Erasing fear from memory
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Chapter 1

General Introduction
Emotional memory is critical for adequate adaptation to future risks. We obtain our knowledge about the emotional significance of objects and events through ‘direct experiences’, ‘observational learning’, and ‘language’ (Rachman, 1977). From an evolutionary perspective, it is extremely functional to never forget the most important events in life. However, ‘emotional memory’ may also become harmful and maladaptive, such as in patients with anxiety disorders. A valuable experimental model for the pathogenesis of anxiety disorders is that they originate from a learned association between a previously neutral or ambiguous event (i.e., Conditioned Stimulus, CS) (e.g., stranger) and an anticipated disaster (i.e., Unconditioned Stimulus, US) (e.g., physical attack). Patients suffering from anxiety disorders feel, think, and act as if a feared stimulus (i.e., CS) predicts the later occurrence of a negative outcome (i.e., US). They either persist in fear responding whilst the acute threat already disappeared or they fear cues that are intrinsically non-threatening.

Anxiety disorders rank among the most prevalent and chronic forms of psychopathology with prevalences up to 12% over the past 12 months (e.g., Vollebergh et al., 2003). Although Cognitive Behavioral Therapy (CBT) is highly effective in treating most anxiety disorders, 19 to 62% of the patients experience a relapse¹ (Craske, 1999). Exposure-based interventions, involving in vivo or imaginary confrontations with the feared object (i.e., unpaired CS presentations), are a crucial component of CBT for anxiety disorders. The prevailing view on the return of fear after apparently successful treatment is that ‘exposure’ can eliminate all fearful responding, but leaves the original fear memory intact (i.e., CS-US). Exposure solely involves the formation of a new inhibitory memory trace (i.e., CS-noUS) that competes with the original fear memory (i.e., CS-US) (Bouton 1993, 2002; LeDoux, 1995). Partial or full reappearance of fear may thus be explained by intact fear memories that resurface. Once a fear memory has been established, it is held to be forever. Fresh insights from neuroscience, however, suggest that it is unnecessarily defeatist to regard fear memory as permanent.

¹ This percentage depends on population, time intervals, and operationalization of the return of fear.
Reconsolidation: An Update Mechanism

Claims about the indelibility of emotional memory originally derived from research on memory ‘consolidation’ (e.g., McGaugh, 1966). Memory consolidation refers to a time-dependent process by which newly learned information is transformed into stable long-term memory (LTM). Crucial for the consolidation of memory is the process of long-term potentiation (LTP), a long-lasting form of synaptic plasticity, which is assumed to rely on the synthesis of proteins (e.g., Davis & Squire, 1984; Bailey & Kandel, 1993, Dudai & Morris, 2000; Kandel, 2001). This assumption followed from research showing that post-training administrations of protein synthesis inhibitors disrupt the formation of LTM (e.g., Flexner et al., 1965). Other treatments such as ‘electroconvulsive shock’ (e.g., Duncan, 1949) or ‘new competing learning’ have also been shown to disrupt LTM formation. Critically, amnesic treatments targeting the process of consolidation are only effective when administered shortly after initial learning. For instance, the protein synthesis inhibitor ‘anisomycin’ is capable of inducing amnesia when administered shortly after training but not after a delay of 6 hr (e.g., Schafe & LeDoux, 2000). Over the years, such time-dependent effects led to the notion that memory exists in two stages (i.e., ‘consolidation theory’): a labile state, in which it is sensitive to disruption, and a stable state, in which memory is thought to be ‘fixed’ and no longer susceptible to previously effective amnesic treatments (McGaugh, 1966).

The assumption that memories are consolidated over time into a permanently ‘fixed’ state was initially challenged in 1968 (Misanen et al., 1968). In line with the earlier studies on memory consolidation (Duncan, 1949), electroconvulsive shock did not affect memory performance when given 24 hr after initial learning. However, if the memory was ‘reactivated’ before electroconvulsive shock administration, the performance on a memory-recall task was impaired 24 hr later (Misanen et al., 1968). The reactivation of the consolidated memory presumably returned it to a labile state during which it was vulnerable to disruption (see Fig. 1.1). This phenomenon is now referred to as ‘reconsolidation’ (Spear, 1973; Przybylslawski & Sara, 1997). Unfortunately, for reasons that remain unclear, the dozens of studies demonstrating the effect across species and tasks had little impact in the field of memory research in those days (e.g., DeVietti & Holliday,
1972; DeVietti & Kirkpatrick, 1976; a detailed review of this literature is provided by Sara, 2000).

