nature neuroscience

Beyond extinction: erasing human fear responses and preventing the return of fear

Merel Kindt, Marieke Soeter & Bram Vervliet

Animal studies have shown that fear memories can change when recalled, a process referred to as reconsolidation. We found that oral administration of the β-adrenergic receptor antagonist propranolol before memory reactivation in humans erased the behavioral expression of the fear memory 24 h later and prevented the return of fear. Disrupting the reconsolidation of fear memory opens up new avenues for providing a long-term cure for patients with emotional disorders.

Since the dawn of psychology at the end of the nineteenth century, psychologists and psychiatrists have tried with dozens of pharmacological and psychological treatments to change undesired emotional memory. However, even the most effective treatments only eliminate fearful responding, leaving the original fear memory intact[1], as is substantiated by the high percentages of relapse after apparently successful treatment[2]. Once emotional memory is established, it appears to last forever. From an evolutionary perspective, it is extremely functional to never forget the most important events in life. However, the putative indelibility of emotional memory can also be harmful and maladaptive, such as in some trauma victims who suffer from dreadful memories and anxiety. If emotional memory could be weakened or even erased, then we might be able to eliminate the root of many psychiatric disorders, such as post-traumatic stress disorder. Recently, it was rediscovered that fear memory in animals is not necessarily permanent but can change when retrieved[3–5]. The reactivation of a consolidated (fear) memory can return it to a labile, supposedly protein synthesis–dependent state, a process that is referred to as reconsolidation[4]. Reconsolidation of fear memory can be influenced by neurobiological manipulations during or shortly after the reactivation period[5]. These manipulations are thought to alter protein synthesis directly[4] or by interacting with the release of neurotransmitters (for example, norepinephrine) in the amygdala[6,7]. At the behavioral level, this may lead to changes in later expressions of that fear memory. In particular, infusion of propranolol into the amygdala of rats shortly after the reactivation period of a previously acquired fear association impaired the fear expression on a long-term test. Apparently, propranolol disrupts the reconsolidation of reactivated fear memories[8]. Animal and human studies have shown that adrenal stress hormones activate adrenergic receptors in the amygdala and that the basolateral amygdala is essential for fear memory[7,9].

In this human study, we tested the hypotheses that the fear response can be weakened by disrupting the reconsolidation process and that disrupting the reconsolidation of the fear memory will prevent the return of fear. To test these hypotheses, we used a differential fear-conditioning procedure with fear-relevant stimuli. Testing included different phases across 3 d: fear acquisition (day 1), memory reactivation (day 2), and extinction followed by a reinstatement procedure and a test phase (day 3) (**Supplementary Figs. 1** and **2** online). The conditioned fear response was measured as potentiation of the eyeblink startle reflex to a loud noise (40 ms, 104 dB) by electromyography of the right orbicularis oculi muscle. Stronger startle responses to the loud noise during the fear-conditioned stimulus ($CS1^+$) as compared with the control stimulus ($CS2^-$) reflects the fearful state of the participant elicited by $CS1^+$. Startle potentiation taps directly into the amygdala, and fear-conditioning procedures yield highly reliable and robust startle potentiation[10]. In addition, declarative knowledge of the contingency between the conditioned stimulus and the unconditioned stimulus was measured through online shock-expectancy ratings during each conditioned stimulus presentation. Reconsolidation of fear memory was manipulated by administration of propranolol (40 mg, $n = 20$), randomized and double-blind placebo controlled ($n = 20$) (see **Supplementary Methods** online). For the additional control condition ($n = 20$), propranolol (40 mg) was administered without memory reactivation.

Analysis of variance showed fear conditioning on day 1 (stimulus × trial, $F_{1,38} = 46.91$, $P < 0.001$, $\eta^2 = 0.55$; **Fig. 1**). We observed no difference in fear learning between the propranolol and placebo group (stimulus × trial × condition, $F_{1,38} < 1.37$; **Supplementary Data** online). On day 2, the two groups expressed comparable levels of startle response during the fear memory reactivation ($t_{38} < 1$). In addition, the conditioned fear memory was equally well consolidated in the two groups, as is indicated by both the absence of a main effect of trial from the last three acquisition trials to the reactivation trial ($F_{1,38} < 1$) and the absence of a trial × condition interaction effect ($F_{1,38} < 1$). These data demonstrate that propranolol did not directly affect the expression of the fear memory. Propranolol also did not reduce the startle response *per se*, as we found no effects of propranolol on the habituation trials (main effect of condition and trial × condition interaction, $F_{1,35} < 1$; **Supplementary Fig. 3** online).

