not complete the entire experiment due non-compliance with instructions (*e.g.*, falling asleep). Exclusion criteria were: current or lifetime history of psychiatric, neurological, or endocrine illness, current treatment with any medication that affects central nervous system or endocrine systems, average use of more than 3 alcoholic beverages daily, average use of recreational drugs weekly or more, habitual smoking, predominant left-handedness, uncorrected vision, intense daily physical exercise, and any contraindications for MRI. Participants gave written informed consent and were paid for their participation. This study was approved by the local ethical review board (CMO region Arnhem-Nijmegen).

Pavlovian fear conditioning paradigm

Participants were tested in a differential delay fear conditioning paradigm (Schiller et al., 2010, 2013) on three consecutive days with 24h in between. The first day comprised an acquisition session, the second day an extinction session, and the third day a recall session. The stimulus set across the three days consisted of four squares as conditioned stimuli (CS) with a different color. The luminance of the stimuli, background, and ISI screen was equalized. On day one, two cues (CS+s, 4s duration) were partially reinforced (37.5% reinforcement rate) with a mild electrical shock to the fingers (*i.e.*, the unconditioned stimulus; UCS). The two other cues (CS-s, 4s duration) were never reinforced. In total there were 64 trials (16 trials per CS). The CS+s reinforced, CS+s unreinforced, and CS-s were presented in a pseudorandom order. The ISI was jittered between 4s and 8s with an average of 6s.

On day two, extinction included 48 CS trials (12 trials per CS, 4s duration) and 24 eye-movement blocks (10 s duration). One CS+ (CS+eye) and one CS- (CS-eye) were always followed by an eye-movement block while the other CS+ (CS+no-eye) and the other CS- (CS-no-eye) were always followed by a fixation block. The ISI between CS and eye movement block was jittered between 0.5s and 1.5s which was done to minimize eye movement anticipation during the CS presentation. With the

duration of 10 s, we stayed on the lower end of what is used in EMDR treatment, in which the duration of eye movements varies between 8 and 96 s (Lee & Cuijpers, 2013). This 10 s duration limits the length of the experiment, while still including the peak of the Blood Oxygenation Level Dependent (BOLD) response within the eye-movement blocks (Heeger & Ress, 2002). As in experiment 1, the speed of the moving dot was 1 Hz, based on previous laboratory models of EMDR (van den Hout et al., 2013). The visual angle was approximately 11°. We verified compliance of participants using eye-tracking measurements (see below). The ISI after the eye-movement block varied between 4s and 8s with an average of 6s. On day three, the experiment started with a re-extinction session (re-extinction1), which included 24 CS trials (6 trials per CS, 4s duration) with an ISI jittered between 4s and 8s (average of 6s). After this session there was a reinstatement procedure (Haaker et al., 2014) consisting of 3 un-signaled UCS presentations (ISI: 10s). Following this, participants underwent a second re-extinction session (re-extinction2), which included 24 CS trials (6 trials per CS, 4s duration). ISI was jittered between 4s and 8s with an average of 6s. See Figure 5.1 for an overview.

Questionnaires and debriefing

Participants completed the Beck Depression Inventory (BDI; Beck et al., 1996) and the trait version of State-Trait Anxiety Inventory (STAI-t; Van der Ploeg, 1980). A BDI score above 13 was used to exclude participants from the analyses, but none of the participants had a score higher than the cut-off. Average BDI score was 3.5 (range: 0-10) and STAI-t was 33.5 (range: 25-48). Participants were debriefed after the completion of the experiment and asked about their contingency knowledge on the occurrence of electrical shocks, as well as the relationship between the CSs and eye-movement blocks. Participants were furthermore asked about their knowledge of EMDR and whether they at some time during the experiment thought of the experiment in the context of EMDR treatment. Five participants reported doing so. We therefore redid the analyses of the two re-extinction phases on day 3 excluding these five participants. The results and conclusions remained the same and therefore the results are reported including all participants.

Peripheral stimulation

Electrical shocks were delivered via two Ag/AgCl electrodes attached to the distal phalanges of the second and third fingers of the right or left hand (counterbalanced between subjects) using a MAXTENS 2000 (Bio-Protech) device. Shock duration was 200 ms, and intensity varied in 10 intensity steps between 0V-40V/0mA-80mA. During a standardized shock intensity adjustment procedure, each participant received and subjectively rated five shocks, allowing shock intensity to converge to a level experienced as uncomfortable, but not painful. The resulting average

intensity step was 4.8 (SD: 1.8) on a scale from 1 to 10. The intensity step was set on day 1 and remained the same on day 3 for the reinstatement procedure.

Peripheral measurements

Electrodermal activity was assessed using two Ag/AgCl electrodes attached to the distal phalanges of the first and second fingers of the left or right hand (counterbalanced between subjects) using a BrainAmp MR system and recorded using BrainVision Recorder software (Brain Products GmbH, Munich, Germany). Data were preprocessed using in-house software; radio frequency (RF) artifacts were removed and a low-pass filter was applied. Skin conductance responses (SCR) were automatically scored with additional manual supervision using Autonomate (Green et al., 2014) implemented in Matlab 7.14 (MathWorks). SCR amplitudes (measured in μSiem) were determined for each trial within an onset latency window between 0.5 and 4.5 s after stimulus onset, with a minimum rise time of 0.5 s and a maximum rise time of 5 s after response onset. Reinforced trials were omitted and all other response amplitudes were square-root transformed prior to statistical analysis. One subject was omitted from the SCR analyses on day 1 because of failed recordings presumably due to motion of the hand.

Analyses on the SCR were performed using SPSS 19 (IBM Corp, Armonk, New York). Four repeated measure ANOVAs were conducted, one for each experimental phase (Acquisition, Extinction, Re-extinction1, and Re-extinction2). Each ANOVA included CS (CS+, CS-) and extinction manipulation (eye movements, no-eye movements) as within-subject factors. During the extinction and re-extinction phases, an additional within-subject factor was included, namely time (early, late). Subsequently, differential SCR were calculated (CS+ minus CS-) to test for differences between the two conditions (eye movement and no-eye movement). To test for spontaneous recovery of fear, the differential response on the last trial of extinction was subtracted from the first differential response during re-extinction1 (Schiller et al., 2010, 2013). The reinstatement recovery index was calculated in a similar way by subtracting the last differential response during re-extinction1 from the first differential response during re-extinction2 (Schiller et al., 2010, 2013). For the spontaneous recovery index and reinstatement recovery index analyses, we covaried the order of the CS+ (CS+eye or CS+no-eye) presentation. Lastly, the amount of amygdala suppression that occurred on day 2 during the eye-movement blocks was added as a covariate to the recovery index analyses on day 3 to test whether amygdala deactivation predicted fear recovery.

Eye tracking was recorded using an MR-compatible eye-tracking system (MEye Track-LR camera unit, SMI, SensoMotoric Instruments). Data were preprocessed using in-house software (Hermans et al., 2013) implemented in Matlab 7.14 (MathWorks). Blinks were removed from the signal using linear interpolation. Eye-

tracking data during the eye-movements blocks were normalized based on a calibration at the start of the experiment.

Physiological noise correction

Finger pulse was recorded using a pulse oximeter affixed to the third finger of the left or right hand (counterbalanced between subjects). Respiration was measured using a respiration belt placed around the participant's abdomen. Pulse and respiration measures were used for retrospective image-based correction (RETROICOR) of physiological noise artifacts in BOLD-fMRI data (Glover et al., 2000). Raw pulse and respiratory data were processed offline using in-house software for interactive visual artifact correction and peak detection, and were used to specify fifth-order Fourier models of the cardiac and respiratory phase-related modulation of the BOLD signal (Van Buuren et al., 2009), yielding 10 nuisance regressors for cardiac noise and 10 for respiratory noise. Additional regressors were calculated for heart rate frequency, heart rate variability, (raw) abdominal circumference, respiratory frequency, respiratory amplitude, and respiration volume per unit time (Birn et al., 2006), yielding a total of 26 RETROICOR regressors.

MRI data acquisition and multi-echo weighting

MRI scans were acquired using a Siemens (Erlangen, Germany) MAGNETOM Avanto 1.5T MR scanner. T2*-weighted blood oxygenation level-dependent (BOLD) images were recorded using a customized multi-echo EPI sequence with ascending slice acquisition (35 axial slices; TR, 2.2 s; TE, 9.4 ms, 21 ms, 33 ms, 44 ms, and 56 ms; Generalized Autocalibrating Partially Parallel Acquisitions (GRAPPA; Griswold et al., 2002) acceleration factor 3; flip angle, 90°; slice matrix size, 64x64; slice thickness, 3.0 mm; slice gap, 0.51 mm; FOV, 212 x 212 mm; bandwidth: 2604 Hz/px; echo spacing: 0.49 ms). A structural image (1 mm isotropic) was acquired using a T1-weighted 3D magnetization-prepared rapid gradient-echo sequence (MP-RAGE; TR, 2.73 s; TE, 2.95 ms; flip angle, 7°; FOV, 256 x 256 x 176 mm). To account for regional variation in susceptibility-induced signal dropout, voxel-wise weighted sums of all echoes were calculated based on local contrast-to-noise ratio (Poser et al., 2006).

MRI data preprocessing of the extinction session in native space and statistical analyses

For the primary fMRI analysis (amygdala response during eye movements), we pre-processed MRI data during extinction in native space (*i.e.*, without stereotactic normalization) using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>; Wellcome Department of Imaging Neuroscience, London, UK). All functional scans were co-registered with structural scans using mutual information maximization. The amygdala was

individually defined in native space using automated anatomical segmentation of T1-weighted images using FSL FIRST (see <http://www.fmrib.ox.ac.uk/fsl/first/index.html>). The Frontal Eye Field (FEF) were defined based on a 5 mm sphere around the MNI peak coordinates reported in a meta-analysis (Jamadar et al., 2013). Subsequently, the FEF masks were transferred back into native space for each individual using the reversed spatial normalization parameters.

For statistical analyses, responses to the eye-movement and no-eye movement blocks were estimated using a finite impulse response (FIR) model which included 9 time bins (TR = 2.2 s) starting one time bin before the onset of the CS (-2.2 s) and ending one time bin after the eye-movement blocks (17.6 s). Therefore, bin numbers 5 until 8 (6.6 s – 15.4 s) always fell within the eye-movement blocks. For the no-eye movement block, the same time frame was used. This first-level model makes no assumptions regarding the HRF shape, and yields independent response estimates for all 9 time bins, which makes it possible to investigate the time course of the responses. The first-level models additionally included six movement parameter regressors (3 translations, 3 rotations) derived from rigid-body motion correction, 26 RETROICOR physiological noise regressors (see above), high-pass filtering (1/128 Hz cut-off), and AR(1) serial correlations correction. We extracted the average beta weights within the amygdala and FEF for each time bin and each CS. A repeated measures ANOVA was conducted for each region with CS (CS+, CS-), extinction manipulation (eye movements, no-eye movements) and time bin (5-8) as within-subject factors. See Figure 5.2 and Figure 5.3.

Statistical testing

Partial eta squared (η_p^2) or Cohen's d effect size estimates are reported for all relevant tests. Alpha was set at .05 throughout and two-tailed t-tests were conducted unless stated otherwise.



Chapter 6

General discussion

Summary of the findings

The aim of this thesis was to investigate to how systems-level interactions play a role during the consolidation of memory for stressful experiences in humans. Furthermore, I have investigated if and how such “offline” processes can be targeted to ultimately alter memory retention. To do so, I have used univariate, multivariate and connectivity analyses on BOLD-fMRI data when participants were performing a task and during awake rest. Stress was induced by means of mild electrical shocks to the fingers, and via stressful movie clips. Fear memory was assessed using physiological responses including pupil dilation, and skin conductance responses and declarative memory was accessed by means of recognition and associative memory. Here, I will first summarize the findings of my thesis, structured according to the questions I set out to answer in **Chapter 1**.

Q1: What are the roles of arousal and amygdala activation during encoding in enhancing declarative memory for stressful experiences?

In **Chapter 2**, we investigated the roles of arousal and the amygdala in the formation of declarative memory for stressful experiences. We found that skin conductance responses and pupil dilation showed a robust differential conditioning effect, but did not predict subsequent item memory. In contrast, we found that amygdala activity did not show this differential conditioning effect, but only predicted subsequent item memory, and did so specifically for CS+ trials. These data indicate a dissociation between the roles of amygdala activation and noradrenergic-sympathetic responses in declarative memory for stressful experiences. This is in line with animal models that indicate that the amygdala mediates the effect of stress-related hormones and neurotransmitters on memory.

Q2: Which systems-level interactions continue to play a role in enhancing declarative memory for stressful experiences?

In **Chapter 3**, we investigated whether amygdala-hippocampal interactions following learning continue to play a role in memory for stressful experiences. We found that memory performance was enhanced under stress, and that stress-induced cortisol responses predicted this memory enhancement. Critically, amygdala-hippocampal connectivity also predicted stress-induced memory enhancement, but did so regardless of context (stress, neutral). Amygdala-hippocampal connectivity during post-encoding awake rest did not differ between the sessions, and positively correlated across participants. Thus, our data indicate that amygdala-hippocampal connectivity during rest facilitates memory enhancement under

stress as trait rather than a state factor.

Q3: Do stressful experiences alter awake memory reactivation in humans?

In **Chapter 4**, we investigated whether spontaneous reactivations across hippocampal-neocortical circuits following learning are stronger for stressful experiences compared to non-stressful experiences. We found increased spontaneous reactivation of category-specific patterns of BOLD signal during post-learning awake rest for a conceptual category that was coupled to a stressful experience. Next, hippocampal connectivity with the regions exhibiting these reactivations and the hippocampus predicted interindividual differences in fear recall (differential pupil dilation) 24 hours later. Lastly, we found that amygdala-hippocampal connectivity was increased during post-learning awake rest compared to baseline. These data indicate that acute stress during learning promotes spontaneous post-learning reactivation of neocortical representations of recent experiences, which leads to better memory when coinciding with hippocampal connectivity. These findings reveal a systems-level mechanism that may explain the persistence of long-term memory for stressful experiences.

Q4: Can memory be altered by disrupting amygdala activation via non-invasive means?

In **Chapter 5**, we investigated whether disrupting amygdala activation with a behavioral manipulation, namely eye movements, following reactivation of a memory for a stressful experience could disrupt memory retention. We found that the execution of eye movements reduced amygdala activation compared to fixation. When these eye movements were executed following memory retrieval during extinction this diminished spontaneous recovery of the extinguished fear (differential skin conductance responses). Lastly, although on average, fear recovered after reinstating the original memory trace, the strength of the recovery was dependent on how strong the amygdala suppression was during the eye movements on day two. Thus, the addition of eye movements during extinction learning is beneficial in attenuating fear recovery as compared to extinction alone. These findings provide novel insights into the reciprocal nature of large-scale brain networks influencing affective and cognitive processes. They furthermore contribute to a mechanistic understanding of a widely used treatment for traumatic symptoms which uses eye movements to enhance therapy outcome.

Integration of the findings and open questions

In this thesis I have investigated systems-level interactions that underlie enhanced memory for stressful experiences and specifically focused on the role of the amygdala in these interactions. In rodents it was shown that the amygdala enhances memory by mediating the effects of stress-related hormones and neurotransmitters on other memory systems, such as the hippocampus (McGaugh, 2002, 2000, 2004; Roozendaal et al., 2009). We have found evidence for this in humans. The amygdala starts to play a relevant role in memory already at the time of encoding (**Chapter 2**) and might continue playing a role after learning via increased connectivity with the hippocampus (**Chapter 4**). Intrinsic connectivity between these regions can furthermore account for better memory under stress, suggesting these effects are not only state-dependent (**Chapter 3**). Following memory reactivation, it was possible to alter implicit associative memory of a stressful experience by manipulating (parts of) these systems (**Chapter 5**). These findings together indicate that the consolidation of stressful memories engage a systems-level mechanism that may explain the persistence of these memories. More importantly, they show that it is possible to alter memories for stressful experiences by disrupting these processes.