**Fig. 1.1.** The process of reconsolidation, the protein synthesis dependent restabilization of a memory upon retrieval, enables the modification of memory representation.

Research on the reconsolidation effect was revitalized by a study on fear memory in the rat (Nader et al., 2000). Nader and colleagues (2000) showed that a reminder cue could bring well-consolidated fear memories back to a labile state, where they could be disrupted by infusing the protein-synthesis inhibitor ‘anisomycin’ directly into the ‘amygdala’, a memory system known to be critical for fear learning (LeDoux, 1996; Davis, 1997). Such impairments after drug administrations were not observed in the absence of memory reactivation. The amnesic treatment was also ineffective 6 hr after memory reactivation, demonstrating that the post-reactivation restabilization process was time-dependent, like ‘consolidation’. Together, these findings strongly support and extend the earlier conclusions from the 1970s that the reactivation of a ‘fixed’ and consolidated memory returns it to a labile state from which it has to restabilize over time via *de novo* protein synthesis (Nader et al., 2000). Since then, numerous animal studies have demonstrated that disrupting the reconsolidation process engaged during retrieval prevents memory restorage and produces amnesia for the original (fear) learning (Eisenberg et al., 2003; Duvarci & Nader, 2004; Suzuki et al., 2004).
Given that reconsolidation provides an update mechanism through which original fear memories (i.e., CS-US) can be permanently changed (Nader et al., 2000), targeting the process of reconsolidation may point to a promising alternative strategy in the treatment of anxiety disorders, such as posttraumatic stress disorder. Hence, one important direction for reconsolidation research is its demonstration in human fear memory. However, in animals, the phenomenon of reconsolidation is typically studied using protein synthesis inhibitors (e.g., ‘anisomycin’) that are directly injected into the amygdala, which is not feasible in humans. Recently, a study in rats demonstrated that the systemic administration of propranolol HCl shortly after the reactivation of a previously acquired fear memory also resulted in amnesia for the original fear learning (Dębiec & LeDoux, 2004). Propranolol HCl, a noradrenergic β-blocker\(^2\), is supposed to specifically act on the β-adrenergic receptors in the basolateral amygdala (McGaugh, 2004), thereby inhibiting the ‘noradrenaline-induced’ CREB phosphorylation (Thonberg et al., 2002). Several lines of evidence suggest that CREB (i.e., cAMP response element binding) is one of the transcription factors regulating the synthesis of proteins necessary for the formation of long-term (fear) memory (e.g., Guzowski & McGaugh, 1997; Josselyn et al., 2001; Davies et al., 2004). Indeed, in humans, the β-blocker propranolol HCl has been shown to impair the formation of memory for emotionally arousing material (Cahill et al., 1994; van Stegeren et al., 1998; Hurlemann et al., 2005). Accordingly, β-blockers are currently being evaluated as potential agents for the secondary prevention of PTSD (Pitman et al., 2002; Vaiva et al., 2003). However, given that it is often not possible to administer a ‘consolidation-blocking’ agent at an initial trauma or triggering event, the possibility of later eliminating fear memory by pharmacologically disrupting reconsolidation is of particular clinical relevance. Thus, taken together, a crucial question is whether propranolol HCl also disrupts the reconsolidation of human fear memory. If we can modify the original fear memory (i.e., CS-US) through targeting the process of reconsolidation, then we might be able to provide a long-term cure for patients suffering from anxiety disorders.

\(^2\) The drug propranolol HCl is a very common ‘β-blocker’ discovered in the late fifties by Nobel Prize winner James W. Black and is prescribed by GPs every day, mainly for the treatment of hypertension.
Experimental Model of Fear

Pavlovian fear conditioning serves a well-controlled experimental model to unravel the processes and mechanisms underlying fear memory in humans. In a typical fear conditioning procedure in the laboratory, a neutral or ambiguous stimulus (i.e., Conditioned Stimulus, CS) (e.g., tone) acquires the capacity to elicit fear responses _after_ the pairing with an intrinsically aversive event (i.e., Unconditioned Stimulus, US) (e.g., electric stimulus). A large body of evidence indicates that the ‘amygdala’ is critically involved in the acquisition and expression of this conditioned fear responding (i.e., CRs) (LeDoux, 1996, 2000; Davis, 1997). By consequence, the presentation of the feared stimulus (i.e., CS) activates the ‘amygdala’ among several other brain regions, which in turn drives fear behavior (i.e., CRs). Even though Pavlovian fear conditioning is not necessarily the means through which human fear originates (Rachman, 1977), it offers an excellent model of ‘associative learning’, which is considered to play an important role in the aetiology of anxiety disorders.

