Disruption of Reconsolidation Erases a Fear Memory Trace in the Human Amygdala

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Memories become labile when recalled. In humans and rodents alike, reactivated fear memories can be attenuated by disrupting reconsolidation with extinction training. Using functional brain imaging, we found that, after a conditioned fear memory was formed, reactivation and reconsolidation left a memory trace in the basolateral amygdala that predicted subsequent fear expression and was tightly coupled to activity in the fear circuit of the brain. In contrast, reactivation followed by disrupted reconsolidation suppressed fear, abolished the memory trace, and attenuated fear-circuit connectivity. Thus, as previously demonstrated in rodents, fear memory suppression resulting from behavioral disruption of reconsolidation is amygdala-dependent also in humans, which supports an evolutionarily conserved memory-update mechanism.

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xiety disorders are common, and they cause great suffering and high societal costs (1). The etiology involves amygdala-dependent memory mechanisms that link stressful events to previously neutral stimuli (2), and the amygdala has been demonstrated to be hyper-responsive across the anxiety disorders (3). Pharmacological and behavioral treatments of anxiety reduce symptomatology and amygdala activity (4) but have limited success because lapses-occur (5). However, fear memories may be erased by recalling them and preventing their reconsolidation (6, 7). In rodents, the amygdala seems vital for fear memory reconsolidation (7, 8), but this has not been investigated in humans.

Fear conditioning, in which a previously neutral stimulus turns into a conditioned stimulus (CS) through pairings with an aversive stimulus, forms a memory trace in the amygdala (2). Memory activation produces behavioral (2, 9) and autonomic fear reactions, such as skin conductance responses (SCRs) (10–12), frequently used to measure fear learning. Studies in animals (13) and anxiety patients (14) demonstrate that extinction weakens, but does not erase, fear memories. In rodents (13) and humans (15) alike, extinction attenuates conditioned fear expression through prefrontal inhibition. Fear can return after stress be renewed when altering the environmental context, or reoccur with the passage of time (16).

By activating memories and disrupting their reconsolidation, through protein synthesis blockade-local in the amygdala (8) or through systemic administration of β-adrenergic receptor antagonists (17, 18), fear memories are inhibited. Fear memory reconsolidation can also be disrupted behaviorally (6, 7, 19). In rodents, extinction of fear conditioning performed 10 or 60 min after presenting a reminder of the conditioned fear, but not after 6 or 24 hours, inhibited fear expression (7). Fear did not return in a new context, after shock exposure, or with time. Thus, extinction conducted within, but not outside, the reconsolidation window resulted in permanent attenuation of the fear memory (7).

In humans, extinction performed within the reconsolidation interval also inhibited fear, whereas extinction training performed outside of the reconsolidation interval spared the memory and fear returned (6). In animals, the neural functions enabling fear memory formation and reconsolidation are located in the amygdala (2, 7–9, 20). In humans, lesion (21) and brain imaging studies (10–12, 22) confirm that the amygdala is a key area for fear memory encoding.

To test the hypothesis that reconsolidation in humans is amygdala-mediated and that disruption of reconsolidation inactivates a memory trace in the basolateral amygdala, we performed a study combining brain imaging with a physiological measure of fear.

On day 1, twenty-two subjects (11 women) aged 24.0 ± 0.48 (mean ± SEM) underwent fear conditioning to establish an associative fear memory (Fig. 1A and fig. S1). On day 2, the fear memory was reactivated by presenting the cue previously paired with the shock (CS+) for 2 min. Subjects were randomized into two groups. One group received extinction, consisting of repeated CS presentations with the shock withheld, 10 min after reactivation and thus within the reconsolidation interval. The other group received extinction 6 hours after the reactivation—i.e., outside of the interval. Fear expression was measured using SCRs (6, 19). On day 3, a renewal session was performed in a new environment, a magnetic resonance scanner, where shock electrodes were attached, although no shocks were delivered. SCRs were not measured for technical reasons. On day 5, subjects were exposed to unsignaled shocks and then re-exposed to CS+. Return of fear was defined as the increase in SCR from the last extinction trial on day 2 to the first reinstatement trial on day 5 (Fig. 1B) (6).