These findings raise again new questions. Amygdala activation has been found in response to threat (LeDoux, 2003), ambiguity (Whalen, 2007), and in rodents conditioned fear is not acquired without a functional amygdala. Here we have primarily focused on the role of the amygdala in memory consolidation (McGaugh, 2000). First, this raises the question what the exact role of the amygdala is in enhancing memory? Second, if the amygdala plays a crucial role in systems-level consolidation, then how does this relate to the role of the amygdala in synaptic consolidation? Third, how does the amygdala alter systems-consolidation? I would like to propose an extension to the standard model of systems consolidation which can explain preferential consolidation of memories for stressful experiences. Forth, consolidation was for a long time considered an irreversible process. This view has changed in recent years which raises the question if and how memories for stressful experiences can be altered or updated. It is crucial to understand this, since this would open up new possibilities to advance therapy for fear-related disorders.

What is the function of the amygdala?

The main region of interest discussed in this thesis is the amygdala. We found that during encoding the amygdala activity predicted subsequent memory, while stress responses were high for both remembered and forgotten items (**Chapter 2**). This means that noradrenergic-sympathetic responses and amygdala activation may not play a uniform role in memory enhancement and that noradrenergic-

sympathetic responses per se are not sufficient to enhance memory. We found that amygdala-hippocampal connectivity was elevated after fear learning (**Chapter 4**). Furthermore, we found that amygdala-hippocampal connectivity during rest predicted enhanced memory under stress in a trait-like manner (**Chapter 3**). There are two important views on what the role of the amygdala is, at least in relation to learning and memory. The first one describes a role for the amygdala in initiating (conditioned) fear responses (LeDoux et al., 1988), which were later proposed to be called threat detection and defense responses (see: LeDoux, 2014). The other view proposes a role for the amygdala in modulating consolidation of memory (McGaugh, 2004). These two views seemingly contradict each other. Moreover, the amygdala has been implicated in numerous other affective and cognitive processes. This raises the question what the function of the amygdala is and I would like to discuss a possibility that would fit both views.

Early studies showed that primates became “fearless” after removal of the amygdala (Brown & Shafer, 1888; Klüver & Bucy, 1937). Furthermore, the amygdala was found to be involved in regulating autonomic (LeDoux et al., 1988) and noradrenergic responses (Reyes et al., 2011). Stimulation of the amygdala leads to changes in autonomic responses in both humans and animals (Chapman et al., 1954; Kaada et al., 1954; Reis & LeDoux, 1987). Human studies have indeed shown that the amygdala responds to arousing material, such as threatening or salient stimuli and faces (Hariri et al., 2002; Morris et al., 1997; Vuilleumier et al., 2001; Whalen et al., 1998). One theory is that the amygdala receives sensory input that bypasses primary sensory cortices (LeDoux, 1996) and thereby escapes conscious awareness (Tamietto & de Gelder, 2010). A recent study found evidence for this fast subcortical pathway in humans (Méndez-Bértolo et al., 2016). The authors found amygdala responses to fearful faces occurring as rapidly as within 74 ms, indicating that visual information could indeed arrive at the amygdala via a faster route than via the visual cortex. The finding that stimulating the amygdala activates autonomic responses shows that when the amygdala is activated it could initiate a stress response. However, this does not mean that all stress responses are initiated by the amygdala. If the amygdala would be the sole initiator of the stress response then anything that is threatening would elicit amygdala activation and in the absence of a functional amygdala, stress responses would not occur. There is evidence showing this is not the case. Human fear conditioning studies do not show robust amygdala activation during the expression of fear (Bach et al., 2011; Fullana et al., 2016; Mechias et al., 2010). Furthermore, patients with Urbach–Wiethe disease that have bilateral amygdala lesions still show stress responses to unconditioned stimuli such as electrical shock (Bechara et al., 1995; Klumpers et al., 2015a; LaBar et al., 1995). This could also mean that amygdala-triggered stress responses do not necessarily drive the enhancing effect of the stress response on memory formation.

Indeed, there are data demonstrating that noradrenergic manipulations are ineffective in modulating memory in the absence of a functional amygdala (Cahill & McGaugh, 1991; Liang et al., 1982). Thus, the amygdala might be able to activate a stress response, there are also other pathways through which stress responses can be initiated. The amygdala is, however, essential for the influence of stress-related hormones and neurotransmitters on memory formation.

It might be the case that for acquiring conditioned fear the amygdala is essential, but for expressing this fear the amygdala may no longer be necessary, at least not in nonhuman primates (Antoniadis et al., 2009). Causal evidence for this in humans does not exist, but patient studies indicate that the amygdala is at least necessary to acquire (uninstructed) conditioned fear (Bechara et al., 1995; Klumpers et al., 2015a; LaBar et al., 1995). However, persistent amygdala responses during the expression measured with BOLD-fMRI of conditioned fear are usually not observed (Bach et al., 2011; Fullana et al., 2016; Mechias et al., 2010). The assumption is that the amygdala detects and links external stimuli to defensive responses (LeDoux, 2003) which could be possible without conscious awareness (Tamietto & de Gelder, 2010). However, at least in humans, learning could also be established via different routes, for example via explicit instructions. A recent study found that amygdala responses were associated with feed-back-driven learning (*i.e.*, learning by experience) rather than with learning driven by instructions. In contrast, stress responses were updated with instructions (Atlas et al., 2016). These findings together indicate that the amygdala is necessary to acquire uninstructed, experience-driven conditioned fear, but does not correlate with stress responses when learning is established through instructions. **This raises the question whether the amygdala is essential for acquiring conditioned fear in case this can be learned via explicit instructions.** This could be tested with UWD patients having amygdala lesions. Patients who underwent a unilateral temporal lobe resection are at least able to acquire conditioned fear when learning goes along with explicit stimulus contingency knowledge (Coppens et al., 2009).

It is also important to note that the amygdala is not a uniform region, but consists of sub-regions. Animal research has indicated that specifically the central nucleus of the amygdala (CeA) is involved in initiating autonomic responses (Swanson & Petrovich, 1998) and the basolateral complex (BLA) in memory modulation (Roozendaal et al., 2009). For example, post-encoding infusion of NE in the BLA enhanced consolidation of memory in a spatial water maze task, while this was not the case when NE was infused into the CeA (Hatfield & McGaugh, 1999). In humans, a comparison between sub-regions of the amygdala using BOLD-fMRI is inherently difficult, because of signal loss and distortions due to magnetic field inhomogeneity. This increases towards the ventral part of the brain, where the BLA is located ((Merboldt et al., 2001; Sladky et al., 2013). Moreover, in humans, the BLA

is much larger than the CeA (Amunts et al., 2005). Spatial smoothing on BOLD-fMRI data is often applied to improve the signal-to-noise ratio and to accommodate the anatomical and functional variability between participants, but this reduces the spatial specificity of the BOLD signal. Thus, when assigning a function to the amygdala it is important to consider these sub-regions and to realize that it is inherently problematic to disentangle them in humans.

A selective role for the amygdala in threat detection has been challenged by numerous studies showing that the amygdala also responds to non-threatening stimuli. For example, the amygdala was shown to be activated by an unpredictable compared to a predictable tone, without any motivational association (Herry et al., 2007). Also, masked eye whites were shown to activate the amygdala (Whalen et al., 2004). These findings indicate that simple perceptual features are sufficient to elicit amygdala responses and suggest that the involvement of the amygdala in threat detection is not per se coupled to threat. One theory states that the amygdala is specifically activated during ambiguous situations and it might be the case that these situations are often threatening (Whalen, 2007). This can be associated with stress responses, but does not necessarily have to be the case. If a situation is irrefutably threatening (*e.g.*, electric shock), or when learning can take places via other means then environmental cues (*e.g.*, verbal instructions) amygdala responses might not correlate with stress responses and/or might thus not be necessary to initiate a stress response. Our results from **Chapter 2** are in line with this idea by showing a dissociation between amygdala and stress responses.

Thus, the role of the amygdala in detecting possible threat seems to account for many of the above explained findings. The ambiguity account (Whalen, 2007) and the memory modulation account (McGaugh, 2004) point towards an integrated role of the amygdala in perception and mnemonic processing in supporting the organism to respond adaptively to future possible threatening encounters. **A prediction following this inference is that, memory modulation specifically occurs for situations that are threatening and ambiguous, compared to events that are unambiguously threatening.**

Linking synaptic and systems consolidation

The main focus of this thesis is systems-level interactions during post-learning rest. We have found increased spontaneous reactivation of category-specific patterns of BOLD signal during post-learning awake rest for a conceptual category that was coupled to a stressful experience (**Chapter 4**). Connectivity with the regions exhibiting these reactivations and the hippocampus predicted inter-individual differences in fear recall 24 hours later (**Chapter 4**). We also found that amygdala-hippocampal connectivity was increased during post-learning awake rest compared

to baseline (**Chapter 4**). Furthermore, we found that these amygdala-hippocampal interactions regardless of state (stressful or neutral) predicted enhanced memory recall due to stress (**Chapter 3**). These systems-level findings occurred during early consolidation, immediately after learning. These changes were thus observed in a time period when synaptic consolidation takes place (Frankland & Bontempi, 2005). The (temporal) relationship between synaptic and systems consolidation is far from clear. Furthermore, these two types of consolidation have been mostly investigated in isolation and direct links are missing, but it is very likely that these two types are related. This raises the question, how are these systems-level changes during consolidation (**Chapter 3** and **Chapter 4**) related to animal studies that show stress hormones and neurotransmitters enhance synaptic consolidation?

Memory replay

A crucial process that might link synaptic consolidation with systems consolidation could be replay. Replay (Wilson & McNaughton, 1994) provides support for early systems-level consolidation theories (Marr, 1970) and could underlie the integration of memory traces, which are initially dependent on the hippocampus, into the neocortex (Frankland & Bontempi, 2005). Hippocampal sharp-wave/ripples form another fundamental mechanism supporting systems-level consolidation. Spontaneous replay of information occurs specifically during hippocampal sharp-wave/ripples (Karlsson & Frank, 2009; Skaggs & McNaughton, 1996) and when synaptic plasticity is experimentally induced (in vitro and in vivo), sharp-wave/ripples are increased (Behrens et al., 2005; Buzsaki, 1984). This suggests that replay could be increased with increasing synaptic plasticity (Carr et al., 2011). There is evidence that molecular and cellular processes in cortical networks are crucially involved in permanent memory traces. For example, synaptic plasticity in the cortex was shown to be important for long-term memory retention while not affecting immediate memory (Frankland et al., 2001). It was furthermore shown that replay in the cortex is increased during hippocampal sharp-wave/ripples (Logothetis et al., 2012; Peyrache et al., 2009). Thus, hippocampal-neocortical interactions are influenced by synaptic changes. **However, it is still an open question whether stress hormones or catecholamines can alter replay processes. For example, would replay of the representation of a spatial environment be increased when a rat was given a foot shock in that environment?**

There are studies in line with the hypothesis that stress enhances replay. Replay was found to be enhanced and with more precision in novel environments compared to familiar ones (Cheng & Frank, 2008; Foster & Wilson, 2006). Specifically this precise coordinated firing underlies synaptic plasticity or LTP (Hebb, 1949). Furthermore, awake replay is enhanced for reward-related experiences (Singer & Frank, 2009). A recent study in humans found that hippocampal representations of

high-reward contexts were preferentially reactivated during post-learning rest (Gruber et al., 2016). Since it is also known that stressors lead to the release of dopamine, typically released by reward and novelty (Berridge & Robinson, 1998), it is possible that stress enhances replay via this pathway. A recent study showed that optogenetic stimulation of hippocampal dopaminergic fibers from midbrain neurons in mice exploring novel environments enhances the reactivation of pyramidal cell assemblies during subsequent sleep/rest (McNamara et al., 2014). Stress hormones and catecholamines were shown to alter basic mechanisms of memory such as facilitating LTP (Thomas et al., 1996), increasing AMPA receptors at postsynaptic synapses (Hu et al., 2007) and increasing synaptic transmission in the hippocampus (Karst et al., 2005). Moreover, the amygdala engages neuromodulatory systems to alter thresholds for synaptic modification (Roozendaal et al., 2009), so this region could play a key role in modulating replay and hippocampal-neocortical integration of memories for stressful experiences. Our findings from **Chapter 4** are the first to link replay-like processes (in humans) to stress responses.

In conclusion, it might be the case that neuromodulators (*e.g.*, stress hormones or catecholamines) facilitate replay by lowering thresholds for synaptic plasticity. In turn this leads to enhanced hippocampal-neocortical interactions. If the systems-level changes we observed are related to initial synaptic systems then it should be possible to manipulate those systems and find altered systems-level interactions. **It is therefore an open question whether NE administration or stimulation would enhance replay, whether a blockade would disrupt this, and if the amygdala mediates these effects. In animal models it could be investigated whether optogenetic stimulation of main sites involved in the release of neuromodulators (*e.g.*, the LC) induces replay and strengthens memory.**

New model of systems consolidation for memories of stressful experiences

The amygdala plays a crucial role in synaptic consolidation of memories for stressful experiences. Animals studies also indicate the amygdala plays a role in systems-consolidation. It was shown that amygdala-hippocampal theta coherence is increased during the expression of conditioned fear in mice (Seidenbecher et al., 2003). Furthermore, after chronic immobilization stress in rats, beta and gamma synchrony was enhanced between the lateral amygdala and the CA1 region of the hippocampus, which lasted up to ten days (Ghosh et al., 2013). Indeed, it was shown that increases in theta coherence between amygdala and hippocampus during sleep after fear learning was predictive for later fear retention (Popa et al., 2010). Thus, together these findings in animals show that the amygdala interacts with the hippocampus, the region critically involved in systems consolidation (Marr,

1970; McClelland et al., 1995; Squire & Alvarez, 1995). The findings in this thesis provide first evidence that the amygdala might also play a role in systems-level consolidation of memories for stressful experiences in humans.

More human evidence for this notion comes from patient studies. Patients with amygdala lesions seem to show the typical emotional enhancement effect when tested immediately, but after a period of consolidation this enhancement is diminished (LaBar et al., 1995). Another important finding in these patients is that they have impairments in the retrieval of (emotional) autobiographical memories (Buchanan et al., 2005). It could therefore be the case that these memories stay longer dependent on the hippocampus. Others have made important additions to the standard model of consolidation and proposed that episodic memories may never become fully hippocampus-independent (Nadel & Moscovitch, 1997; Nadel et al., 2007). This might be specifically the case for memories for stressful experiences that are episodic in nature. These findings in patients and the findings presented in this thesis call for an adjustment of the standard model of consolidation for memories of stressful experiences.

The newly proposed model for memories of stressful experiences will thus entail an extension of the standard model of systems-consolidation (Marr, 1970; McClelland et al., 1995; Squire & Alvarez, 1995) and the multiple trace theory (Nadel & Moscovitch, 1997; Nadel et al., 2007) by adding the amygdala. The standard model of system consolidation (Marr, 1970; McClelland et al., 1995; Squire & Alvarez, 1995) states that memory traces in the neocortex are initially dependent on the hippocampus, but over time become independent of the hippocampus. The hippocampus thus serves a “binding” function which over time is no longer necessary (Marr, 1970). If the amygdala interacts with this system then this should have consequences for hippocampal-neocortical integration and the binding function of the hippocampus.

The new model (see Figure 6.1) would make the following predictions: (1) stressful experiences are stronger encoded, (2) memories for stressful experiences would stay longer dependent on the hippocampus due to the involvement of the amygdala and consequently, (3) this should affect other functions known to be dependent on the hippocampus such as pattern separation (Wiltgen et al., 2010; Yassa & Stark, 2011), (4) this hippocampal dependency might have consequences for the possibility to alter them. Indeed, it was previously shown that specifically remote memories are difficult to reactivate and less vulnerable for disruption (Alberini & Ledoux, 2013). And (5), due to strong encoding more cues could reactivate the memory, including stressors, strengthening the memory even further.