The effect of ‘exposure therapy’ on anxiety disorders is often attributed to the Pavlovian extinction of fear responding (Bouton, 1988). In a typical extinction procedure, a stimulus (CS; e.g., tone) that has acquired the ability to elicit fear reactions through conditioning is repeatedly presented in the absence of the aversive event. Though extinction is highly effective in eliminating all fearful responding, it does not destroy the original learning (i.e., CS-US) but instead generates new learning (i.e., CS-noUS) that acts to inhibit or competes with the original fear association (Bouton, 1993; LeDoux, 1995). That is, during the retrieval of extinction learning (i.e., CS-noUS), the ventral medial prefrontal cortex (vmPFC) is thought to inhibit the amygdala so that the feared stimulus (i.e., CS) is prevented from eliciting conditioned fear responding (i.e., CRs) (Milad et al., 2006; Rauch et al., 2006; Sotres-Bayon et al., 2006; Milad et al., 2007). However, several animal as well as human experimental studies show that a variety of post extinction processes can prevent the vmPFC from inhibiting the amygdala, thereby allowing the original fear memory to recover (i.e., CS-US) (Bouton, 2002, 2004; Hermans et al., 2006). For instance, if the aversive event (i.e., US) is presented on its own following extinction learning, it can cause ‘reinstatement’ of the extinguished fear responding to the conditioned stimulus (i.e., CS; e.g., Rescorla & Heth, 1975). A return of fear can also occur when the context is changed after extinction learning.
(i.e., ‘renewal’; e.g., Bouton & Bolles, 1979). Moreover, if time elapses following fear extinction, the extinguished responding can recover ‘spontaneously’ (i.e., ‘spontaneous recovery’, e.g., Baum, 1988). The rapid ‘reacquisition’ of fear post extinction learning further demonstrates that the original fear association (i.e., CS-US) is not destroyed but rather ‘saved’ (e.g., Napier et al., 1992; Ricker & Bouton, 1996). From a clinical perspective, post extinction retrieval effects may provide the mechanisms of relapse after successful exposure therapy (Bouton, 1988). At the same time, post extinction retrieval techniques (i.e., ‘reinstatement’, ‘renewal’, ‘spontaneous recovery’, and ‘reacquisition’) offer a potent means to trigger the original fear memory in the experimental setting. For that reason, the Pavlovian fear conditioning paradigm is well suited to investigate whether targeting the process of reconsolidation results in a permanent reduction of fear responding.

**Outcome Measures of Human Fear Conditioning:**

Conditioned fear responding in humans is typically assessed on a subjective as well as a physiological level. On the subjective level, participants are for instance asked to what extent they expect a stimulus (CS) to be followed by an aversive event (US) (i.e., US expectancy ratings) or to rate their subjectively experienced distress (i.e., anxiety, tension, or nervousness) during a CS presentation (i.e., distress ratings). The physiological component of fear is usually measured by the ‘eyeblink’ startle reflex or skin conductance responding.

Startle in response to an intense stimulus with a sudden onset is a universal reflex that serves a protective function and involves multiple motor actions, the most robust of which is the eyeblink (Landis & Hunt, 1939). The amplitude of the eyeblink is therefore typically used to index startle magnitude in humans. Startle procedures involve electromyogram (EMG) measurements in which muscle activity is assessed from electrodes placed over the orbicularis oculi muscle (i.e., just beneath the lower eyelid). The most commonly used startle-eliciting stimulus is the ‘startle probe’, a loud noise that is presented during a stimulus or in the interval between two stimulus presentations (i.e., intertrial interval). Eyeblink reflexes to the loud noise elicited during aversive states (e.g., in anticipation of an electric stimulus) are potentiated as compared to responses evoked during neutral states (Lang et al., 1990). Conditioned fear responding is indexed by a larger eyeblink to probes presented during a conditioned stimulus (i.e., CS1’) relative to a
control stimulus (i.e., CS2') that is never paired with the US (e.g., electric stimulus). Given that human startle potentiation is only observed during aversive fear conditioning (Weike et al., 2007), it is considered to be a reliable and specific index of fear (Hamm & Weike, 2005). Startle potentiation is directly connected with and modulated by the ‘amygdala’ and fear-conditioning procedures yield highly reliable and robust startle potentiation (Davis, 2006).