First, we evaluated if the predicted behavioral reinstatement effect was present on day 5. Confirming this, increased fear responding was observed in the 6 hours, but not the 10 min group

References

5. Materials and methods are available as supplementary materials on Science Online.
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14. First, we evaluated if the predicted behavioral reinstatement effect was present on day 5. Confirming this, increased fear responding was observed in the 6 hours, but not the 10 min group.
Groups were indistinguishable in acquisition and extinction (Fig. 1B and fig. S1). Next, we tested the hypothesis that the fear memory representation is localized to the amygdala. Significantly greater activity was evident bilaterally in the basolateral amygdala in the 6 hours group as compared with the 10 min group (Fig. 1B).

We then tested if the amygdala-localized memory predicted return of fear. Positive correlations were present between return of fear and blood oxygen level-dependent (BOLD) activity bilaterally in the basolateral amygdala in the 6 hours group (Fig. 2A). In the 10 min group, a cluster in the right claustrum extending into the amygdala correlated significantly with SCRs (Fig. 2A). BOLD activity reflecting the amygdala-localized memory trace also correlated with fear recall during extinction the previous day in the 6 hours, but not the 10 min, group (Fig. 2B).

**Fig. 1.** Extinction during reconsolidation blocks reinstatement of fear and abolishes a memory trace in the amygdala. (A) After fear conditioning on day 1, when 16 shocks were paired with a visual cue, a memory reminder was given on day 2, and extinction was performed after 10 min or 6 hours, by exposure to eight conditioned cues with no shocks. On day 3, amygdala activity was assessed with functional magnetic resonance imaging (fMRI) during renewal-induced fear. On day 5, extinction was performed after 10 min or 6 hours, but not the 10 min, group (Fig. 1B). The autonomic nervous system measure of fear is the SCR. The CNS measure of amygdala activity is BOLD activity. Brain coordinates are according to the Montreal Neurological Institute (MNI). Error bars are standard error of means.

**Fig. 2.** Amygdala activity predicts return of fear and correlates with recall of fear. (A) In the 6 hours group (top), activity bilaterally in the basolateral amygdala predicted return of fear 2 days later ($x,y,z=21,-1,-17$; $Z=2.06$; $P=0.002$; $999$ mm$^3$; $x,y,z=-21,-4,-14$; $Z=2.38$; $P=0.009$; $1107$ mm$^3$). In the 10 min group (bottom), an area in the right temporal claustrum extending into the amygdala was also related to SCR ($x,y,z=33,2,-23$; $Z=2.49$; $P=0.006$; $324$ mm$^3$). Because fear did not return in the 10 min group, the correlation may reflect individual brain–behavior relations unrelated to fear and the experimental manipulation. (B) In the 6 hours group (top), recall of fear during extinction covaried with the strength of amygdala activity bilaterally ($x,y,z=24,-1,-20$; $Z=2.35$; $P=0.009$; $378$ mm$^3$; $x,y,z=-15,4,-17$; $Z=2.27$; $P=0.012$; $189$ mm$^3$). No covariation existed in the 10 min group (bottom).
Amygdala areas harboring the memory trace (Fig. 1B) and areas covarying with return of fear (Fig. 2A) overlapped in the 6 hours group only (Fig. 3A). Moreover, the memory trace was colocalized to areas involved in fear memory recall during extinction (Fig. 3B). Finally, all these areas overlapped with each other only in the 6 hours group (Fig. 3C). Thus, the localization of the memory trace in the amygdala overlapped bilaterally with areas related both to recall of fear during extinction and return of fear during reinstatement. The hypothesis that memory was not erased, but only suppressed, by extinction-mediated prefrontal inhibition was not supported because the theoretically predicted $13,15$ negative coupling between activity in the ventromedial prefrontal cortex (vmPFC) and return of fear was absent because vmPFC activity did not correlate negatively with fear in either group ($Z$-scores of $<1$).