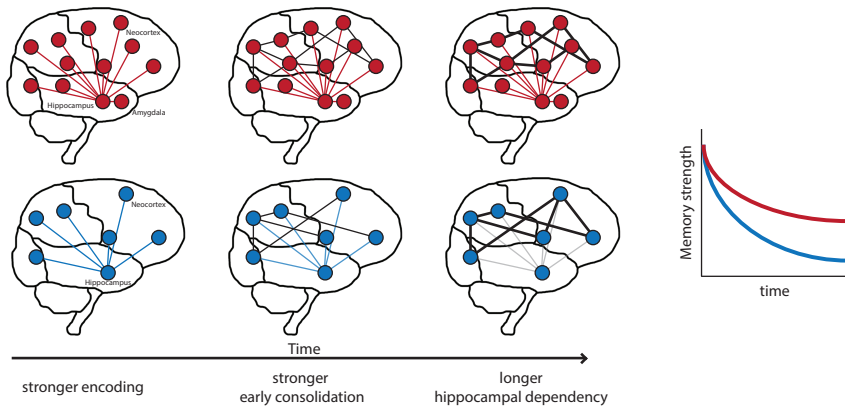


Figure 6.1: New model of systems-consolidation for memories of stressful experiences

Top represents the standard model of consolidation (Marr, 1970; McClelland et al., 1995; Squire & Alvarez, 1995) and multiple trace theory (Nadel & Moscovitch, 1997; Nadel et al., 2007). The bottom represents the new model of systems-consolidation for memories of stressful experiences. The model predicts that there is initially stronger encoding. Over time, there is stronger consolidation and memory traces stay longer dependent on the hippocampus. As a consequence, memories for stressful experiences are more persistent.

The relationship between post-encoding and post-reactivation processes

In **Chapter 5**, we found that the execution of eye movements reduced amygdala activation compared to fixation. When the eye movements were executed following memory retrieval during extinction this diminished spontaneous recovery of the extinguished fear. The strength of the recovery following a reinstatement of the original memory was dependent on the amount of amygdala suppression during the eye movements. In **Chapter 4** we found that post-encoding systems level changes were related to memory recall 24 hours later. This was, however, immediately after learning and not after reactivation of that memory. A crucial question is then, how are the processes we observed in **Chapter 3** and **Chapter 4** related to the processes we disrupted in **Chapter 5**?

The reconsolidation hypothesis

For a long time it was assumed that once memories have undergone consolidation they remain in a fixated state (McGaugh, 1966). This means that it would not be possible to alter memories after consolidation. However, there were also studies showing that it was actually possible for consolidated memories to be destabilized and disrupted after a brief reminder (Misanin et al., 1968), but have gotten less attention. Decades later the reconsolidation hypothesis was revived by a more well-known study by Nader and colleagues (2000) who showed that, by blocking

protein synthesis following a brief reminder, memories could be disrupted (also see: Alberini, 2005; Dudai, 2004; Sara, 2000). There is more evidence for this in humans using other types of drug administration. For example, it was found that propranolol (beta-adrenergic receptor blocker) can have similar effects as a protein synthesis inhibitor in rodents (Kindt et al., 2009, but see (Bos et al., 2012)). Thus, these data are in line with the idea that memories can become labile following reactivation and this reactivation might elicit a similar process as consolidation.

Reconsolidation and extinction

Reconsolidation might be triggered by reminders, but if these reminders are repeated often, the consequence could be the formation of a new memory trace rather than the reactivation of the old memory (Bouton, 1993; Maren, 2001). Since in **Chapter 5** we investigated whether we could manipulate implicit associative memory (*i.e.*, conditioned fear) during extinction, both mechanisms have to be considered in order to understand what is being manipulated. Indeed, the dominant view on fear and extinction learning is that extinction does not overwrite old memories, but leads to the formation of a new memory trace (Bouton, 1993). This is interesting, because in the field of (cognitive) psychology it is widely assumed that new information can get integrated into old memories (Dudai et al., 2015; Van Kesteren et al., 2012; Wang & Morris, 2010). One of the first studies describing this concept is a study by Bartlett (1932) showing that retelling folktales led to restructuring of the story based on cultural schema's. Other evidence that new information can get integrated into old memories comes from studies performed by Loftus and colleagues (1991) which showed that suggestive questions about a crime scene can lead to false memories of that crime scene (Christianson & Loftus, 1991). Indeed, in humans episodic memories have been shown to be malleable following reactivation which can lead to the incorporation of new information in memory (Hupbach et al., 2007). A critical point regarding the reconsolidation hypothesis is also that this hypothesis would predict that memories could potentially be erased. Especially that aspect has been subject to debate (Clem & Schiller, 2016), because most evidence of this relies on the absence of a behavioral output. An alternative explanation could therefore be that retrieval is blocked. Indeed, a recent study showed that amnesia via protein synthesis inhibitors can be undone by providing the amnesic treatment as a reminder (Gisquet-Verrier et al., 2015). This finding was not new, however, because already in the 1960s it was shown that memories can be recovered following amnesic treatment after a brief reminder of such a memory trace (Misanin et al., 1968).

These findings together challenge the dominant view in the field of fear conditioning that reminders can either reactivate the old memory trace or form a new memory trace and calls for more nuance. In conclusion, our findings in **Chapter**

5 cannot be explained by reconsolidation blockade, but also not by new learning alone.

Systems-level interactions following retrieval

If reactivation leads to a reinitiating of consolidation, it is expected that amygdala-hippocampal connectivity post-reactivation is stronger compared to pre-reactivation. This is unknown, but there are indications this could be the case. For example, electrophysiology studies in rodents demonstrated that amygdala-hippocampal theta coherence does not only increase following learning (Popa et al., 2010), but also during the expression of conditioned fear, hence memory retrieval (Seidenbecher et al., 2003). Also in humans it was shown that regions involved during encoding (**Chapter 2**; Dolcos et al., 2005, 2004; Hamann et al., 1999) and consolidation (**Chapter 4**) are involved in the retrieval of emotional memories (Smith et al., 2006). A previous study investigated the consolidation of conditioned fear demonstrated that indeed during rest following reactivation 24h later, multivoxel connectivity patterns in the amygdala obtained during acquisition return (Hermans et al., 2016). The data from **Chapter 4** did not include post-reactivation rest blocks so we could not investigate this in that chapter.

In conclusion, these findings together indicate that the processes we observed in **Chapter 4** are related to the processes we disrupted in **Chapter 5** and show that systems-level processes that occur during or following retrieval could be comparable to processes taking place during or following encoding.

Clinical perspective and implications

In the last paragraph I will discuss the clinical implications of the findings I presented in this thesis. In **Chapter 4** we found enhanced memory trace reactivation for memories with a stressful connotation. If this is the mechanism through which memories for stressful experiences get consolidated more strongly, one could speculate on how this mechanism might underlie extremely persistent and intrusive memories as well. Stronger (re)consolidation has indeed been proposed as a mechanism by which traumatic memories develop over time (Pitman, 1989) and it is possible that this occurs through the processes we observed in **Chapter 4**. Memory retrieval of traumatic events can be due to externally or internally driven reminders of the traumatic event (Ehlers et al., 2004). It was proposed previously that awake replay could be a potential mechanism through which retrieval is facilitated (Karlsson & Frank, 2009). Memory reactivation can be triggered by cues (Carr et al., 2011), subsequently lower the threshold for spontaneous memory recall, and start a vicious cycle of re-experiencing memories for stressful experiences.

I have argued in this thesis extensively that stress and memory are two inter-

twined processes. This means that the reactivation of a memory can also reactivate the stress response (*i.e.*, a reminder of a stressful experience could serve as an unconditioned stimulus). According to the new model explained above, the consequence of this could be a further strengthening of the memory trace via prolonged dependency on the hippocampus. Altering memories for stressful experiences could be achieved on the one hand via disrupting the memory trace. An alternative view, however, I would like to propose is that these manipulations via one way or another also alter the associated stress response. This reduction in stress responses and neuromodulators could then in turn have similar consequences on memory reconsolidation as it has on consolidation. Namely, blocking stress hormones during or following learning blocks the emotional enhancement effect on memory (Strange and Dolan, 2004). The latter perspective could explain our findings from **Chapter 5** and have new implications for therapy aiming to reduce symptoms of traumatic memories.

Emotion regulation

One option to reduce stress responses would be via pharmacological treatments such as propranolol. Propranolol has indeed been used to target memory processes and it could do so via inhibition of protein synthesis which thereby targets the memory trace following reactivation (Kindt et al., 2009; Vogel et al., 2015). However, propranolol is actually an anxiolytic medication, thus propranolol could also exert its effects via reducing the stress response associated with the memory. This is relevant, because stress responses can also be altered via other, behavioral means, namely through top-down regulation for example. Indeed, cognitive control is an effective way to regulate emotions (Ochsner & Gross, 2005). This means that potentially improving the ability to regulate emotions could be beneficial in reducing traumatic symptoms as well. This idea is not so strange considering the large body of studies showing that a high cognitive load can alter stress responses. For example, fMRI-BOLD responses during the processing of faces is dependent on attention (Pessoa et al., 2002). Moreover, startle responses were shown to be reduced during under high attentional load compared to baseline (Vytal et al., 2012). Even conditioned fear responses are reduced when participants are instructed to regulate their emotions (Delgado et al., 2008). Unrelated stress induction was, however, shown to disrupt this learned emotion regulation (Raio et al., 2013) suggesting that this trained cognitive regulation of emotion is not per se permanently effective. Finally, this top-down control also affects responsivity in the amygdala (Delgado et al., 2008; Kellermann et al., 2012). Thus, the goal-directed eye movement in EMDR treatment and **Chapter 5** might work via the same pathways as emotion regulation.

There is indeed a commonality between neural circuits involved in affective cognitive control (Duncan, 2010; Wager et al., 2008) and working memory (Wager

& Smith, 2003), namely activation of the frontal-parietal network. Regions in this network are involved in down regulating other regions such as the amygdala (Etkin et al., 2015; Ochsner & Gross, 2005; Wager et al., 2008). We have found in **Chapter 5** that both a working memory task and goal-directed eye movements suppress activity in the amygdala. Interestingly, it was also found that training working memory improves the ability to regulate emotions and this was coupled to down regulation in regions such as the amygdala (Schweizer et al., 2013). **It is unknown if emotion regulation, via down-regulation of regions that are involved in the formation of memory, is an effective way to alter memories for stressful experiences.** However, patients with fear-related disorders have impairments in emotion regulation (Gross, 2013). Thus, engaging in an endogenous attention might be easier than regulating emotions via top-down control. In conclusion, tasks that require endogenous attention provide a useful tool to regulate emotion.

Conclusion

Stress and memory are two inseparable processes. On the one hand, stress enhances the storage of memories. On the other hand, memories for such events can be stressful by themselves. Long-lasting memories are not created instantly and our results contribute to the understanding of how consolidation processes strengthen memories for stressful experiences following learning. Also, we showed that it is possible to interfere with this strengthening and have revealed a mechanism through which long-lasting memories can be altered. This insight yields a novel, parsimonious account of the neural basis of a poorly understood, but widely used treatment, namely EMDR. It furthermore provides a strong foundation for improvement of therapies for patients suffering from memories of stressful experiences.

References

- Abe, K. (2001). Modulation of hippocampal long-term potentiation by the amygdala: a synaptic mechanism linking emotion and memory. *Japanese journal of pharmacology*, 86(1), 18–22.
- Alberini, C. M. (2005). Mechanisms of memory stabilization: Are consolidation and reconsolidation similar or distinct processes?
- Alberini, C. M. & Ledoux, J. E. (2013). Memory reconsolidation. *Current Biology*, 23(17), R746–R750.
- Amunts, K., Kedo, O., Kindler, M., Pieperhoff, P., Mohlberg, H., Shah, N. J., Habel, U., Schneider, F., & Zilles, K. (2005). Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: Intersubject variability and probability maps. In *Anatomy and Embryology*, volume 210 (pp. 343–352).
- Anderson, M. L. & Oates, T. (2004). A critique of multi-voxel pattern analysis. *Most*, (July), 1511–16.
- Andrade, J., Kavanagh, D. J., & Baddeley, A. D. (1997). Eye-movements and visual imagery: a working memory approach to the treatment of post-traumatic stress disorder. *The British journal of clinical psychology / the British Psychological Society*, 36 (Pt 2), 209–23.
- Antoniadis, E. a., Winslow, J. T., Davis, M., & Amaral, D. G. (2009). The Nonhuman Primate Amygdala Is Necessary for the Acquisition but not the Retention of Fear-Potentiated Startle. *Biological Psychiatry*, 65(3), 241–248.
- Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *NeuroImage*, 38, 95–113.
- Ashburner, J. & Friston, K. J. (2005). Unified segmentation. *NeuroImage*, 26, 839–851.
- Aston-Jones, G. & Bloom, F. E. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *The 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(1), 403–450.
- Atlas, L. Y., Doll, B. B., Li, J., Daw, N. D., & Phelps, E. A. (2016). Instructed knowledge shapes feedback-driven aversive learning in striatum and orbitofrontal cortex, but not the amygdala. *Elife*, (September), 332–335.
- Bach, D. R., Weiskopf, N., & Dolan, R. J. (2011). A stable sparse fear memory trace in human amygdala. *Journal of Neuroscience*, 31(25), 9383–9389.
- Bass, D. I. & Manns, J. R. (2015). Memory-Enhancing Amygdala Stimulation Elicits Gamma Synchrony in the Hippocampus. *Behavioral Neuroscience*, 129(3), 244–256.
- Bauer, E. P., Paz, R., & Paré, D. (2007). Gamma oscillations coordinate amygdalo-rhinal interactions during learning. *Journal of Neuroscience*, 27(35), 9369–9379.
- Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science*, 269, 1115–1118.

- Beck, A., Steer, R., & Brown, G. (1996). *Beck Depression Inventory-II*.
- Behrens, C. J., van den Boom, L. P., de Hoz, L., Friedman, A., & Heinemann, U. (2005). Induction of sharp wave-ripple complexes in vitro and reorganization of hippocampal networks. *Nature neuroscience*, 8, 1560–1567.
- 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(April), 108.
- Berkers, R. M. W. J., Klumpers, E., & Fernández, G. (2016). Medial prefrontal-hippocampal connectivity during emotional memory encoding predicts individual differences in the loss of associative memory specificity. *Neurobiology of Learning and Memory*.
- Berntson, G. G., Cacioppo, J. T., & Quigley, K. S. (1991). Autonomic determinism: The modes of autonomic control, the doctrine of autonomic space, and the laws of autonomic constraint. *Psychological Review*, 98(4), 459–487.
- Berridge, K. C. & Robinson, T. E. (1998). What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience?
- Birn, R. M., Diamond, J. B., Smith, M. A., & Bandettini, P. A. (2006). Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI. *NeuroImage*, 31(4), 1536–1548.
- Bisson, J. I., Roberts, N. P., Andrew, M., Cooper, R., & Lewis, C. (2013). Psychological therapies for chronic post-traumatic stress disorder (PTSD) in adults. *Cochrane Database of Systematic Reviews*, (12), 4–7.
- Bonnet, L., Comte, A., Tatu, L., Millot, J. L., Moulin, T., & Medeiros de Bustos, E. (2015). The role of the amygdala in the perception of positive emotions: an "intensity detector". *Front Behav Neurosci*, 9, 178.
- Bos, M. G. N., Beckers, T., & Kindt, M. (2012). The effects of noradrenergic blockade on extinction in humans. *Biological Psychology*, 89(3), 598–605.
- Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning.
- Bradley, M. M., Greenwald, M. K., Petry, M. C., & Lang, P. J. (1992). Remembering pictures: pleasure and arousal in memory. *Journal of experimental psychology. Learning, memory, and cognition*, 18(2), 379–390.
- Bradley, M. M., Miccoli, L., Escrig, M. a., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*, 45(4), 602–607.
- Brewin, C. R., Gregory, J. D., Lipton, M., & Burgess, N. (2010). Intrusive images in psychological disorders: characteristics, neural mechanisms, and treatment implications. *Psychological review*, 117, 210–232.
- Brown, M. W. & Aggleton, J. P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nature reviews. Neuroscience*, 2(1), 51–61.
- Brown, S. & Shafer, E. (1888). An investigation into the functions of the occipital and temporal lobes of the monkey's brain. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 179, 303–327.
- Brunet, A., Orr, S. P., Tremblay, J., Robertson, K., Nader, K., & Pitman, R. K. (2008). Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *Journal of Psychiatric Research*, 42(6), 503–506.
- Buchanan, T. W., Etzel, J. a., Adolphs, R., & Tranel, D. (2006). The influence of autonomic arousal and semantic relatedness on memory for emotional words. *International Journal of Psychophysiology*, 61(1), 26–33.
- Buchanan, T. W., Tranel, D., & Adolphs, R. (2005). Emotional autobiographical memories in amnesic patients with medial temporal lobe damage. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25(12), 3151–60.