Skin conductance responding (SCR) refers to electrodermal activity caused by the activity of sweat glands (Lykken & Venables, 1971). The skin conductance response is typically measured by electrodes attached to the first and third fingers of the non-preferred hand. Similar to the startle reflex, conditioned responding is reflected by larger skin conductance responses in reaction to the CS1+ as compared to the CS2-. However, a main disadvantage of SCR discrimination is that it is not only observed for aversive conditioning but also for nonaversive, but arousing events (e.g., reaction time tasks or positive pictures) (e.g., Hamm & Vaitl, 1996). Thus, electrodermal conditioning seems to primarily reflect ‘anticipatory arousal’ rather than emotional learning (Weike et al., 2007).

**Outline of the Present Thesis**

The aim of the present thesis is to test whether targeting the process of reconsolidation results in a permanent reduction of fear. We addressed this issue by using a differential fear conditioning paradigm with fear-relevant stimuli (e.g., pictures of spiders or guns). We employed fear-relevant stimuli because they lead to a superior conditioning of aversive associations and are especially resistant to extinction learning compared with fear-irrelevant cues (Mineka & Öhman, 2002; Lang et al., 2005). Moreover, given that most anxiety disorders are associated with these categories of stimuli, we are specifically interested in targeting stronger fear memory. In order to maximize the likelihood of fear memory expression, we used all of the well-established post-extinction retrieval techniques (i.e., ‘reinstatement’, ‘spontaneous recovery’, ‘reacquisition’, and ‘renewal’) over the various experiments.

In **Chapter 2** we tested whether disrupting reconsolidation by the oral administration of propranolol HCl prior to memory reactivation would diminish fear responding and prevent the return of fear relative to placebo pill. Next, in **Chapter 3**, we tested if the positive findings from **Chapter 2** could be replicated
and whether the fear-erasing effects would persist over time or recover spontaneously. However, in Chapter 2 and Chapter 3, the typical differential fear conditioning paradigm (i.e., CS1-US vs. CS2) did not allow any inference about the nature of the fear memory ‘erasure’. That is, due to the systemic (as opposed to intra-amygdala) drug administration, the erasure of the startle fear responding could also have resulted from a more diffuse effect of the propranolol HCl manipulation by reducing the fear-provoking aspects of the aversive consequence itself (i.e., US). In considering clinical implications, disrupting reconsolidation should not radically alter functional reactions to potentially dangerous situations (i.e., US), but ‘selectively’ weaken the underlying maladaptive fear association (i.e., CS1-US). On the other hand, disrupting reconsolidation should not be restricted to the feared cue itself but rather spread to category-related information considering that fear generalization is a main characteristic of anxiety disorders. In Chapter 4, we addressed these issues by using a within-subject differential fear conditioning paradigm allowing selective reactivation of one of two categorically distinct fear associations sharing the same aversive outcome (i.e., US) and a test of fear generalization. We further tested in Chapter 4 whether a behavioral approach targeting the process of reconsolidation through extinction learning was also effective in weakening the original fear memory. Given that ‘strong’ fear memory has been proposed to prevent reconsolidation from occurring, we first tested in Chapter 5 whether we could strengthen fear memory by stimulating noradrenergic activity during fear learning (i.e., yohimbine HCl). Next, we tested in Chapter 6 whether stimulation of the noradrenergic system during memory formation would impair the disruption of reconsolidation. We also tested in Chapter 6 whether the noradrenaline-induced strengthening of fear memory would broaden the generalization of fear responding. In order to discard the effect of the propranolol HCl manipulation on the memory retrieval itself, we further tested in Chapter 6 whether the β-blocker could also be administered after reactivation of the memory. As disrupting reconsolidation had thus far only been shown to diminish the emotional expression of fear memories (i.e., startle fear responding), we tested in Chapter 7 whether targeting the process of reconsolidation would diminish the subjective feelings of anxiety. Moreover, considering that patients with anxiety disorders often fear objects and situations that they have never actually experienced, we tested in Chapter 7 whether an aversive event that was only ‘imagined’ (i.e., instructed fear learning) instead of really experienced (i.e.,
Pavlovian fear conditioning) would also undergo reconsolidation. Finally, in Chapter 8, the main findings of the studies presented in this thesis are summarized and discussed.

References


Chapter 1


