Chapter 1

General Introduction
Graduation, the birth of a child, a wedding, but also the death of a beloved one, a car accident or physical assault are examples of emotional experiences that one may encounter during life. Even after decades, emotional experiences are often vividly remembered, whereas the bulk of daily life experiences are forgotten. The ability to recall the important events in life is crucial to adaptively respond to future situations. Nevertheless, the strength of emotional memories may also become harmful and maladaptive when it constraints daily functioning, such as in patients suffering from anxiety disorders or post-traumatic stress disorder.

From an evolutionary perspective, stability of our memory system is essential to recognize important predictors (i.e., stimuli and contexts) of imminent threat and reward. However, since no stimulus or context will keep its predictive value forever, memories need to be malleable as well. Evidence from neuroscience suggests that upon recall, memories can enter a labile state where they are sensitive to change before being restabilized again (Nader, Schafé, & Le Doux, 2000; Nader, 2003; Sara, 2000). This process is referred to as reconsolidation. This temporary period of plasticity provides the opportunity to interfere with unwanted and excessive emotional memories. Empirical findings highlight this plasticity and indicate that emotional memories can be weakened through pharmacological interference with reconsolidation (e.g., Dębiec, LeDoux, & Nader, 2002; Kindt, Soeter, & Vervliet, 2009; Nader et al., 2000; Soeter & Kindt, 2010). These findings raise the prospect that disrupting reconsolidation may be a promising technique to provide long-term cure for patients with several psychiatric conditions. The observed plasticity of emotional memory was the starting point of this thesis. Here, we aimed to gain more insight into the conditions under which memory can be altered by experimentally investigating different components of emotional memory in healthy participants.

Plasticity of memory

The formation of a memory trace starts with the encoding of new information (acquisition), after which it is consolidated and stored into long-term memory (LTM)(McGaugh, 2000). Directly after acquisition a memory trace is labile and prone to interference by pharmacological agents, brain injuries and behavioral manipulations. It has been suggested that with the passage of time newly formed memory traces enter a stable and permanent state because these modulatory factors lose their effectiveness (Dudai, 2004). The process of memory stabilization is referred to as consolidation and consists of two stages: synaptic consolidation and system consolidation. Changes in synaptic efficacy (i.e., the capacity of a presynaptic input to influence postsynaptic output) occur in the first minutes to hours after encoding of the new information and regulate the structural synaptic changes that underlie the formation
of memory (synaptic consolidation) (Lampecht & LeDoux, 2004; McGaugh, 1966). System consolidation refers to a time-dependent shift in brain systems that support memory storage and retrieval (McGaugh, 2000). Newly formed memories are dependent on the hippocampus; gradually, there is a shift to more involvement of cortical areas (for a review, see Dudai, 2004; Squire & Alvarez, 1995). This reorganization process takes weeks, months or even years to accomplish. For years LTM was conceived as a ‘fixed’ state and insensitive to change.

The hypothesis that LTM was no longer malleable after consolidation dominated the field of learning and memory until the end of the 20th century (Nader & Hardt, 2009; Sara, 2000). However, both in cognitive psychology and behavioral neuroscience there was already ample evidence that consolidated memory is much more dynamic than originally assumed. Based on the observations of systematic distortions of episodic memory over time, memory has been conceived as a continuously reconstructive process in cognitive psychology (e.g., Bartlett, 1932; Loftus, 2005). In behavioral neuroscience the consolidation hypothesis was challenged in the late 1960s. Misanin and colleagues (1968) showed that memory may not only be vulnerable to change directly after encoding, but also following a consolidation period. In this study, rats received a reminder of a previously formed fear memory followed by electroconvulsive shock. At test, memory performance of these rats was impaired, whereas the memory of control rats that only received an electroconvulsive shock - was still intact. Hence, memory reactivation was a necessary condition to interfere with the original memory trace. A few other studies at that time showed similar results (Devietti & Hopfer, 1974; Lewis, Bregman, & Mahan, 1972; for a review, see Sara, 2000). These findings indicated a retrieval-induced labile period during which a consolidated memory is susceptible to change again. This process was later called reconsolidation. Remarkably, these findings were for a long time basically neglected in the field (Sara, 2000). The interest in memory reconsolidation was renewed by research of Nader, Schafé and Ledoux (2000). In their seminal paper, the authors showed that infusion of anisomycin - a protein synthesis inhibitor - into the amygdala after memory reactivation resulted in amnesia on later tests. Critically, the reminder of the consolidated fear memory prior to protein synthesis blockade was crucial to induce amnesia; anisomycin alone left the fear memory intact. Moreover, memory performance was not affected directly after protein synthesis blockade, but only several hours after the manipulation. Infusion of anisomycin 6 hours after memory reactivation did, however, not impair memory performance, indicating that there is a certain time-window in which the reconsolidation process requires protein synthesis. Taken together, these findings demonstrated that upon recall a consolidated memory can return to a labile state requiring de novo protein synthesis to restabilize (i.e., reconsolidation). Since the paper of Nader et al., (2000), the number of studies on reconsolidation has grown exponentially and reconsolidation has been demonstrated in several species from the medaka
fish to humans (e.g., Eisenberg, Kobilo, Berman, & Dudai, 2003; Kindt et al., 2009; Walker, Brakefield, Hobson, & Stickgold, 2003).