- Büchel, C. & Dolan, R. J. (2000). Classical fear conditioning in functional neuroimaging. *Current Opinion in Neurobiology*, 10(2), 219–223.
- Buzsaki, G. (1984). Long-term changes of hippocampal sharp-waves following high frequency afferent activation. *Brain Research*, 300, 179–182.
- Cahill, L., Gorski, L., & Le, K. (2003). Enhanced Human Memory Consolidation With Post-Learning Stress: Interaction With the Degree of Arousal at Encoding. *Learning & 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 of the United States of America*, 93(15), 8016–21.
- Cahill, L. & McGaugh, J. L. (1991). NMDA-induced lesions of the amygdaloid complex block the retention-enhancing effect of posttraining epinephrine. *Psychobiology*, 19(3), 206–210.
- Cahill, L. & McGaugh, J. L. (1995). A novel demonstration of enhanced memory associated with emotional arousal. *Consciousness and cognition*, 4, 410–421.
- Cahill, L., Prins, B., Weber, M., & McGaugh, J. L. (1994). Beta-adrenergic activation and memory for emotional events.
- Canli, T., Zhao, Z., Brewer, J., Gabrieli, J. D., & Cahill, L. (2000). Event-related activation in the human amygdala associates with later memory for individual emotional experience. *Journal of Neuroscience*, 20, RC99.
- Cannon, W. B. (1929). Organization for physiological homeostasis. *Physiological Reviews*, 9(3), 399–431.
- Carr, M. E., Jadhav, S. P., & Frank, L. M. (2011). Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval. *Nature neuroscience*, 14(2), 147–153.
- Chapman, W. P., Schroeder, H. R., Geyer, G., Brazier, M. a., Fager, C., Poppen, J. L., Solomon, H. C., & Yakovlev, P. I. (1954). Physiological evidence concerning importance of the amygdaloid nuclear region in the integration of circulatory function and emotion in man. *Science*, 120(1), 949–950.
- Cheng, S. & Frank, L. M. (2008). New Experiences Enhance Coordinated Neural Activity in the Hippocampus. *Neuron*, 57(2), 303–313.
- Christianson, S.-å. & Loftus, E. F. (1991). Remembering emotional events : The fate of detailed information. *Cognition & Emotion*, 5(2), 81–108.
- Clem, R. L. & Schiller, D. (2016). New Learning and Unlearning: Strangers or Accomplices in Threat Memory Attenuation?
- Coelho, C. A. O., Dunsmoor, J. E., & Phelps, E. A. (2015). Compound stimulus extinction reduces spontaneous recovery in humans. *Learning & memory (Cold Spring Harbor, N.Y.)*, 22(12), 589–93.
- Coppens, E., Spruyt, A., Vandenbulcke, M., Van Paesschen, W., & Vansteenwegen, D. (2009). Classically conditioned fear responses are preserved following unilateral temporal lobectomy in humans when concurrent US-expectancy ratings are used. *Neuropsychologia*, 47(12), 2496–2503.
- Corbetta, M. & Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience*, 3(3), 215–229.
- Cousijn, H., Rijpkema, M., Qin, S., van Wingen, G. A., & Fernández, G. (2012). Phasic deactivation of the medial temporal lobe enables working memory processing under stress. *NeuroImage*, 59(2), 1161–7.
- Davis, H. P. & Squire, L. R. (1984). Protein Synthesis and Memory : A Review. *Psychological Bulletin*, 96(3), 518–559.
- Davis, M. & Whalen, P. J. (2001). The amygdala: vigilance and emotion. *Molecular psychiatry*, 6(1), 13–34.
- de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience*, 6(6), 463–475.
- de Voogd, L. D., Fernández, G., & Hermans, E. J. (2016a). Awake reactivation of emotional memory traces through hippocampal–neocortical interactions. *NeuroImage*, 134, 563–572.

- de Voogd, L. D., Fernández, G., & Hermans, E. J. (2016b). Disentangling the roles of arousal and amygdala activation in emotional declarative memory. *Social Cognitive and Affective Neuroscience*, 11(9), 1471–1480.
- de Voogd, L. D., Klumpers, F., Fernández, G., & Hermans, E. J. (2017). Intrinsic functional connectivity between amygdala and hippocampus during rest predicts enhanced memory under stress. *Psychoneuroendocrinology*, 75, 192–202.
- Delgado, M. R., Nearing, K. I., Ledoux, J. E., & Phelps, E. A. (2008). Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron*, 59(5), 829–38.
- Deuker, L., Olligs, J., Fell, J., Kranz, T. a., Mormann, F., Montag, C., Reuter, M., Elger, C. E., & Axmacher, N. (2013). Memory consolidation by replay of stimulus-specific neural activity. *Journal of Neuroscience*, 33(49), 19373–83.
- Dolcos, F., Labar, K. S., & Cabeza, R. (2004). Interaction between the amygdala and the MTL memory system predicts better memory for emotional events. *Neuron*, 42, 855–863.
- Dolcos, F., LaBar, K. S., & Cabeza, R. (2005). Remembering one year later: role of the amygdala and the medial temporal lobe memory system in retrieving emotional memories. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 2626–2631.
- Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annual Review of Psychology*, 55, 51–86.
- Dudai, Y., Karni, A., & Born, J. (2015). The Consolidation and Transformation of Memory. *Neuron*, 88(1), 20–32.
- Duncan, J. (2010). The multiple-demand (MD) system of the primate brain: mental programs for intelligent behaviour.
- Dunsmoor, J. E., Kragel, P. a., Martin, A., & Labar, K. S. (2014). Aversive Learning Modulates Cortical Representations of Object Categories. *Cerebral cortex (New York, N.Y. : 1991)*, (pp. bht138–).
- Dunsmoor, J. E., Martin, A., & LaBar, K. S. (2012). Role of conceptual knowledge in learning and retention of conditioned fear. *Biological Psychology*, 89(2), 300–305.
- Dunsmoor, J. E., Murty, V. P., Davachi, L., & Phelps, E. a. (2015). Emotional learning selectively and retroactively strengthens memories for related events. *Nature*.
- Dupret, D., O'Neill, J., Pleydell-Bouverie, B., & Csicsvari, J. (2010). The reorganization and reactivation of hippocampal maps predict spatial memory performance. *Nature neuroscience*, 13(8), 995–1002.
- Eger, E., Michel, V., Thirion, B., Amadon, A., Dehaene, S., & Kleinschmidt, A. (2009). Deciphering Cortical Number Coding from Human Brain Activity Patterns. *Current Biology*, 19(19), 1608–1615.
- Ehlers, A. (2010). Understanding and Treating Unwanted Trauma Memories in Posttraumatic Stress Disorder.
- Ehlers, A., Hackmann, A., & Michael, T. (2004). Intrusive re-experiencing in post-traumatic stress disorder: phenomenology, theory, and therapy. *Memory (Hove, England)*, 12, 403–415.
- Eisenberg, M., Kobil, T., Berman, D. E., & Dudai, Y. (2003). Stability of retrieved memory: inverse correlation with trace dominance. *Science (New York, N.Y.)*, 301(5636), 1102–1104.
- Engelhard, I. M., van Uijen, S. L., & van den Hout, M. A. (2010). The impact of taxing working memory on negative and positive memories. *European Journal of Psychotraumatology*, 1(0), 1–8.
- Erk, S., Kiefer, M., Grothe, J., Wunderlich, A. P., Spitzer, M., & Walter, H. (2003). Emotional context modulates subsequent memory effect. *NeuroImage*, 18, 439–447.
- Ethofer, T., Van De Ville, D., Scherer, K., & Vuilleumier, P. (2009). Decoding of Emotional Information in Voice-Sensitive Cortices. *Current Biology*, 19, 1028–1033.
- Etkin, A., Büchel, C., & Gross, J. J. (2015). The neural bases of emotion regulation. *Nature Reviews Neuroscience*, 16(11), 693–700.
- Everaerd, D., Klumpers, F., van Wingen, G., Tendolkar, I., & Fernández, G. (2015). Association between neuroticism and amygdala responsivity emerges under stressful conditions. *NeuroImage*, 112, 218–224.

- Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychonomic bulletin & review*, 1(4), 429–38.
- Fastenrath, M., Coynel, D., Spalek, K., Milnik, A., Gschwind, L., Roozendaal, B., Papassotiropoulos, A., & de Quervain, D. J. F. (2014). Dynamic Modulation of Amygdala-Hippocampal Connectivity by Emotional Arousal. *Journal of Neuroscience*, 34(42), 13935–13947.
- Feinstein, J. S. (2013). Lesion studies of human emotion and feeling. *Current Opinion in Neurobiology*, 23(3), 304–309.
- Feinstein, J. S., Adolphs, R., Damasio, A., & Tranel, D. (2011). The Human Amygdala and the Induction and Experience of Fear. *Current Biology*, 21(1), 34–38.
- 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.
- Foote, S. L., Freedman, R., & Oliver, A. P. (1975). Effects of putative neurotransmitters on neuronal activity in monkey auditory cortex. *Brain Research*, 86(2), 229–242.
- Foster, D. J. & Wilson, M. A. (2006). Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature*, 440, 680–683.
- Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 9673–9678.
- Frankland, P. W. & Bontempi, B. (2005). The organization of recent and remote memories. *Nature reviews. Neuroscience*, 6, 119–130.
- Frankland, P. W., O'Brien, C., Ohno, M., Kirkwood, A., & Silva, a. J. (2001). Alpha-CaMKII-dependent plasticity in the cortex is required for permanent memory. *Nature*, 411(6835), 309–313.
- Friston, K. J., Holmes, A., Poline, J. B., Price, C. J., & Frith, C. D. (1996). Detecting activations in PET and fMRI: levels of inference and power. *NeuroImage*, 4, 223–235.
- Fullana, M. A., Harrison, B. J., Soriano-Mas, C., Vervliet, B., Cardoner, N., Àvila-Parcet, A., & Radua, J. (2016). Neural signatures of human fear conditioning: an updated and extended meta-analysis of fMRI studies. *Molecular psychiatry*, 21(4), 500–8.
- Gabrieli, J. D. E., Brewer, J. B., Desmond, J. E., & Glover, G. H. (1997). Separate Neural Bases of Two Fundamental Memory Processes in the Human Medial Temporal Lobe. 264, 264–266.
- Gallagher, M. & Kapp, B. S. (1981). Effect of phentolamine administration into the amygdala complex of rats on time-dependent memory processes. *Behavioral and Neural Biology*, 31(1), 90–95.
- Geerligs, L., Rubinov, M., Cam-CAN, & Henson, R. N. (2015). State and Trait Components of Functional Connectivity: Individual Differences Vary with Mental State. *Journal of Neuroscience*, 35(41), 13949–13961.
- Gerraty, R. T., Davidow, J. Y., Wimmer, G. E., Kahn, I., & Shohamy, D. (2014). Transfer of Learning Relates to Intrinsic Connectivity between Hippocampus, Ventromedial Prefrontal Cortex, and Large-Scale Networks. *Journal of Neuroscience*, 34(34), 11297–11303.
- Gerritsen, L., Rijpkema, M., van Oostrom, I., Buitelaar, J., Franke, B., Fernández, G., & Tendolkar, I. (2012). Amygdala to hippocampal volume ratio is associated with negative memory bias in healthy subjects. *Psychological Medicine*, 42, 335–343.
- Ghosh, S., Laxmi, T. R., & Chattarji, S. (2013). Functional connectivity from the amygdala to the hippocampus grows stronger after stress. *Journal of Neuroscience*, 33(17), 7234–7244.
- Gilzenrat, M. S., Nieuwenhuis, S., & Cohen, J. D. (2010). Pupil diameter tracks changes in control state predicted by the adaptive gain theory of locus coeruleus function. 10(2), 252–269.
- Gisquet-Verrier, P., Iii, J. F. L., Cutolo, P., Toledano, D., Ulmen, A., Jasnow, A. M., & Riccio, D. C. (2015). Integration of New Information with Active Memory Accounts for Retrograde Amnesia : A Challenge to the Consolidation / Reconsolidation Hypothesis ? 35(33), 11623–11633.
- Gläscher, J. & Adolphs, R. (2003). Processing of the arousal of subliminal and supraliminal emotional stimuli by the human amygdala. *Journal of Neuroscience*, 23(32), 10274–10282.

- Glover, G. H., Li, T. Q., & Ress, D. (2000). Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. *Magnetic Resonance in Medicine*, 44(1), 162–167.
- Gluck, M. A. & Myers, C. E. (1993). Hippocampal mediation of stimulus representation: a computational theory. *Hippocampus*, 3(4), 491–516.
- Gold, P. E., Hankins, L., Edwards, R. M., Chester, J., & McGaugh, J. L. (1975). Memory interference and facilitation with posttrial amygdala stimulation: effect on memory varies with footshock level. *Brain research*, 86, 509–513.
- Green, S. R., Kragel, P. A., Fecteau, M. E., & LaBar, K. S. (2014). Development and validation of an unsupervised scoring system (Autonomate) for skin conductance response analysis. *International Journal of Psychophysiology*, 91(3), 186–93.
- Griswold, M. A., Jakob, P. M., Heidemann, R. M., Nittka, M., Jellus, V., Wang, J., Kiefer, B., & Haase, A. (2002). Generalized Autocalibrating Partially Parallel Acquisitions (GRAPPA). *Magnetic Resonance in Medicine*, 47, 1202–1210.
- Gross (2013). Emotion regulation: Taking stock and moving forward. *Emotion*, 13(3), 359–365.
- Gruber, M. J., Ritchey, M., Wang, S. F., Doss, M. K., & Ranganath, C. (2016). Post-learning Hippocampal Dynamics Promote Preferential Retention of Rewarding Events. *Neuron*, 89(5), 1110–1120.
- Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., McGaugh, J. L., Worley, P. F., & Barnes, C. a. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *Journal of Neuroscience*, 20(11), 3993–4001.
- Haaker, J., Golkar, A., Hermans, D., & Lonsdorf, T. B. (2014). A review on human reinstatement studies : an overview and methodological challenges. *Learning & Memory*.
- Hamann, S. B. (2001). Cognitive and neural mechanisms of emotional memory. *Trends in cognitive sciences*, 5(9), 394–400.
- Hamann, S. B., Ely, T. D., Grafton, S. T., & Kilts, C. D. (1999). Amygdala activity related to enhanced memory for pleasant and aversive stimuli. *Nature neuroscience*, 2, 289–293.
- Hariri, A. R., Tessitore, A., Mattay, V. S., Fera, F., & Weinberger, D. R. (2002). The amygdala response to emotional stimuli: a comparison of faces and scenes. *NeuroImage*, 17(1), 317–323.
- 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.
- Haxby, J. V., Gobbini, M. I., Furey, M. L., Ishai, A., Schouten, J. L., & Pietrini, P. (2001). Distributed and overlapping representations of faces and objects in ventral temporal cortex. *Science*, 293, 2425–2430.
- Hebb, D. O. (1949). *The Organization of Behavior*, volume 911.
- Heeger, D. J. & Ress, D. (2002). What does fMRI tell us about neuronal activity? *Nature Reviews Neuroscience*, 3(February), 1–10.
- Henckens, M. J. A. G., Hermans, E. J., Pu, Z., Joëls, M., & Fernández, G. (2009). Stressed memories: how acute stress affects memory formation in humans. *Journal of Neuroscience*, 29(32), 10111–10119.
- Henckens, M. J. A. G., Klumpers, E., Everaerd, D., Kooijman, S. C., Van Wingen, G. A., & Fernández, G. (2016). Inter-individual differences in stress sensitivity: Basal and stress-induced cortisol levels differentially predict neural vigilance processing under stress. *Social cognitive and affective neuroscience*, 11(4), 663–673.
- Henckens, M. J. A. G., van Wingen, G. A., Joëls, M., & Fernández, G. (2010). Time-Dependent Effects of Corticosteroids on Human Amygdala Processing. *The Journal of Neuroscience*, 30(38), 12725–12732.
- Henckens, M. J. A. G., van Wingen, G. A., Joëls, M., & Fernández, G. (2011). Time-dependent corticosteroid modulation of prefrontal working memory processing. *Proceedings of the National Academy of Sciences*, 108(14), 5801–5806.
- Henke, K. (2010). A model for memory systems based on processing modes rather than consciousness. *Nature reviews. Neuroscience*, 11(7), 523–532.