In contrast with the pill placebo condition, the administration of propranolol significantly decreased the differential startle response 48 h later (**Fig. 1a**,**c**), that is, from acquisition (trial 6–8, day 1) to extinction (trial 1–3, day 3; stimulus × trial × condition, $F_{1,38} = 17.17$, $P < 0.001$, $\eta^2 = 0.31$). *Post hoc* comparisons showed that propranolol strongly reduced the expression of fear memory (stimulus × trial, $F_{1,19} = 25.47$, $P < 0.001$, $\eta^2 = 0.57$), whereas the differential startle response remained stable in the pill placebo condition (stimulus × trial, $F_{1,19} < 1$). In the propranolol condition, the conditioned

Department of Clinical Psychology, University of Amsterdam, Roetersstraat 15, 1018 WB Amsterdam, The Netherlands. Correspondence should be addressed to M.K. (m.kindt@uva.nl).

Received 3 November 2008; accepted 9 January 2009; published online 15 February 2009; doi:10.1038/nn.2271

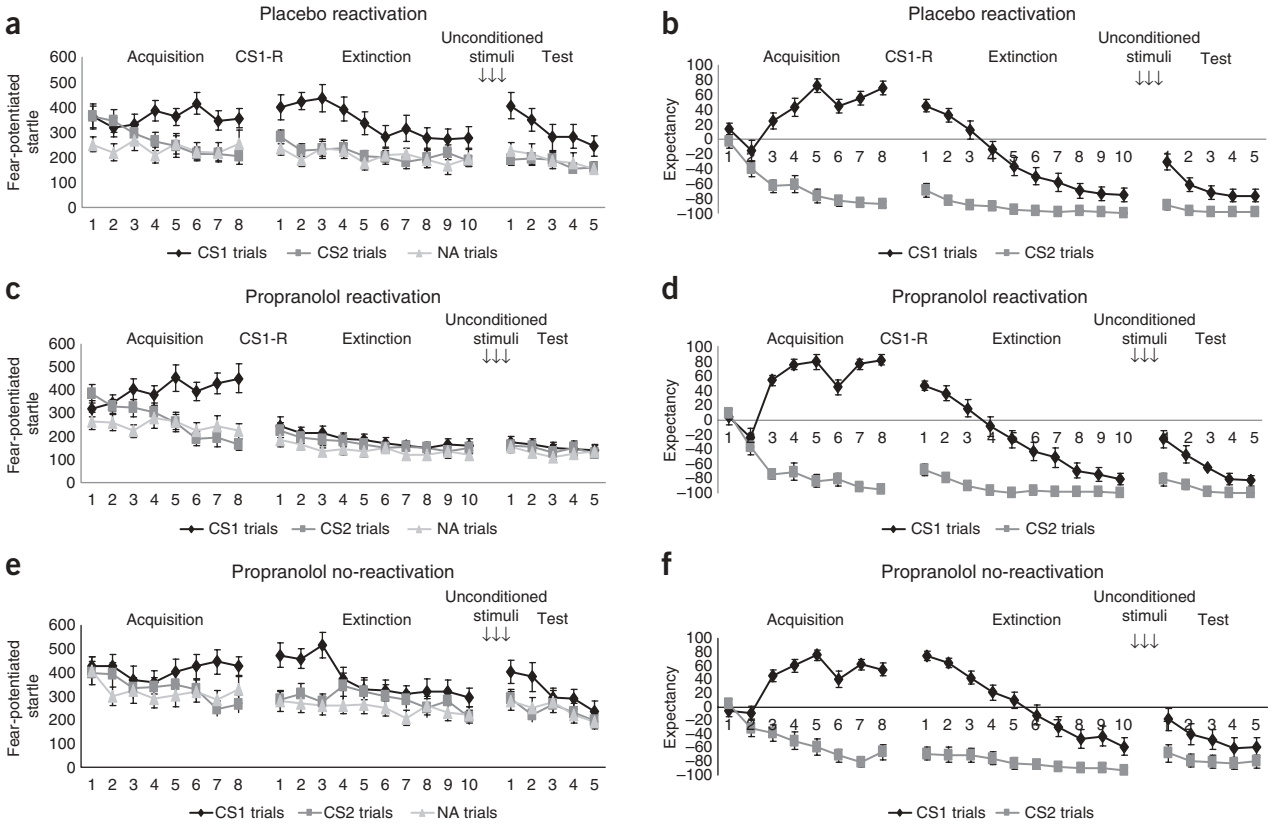

Figure 1 Propranolol disrupts the reconsolidation of a fear memory, but not declarative memory. (**a**–**f**) Mean startle potentiation to the fear-conditioned stimulus (CS1), the control stimulus (CS2) and noise alone (NA) trials (left) and mean expectancy scores of the unconditioned stimulus to CS1 and CS2 trials (right) during acquisition (trial 1–8), extinction (trial 1–10) and test (trial 1–5) for the placebo ($n = 20$, **a**,**b**), propranolol reactivation ($n = 20$, **c**,**d**) and propranolol without reactivation ($n = 20$, **e**,**f**) group. $CS1^+$ refers to the fear conditioned stimulus during acquisition, $CS1^-$ refers to the fear conditioned stimulus during extinction and test, CS1-R refers to the reactivation of the fear conditioned stimulus and $CS2^-$ refers to the control stimulus during all phases of the experiment. Error bars represent s.e.m.

fear response was not only reduced but even eliminated, as we no longer observed the differential startle response (extinction trial 1–3, day 3; $t_{19} < 1.22$). In contrast, the differential startle response remained significant in the placebo condition ($t_{19} = 5.26$, $P < 0.001$, two tailed).