How to study memory reconsolidation?

Reconsolidation can be seen as an updating process that keeps memory relevant (Lee, 2009; Tronson & Taylor, 2007). As pointed out above, this process is highly dependent on complex molecular mechanisms (e.g., new protein synthesis) that cannot directly be observed. Typically, evidence for the reconsolidation process is provided by demonstrating changes in the behavioral expression of memory. However, to experimentally demonstrate reconsolidation, there are a few prerequisites (Lewis, 1979; Tronson & Taylor, 2007). First, a memory for specific information needs to be consolidated. Next, the memory trace should be reactivated and contiguously some form of manipulation must be administered, like pharmacological agents, brain injury, behavioral procedures or new learning. This manipulation can be administered before or after memory reactivation. Finally, changes in the expression of memory must be observed at a long-term test and not during or directly following the manipulation, because the required protein synthesis for reconsolidation takes at least several hours (Duvarci & Nader, 2004; Walker et al., 2003). A frequently used and well-suited paradigm to study reconsolidation both in animals and humans is Pavlovian fear conditioning.

Fear conditioning as an experimental model for associative fear learning and memory

In Pavlovian conditioning, fear for an initially neutral stimulus, or conditioned stimulus (CS; e.g., tone or picture), is acquired by temporally pairing the CS with an aversive outcome, or unconditioned stimulus (US; e.g., shock). As a result, presentation of the CS alone elicits a conditioned response (CR) that is indicative of fear. The CR can be measured by several physiological and subjective indices (see box 1). Memory of this fear association (CS-US) can be tested by presenting the CS without the US. To substantiate memory consolidation, memory performance is often assessed 24 hours after encoding. Retention of the fear memory is inferred from the behavioral or neural expression of the CR.

The classical approach for reducing the expression of fear is extinction training, which involves repeated unreinforced re-exposure to the CS to promote the formation of a new ‘inhibitory’ memory trace (CS-noUS)(Bouton, 2002; Rescorla, 2001). Extinction training leaves
Box 1 – Measurements to index conditioned fear
In human fear conditioning research various measurements are used to index the conditioned fear response. The most frequently used indices of conditioned fear are US expectancy ratings, skin conductance and startle response.

**US-expectancy ratings.** A trial-by-trial rating of participants’ expectation to receive the US. This online rating method draws the attention towards the association between the CS and the US and indexes fear learning on a cognitive level (Boddez et al., 2013).

**Skin conductance response (SCR).** Changes in electrodermal responding by autonomic innervation of the sweat glands at the surface of the skin are measured using electrodes attached to the medial phalanx surface of the middle and fourth finger (Critchley, 2002; Dawson et al., 2007). The CR is indexed by larger skin conductance response during the conditioned stimulus (CS) compared to a control stimulus that is not followed by an US (e.g., electric shock). SCR is supposed to primarily reflect anticipatory arousal, regardless of whether the anticipated stimulus is positive or negative (Hamm & Vaitl). As such, in fear conditioning studies SCR is often related to the expectancy of the US and may express the more cognitive level of fear learning (Hamm & Vaitl, 1996; Hamm & Weike, 2005; Sevenster et al., 2014; Soeter & Kindt, 2010; Weike et al., 2007).