- Herbert, J. D., Lilienfeld, S. O., Lohr, J. M., Montgomery, R. W., O'Donohue, W. T., Rosen, G. M., & Tolin, D. F. (2000). Science and pseudoscience in the development of eye movement desensitization and reprocessing: implications for clinical psychology. *Clinical psychology review*, 20(8), 945–971.
- Hermans, E. J., Battaglia, F. P., Atsak, P., de Voogd, L. D., Fernández, G., & Roozendaal, B. (2014). 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. A. G., Roelofs, K., & Fernández, G. (2013). Fear bradycardia and activation of the human periaqueductal grey. *NeuroImage*, 66, 278–287.
- Hermans, E. J., Kanen, J. W., Tambini, A., Fernández, G., Davachi, L., & Phelps, E. A. (2016). Persistence of Amygdala–Hippocampal Connectivity and Multi-Voxel Correlation Structures During Awake Rest After Fear Learning Predicts Long-Term Expression of Fear. *Cerebral Cortex*.
- Hermans, E. J., van Marle, H. J. F., Ossewaarde, L., Henckens, M. J. A. G., Qin, S., van Kesteren, M. T. R., Schoots, V. C., Cousijn, H., Rijpkema, M., Oostenveld, R., & Fernandez, G. (2011). Stress-Related Noradrenergic Activity Prompts Large-Scale Neural Network Reconfiguration. *Science*, 334(6059), 1151–1153.
- Herry, C., Bach, D. R., Esposito, F., Di Salle, F., Perrig, W. J., Scheffler, K., Lüthi, A., & Seifritz, E. (2007). Processing of temporal unpredictability in human and animal amygdala. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(22), 5958–66.
- Herweg, N. A., Weber, B., Kasparbauer, A., Meyh??fer, I., Steffens, M., Smyrnis, N., & Ettinger, U. (2014). Functional magnetic resonance imaging of sensorimotor transformations in saccades and antisaccades. *NeuroImage*, 102(P2), 848–860.
- Hu, H., Real, E., Takamiya, K., Kang, M. G., Ledoux, J., Huganir, R. L., & Malinow, R. (2007). Emotion Enhances Learning via Norepinephrine Regulation of AMPA-Receptor Trafficking. *Cell*, 131(1), 160–173.
- Hupbach, A., Gomez, R., Hardt, O., & Nadel, L. (2007). Reconsolidation of episodic memories: A subtle reminder triggers integration of new information. *Learning & Memory*, 14(1-2), 47–53.
- Hurlemann, R., Walter, H., Rehme, a. K., Kukolja, J., Santoro, S. C., Schmidt, C., Schnell, K., Musshoff, F., Keyzers, C., Maier, W., Kendrick, K. M., & Onur, O. a. (2010). Human amygdala reactivity is diminished by the β -noradrenergic antagonist propranolol. *Psychological Medicine*, 40, 1839–1848.
- Hutton, C., Bork, A., Josephs, O., Deichmann, R., Ashburner, J., & Turner, R. (2002). Image distortion correction in fMRI: A quantitative evaluation. *NeuroImage*, 16, 217–240.
- Irwin, W., Anderle, M. J., Abercrombie, H. C., Schaefer, S. M., Kalin, N. H., & Davidson, R. J. (2004). Amygdalar interhemispheric functional connectivity differs between the non-depressed and depressed human brain. *NeuroImage*, 21(2), 674–686.
- Jamadar, S. D., Fielding, J., & Egan, G. F. (2013). Quantitative meta-analysis of fMRI and PET studies reveals consistent activation in fronto-striatal-parietal regions and cerebellum during antisaccades and prosaccades. *Frontiers in Psychology*, 4(October), 1–15.
- Ji, D. & Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nature neuroscience*, 10, 100–107.
- Joels, M., Sarabdjitsingh, R. A., & Karst, H. (2012). Unraveling the Time Domains of Corticosteroid Hormone Influences on Brain Activity: Rapid, Slow, and Chronic Modes. *Pharmacological Reviews*, 64(4), 901–938.
- Kaada, B. R., Andersen, P., & Jansen, J. (1954). Stimulation of the amygdaloid nuclear complex in unanesthetized cats. *Neurology*, 4(1), 48–64.
- Kanwisher, N., McDermott, J., & Chun, M. M. (1997). The fusiform face area: a module in human extrastriate cortex specialized for face perception. *Journal of Neuroscience*, 17, 4302–4311.
- Karlsson, M. P. & Frank, L. M. (2009). Awake replay of remote experiences in the hippocampus. *Nature neuroscience*, 12, 913–918.
- Karst, H., Berger, S., Turiault, M., Tronche, F., Schutz, G., & Joels, M. (2005). Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission

- by corticosterone. *Proceedings of the National Academy of Sciences of the United States of America*, 102(52), 19204–19207.
- Kellermann, T. S., Sternkopf, M. A., Schneider, F., Habel, U., Turetsky, B. I., Zilles, K., & Eickhoff, S. B. (2012). Modulating the processing of emotional stimuli by cognitive demand. *Social Cognitive and Affective Neuroscience*, 7(3), 263–273.
- Kindt, M., Soeter, M., & Vervliet, B. (2009). Beyond extinction: erasing human fear responses and preventing the return of fear. *Nature Neuroscience*, 12(3), 256–8.
- Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic medicine*, 61(2), 154–62.
- Kleinsmith, L. J. & Kaplan, S. (1963). Paired-associate learning as a function of arousal and interpolated interval. *Journal of experimental psychology*, 65(2), 190–193.
- Klumpers, F., Kroes, M. C., Heitland, I., Everaerd, D., Akkermans, S. E. A., Oosting, R. S., Van Wingen, G., Franke, B., Kenemans, J. L., Fernández, G., & Baas, J. M. P. (2015a). Dorsomedial prefrontal cortex mediates the impact of serotonin transporter linked polymorphic region genotype on anticipatory threat reactions. *Biological Psychiatry*, 78(8), 582–589.
- Klumpers, F., Kroes, M. C., Heitland, I., Everaerd, D., Akkermans, S. E. A., Oosting, R. S., Van Wingen, G., Franke, B., Kenemans, J. L., Fernández, G., & Baas, J. M. P. (2015b). Dorsomedial prefrontal cortex mediates the impact of serotonin transporter linked polymorphic region genotype on anticipatory threat reactions. *Biological Psychiatry*, 78(8), 582–589.
- Klüver, H. & Bucy, P. C. (1937). "Psychic blindness" and other symptoms following bilateral temporal lobectomy in Rhesus monkeys. *American Journal of Physiology*, 119, 352–353.
- Kriegeskorte, N., Mur, M., Ruff, D. a., Kiani, R., Bodurka, J., Esteky, H., Tanaka, K., & Bandettini, P. a. (2008). Matching Categorical Object Representations in Inferior Temporal Cortex of Man and Monkey. *Neuron*, 60(6), 1126–1141.
- Kroes, M. C. W., Tona, K.-D., den Ouden, H. E., Vogel, S., van Wingen, G. A., & Fernández, G. (2015). How Administration of the Beta-Blocker Propranolol Prior to Extinction can Prevent the Return of Fear. *Neuropsychopharmacology*, (pp. 1–10).
- Krugers, H. J., Karst, H., & Joels, M. (2012). Interactions between noradrenaline and corticosteroids in the brain: from electrical activity to cognitive performance. *Frontiers in Cellular Neuroscience*, 6(April), 1–10.
- Kudrimoti, H. S., Barnes, C. a., & McNaughton, B. L. (1999). Reactivation of hippocampal cell assemblies: effects of behavioral state, experience, and EEG dynamics. *Journal of Neuroscience*, 19(10), 4090–4101.
- Kuhlmann, S. & Wolf, O. T. (2006). Arousal and Cortisol Interact in Modulating Memory Consolidation in Healthy Young Men. *Behavioral Neuroscience*, 120(1), 217–223.
- LaBar, K. & Cabeza, R. (2006). Cognitive neuroscience of emotional memory. *Nature reviews. Neuroscience*, 7(January), 54–64.
- LaBar, K. S., Gatenby, J. C., Gore, J. C., LeDoux, J. E., & Phelps, E. A. (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron*, 20(5), 937–945.
- LaBar, K. S., LeDoux, J. E., Spencer, D. D., & Phelps, E. a. (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. *Journal of Neuroscience*, 15(October), 6846–6855.
- Lang, P. J., Greenwald, M. K., Bradley, M. M., & Hamm, a. O. (1993). Looking at pictures: affective, facial, visceral, and behavioral reactions. *Psychophysiology*, 30(3), 261–273.
- Lechner, H. A., Squire, L. R., & Byrne, J. H. (1999). 100 Years of Consolidation—Remembering Müller and Pilzecker. *Learning & Memory*, 6(2), 77–87.
- LeDoux, J. E. (1996). *The Emotional Brain*. Simon & Schuster; New York.
- LeDoux, J. E. (2003). The emotional brain, fear, and the amygdala. *Cellular and Molecular Neurobiology*, 23(4-5), 727–738.

- LeDoux, J. E. (2012). Rethinking the Emotional Brain. *Neuron*, 73(4), 653–676.
- LeDoux, J. E. (2014). Coming to terms with fear. *Proceedings of the National Academy of Sciences of the United States of America*, 111(4), 2871–2878.
- LeDoux, J. E., Iwata, J., Cicchetti, P., & Reis, D. J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *Journal of Neuroscience*, 8(July), 2517–2529.
- Lee, C. W. & Cuijpers, P. (2013). A meta-analysis of the contribution of eye movements in processing emotional memories. *Journal of Behavior Therapy and Experimental Psychiatry*, 44(2), 231–239.
- Li, J., Schiller, D., Schoenbaum, G., Phelps, E. a., & Daw, N. D. (2011). Differential roles of human striatum and amygdala in associative learning. *Nature Neuroscience*, 14(10), 1250–1252.
- Li, S., Weerda, R., Guenzel, F., Wolf, O. T., & Thiel, C. M. (2013). ADRA2B genotype modulates effects of acute psychosocial stress on emotional memory retrieval in healthy young men. *Neurobiology of Learning and Memory*, 103, 11–18.
- Liang, K. C., McGaugh, J. L., Martinez, J. L., Jensen, R. a., Vasquez, B. J., & Messing, R. B. (1982). Post-training amygdaloid lesions impair retention of an inhibitory avoidance response. *Behavioural brain research*, 4(3), 237–49.
- Liao, W., Qiu, C., Gentili, C., Walter, M., Pan, Z., Ding, J., Zhang, W., Gong, Q., & Chen, H. (2010). Altered effective connectivity network of the amygdala in social anxiety disorder: A resting-state fMRI study. *PLoS ONE*, 5(12).
- Logothetis, N. K., Eschenko, O., Murayama, Y., Augath, M., Steudel, T., Evrard, H. C., Besserve, M., & Oeltermann, a. (2012). Hippocampal-cortical interaction during periods of subcortical silence. *Nature*, 491(7425), 547–53.
- LØmo, T. (1966). Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. *Acta Physiol. Scand.*, 68 (Suppl.
- MacMillan, S., Szeszko, P. R., Moore, G. J., Madden, R., Lorch, E., Ivey, J., Banerjee, S. P., & Rosenberg, D. R. (2003). Increased amygdala: hippocampal volume ratios associated with severity of anxiety in pediatric major depression. *J Child Adolesc Psychopharmacol*, 13(1), 65–73.
- Maren, S. (1999). Neurotoxic Basolateral Amygdala Lesions Impair Learning and. 19(19), 8696–8703.
- Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annual review of neuroscience*, 24, 897–931.
- Maren, S. (2011). Seeking a Spotless Mind: Extinction, Deconsolidation, and Erasure of Fear Memory. *Neuron*, 70(5), 830–845.
- Marr, D. (1970). A theory for cerebral neocortex. *Proceedings of the Royal Society of London. Series B, Containing papers of a Biological character. Royal Society (Great Britain)*, 176, 161–234.
- McClelland, J. L., McNaughton, B. L., & O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychological review*, 102, 419–457.
- McGaugh, J. L. (1966). Time-dependent processes in memory storage. *Science*, 153(742), 1351–1358.
- McGaugh, J. L. (2000). Memory—a century of consolidation. *Science*, 287(2000), 248–251.
- McGaugh, J. L. (2002). Memory consolidation and the amygdala: A systems perspective. *Trends in Neurosciences*, 25(9), 456–461.
- 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 of the United States of America*, 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 of the United States of America*, 93(24), 13508–13514.

- McIntyre, C. K., Miyashita, T., Setlow, B., Marjon, K. D., Steward, O., Guzowski, J. E., & 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 of the United States of America*, 102, 10718–10723.
- McKenzie, S. & Eichenbaum, H. (2011). Consolidation and Reconsolidation: Two Lives of Memories? *Neuron*, 71(2), 224–233.
- McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. *Journal of Psychiatric Research*, 45(8), 1027–1035.
- McNamara, C. G., Tejero-cantero, Á., Trouche, S., Campo-urriza, N., & Dupret, D. (2014). Dopaminergic neurons promote hippocampal reactivation and spatial memory persistence. *Nature neuroscience*, 17(12), 1658–1660.
- Mechias, M. L., Etkin, A., & Kalisch, R. (2010). A meta-analysis of instructed fear studies: Implications for conscious appraisal of threat. *NeuroImage*, 49(2), 1760–1768.
- Méndez-Bértolo, C., Moratti, S., Toledano, R., Lopez-Sosa, E., Martínez-Alvarez, R., Mah, Y. H., Vuilleumier, P., Gil-Nagel, A., & Strange, B. A. (2016). A fast pathway for fear in human amygdala. *Nature Neuroscience*, (June).
- Merboldt, K. D., Fransson, P., Bruhn, H., & Frahm, J. (2001). Functional MRI of the human amygdala? *NeuroImage*, 14(2), 253–257.
- Misanin, J. R., Miller, R. R., & Lewis, D. J. (1968). Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science*, 160(3827), 554–555.
- Monfils, M.-H., Cowansage, K. K., Klann, E., & LeDoux, J. E. (2009). Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. *Science*, 324(5929), 951–955.
- Morgan, B., Terburg, D., Thornton, H. B., Stein, D. J., & Honk, J. (2012). Paradoxical facilitation of working memory after basolateral amygdala damage. *PLoS ONE*, 7(6).
- Morris, J. S., Friston, K. J., & Dolan, R. J. (1997). Neural responses to salient visual stimuli. *Proceedings. Biological sciences / The Royal Society*, 264(1382), 769–775.
- Moscarello, J. M. & LeDoux, J. E. (2013). Active Avoidance Learning Requires Prefrontal Suppression of Amygdala-Mediated Defensive Reactions. *Journal of Neuroscience*, 33(9), 3815–3823.
- Muris, P. & Merckelbach, H. (1999). Traumatic memories, eye movements, phobia, and panic: A critical note on the proliferation of EMDR. *Journal of Anxiety Disorders*, 13(1), 209–223.
- Murray, L. J. & Ranganath, C. (2007). The dorsolateral prefrontal cortex contributes to successful relational memory encoding. *Journal of Neuroscience*, 27(20), 5515–5522.
- Murty, V. P., Ritchey, M., Adcock, R. A., & LaBar, K. S. (2011). Reprint of: fMRI studies of successful emotional memory encoding: A quantitative meta-analysis. *Neuropsychologia*, 49(12), 695–705.
- Nadel, L. & Moscovitch, M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex.
- Nadel, L., Winocur, G., Ryan, L., & Moscovitch, M. (2007). Systems consolidation and hippocampus: Two views. *Debates in Neuroscience*, 1(2-4), 55–66.
- Nader, K., Schafe, G. E., & LeDoux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*, 406(6797), 722–726.
- Nieuwenhuis, S., Aston-Jones, G., & Cohen, J. D. (2005). Decision making, the P3, and the locus coeruleus-norepinephrine system. *Psychological bulletin*, 131(4), 510–532.
- Ochsner, K. N. & Gross, J. J. (2005). The cognitive control of emotion.
- Öhman, A. & Mineka, S. (2001). Fears, phobias, and preparedness: toward an evolved module of fear and fear learning.
- O'Keefe, J. & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Research*, 34(1), 171–175.
- O'Keefe, J. & Nadel, L. (1978). *The Hippocampus as a Cognitive Map*. Oxford University Press.