Finally, we evaluated if activation of the fear memory in the amygdala was linked to activity in other nodes of the fear network (23) by calculating the covariation between memory-associated amygdala activity and activity in the remaining network. Our amygdala seed of interest correlated strongly with activity bilaterally in the insula, hippocampus, and the midline anterior cingulate cortex and significantly more so in the 6 hours than in the 10 min group (Fig. 4). No clusters showed a better correlation with the amygdala seed in the 10 min group. This suggests that the amygdala could be the primary site of memory plasticity, but also influence reconsolidation by affecting other regions of the fear network. The amygdala could thus play a modulatory, rather than a solitary, role in human fear reconsolidation processes.

In summary, whereas the amygdala memory representation after activation and undisrupted reconsolidation predicted return of fear and was functionally coupled to other nodes of the brain’s fear network, disruption of reconsolidation significantly weakened the amygdala memory and its coupling, rendering it unrelated to both recall and return of fear. We conclude that extinction training initiated during reconsolidation abolishes fear expression by erasing a memory trace in the amygdala. Reactivated fear memories are sensitive to behavioral disruption (6, 7, 19), and the amygdala proves to be a key neurobiological substrate for this process also in humans. This mechanism holds great clinical promise in anxiety treatment (6, 17–19) in order to dissociate fear from cognitive memory.
Supplementary Materials for

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This PDF file includes:

- Materials and Methods
- Supplementary Text
- Fig. S1
- Full References
Supplementary Information

Methods

Participants and general design outline

Thirty participants (16 females) were recruited by public advertisements. A fear conditioning session was completed on day 1. Subjects who acquired fear conditioning (n = 22, 11 females) continued the experiment, while those who did not were excluded from further participation. Two stimuli consisting of lamp-photos lit either in red or blue signaled the presence (CS+) or absence (CS-) of an aversive electric shock. The 16 CS+ were always paired with an electric shock to the dorsal right lower arm, while the 16 CS- were never paired with shock. On day 2, subjects were randomly assigned to one of two groups. In the 10 min group, extinction training was performed after 10 min and thus inside the reconsolidation interval following a 2 min fear memory reminder (n = 11). The 6 hrs group (n = 11) extinguished after 6 hrs and thus outside the reconsolidation interval. On day 3, a renewal session was performed in the fMRI environment and after another two days, on day 5, a reinstatement session included unsignedaled shock presentations and then re-exposure to conditioning cues. Data from the reinstatement session were lost for two subjects due to technical difficulties. Subjects received SEK 300 for participation. The local ethics committee approved the study and written informed consent was obtained from all subjects.

Materials

Stimuli

Stimuli were projected on a 17” computer screen and consisted of a photo of a neutral environment containing a lamp that was lit either in red or blue. One color was paired with
the unconditioned stimulus (US) and became the CS+ while the other color was unpaired (CS-). CS color was counterbalanced across subjects. Each stimulus was shown for 6 s with 14 s between trials when the environment was displayed with the lamp unlit. A 500 ms mild electric shock was delivered 250 ms before the CS+ ended.

**Psychophysiology equipment**

The electric shocks were administered by a stimulator and had a maximum strength of 5mA. Electrodes, prepared with electrolyte medium to facilitate shock conduction were attached with surgical tape to the dorsal right lower arm of the participants. Skin conductance responses (SCRs) were measured in µSiemens using two 8 mm Ag/AgCl electrodes filled with isotonic electrolyte gel attached to the hypothenar eminence of the left hand (24).

**Brain imaging**

Data were acquired using a 3T whole body scanner with an 8 channel head coil. Head movement was restricted using foam cushions. Initial scanning was performed to create an anatomical T₁-weighted reference data set with a voxel size of 0.8×1.0×2.0 mm³ and 60 slices. During visual presentations blood oxygen level dependent (BOLD) imaging was performed using a single shot EPI sequence with parameters TE/TR 35/3000 ms, flip angle 90°, acquisition matrix 76×77, acquired voxel size 3.0×3.0×3.0. A total of 30 slices were sampled for whole brain coverage. BOLD data were motion corrected, temporally and spatially smoothed using a 6 mm FWHM kernel.
Procedure

Day 1

Participants signed informed consent and determined the strength of the unconditioned electric shock by following the instruction that it should be unpleasant, but endurable. They then underwent an acquisition session in which 16 CS+ and 16 CS- were presented in a random order with the limitation that no stimulus type was repeated more than two times in a row. CS+ was always paired with US.