**Acoustic startle reflex.** Startle responding can be evoked by a loud noise and is characterized by an integrative, reflex contraction of the skeletal musculature. In humans, the startle reflex is assessed by the eye-blink component of the reflex, which consists of a rapid contraction of the orbicularis oculi muscle. This contraction is measured by electromyography (EMG) and the electrodes are placed over the right orbicularis oculi, which is just below the eye. CR is reflected by larger startle amplitude to loud noises during the CS than during the control stimulus that is not followed by an US. In contrast to SCR, potentiation of the startle response is considered as a specific measure of fear. Eye-blink reflexes to the loud noise evoked during aversive states are potentiated, whereas responses elicited during pleasant states are attenuated (Lang, Bradley, & Cuthbert, 1990; Vrana et al., 1988). Furthermore, the neuroanatomical pathway of startle potentiation is directly connected with the amygdala (Davis et al., 1993; Davis, 2006), which is considered the emotional center of the brain (Lang et al., 1997; LeDoux, 2000).

Even though the different physiological indices of conditioned fear seems to represent different underlying mechanisms, fear conditioning studies tend to use SCR and startle response interchangeable to measure the conditioned fear. In Chapter 4, we examined whether it is appropriate to use both physiological response systems to index the same underlying fear process.
the original fear memory (CS-US) intact, even though the fear is behaviorally silenced. The intact fear memory may promote the return of fear as triggered by external factors, such as a change of context (i.e., renewal, Bouton & Bolles, 1979), passage of time (i.e., spontaneous recovery, Brooks & Bouton, 1993) or re-exposure to the US (i.e., reinstatement, Rescorla & Heth, 1975). Extinction training is the experimental equivalent of exposure, which is a core component of cognitive behavioral therapy (CBT).

Another approach to reduce the expression of fear is to target the original fear memory directly by interference with reconsolidation. In animal research, disruption of reconsolidation is often accomplished by inhibiting protein synthesis using pharmacological agents like anisomycin (e.g., Dębiec et al., 2002; Duvarci & Nader, 2004; Nader et al., 2000). These protein-synthesis inhibitors are however not suitable for humans. Fortunately, inhibiting specific neurotransmission, like noradrenergic or gamma aminobutyric acid (GABA) transmission, can disrupt the reconsolidation process as well (Bustos, Maldonado, & Molina, 2006; Dębiec & LeDoux, 2004). Indeed, systemic administration of the noradrenergic β-blocker propranolol HCl after memory reactivation diminished the expression of fear during long-term memory test in rats (Dębiec & LeDoux, 2004). These findings hold considerable promise for the use of pharmacological agents to disrupt reconsolidation of fear memory in humans.

Disrupting reconsolidation of fear memory in humans

A study from our lab was the first to demonstrate that fear memory expression could be weakened in humans by disrupting reconsolidation with the noradrenergic β-blocker propranolol HCl (Kindt et al., 2009). This initial study showed that oral administration of 40 mg propranolol HCl prior to memory reactivation eliminated the behavioral expression of fear (i.e., startle reflex) 24 hours later and prevented the return of fear. Interestingly, the declarative part of the fear memory remained intact but no longer produced fearful responding. Other studies from our lab replicated this initial finding and showed that this fear-reducing effect was long-lasting (Soeter & Kindt, 2010) and generalized to semantically related stimuli (Soeter & Kindt, 2011b; 2011a) as well as to other contexts (Soeter & Kindt, 2012a). These findings open up new avenues to eliminate excessive emotional memory in humans.
Transition from reconsolidation to extinction

In a typical reconsolidation study using the fear conditioning paradigm, memory is reactivated by presenting the CS without the US (CS-noUS). This procedure is equivalent to the first extinction trial. An unresolved question is how the transition from reconsolidation to extinction proceeds. Molecular mechanisms that are involved in reconsolidation, like noradrenergic signaling, are also involved in extinction consolidation (Mueller & Cahill, 2010; Pape & Pare, 2010; Tronson & Taylor, 2007). Thus, reconsolidation and extinction share common molecular mechanisms that may facilitate a molecular competition between the two processes during memory reactivation. Memory reactivation may initially trigger reconsolidation that serves to maintain or adapt the original memory (e.g., Lee, 2009; Nader, 2003; Tronson & Taylor, 2007), whereas prolonged or repeated memory retrieval may initiate the formation of a new inhibitory memory trace. It has been suggested that the most dominant process of the reactivation session (i.e., reconsolidation or extinction) will be the one most affected by the pharmacological or behavioral manipulation (Eisenberg et al., 2003; Nader, 2003). An important question is whether pharmacological agents – such as propranolol HCl – intend to interfere with memory reconsolidation can also affect extinction learning. This issue is also clinically important for the applicability of reconsolidation-based treatments in clinical practice. In contrast to experimental procedures, the control over timing parameters during memory reactivation is rather limited in a therapeutic setting. One could imagine that after a single, time-limited reactivation session a patient further rehearse this retrieval experience and engage in imaginary exposure.