- Osada, T., Adachi, Y., Kimura, H. M., & Miyashita, Y. (2008). Towards understanding of the cortical network underlying associative memory. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 363(March), 2187–2199.
- 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 of the United States of America*, 91(18), 8477–8481.
- Pape, H.-C. & Pare, D. (2010). Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiological reviews*, 90, 419–463.
- Paz, R., Pelletier, J. G., Bauer, E. P., & Paré, D. (2006). Emotional enhancement of memory via amygdala-driven facilitation of rhinal interactions. *Nature neuroscience*, 9(10), 1321–1329.
- Pessoa, L., McKenna, M., Gutierrez, E., & Ungerleider, L. G. (2002). Neural processing of emotional faces requires attention. *Proceedings of the National Academy of Sciences*, 99(17), 11458–11463.
- Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S. I., & Battaglia, F. P. (2009). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nature neuroscience*, 12(April), 919–926.
- Pitkänen, A., Pikkarainen, M., Nurminen, N., & Ylinen, A. (2000). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Annals of the New York Academy of Sciences*, 911, 369–391.
- Pitman, R. K. (1989). Post-traumatic stress disorder, hormones, and memory.
- Popa, D., Duvarci, S., Popescu, A. T., Léna, C., & Paré, D. (2010). Coherent amygdalocortical theta promotes fear memory consolidation during paradoxical sleep. *Proceedings of the National Academy of Sciences of the United States of America*, 107(14), 6516–9.
- Poser, B. A., Versluis, M. J., Hoogduin, J. M., & Norris, D. G. (2006). BOLD contrast sensitivity enhancement and artifact reduction with multiecho EPI: Parallel-acquired inhomogeneity-desensitized fMRI. *Magnetic Resonance in Medicine*, 55, 1227–1235.
- Power, J. D., Schlaggar, B. L., & Petersen, S. E. (2015). Recent progress and outstanding issues in motion correction in resting state fMRI. *NeuroImage*, 105(0), 536–551.
- Pu, Z., Krugers, H. J., & Joëls, M. (2007). Corticosterone time-dependently modulates beta-adrenergic effects on long-term potentiation in the hippocampal dentate gyrus. *Learning & memory (Cold Spring Harbor, N.Y.)*, 14(5), 359–367.
- Qin, S., Hermans, E. J., van Marle, H. J. F., Luo, J., & Fernández, G. (2009). Acute psychological stress reduces working memory-related activity in the dorsolateral prefrontal cortex. *Biological Psychiatry*, 66(1), 25–32.
- Qin, Y. L., McNaughton, B. L., Skaggs, W. E., & Barnes, C. A. (1997). Memory reprocessing in corticocortical and hippocampocortical neuronal ensembles. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 352, 1525–1533.
- Raio, C. M., Orederu, T. a., Palazzolo, L., Shurick, A. a., & Phelps, E. a. (2013). Cognitive emotion regulation fails the stress test. *Proceedings of the National Academy of Sciences of the United States of America*, 110(37), 15139–44.
- Ramirez, S., Liu, X., Lin, P.-A., Suh, J., Pignatelli, M., Redondo, R. L., Ryan, T. J., & Tonegawa, S. (2013). Creating a false memory in the hippocampus. *Science*, 341, 387–91.
- Rasch, B. & Born, J. (2007). Maintaining memories by reactivation. *Current Opinion in Neurobiology*, 17, 698–703.
- Reinders, A. A. T. S., Den Boer, J. A., & Büchel, C. (2005). The robustness of perception. *European Journal of Neuroscience*, 22, 524–530.
- Reis, D. J. & LeDoux, J. E. (1987). Some central neural mechanisms governing resting and behaviorally coupled control of blood pressure. *Circulation*, 76(1 Pt 2), I2–9.
- Reyes, B. a. S., Carvalho, a. F., Vakharia, K., & Van Bockstaele, E. J. (2011). Amygdalar peptidergic circuits regulating noradrenergic locus coeruleus neurons: Linking limbic and arousal centers. *Experimental Neurology*, 230(1), 96–105.

- 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., Phelps, E. a., Petrov, R., Dougal, S., & Phelps, E. a. (2011). Emotion enhances the subjective feeling of remembering, despite lower accuracy for contextual details. *Emotion*, 11(3), 553–562.
- Rogers, S. & Silver, S. M. (2002). Is EMDR an exposure therapy? A review of trauma protocols. *Journal of Clinical Psychology*, 58(1), 43–59.
- Roozendaal, B., Castello, N. A., Vedana, G., Barseggyan, A., & McGaugh, J. L. (2008). Neurobiology of Learning and Memory Noradrenergic activation of the basolateral amygdala modulates consolidation of object recognition memory q. 90, 576–579.
- Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature reviews. Neuroscience*, 10(juNE), 423–433.
- Roozendaal, B. & McGaugh, J. L. (2011). Memory modulation. *Behavioral Neuroscience*, 125(6), 797–824.
- 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), 6741–6.
- Roy, A. K., Shehzad, Z., Margulies, D. S., Kelly, A. M. C., Uddin, L. Q., Gotimer, K., Biswal, B. B., Castellanos, F. X., & Milham, M. P. (2009). Functional connectivity of the human amygdala using resting state fMRI. *NeuroImage*, 45, 614–626.
- Sandi, C. & Richter-Levin, G. (2009). From high anxiety trait to depression: a neurocognitive hypothesis. *Trends in Neurosciences*, 32(6), 312–320.
- Sara, S. J. (2000). Retrieval and reconsolidation: Toward a neurobiology of remembering. *Learning & Memory*, 7(2), 73–84.
- Sara, S. J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nature reviews. Neuroscience*, 10(MARCH), 211–223.
- Schachter, S. & Singer, J. E. (1962). Cognitive, Social, and Physiological Determinants of Emotional State. *American Psychologist*, 69(5), 379–399.
- Schacter, D. L. (1999). The seven sins of memory. Insights from psychology and cognitive neuroscience. *The American Psychologist*, 54(3), 182–203.
- Schiller, D., Kanen, J. W., LeDoux, J. E., Monfils, M.-H., & Phelps, E. a. (2013). Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. *Proceedings of the National Academy of Sciences of the United States of America*, 110(50), 20040–5.
- Schiller, D., Monfils, M.-H., Raio, C. M., Johnson, D. C., Ledoux, J. E., & Phelps, E. a. (2010). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature*, 463(7277), 49–53.
- Schmolck, H., Kensinger, E. A., Corkin, S., & Squire, L. R. (2002). Semantic knowledge in patient H.M. and other patients with bilateral medial and lateral temporal lobe lesions. *Hippocampus*, 12(4), 520–533.
- Schweizer, S., Grahn, J., Hampshire, A., Mobbs, D., & Dalgleish, T. (2013). Training the emotional brain: Improving affective control through emotional working memory training. *Annals of Internal Medicine*, 158(6), 5301–5311.
- Scoville, W. B. & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery & Psychiatry*, 20(1), 11–21.
- Seidenbecher, T., Laxmi, T. R., Stork, O., & Pape, H.-C. (2003). Amygdalar and hippocampal theta rhythm synchronization during fear memory retrieval. *Science*, 301(2003), 846–850.
- Selye, H. (1936). A Syndrome Produced by Diverse Nocuous Agents. *The Journal of Neuropsychiatry and Clinical Neurosciences*, 10(2), 230–231.
- Selye, H. & Fortier, C. (1950). Adaptive reaction to stress. *Psychosomatic medicine*, 12, 149–157.
- Siegle, G. J., Steinhauser, S. R., Stenger, V. A., Konecky, R., & Carter, C. S. (2003). Use of concurrent pupil dilation assessment to inform interpretation and analysis of fMRI data. *NeuroImage*, 20, 114–124.

- Singer, A. C. & Frank, L. M. (2009). Rewarded Outcomes Enhance Reactivation of Experience in the Hippocampus. *Neuron*, 64(6), 910–921.
- Sirota, A., Csicsvari, J., Buhl, D., & Buzsáki, G. (2003). Communication between neocortex and hippocampus during sleep in rodents. *Proceedings of the National Academy of Sciences of the United States of America*, 100(4), 2065–2069.
- Skaggs, W. E. & McNaughton, B. L. (1996). Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science*, 271, 1870–1873.
- Sladky, R., Baldinger, P., Kranz, G. S., Tr??stl, J., H??flich, A., Lanzenberger, R., Moser, E., & Windischberger, C. (2013). High-resolution functional MRI of the human amygdala at 7 T. *European Journal of Radiology*, 82(5), 728–733.
- 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, A. P. R., Stephan, K. E., Rugg, M. D., & Dolan, R. J. (2006). Task and content modulate amygdala-hippocampal connectivity in emotional retrieval. *Neuron*, 49(4), 631–638.
- Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychological review*, 99, 195–231.
- Squire, L. R. & Alvarez, P. (1995). Retrograde amnesia and memory consolidation: a neurobiological perspective. *Current Opinion in Neurobiology*, 5(2), 169–177.
- Staresina, B. P., Alink, A., Kriegeskorte, N., & Henson, R. N. (2013). Awake reactivation predicts memory in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 21159–64.
- Strange, B. a. & Dolan, R. J. (2004). Beta-adrenergic modulation of emotional memory-evoked human amygdala and hippocampal responses. *Proceedings of the National Academy of Sciences of the United States of America*, 101(31), 11454–11458.
- Swanson, L. & Petrovich, G. (1998). What is the amygdala? *Trends in neurosciences*, 21(8), 323–331.
- Takashima, A., Petersson, K. M., Rutters, F., Tendolkar, I., Jensen, O., Zwarts, M. J., McNaughton, B. L., & Fernandez, G. (2006). Declarative memory consolidation in humans: a prospective functional magnetic resonance imaging study. *Proceedings of the National Academy of Sciences of the United States of America*, 103(3), 756–761.
- Tambini, A. & Davachi, L. (2013). Persistence of hippocampal multivoxel patterns into postencoding rest is related to memory. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 19591–6.
- Tambini, A., Ketz, N., & Davachi, L. (2010). Enhanced Brain Correlations during Rest Are Related to Memory for Recent Experiences. *Neuron*, 65(2), 280–290.
- Tamietto, M. & de Gelder, B. (2010). Neural bases of the non-conscious perception of emotional signals. *Nature reviews. Neuroscience*, 11(10), 697–709.
- Tanaka, K. Z., Pevzner, A., Hamidi, A. B., Nakazawa, Y., & Graham, J. (2014). Cortical Representations Are Reinstated by the Hippocampus during Memory Retrieval. *Neuron*, 84(2), 347–354.
- Terburg, D., Morgan, B. E., Montoya, E. R., Hooge, I. T., Thornton, H. B., Hariri, A. R., Panksepp, J., Stein, D. J., & van Honk, J. (2012). Hypervigilance for fear after basolateral amygdala damage in humans. *Translational psychiatry*, 2(5), e115.
- Thomas, M. J., Moody, T. D., Makhinson, M., & O'Dell, T. J. (1996). Activity-dependent beta-adrenergic modulation of low frequency stimulation induced LTP in the hippocampal CA1 region. *Neuron*, 17(3), 475–482.
- Tranel, D., Damasio, H., & Damasio, A. R. (1997). A neural basis for the retrieval of conceptual knowledge. *Neuropsychologia*, 35, 1319–1327.
- Turk-Browne, N. B., Yi, D. J., & Chun, M. M. (2006). Linking implicit and explicit memory: Common encoding factors and shared representations. *Neuron*, 49(6), 917–927.

- Tye, K. M., Prakash, R., Kim, S.-Y., Fenno, L. E., Grosenick, L., Zarabi, H., Thompson, K. R., Gradinaru, V., Ramakrishnan, C., & Deisseroth, K. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature*, 471(7338), 358–62.
- Tyler, L. K., Chiu, S., Zhuang, J., Randall, B., Devereux, B. J., Wright, P., Clarke, A., & Taylor, K. I. (2013). Objects and categories: feature statistics and object processing in the ventral stream.
- Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., & Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage*, 15, 273–289.
- Van Bockstaele, E. J., Bajic, D., Proudfit, H., & Valentino, R. J. (2001). Topographic architecture of stress-related pathways targeting the noradrenergic locus coeruleus. *Physiology and Behavior*, 73(3), 273–283.
- Van Buuren, M., Gladwin, T. E., Zandbelt, B. B., Van Den Heuvel, M., Ramsey, N. F., Kahn, R. S., & Vink, M. (2009). Cardiorespiratory effects on default-mode network activity as measured with fMRI. *Human Brain Mapping*, 30(December 2008), 3031–3042.
- van den Heuvel, M. P., Mandl, R. C. W., Kahn, R. S., & Hulshoff Pol, H. E. (2009). Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. *Human Brain Mapping*, 30(10), 3127–3141.
- van den Hout, M. A., Bartelski, N., & Engelhard, I. M. (2013). On EMDR: Eye movements during retrieval reduce subjective vividness and objective memory accessibility during future recall. *Cognition & Emotion*, 27(1), 177–183.
- van den Hout, M. A., Muris, P., Salemink, E., & Kindt, M. (2001). Autobiographical memories become less vivid and emotional after eye movements. *The British journal of clinical psychology / the British Psychological Society*, 40(Pt 2), 121–130.
- Van der Ploeg, H. M. (1980). Validity of the Zelf-Beoordelings-Vragenlijst (A Dutch version of the Spielberger State-Trait Anxiety Inventory). *Nederlands Tijdschrift voor de Psychologie en haar Grensgebieden.*, 35, 243–249.
- Van Dijk, K. R. A., Hedden, T., Venkataraman, A., Evans, K. C., Lazar, S. W., & Buckner, R. L. (2010). Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. *Journal of neurophysiology*, 103, 297–321.
- Van Kesteren, M. T. R., Fernández, G., Norris, D. G., & Hermans, E. J. (2010). Persistent schema-dependent hippocampal-neocortical connectivity during memory encoding and postencoding rest in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 107(16), 7550–5.
- Van Kesteren, M. T. R., Ruiter, D. J., Fernández, G., & Henson, R. N. (2012). How schema and novelty augment memory formation. *Trends in Neurosciences*, 35(4), 211–219.
- Van Stegeren, A. H., Everaerd, W., Cahill, L., McGaugh, J. L., & Gooren, L. J. G. (1998). Memory for emotional events: Differential effects of centrally versus peripherally acting β -blocking agents. *Psychopharmacology*, 138, 305–310.
- Viau, V. (2002). Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. *Journal of neuroendocrinology*, 14(6), 506–13.
- Visser, R. M., Scholte, H. S., Beemsterboer, T., & Kindt, M. (2013). Neural pattern similarity predicts long-term fear memory. *Nature neuroscience*, 16(October 2012), 388–90.
- Vogel, S., Klumpers, F., Kroes, M. C., Oplaat, K. T., Krugers, H. J., Oitzl, M. S., Joëls, M., & Fernández, G. (2015). A Stress-Induced Shift from Trace to Delay Conditioning Depends on the Mineralocorticoid Receptor. *Biological Psychiatry*, 78(12), 1–10.
- Vuilleumier, P., Armony, J. L., Driver, J., & Dolan, R. J. (2001). Effects of attention and emotion on face processing in the human brain: an event-related fMRI study. *Neuron*, 30(3), 829–841.
- Vytal, K., Cornwell, B., Arkin, N., & Grillon, C. (2012). Describing the interplay between anxiety and cognition: from impaired performance under low cognitive load to reduced anxiety under high load. *Psychophysiology*, 49(6), 842–52.