Given that the differential startle response was already eliminated in the propranolol condition, the two groups differed over the course of extinction training on day 3 (stimulus × trial × condition, $F_{1,38} = 5.38$, $P < 0.05$, $\eta^2 = 0.12$). *Post hoc* comparisons showed a significant decrease of the differential startle response in the placebo condition (stimulus × trial, $F_{1,19} = 11.31$, $P < 0.005$, $\eta^2 = 0.37$), but no change of the differential startle response in the propranolol condition (stimulus × trial, $F_{1,19} < 1$) (**Fig. 1a**,**c**). At the end of extinction (trial 8–10), the differential startle response was still lower in the propranolol condition than in the placebo condition (stimulus × condition, $F_{1,38} = 7.94$, $P < 0.01$, $\eta^2 = 0.17$).

Exposure to the aversive stimulus (unconditioned stimulus) following extinction has been shown to reinstate the expression of the original fear memory in animals[1] and humans[11]. Evidence for a reinstatement effect is indicated by an increase of the differential startle response from the last extinction trials (trial 8–10) to the first test trial. Comparison of the reinstatement effect between the propranolol and placebo condition showed significantly more reinstatement in the placebo condition (stimulus × trial × condition, $F_{1,37} = 8.72$, $P < 0.01$, $\eta^2 = 0.19$). We observed a significant reinstatement effect in the placebo condition (stimulus × trial, $F_{1,18} = 10.33$, $P < 0.01$, $\eta^2 = 0.37$; **Fig. 1a**) but not in the propranolol condition (stimulus × trial, $F_{1,19} < 1$; **Fig. 1c**). The reinstatement procedure did even not reveal any differential startle response to the first test trial in the propranolol group ($t_{19} < 1$).

To determine whether the effect of propranolol requires active retrieval of the fear memory, we administered propranolol to another fear-conditioned group ($n = 20$) without memory reactivation. Omission of memory reactivation after propranolol intake yielded normal fear responses and a return of fear 48 h after acquisition (stimulus × trial × condition, $F_{1,38} < 1.2$; **Fig. 1e** and **Supplementary Data**). Analysis of variance showed a different pattern for the contingency learning data, with no effects of propranolol (stimulus × trial × condition, $F_{1,38} < 1$; **Fig. 1b**,**d**,**f**).