The first aim of the current thesis was to disentangle this memory trace competition between reconsolidation and extinction. Chapter 2 presents a study that tests the basic finding of disrupting reconsolidation with propranolol HCl, as shown in the studies by Kindt et al., (2009) and Soeter and Kindt (2010). In Chapter 3 we examined whether propranolol HCl would disrupt extinction learning in humans. In the next chapters our focus is on reconsolidation of declarative memory.

Multiple memory systems

As already stated, expression of memory can be assessed by different behavioral and neural expressions. These different response systems support the idea that memory is not a single entity but is composed of different functional and anatomical systems. Even though the different response systems often converge for example during fear learning, several studies demonstrate that the different response systems do not necessarily act in concert (e.g., Hamm
& Weike, 2005; Sevenster, Beckers, & Kindt, 2012a; Soeter & Kindt, 2010; Weike, Schupp, & Hamm, 2007). As stated above, propranolol HCl administration prior or after memory reactivation disrupted the emotional expression of fear memory, while leaving the declarative part of fear memory intact (Kindt et al., 2009; Sevenster, Beckers, & Kindt, 2012b; Soeter & Kindt, 2010; Soeter & Kindt, 2012b). The distinction between declarative and non-declarative memory is well supported by studies from patients with brain damage (Adolphs, Tranel, & Buchanan, 2005; Bechara et al., 1995; Bohbot, Iaria, & Petrides, 2004; LaBar, LeDoux, Spencer, & Phelps, 1995; Weike et al., 2005) and neuroimaging studies in humans (e.g., Bohbot et al., 2004; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003). For example, the cognitive and more declarative part of fear memory is associated with the hippocampal complex (Eichenbaum, 2004; Squire, Stark, & Clark, 2004), whereas the emotional expression of fear memory (startle reflex) is associated with the amygdala (Davis, 2006; LeDoux, 2000). This double dissociation between declarative and non-declarative memory relative to hippocampus and amygdala underscores the independent function of both memory systems. Note that this does not suggest that these systems do not interact (Dolcos, LaBar, & Cabeza, 2004; Phelps, 2004). Nevertheless, this double dissociation may explain why pharmacological and behavioral manipulations sometimes exert different effects on different memory systems. In Chapter 4 we further examined whether the different physiological response systems that are typically used in fear conditioning studies (e.g., skin conductance and startle reflex) represent different underlying mechanisms.

Reconsolidation of declarative memory in humans

The alleged function of reconsolidation is to keep our memories up to date by either altering their strength (e.g., Frenkel, Maldonado, & Delorenzi, 2005; Kindt et al., 2009; Nader et al., 2000; Soeter & Kindt, 2010; Soeter & Kindt, 2011a; Soeter & Kindt, 2012b) or by incorporating new information into memory (e.g., Forcato, Rodríguez, Pedreira, & Maldonado, 2010; Hupbach, Gomez, Hardt, & Nadel, 2007). Hence, reconsolidation provides the opportunity to rewrite a previously formed memory. Research on reconsolidation of declarative memory demonstrated that memories can indeed incorporate new information into a consolidated memory (Forcato et al., 2010; Hupbach et al., 2007; Hupbach, Gomez, & Nadel, 2011; Hupbach, Gomez, & Nadel, 2009). In these kinds of studies, participants have to learn a set of items (e.g., words, objects) in the first session. In the next session, participants are briefly reminded of the first list of items followed by learning a new set of items (list 2). At test (at least 24 hours later), participants are asked to recall items of list 1, but they mistakenly recalled items of list 2 as well. These findings
indicate that the words of list 1 and list 2 are now intermixed and stored into one memory trace instead of representing two independent memory entities. Importantly, memory reactivation before learning list 2 was crucial to incorporate new information (i.e., items of list 2) into the initial memory (i.e., items of list 1) (Forcato et al., 2010; Hupbach et al., 2007; Hupbach et al., 2011; Hupbach et al., 2009).

Other studies on reconsolidation of declarative memory have focused on the possibility of altering the strength of declarative memories (Coccoz, Maldonado, & Delorenzi, 2011; Coccoz, Sandoval, Stehberg, & Delorenzi, 2013; Forcato et al., 2007; Kroes et al., 2014; Schwabe & Wolf, 2010b). Abundant evidence shows that stress and the release of stress hormones (see box 2) strongly affect learning and memory processes (for reviews, see Joëls & Baram, 2009; Schwabe & Wolf, 2010a). However, studies on the effect of stress on reconsolidation are relatively sparse. Detailed insight in the effect of post-reactivation stress exposure on memory performance may increase our understanding of why some memories are more persistent than others. Retrieval of traumatic experiences is often accompanied by strong feelings of distress. One explanation for the strong persistence of those memories might be that post-reactivation stress strengthens the process of reconsolidation and thereby facilitates the maintenance of those memories.