- Wager, T. D., Davidson, M. L., Hughes, B. L., Lindquist, M. A., & Ochsner, K. N. (2008). Prefrontal-Subcortical Pathways Mediating Successful Emotion Regulation. *Neuron*, 59(6), 1037–1050.
- Wager, T. D. & Smith, E. E. (2003). Neuroimaging studies of working memory : A meta-analysis. *Cognitive, Affective, & Behavioral Neuroscience*, 3(4), 255–274.
- Wagner, A. D., Schacter, D. L., Rotte, M., Koutstaal, W., Maril, A., Dale, a. M., Rosen, B. R., & Buckner, R. L. (1998). Building memories: remembering and forgetting of verbal experiences as predicted by brain activity. *Science*, 281(5380), 1188–1191.
- Wang, S.-H. & Morris, R. G. M. (2010). Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation. *Annual review of psychology*, 61, 49–79, C1–4.
- Whalen, P. J. (2007). The uncertainty of it all. *Trends in Cognitive Sciences*, 11(12), 499–500.
- Whalen, P. J., Kagan, J., Cook, R. G., Davis, F. C., Kim, H., Polis, S., McLaren, D. G., Somerville, L. H., McLean, A. A., Maxwell, J. S., & Johnstone, T. (2004). Human amygdala responsivity to masked fearful eye whites. *Science*, 306(December), 2061.
- Whalen, P. J., Rauch, S. L., Etcoff, N. L., McInerney, S. C., Lee, M. B., & Jenike, M. a. (1998). Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. *Journal of Neuroscience*, 18(1), 411–418.
- Wiemers, U. S., Sauvage, M. M., Schoofs, D., Hamacher-Dang, T. C., & Wolf, O. T. (2013). What we remember from a stressful episode. *Psychoneuroendocrinology*, 38(10), 2268–2277.
- Wilson, M. A. & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science*, 265, 676–679.
- Wiltgen, B. J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M. G., Parivash, S. N., Li, W., & Silva, A. J. (2010). The Hippocampus Plays a Selective Role in the Retrieval of Detailed Contextual Memories. *Current Biology*, 20(15), 1336–1344.
- Wimber, M., Alink, A., Charest, I., Kriegeskorte, N., & Anderson, M. C. (2015). Retrieval Induces Adaptive Forgetting of Competing Memories via Cortical Pattern Suppression. *Nature Publishing Group*, 18(4), 582–589.
- Windmann, S. & Kutas, M. (2001). Electrophysiological correlates of emotion-induced recognition bias. *Journal of cognitive neuroscience*, 13(5), 577–592.
- Wohlschläger, A. M., Specht, K., Lie, C., Mohlberg, H., Wohlschläger, A., Bente, K., Pietrzyk, U., Stöcker, T., Zilles, K., Amunts, K., & Fink, G. R. (2005). Linking retinotopic fMRI mapping and anatomical probability maps of human occipital areas V1 and V2. *NeuroImage*, 26, 73–82.
- Wood, N. E., Rosasco, M. L., Suris, A. M., Spring, J. D., Marin, M. F., Lasko, N. B., Goetz, J. M., Fischer, A. M., Orr, S. P., & Pitman, R. K. (2015). Pharmacological blockade of memory reconsolidation in posttraumatic stress disorder: Three negative psychophysiological studies. *Psychiatry Research*, 225(1-2), 31–39.
- Yassa, M. A. & Reagh, Z. M. (2013). Competitive Trace Theory: A Role for the Hippocampus in Contextual Interference during Retrieval. *Frontiers in Behavioral Neuroscience*, 7(August), 1–13.
- Yassa, M. A. & Stark, C. E. (2011). Pattern separation in the hippocampus. *Trends in Neurosciences*, 34(10), 515–525.
- Yonelinas, A. P. & Ritchey, M. (2015). The slow forgetting of emotional episodic memories: an emotional binding account. *Trends in cognitive sciences*, 19(5), 259–67.

Appendix

Nederlandse samenvatting

Het doel van dit proefschrift was om te onderzoeken hoe interacties in het brein op system niveau een rol spelen bij de consolidatie van herinneringen voor stressvolle ervaringen bij mensen. Daarnaast heb ik onderzocht of en hoe deze “offline” processen gemanipuleerd kunnen worden om zo deze herinneringen te kunnen doen verminderen. Om dit alles te onderzoeken heb ik gebruik gemaakt van univariate analyses, multivariate analyses en analyses waarbij je naar connectiviteit tussen hersengebieden kan kijken. Deze heb ik toegepast op BOLD-FMRI data wanneer de proefpersonen een opdracht aan het uitvoeren waren of in een rust toestand waren. Ik heb stress toegediend door middel van milde elektrische schokjes aan de vingers en via stressvolle filmfragmenten. Herinneringen voor deze stressvolle ervaringen heb ik met fysiologische maten gemeten, zoals verwijdingen van de pupil en geleiding van de huid (in andere woorden; zweten van de vingertoppen). Declaratief geheugen heb ik gemeten door middel van herkenning van plaatjes en associaties. Ik zal eerst een samenvatting geven van de bevindingen in mijn proefschrift aan de hand van de vragen die ik in het begin van dit proefschrift heb gesteld.

Vraag 1: Welke rol spelen arousal en amygdala activiteit bij het verbeteren van het geheugen voor stressvolle ervaringen tijdens leren?

In **Chapter 2** hebben we onderzocht welke rol arousal en amygdala activiteit speelt in het vormen van declaratieve herinneringen voor stressvolle gebeurtenissen. We hebben gevonden dat de geleiding van de huid omhoogging en de pupil wijder werd, een duidelijk teken van een conditioneringeffect. Echter, deze twee maten voorspelde niet het geheugen voor de plaatjes. Dit in tegenstelling tot de activiteit in de amygdala die geen conditioneringeffect liet zien, maar wel het geheugen voor specifieke plaatjes van de geconditioneerde categorie voorspelde. Deze data laat zien dat er een dissociatie is tussen de rol die de amygdala speelt en de rol die noradrenaline gedreven sympathische reacties spelen in declaratief geheugen voor stressvolle herinneringen. Dit is in lijn met diermodellen die laten zien dat de amygdala de effecten van stress op geheugen beïnvloed.

Vraag 2: Welke interacties op system niveau spelen een rol in het verbeteren van declaratief geheugen voor stressvolle ervaringen tijdens rust na leren?

In **Chapter 3**, hebben we onderzocht of interacties tussen de amygdala and hippocampus na dat leren plaats heeft gevonden, een rol blijven spelen in het vormen van herinneringen voor stressvolle ervaringen. We hebben gevonden dat

geheugenprestatie beter was in een stressvolle context, en cortisol reacties door de stress manipulatie voorspelde deze geheugenverbetering. Connectiviteit tussen de amygdala en de hippocampus voorspelde ook verbetering in geheugen, maar dit was onafhankelijk van de context (stress of neutraal). Vervolg analyses lieten zien dat deze connectiviteit tijdens rust niet verschilde tussen de context (stress of neutraal). Deze was ook positief gecorreleerd over proefpersonen. In conclusie, onze data laten zien dat connectiviteit tussen de amygdala en hippocampus tijdens rust het geheugen versterkt in een stressvolle context, maar als karaktertrek en niet als het gevolg van stress.

Vraag 3: Veranderde stressvolle gebeurtenissen het reactiveren van geheugenrepresentaties?

In **Chapter 4**, hebben we onderzocht of geheugenrepresentaties in de hippocampus en neocorticale gebieden sterker spontaan worden gereactiveerd voor stressvolle ervaringen dan voor neutrale ervaringen. We vonden inderdaad dat BOLD-fMRI patronen van een specifieke conceptuele categorie (de categorie dieren of fruit/groenten) vaker spontaan werden gereactiveerd tijdens rust als deze geassocieerd waren met een stressvolle ervaring. Vervolgens vonden we dat de connectiviteit tussen deze gebieden waar de patronen werden gereactiveerd en de hippocampus, een gebied dat een cruciale rol speelt bij geheugen, individuele verschillen in geheugen voorspelde. Dit hadden we 24 uur later gemeten aan de hand van het meten van de verwijding van de pupil, een maat voor angst herinneringen. Als laatste vonden we dat connectiviteit tussen de amygdala en hippocampus verhoogd was tijdens rust na leren als we dit vergeleken met voor het leren. Deze bevindingen samen, laten zien dat stress ervoor zorgt dat geheugen representaties spontaan opnieuw worden afgespeeld tijdens rust. Dit leidt tot beter geheugen wanneer er een koppeling is met de hippocampus. Deze bevindingen onthullen dat er op system niveau een mechanisme is dat verklaart waarom stressvolle gebeurtenissen sterker worden onthouden dan niet stressvolle gebeurtenissen.

Vraag 4: Kunnen herinneringen veranderd worden door de activiteit in de amygdala op een non-invasieve manier te beïnvloeden?

In **Chapter 5**, hebben we onderzocht of activiteit in de amygdala kunnen verminderen door middel van een gedragsmanipulatie, namelijk oogbewegingen. We hebben onderzocht of dit het geheugen beïnvloed wanneer we dit doen na het ophalen van bestaande stressvolle herinneringen. We vonden dat het maken van oogbewegingen inderdaad activiteit in de amygdala vermindert. Wanneer deze oogbewegingen plaatsvonden na het ophalen van herinneringen tijdens extinctie

leren, zorgde dit van een vermindering in het spontaan terug komen van deze herinneringen 24 uur later. Dit hebben we gemeten aan de hand van een vermindering in huidgeleiding. Hoewel deze herinnering over de groep gemiddeld wel terugkwam na een directe herinnering van de stressvolle ervaring (we gaven de proefpersonen nogmaals een elektrisch schokje), was dit afhankelijk van hoe goed de activiteit in de amygdala was onderdrukt. We kunnen concluderen dat wanneer oogbewegingen worden toegevoegd aan extinctieleren dit een toegevoegde waarde heeft. Deze bevindingen zijn nieuw, omdat ze inzicht geven in de wederkerige eigenschappen van grote netwerken in het brein die affectieve en cognitieve processen kunnen beïnvloeden. Daarnaast dragen ze bij aan het begrijpen van een veel gebruikte therapie die oogbewegingen gebruikt om traumatische herinneringen te doen verminderen genaamd Eye Movement Desensitization and Reprocessing (EMDR).

In mijn proefschrift heb ik onderzocht hoe interacties tussen hersengebieden op systeemniveau herinneringen voor stressvolle ervaringen voorspellen en vooral hoe de amygdala hierbij een rol speelt. Vanuit onderzoek bij knaagdieren weten we dat de amygdala de effecten van stress beïnvloedt in andere geheugensystemen, zoals de hippocampus. We vonden hiervoor bewijs in mensen. De amygdala speelt al tijdens de leerervaring een rol in het vormen van herinneringen (**Chapter 2**) maar speelt ook na het leren, tijdens consolidatie, een rol via verhoogde connectiviteit met de hippocampus (**Chapter 4**). Deze connectiviteit als karaktertrek verklaart eveneens een verbetering in geheugen door stress. Dit suggereert dat dit niet alleen tijdelijke effecten zijn (**Chapter 3**). Na het ophalen van dit type herinneringen bleek het mogelijk te zijn om impliciet geheugen voor stressvolle ervaringen te beïnvloeden door (een deel van) deze systemen te manipuleren (**Chapter 5**). Deze bevindingen samen laten zien dat de consolidatie van stressvolle herinneringen op systeemniveau anders zijn dan voor neutrale herinneringen en dit mogelijk verklaart waarom dit soort herinneringen zo sterk worden onthouden. De bevindingen in dit proefschrift laten ook zien dat het mogelijk is deze processen te beïnvloeden en hiermee deze herinneringen te doen verminderen.

Dankwoord

Everything has to come to an end, also this book which you of course reached by reading everything before this part. This book would have never been here if it were not for you! Writing this might have been the most difficult part, because I am really afraid to forget someone or not express myself well enough. Please forgive me, I mean well.

Ten eerste dank aan alle proefpersonen die zich door mij hebben laten martelen met elektrische schokken en verkrachtingsfilmpjes. *#explainyourjobbadly*

Erno, ergens in in de herfst van 2011 kwam Peter Bos onze stage kamer binnen lopen en zei dat hij de perfecte PhD plek voor mij wist. Hij noemde jouw naam en zei dat wij veel gemeen hadden en volgens hem moest ik mijn PhD bij jou gaan doen. Het had niet veel gescheeld of alles was is heel anders gelopen, in mijn leven althans. Nu een PhD-time verder bleek het een goede match. Ik heb veel van je geleerd en heb altijd veel ontzettend plezier gehaald uit onze wetenschappelijk, inhoudelijke discussies. Al is het vaak met een omweg, ja dit heeft zeker met mijn koppigheid te maken (je moeten weten dat de eerste woorden die ik ooit uitsprak waren “zelf doen” ;-), true story), of op een ander tempo, denk ik dat we in de essentie hetzelfde over dingen denken en dat is heel veel waard. Ik wil je bedanken voor jouw vertrouwen in mij en de vrijheid die je mij hebt gegeven om mijn eigen PhD vorm te geven. Ik hoop van ganser harte dat dit slechts het begin is van een levenslange samenwerking.

Guillén, we did not meet so often, but when we did your input was always useful and something I never thought of myself. If I have anything to complain about, it would be that the moments my manuscript was off my desk and in your mailbox were waaay too short. With a lot of joy I listened to your try-out pitches in the pizza meeting about how science and the Donders could change to make things better. Mostly I would like to thank you for making me feel welcome in, and part of, your group.

Memory and Emotion lab, dear emo's and memo's. The first group email I ever received from you was an email about the amounts of cake people brought to the lab meetings, and that this was uncoordinated, too many people were bringing cakes and it was getting out of hand. I think this was the strangest group announcement I have ever read and thought if this was the biggest problem they had to deal with, I would really like to be part of that group. Thank you for the nice “knutsel-avondjes”, pizza meetings (still cannot get over the fact they are called meetings), group lunches, wine-and-cheese evenings, bowling evenings, the retreat at the Veluwe and many more activities we did. Thank you, **Guillén, Erno, Geeralien, Sabine, Mariët, Isabella, Marieke, Floris, Martin, Nils K, Atsuko, Gabi, David, Boris, Klodiana, Susanne, Noortje, Ruud, Frauke, Ruud, Nils M, Christina, Jasper, Yu, Hongxia, Marloes, Marijn, Marlieke, Eelco, Lisa, Leonore, Daphne** for making

me a part of all this.

Also thanks to my new lab, **the CANlab, Erno, Maria** (thanks for all the nice conversations about science and cats), **Savi, Hongxia, Judith, Sara, Debora, Lieke, Ilaria, Yannick, Lieke**, thank you all for listening to and helping me with my presentation and always asking me how my book is doing. Well, if you are reading this, it's done :). **Lotte**, speciale dank aan jou natuurlijk. Al was het maar van tijdelijke duur, je hebt een grote rol gespeeld in het begin van onze groep.

Of course I did not do everything myself and received a lot of help from students as well. **Laura**, jij was de eerste student die ik mocht begeleiden. Naast dat je het geweldig hebt gedaan en ik van je op aan kon, was het ook erg gezellig met jou erbij. **Marit en Jennifer**, al waren jullie maar kort hier, ik heb genoten van onze samenwerking binnen ons "EMDR clubje". **Hanjian**, you were the first master student I was going to supervise for a whole year. This was also the last year of my PhD, which gave you a unique insight into the most stressful period of my life ;-). You did a great job and I really would not know how I would have done this study without your help. It was great having you around and I am proud you were able to get into a graduate program in Canada. **Anouk**, al was ik zijdelings bij jouw stage betrokken was het leuk om met jou samen te werken. Ik gun je het allerbeste voor in de toekomst. **Yannick**, ik vond het ontzettend leuk om jou erbij te hebben in onze groep en daarom des te jammer dat alles wat anders gelopen is dan dat jij, en wij dus ook, van te voren hadden gedacht. Ik bewonder je doorzettingsvermogen en wens je het allerbeste voor de volgende stap! **Lieke**, je hebt me geholpen bij een idee dat ik had zonder een al te duidelijke stappen plan voor de uitvoering. Ik ben onder de indruk van hoe je in korte tijd al zoveel werk hebt verricht. Geniet van je reis in Sri Lanka voordat je de master van je keuze gaan doen! **Jon**, you were not a student, but thank you for your "short but powerful" (as we say in Dutch) visit and help with scanning. I hope to visit you in Cambridge soon. And thanks to all the students that were only shortly doing a lab rotation, but always were of great help **Lee Anne, Naomi, Valerie, Kalli, Fleur, Giulia**.

Ook dank aan **Karin**, als jij niet zo enthousiast was geweest had ik waarschijnlijk nooit mijn onderzoeksídee naar EMDR mogen uitvoeren. Ik vind het heel leuk dat je met ons onderzoek mee wilde doen en daarbij altijd zo'n positieve insteek had. **Benno**, dankzij Erika mocht ik met jou samenwerken. Gelukkig klikte het tussen jou en Erno heel goed, en dat lijkt me nogal een understatement. Ik vind het altijd weer leuk om je in de gang tegen te komen en hoop dat ons project een succes wordt.

Isi, my dearest memo and little sister from a different mister ;-). I do not think I can say anything here to you that I have not said before. Isi, without you my PhD would have been quite boring. It took us one very small conversation to become each other's best friend. [Me: I play guitar. You: I play the drums.] Done.