In sum, oral administration of the β-adrenergic receptor antagonist propranolol before reactivation of a fear memory resulted in a substantial weakening of the fear response. We used fear-relevant stimuli (pictures of spiders) because these are especially resistant to extinction following fear conditioning[12]. Even more notable is our finding that one reactivation trial combined with the administration of propranolol completely eliminated the behavioral expression of the fear memory 24 h later. Second, our finding that a well-established retrieval technique for fear memories (reinstatement) failed to uncover any fear response suggests that the fear memory may either be erased (storage theory) or may be unavailable as a result of retrieval failure (retrieval theory)[5]. Note that no behavioral

procedure is currently available that differentiates between these two views of amnesia[13]. Notably, the propranolol manipulation left the declarative memory for the acquired contingency between the conditioned and unconditioned stimulus intact, but this knowledge no longer produced emotional effects. Our finding that propranolol eliminated the fear response, without affecting declarative memory, is consistent with the observed double dissociation of fear conditioning and declarative knowledge relative to the amygdala and hippocampus in humans[14]. Propranolol selectively acts on the β-adrenergic receptors in the amygdala during emotional information processing in animals and humans[7,9]. It may be hypothesized that beta-adrenergic blockade during reconsolidation may selectively disrupt the protein synthesis of the amygdalar fear memory, resulting in deconsolidation of the fear memory trace while leaving the declarative memory in the hippocampus untouched.

Our findings are consistent with those of a recent preliminary study of patients with post-traumatic stress disorder in which post-retrieval propranolol seemed to reduce subsequent physiological responding to traumatic memory[15]. Together, these results strongly suggest that β-adrenergic receptors are critically involved in the reconsolidation process of conditioned fear memories in humans. It is clear that β-adrenergic blockade during reconsolidation outperformed the traditional extinction procedure. But most importantly, and in contrast with the traditional extinction procedure, disrupting reconsolidation of fear memory prevented the return of fear. Millions of people suffer from emotional disorders and the relapse of fear, even after successful treatment. Our findings may have important implications for the understanding and treatment of persistent and self-perpetuating memories in individuals suffering from emotional disorders.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We thank B. Molenkamp for technical help. This work was supported by a Vici grant (M.K.) from The Netherlands Organization for Scientific Research.

AUTHOR CONTRIBUTIONS

M.K and M.S. designed the study. M.S. collected data. M.K. and M.S. analyzed the data, wrote the initial manuscript and were involved in the revision process. All authors discussed the results and commented on the manuscript.

Published online at http://www.nature.com/natureneuroscience/
Reprints and permissions information is available online at http://www.nature.com/reprintsandpermissions/

1. Bouton, M.E. *Biol. Psychiatry* **52**, 976–986 (2002).
2. Craske, M.G. *Anxiety Disorders: Psychological Approaches to Theory and Treatment* (Westview Press, Boulder, Colorado, 1999).
3. Dudai, Y. *Curr. Opin. Neurobiol.* **16**, 174–178 (2006).
4. Nader, K., Schafe, G.E. & LeDoux, J.E. *Nature* **406**, 722–726 (2000).
5. Tronson, N.C. & Taylor, J.R. *Nat. Rev. Neurosci.* **8**, 262–275 (2007).
6. Canal, C.E. & Gold, P.E. *Behav. Neurosci.* **121**, 732–741 (2007).
7. McGaugh, J.L. *Annu. Rev. Neurosci.* **27**, 1–28 (2004).
8. Debiec, J. & LeDoux, J.E. *Neuroscience* **129**, 267–272 (2004).
9. van Stegeren, A.H. *et al. Neuroimage* **24**, 898–909 (2005).
10. Davis, M. *Am. Psychol.* **61**, 741–756 (2006).
11. Norrholm, S.D. *et al. Learn. Mem.* **13**, 681–685 (2006).
12. Mineka, S. & Ohman, A. *Biol. Psychiatry* **52**, 927–937 (2002).
13. Nader, K. & Wang, S.-H. *Learn. Mem.* **13**, 530–535 (2006).
14. Phelps, E.A. *Curr. Opin. Neurobiol.* **14**, 198–202 (2004).
15. Brunet, A. *et al. J. Psychiatr. Res.* **42**, 503–506 (2008).