A second aim of this thesis was to examine the effect of post-reactivation stress on emotional declarative memory. In Chapter 5, we tested whether post-reactivation stress would differentially enhance memory of emotional versus neutral content. In line with previous studies on reconsolidation of declarative memory, we focused in Chapter 5 on rather ‘isolated’ memories such as the memory for single items (words). Yet memories are typically embedded in a contextual setting such as time and place. Memories are usually better retrieved in their encoding context than in an unrelated situation (i.e., memory contextualization, Godden & Baddeley, 1975). Interestingly, patients with PTSD typically show impaired contextualization of trauma memory (Elzinga & Bremner, 2002; Liberson & Sripada, 2007). That is, memory of the traumatic event is easily retrieved in other situations that are unrelated to the trauma-situation. Whether post-reactivation stress would also affect memory contextualization is however unknown; this issue is addressed in Chapter 6.
Confrontation with a stressful situation activates a wide range of physiological systems aimed at restoring homeostasis and coping with the environmental demand at hand. The stress response involves activation of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. Within seconds after stress exposure the sympathetic branch of the ANS is activated, which eventually results in the release of adrenaline from the adrenal medulla and noradrenaline from activation of sympathetic nerve endings via activation of the locus coeruleus. The release of catecholamines ((nor)adrenaline) results in an increase in heart rate and blood pressure and stimulates the fight-flight response. The HPA-axis is a somewhat slower response system and is responsible for hormonal changes that enable the restoration of homeostasis and facilitate storage of relevant information into memory (De Kloet, Joëls, & Holsboer, 2005). Cortisol release from the adrenal cortex is regulated by the adrenocorticotropic hormone (ACTH) released by the anterior pituitary. ACTH is regulated by the neuropeptides corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) that are both secreted by the paraventricular nucleus of the hypothalamus. After the initial cortisol response, a negative feedback mechanism mediated by cortisol receptors (glucocorticoid receptor (GR) and mineralocorticoid receptor (MR)) inhibits the release of CRF and ACTH and dampens the stress response.

The stress hormones noradrenaline and cortisol are known to affect many brain areas that are also involved in various memory processes. Even though the brain consists of a complex network of interacting structures, there are some key structures that are both highly sensitive for stress hormones and critically involved in emotional memory. The amygdala is part of the limbic system and is crucial for processing emotional information (LeDoux, 2000). It has been suggested that adrenal stress hormones influence memory processes via interactions with arousal-induced activation of noradrenergic mechanisms within the amygdala (McGaugh, 2004; Roozendaal et al., 2009). Cortisol exerts its effects by binding to the high affinity MR and the lower affinity GR. MR and GR are co-localized in the hippocampus, a brain area that is also part of the limbic system and is essential for the formation of declarative memory (Eichenbaum, 2004; Squire et al., 2004). Specifically, the hippocampus is important for the contextual embedding of memory and facilitates the binding of multiple elements of an experience into a conjunctive representation (O’Reilly & Rudy, 2001). Given that the hippocampus has a high density of MR and GR, this area is highly sensitive to stress effects (De Kloet et al., 2005; Joëls & Baram, 2009).
Outline of the thesis

This thesis addresses various topics related to the malleable nature of emotional memory in humans. We used different experimental paradigms to test the conditions under which memory can be altered. In the first two empirical chapters (Chapter 2 and 3) we used classical fear conditioning to study the malleable nature of fear memory and its reliance on noradrenergic signaling. We used skin conductance and the acoustic startle reflex to assess the CR on a physiological level and US-expectancy ratings to assess the cognitive level of the CR. In Chapter 4 we examined the differences between these physiological response systems (i.e., startle reflex and SCR) more thoroughly and focused on the question whether both response systems reflect a defensive, fearful state. In the last two empirical chapters we investigated the effects of stress exposure on reconsolidation of declarative memory. In Chapter 5 we examined whether stress would differentially affect reconsolidation of emotional versus neutral declarative memory. In Chapter 6 we tested whether post-retrieval stress would affect the contextualization of emotional memory. The experimental findings of this thesis are discussed in Chapter 7.