Thank you for introducing me to Austria, a country I never really thought about so much, but now feels like a second home. Never could I have imagined to be such good friends with someone from a different country. Funny he ;). Funny haha not funny weird. You taught me Schi zu foan, introduced me to Kürbiskernöl, Sturm, Oachkatzlschwoaf, Knittelfeld, Sissi's gardenhouse, and Kaiserschmarrn (although technically your mom did). Apart from all the fun things we did that help us to stay sane, you would almost forget that a big part of our friendship is also based on our joined interest in science. It was nice to have you as a sparring partner and discuss supervisor issues, mvpa implementations, avoid overloading the torque system etc.. I still miss you every day at our beloved D. In science it is hard to keep your good friend around, but at the same time allows us to keep in touch through collaborations and conferences, I think we already fully explored these possibilities :-). Auch danke an deine Eltern, **Hildegard und Jimmy**, dank für die Gastfreundschaft. Ich werde nie vergessen unsere Reis nach die zotter Schokoladen Fabrik und die Weltmaschine, die gar niks macht. Isi, thank you for being my friend.

Floris, mede emo, onze samenwerking begon in Utrecht toen ik vanwege scanner problemen ineens geen stage project meer had en aan jouw project kon werken, daarna ook nog als RA. Ik was heel blij dat jij naar de groep ging als postdoc waar ik uiteindelijk mijn PhD ging doen. Je bent een ontzettend fijne collega en bedankt voor je niet klein te krijgen optimisme!

Jack, door jouw colleges is mijn interesse voor emotie onderzoek ontstaan. Bedankt hiervoor en voor de mogelijkheid om ook nog een stage bij jou te lopen. Ik ben vereerd dat je ook in mijn corona plaats wilt nemen. **Estrella, David en Peter**, door jullie heb ik voor altijd een ideaal beeld van hoe je binnen een groep kan samenwerken. Bedankt voor alles wat ik van jullie heb mogen leren en dat jullie mij vertrouwen hebben gegeven in mijn eigen kunnen.

Bart, mede stagiaire, bedankt voor de inhoudelijk discussies die we hadden, dat je je plezier in wetenschap, de nieuwste snufjes op technologie gebied (haha die tablet gebruik ik al lang niet meer) of je nieuwste ideeën om alles nog efficiënter aan te pakken met mij wilde delen. Met veel plezier denk ik terug aan ons uitstapje als "jonge" masterstudenten tussen alle "volwassen" PhD studenten bij de Donders Discussions.

James, thanks for letting me be part of your lab. This might not be something you have realized but you gave me a lot of confidence by the way you accepted me as a student in your group. I would like to thank you for your encouraging words and support. Thanks **Harma, Stuart, Roberta, Cindy, Craig, Chris** and of course **Sarah** for making my time at the NIH unforgettable. **Laura**, als jij mij toen niet had "gedwongen" om dat ene telefoontje te maken had het er voor mij allemaal anders uitgezien en daar ben ik je heel dankbaar voor. Al was het maar heel kort dat we samen bij het NIH waren, houd ik hier hele warme herinneringen aan over.

Bedankt voor alle leuke gesprekken waarin we altijd alles en iedereen proberen te doorgronden :-).

Paul, mijn experimenten waren nooit zo goed verlopen als ik dit zonder jouw expertise had moeten doen. Ik heb ontzettend veel van je geleerd, bedankt hiervoor. Daarnaast waren al die uren in de kelder ook een stuk saaier geweest zonder jouw aanwezigheid en gevoel voor humor.

Tildie, bedankt voor al je hulp bij alle, tja soms niet te categoriseren vragen die ik hoe dan ook altijd aan jou kon stellen.

Dank aan, **Marek, Mike, Jessica, Sander, Erik, Uriel, Arthur, Berend, Sandra, Nicole, Ayse, Renee**, bedankt dat jullie er altijd waren als ik jullie hulp nodig had. Het Donders was het Donders niet zonder jullie. **Lucia**, het is altijd gezellig als jij in het kantoor bent en ik denk dat ik je vooral moet bedanken voor het in leven houden van mijn plant.

Erika, thanks to you for the rest of my life I am able to say that one of the ideas for one of my studies started in a bar! Unfortunately it did not make it into the thesis, but I am sure something nice will come out of it. It was great to have met you via Peter and I hope we can continue to collaborate and discuss science in bars wherever they may be ;).

Roomies, **Ashley**, thanks for the tennis games, **Alex**, thanks for the conversations on memory and classifiers, **Peter**, thanks for all the conversations on, yeah what not ;-), **Jenny**, sorry I was not always the most talkative person in the last year of my PhD, but I was really happy to have you sitting next to me in my new, now old, office, **Reinout**, eindelijk iemand die ook iets ook iets met emotie doet :-), **Matthias, Ilke, Kim** and newest roomie **Sam**.

Ruudi, memo, buurman en vriend. Jij, Isi en ik werden vaak in een adem genoemd. Daarom is het nog steeds raar om “alleen” op het Donders rond te lopen. Wetenschap kan erg alleen zijn en ik denk dat we heel blij mogen zijn dat we samen onze PhD's konden doen, ik heb hier in ieder geval veel steun uit gehaald. Maarrrrrr, haha niet zo serieus, natuurlijk ook uit al onze concert bezoeken, etentjes, roadtrips, volleybal avonden, de keren dat je 10 kilometer voor mij uit rende, spelletjes en knutselavonden, onze weergaloze dansmoves *insert random dancebeat*, grapjes en zoveel meer. Het ga je goed in Leipzig.

Matthi, me and Isi met you only 4 weeks before you moved to Norway. Our friendship was of short duration, but you fitted right in. We like the same music, we eat the same food, we find the same things funny (funny haha not funny weird), and mostly I find a good colleague in you with which it was great to talk, although may it over a beer at Frohwijn. I could say a lot about how nice it would have been if only, but the nice thing about science is you can have colleagues, and friends, across the entire world. I genuinely hope we can stay in touch and one day visit you in Norway ;-).

Tjerk, bandmate en paranimf, zoals je het zelf ooit verwoordde; band practice on Sunday is the highlight of my week. Was het toeval, of misschien niet, ik ben heel blij dat wij jou zijn tegengekomen en dat je mee wilde doen in onze band. Pas vanaf dat moment waren we compleet. Stiekem tussen alle muzikaliteit door had je ook soms een luisterend oor voor dingen waar ik in mijn PhD, of wetenschap in het algemeen, tegenaan liep. Tja van ons drie (jij, Isi en ik) was je toch de volwassenste onderzoeker. Jouw kalmerende woorden en onnavolgbaar vermogen om je niet druk te maken zorgde er bij mij voor dat ik dit ook niet deed. Bedankt dat je ook nog eens mijn paranimf wilt zijn.

Ries, ik heb je al vrij snel leren kennen nadat ik naar Nijmegen verhuisde. Jaren lang hebben we elke zondag, of soms dinsdag, maar ook op zaterdag, tja het maakte eigenlijk niet uit, jij kon altijd wel, in de kelder van de Plu muziek gemaakt. Het is jammer dat je uiteindelijk met 3 wetenschappers in een band zat die zelden langer dan een paar jaar op dezelfde plek blijven en de band dus uit elkaar is gevallen. Bedankt voor alle muzikaliteit, gezelligheid en dat je altijd voor ons klaar stond.

Rien, toch ook een bedankje aan jou. Ik ben ontzettend genoten van jouw judolessen en al was dit misschien maar kort en woon je nu ver weg, ik ben heel blij dat ik jou heb leren kennen. Ik hoop dat ons onderzoeks idee / samenwerking ooit nog eens van de grond kan komen.

Although the tradition is way gone, thanks to all the FADs (I already hear some of you thinking, what does that mean FAD?) and people I met there, **Winke**, bedankt voor het skiën, de asperges, jongleren, wadlopen, maar vooral bedankt voor je relaxtheid en kalmte (niet te verwarren met pretentieloosheid!) en vooral je humor. Ik ben blij dat jij er was. **Paul**, I hope we can keep going to many random bands or festivals in many random cities. **Izabela**, thanks for introducing me to gimbap (had to google how it was spelled) and South Korean boy bands, but mostly thanks for being such a kind person. **Thomas**, I really always enjoy our scientific discussions and I hold a lot of value in hearing your opinion. You are a great scientist with great ideas. **Daphne**, bedankt dat je zo'n fijne collega bent en ik hoop dat als je dit leest onze "project" van de grond is gekomen ;-). Bedankt aan zoveel meer mensen die mijn PhD tijd tot een van de leukste periodes uit mijn leven hebben gemaakt, **Miriam** (voor de thee avondjes en BBQs in ons fijne huis), **Jeanette**, **Anke Marit**, **Sander** (voor je humor, luchtigheid en latex template), **Jolien**, **Mirjam**, **René**, **Lieneke**, **Richard**, **Lorijn**, **Aline**, **Tim**, **Natalia**, **Erik**, **Corine**, **Marianne**, **Veerle**, **Flora**, **Ruben**, **Thomas**, **Iske**, **Monja**, **Jacob**, **Lonja**, **Tobias** (for the nice conversations on amygdala recording possibilities and politics), **Thomas S**, **Verena**, **Lisa**, **Inge**, **Marianne**, **Roselyn**, **Sybrine**, **Sanne**, **Fenny**, **Xiaochen**, **Simon Jan**, **Dan**, **Tjerk**, **Iris**, **Johanna**, **Larry and Matthias** (thanks for the best housewarming party ever!), **Irati**, **Izabela**, **Martin**, **Annemiek**, **Piray**, **Achiël**, **Giacomo**, **Sandra**, **Alexis**, **Sara**, **Clauudia**, **Catalina**, and I am sure I am forgetting a lot of people.

Fred en Juul, bedankt voor al die gezellig daagjes en weekendjes weg en voor al die goede gesprekken over mensen en hun gedragingen, politiek, hippe koffietentjes, en EMDR.

Lieve **Anneke, Famke, Marja, Marike, Victoria en Marit**, we kennen elkaar al sinds de middelbare school en kan soms niet geloven wat er in onze levens al niet is veranderd, en jullie hebben dit allemaal meegemaakt. Wat er ook gebeurt, jullie zullen altijd degene zijn die echt snappen waar ik vandaan kom, wat ik heb moeten doen om hier te komen en dus ook hoeveel het voor mij betekent dat dit boek nu bestaat. Het is soms moeilijk over te brengen wat het nu precies is om een PhD doen, jullie hebben altijd een luisterend oor en begrip. Hoeveel werk het soms ook is, hoe druk ik soms ook ben, hoe erg ik mij soms misschien ook aanstel, hoe ver weg we nu ook van elkaar wonen, en hoe verder weg dat nog zal worden, ik weet dat jullie er altijd voor mij zullen zijn. Hoewel het misschien niet altijd zo lijkt in onze dagelijks activiteit, we zien elkaar te weinig, ik hoop dat jullie weten hoeveel jullie voor mij betekenen. **An**, bedankt dat je onze groep zo goed bij elkaar houdt. Ik hoop dat ik er ook voor jou kan zijn zoals jij er voor mij bent. **Fam**, bedankt dat je mijn artikelen uitprint en er met een stift dingen in markeert, ik weet niet of je dit echt doet maar ik zie het zo voor me ;-). Bedankt dat je mijn paranimf wilt zijn. **Miep**, bedankt voor de altijd gezellige lunchen en je heerlijke nuchterheid, de spreekwoordelijke dan he ;-).

Als laatste wil ik natuurlijk mijn familie bedanken. **Dik**, we lijken zo op elkaar dat het soms wel eens botst. Ik denk omdat we allebei het beste voor elkaar willen, maar dat we dit ook op onze eigen manier willen doen. Weet echter dat ik ontzettend trots ben op wie jij bent en wie je bent geworden. Iedere leerling mag blij mag zijn met zo'n lieve en betrokken docent als jij. **Rob**, mijn nog kleinere, maar nu bijna 2 meter lange, broertje. Niemand vraagt zo vaak hoe het met mij gaat als jij. Bedankt voor al je lieve e-mails, foto's en je hulp bij mijn computer issues. Ik hoop dat je op een dag vindt wat je gelukkig maakt.

Pa en ma, ik weet dat het voor jullie niet altijd makkelijk moet zijn geweest, een kind die er altijd op uit wil, het liefst zo ver mogelijk en het altijd anders wil doen dan dat jullie het zouden doen. Bedankt dat jullie dit ondanks alles toch hebben geaccepteerd. Bedankt dat jullie altijd mijn keuzes hebben ondersteund, ook al waren jullie misschien bang dat ik het verkeerde zou kiezen. Zie, het is toch goed gekomen ;-). Mam, bedankt voor het uitknippen van alle krantenartikelen die ook maar iets met hersenen te maken hebben en bedankt dat ook wanneer het misschien niet altijd te volgen is, je het toch altijd probeert. Ik heb jullie lief.

Curriculum Vitae

Lycia Dienneke de Voogd was born in Utrecht on March 21, 1983. Most people know her under the name of Linda and this is because her parents gave her that name. Linda does not like to talk about herself in the third person, but at the same time complies with what is expected of her and chooses to do so anyway.



After completing a study in Social Work at the University of Applied Sciences in Utrecht, Linda worked as a social worker in organizations such as the Salvation Army. Later she worked as a high school teacher at a school for young adolescents with learning disabilities. Although she loved the interactions with real human beings, this work also made her realize that to really understand human behavior one must study the brain. In 2007 she therefore decided to study Psychology at the Utrecht University and continued with a masters in Neuroscience and Cognition.

She was interested in studying aggressive behavior and therefore did an internship with Prof. Dr. Jack van Honk where she was involved in setting up a research environment at the Pieter Baan Centrum, a forensic psychiatric observation clinic in Utrecht. To study this type of behavior even further, she went abroad to the United States of America to work as a trainee in the lab of Prof. Dr. James Blair at the National Institute of Health (NIH) studying the neural correlates of psychopathy.

In 2012 she started her PhD with Prof. Dr. Guillén Fernández and Dr. Erno Hermans to study the effects of stress on memory consolidation, as can be read in the pages preceding this one. Since August 2016 she is a postdoctoral researcher in the Cognitive Affective Neuroscience lab with Dr. Erno Hermans.

She pursues a career in science, but if this does not work out, her back-up plan is to become a rock star.

List of publications

Published

- **de Voogd L. D.**, Klumpers F, Fernández G, Hermans E. J. (2017) Intrinsic functional connectivity between amygdala and hippocampus during rest predicts enhanced memory under stress. *Psychoneuroendocrinology* 75:192–202
- **de Voogd L. D.**, Fernández G, Hermans EJ (2016) Disentangling the roles of arousal and amygdala activation in emotional declarative memory. *Social Cognitive Affective Neuroscience* 11:1471–1480
- **de Voogd L. D.**, Fernández G, Hermans E. J. (2016) Awake reactivation of emotional memory traces through hippocampal–neocortical interactions. *Neuroimage* 134:563–572
- Roozendaal, B., Luyten, L., **de Voogd, L. D.**, Hermans, E. J. (2016). Importance of amygdala noradrenergic activity and large-scale neural networks in regulating emotional arousal effects on perception and memory, *Behavioral and Brain Sciences* 31(0), 1–10.
- Hermans, E. J., Battaglia, F P, Atsak, P, **de Voogd, L. D.**, Fernández, G., Roozendaal, B. (2014). How the amygdala affects emotional memory by altering brain network properties. *Neurobiology of Learning and Memory* 112:2–16.

Under review

- **de Voogd, L. D.**, Kanen, J., Roelofs, K., Fernández, G., Hermans, E. J. Eye-movement intervention prevents fear recovery via amygdala deactivation.

In preparation

- **de Voogd, L. D.**, Murray, Y., Fernández, G., Doeller, C., Hermans, E. J. Neural mechanisms of context-dependent fear memory.
- **de Voogd, L. D.***, Collin, S.*, Barth M., Fernandez, G., Hermans, E. J. Phasic BOLD activity in the locus coeruleus and pupil dilation at different levels of tonic arousal.
- **de Voogd, L. D.**, Atutch, E., Roozendaal, B. Fernández, G., Hermans, E. J. Temporal dynamics of emotional memory consolidation.
- **de Voogd, L.D.**, Hilderink, L., Roelofs, K., Fernández, G., Hermans, E. J. Altering memory through eye-movement induced medial temporal lobe suppression following memory reactivation

* denotes equal contributions.

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/>

DONDERS

I N S T I T U T E



Max Planck Institute
for Psycholinguistics

ISBN 978-94-6284-107-9

Radboud University



Radboudumc