Day 2

Twenty-four hours after acquisition the participants returned, electrodes were attached in the same way and the CS+ reminder was shown for 2 min to activate memory. One group then received extinction after 10 min following memory activation (the 10 min group), while the other group underwent extinction after 6 hrs (the 6 hrs group). Extinction consisted of eight presentations of CS+ and CS-. Shock electrodes were applied, but no shock was administered.

Day 3

Subjects appeared in the scanner-environment for a renewal session, and were exposed to 8 presentations of CS+ and CS- while BOLD signal changes were measured. The renewal effect occurs when a contextual change produces fear recovery in response to a conditioned, but extinguished stimulus. Shock electrodes were applied, but no shock was administered. With their heads lightly fixated subjects were shown the visual stimuli in head coil mounted goggles. They were instructed to keep their eyes open and pay attention to the stimulation. A syncbox synchronized the stimulus presentations with the MR-scanner.
Day 5

Two days after completing extinction, participants returned to the psychophysiology laboratory for reinstatement. Four presentations of CS+ and CS- followed 4 unsigned shocks given within inter-trial intervals of about 30 s. SCRs were measured as previously.

SCR-data reduction and analyses

Stimulus induced SCRs were calculated by taking the maximum of the skin conductance deflection in the 1.5-5.75 s interval after stimulus onset subtracted by the immediate preceding baseline (24). All SCRs were range-corrected by dividing each reaction for every individual with that individual’s maximum deflection (irrespective of stimuli and experimental phase) (25). Because participants that do not acquire a fear memory cannot be used to study reconsolidation processes, these were excluded from further analyses. In order to characterize those who did and those who did not acquire a conditioned reaction, every individual’s 16 reactions to CS+ and CS- during acquisition were ordered in pairs and sixteen delta scores were calculated as follows: CS+1-CS-1, CS+2-CS-2...CS+16-CS-16. The average delta scores were tested against zero using a one-tailed t-test with a statistical cutoff offset at p<.10 as the within subject conditioning criterion. This left 22 subjects (11 women) eligible for participation in the extinction, renewal and reinstatement sessions. Due to equipment failure SCR-data are missing for two subjects in the 10 min group (experimental day 5).

To evaluate fear conditioning and extinction, mean values of CS+, CS- as well as delta scores (CS+ minus CS-) were calculated for acquisition and extinction, for each individual respectively. Acquisition and extinction were evaluated by comparing delta scores, and
return of fear was defined as the increase in SCRs from the last extinction trial to the first reinstatement trial, identical to previous reconsolidation studies (6).

Regions of interest

Data were evaluated using SPM 8 and regions of interests (ROIs) created using the definitions from the Wake Forest University School of Medicine PickAtlas (26) and included the bilateral amygdala, hippocampus and insula cortex. Based on (27) and (15) the ventromedial prefrontal cortex (vmPFC) was defined as a 10 mm spherical ROI centered on the Montreal Neurological Imaging (MNI) coordinates xyz = 4, 32, -5. Due to the a priori hypotheses (6, 7, 8, 17, 19) and the anatomically restricted and predicted (2, 6, 7, 8, 17, 19) nature of reconsolidation, we used directed statistical tests uncorrected for multiple comparisons. This approach protects against false negatives, and to protect against false positives, we used a voxel extension criteria of five consecutive voxels (135 mm$^3$) at the p< .05 threshold. Connectivity analyses between BOLD activity in the amygdala and the remaining fear-circuit utilized an amygdala seed based on the empirically defined amygdala activation reflecting the memory trace. The bilateral amygdala activations were linked because we had no a priori hypothesis on laterality. The analyses testing the covariation between brain activity and fear memory on the one hand and brain activity and return of fear on the other included the SCRs as a regressor of interest in SPM 8. To test the hypothesis that vmPFC activity inhibits conditioned fear reactions, a 10 mm spherical ROI centered on xyz = 4, 32, -5 was created and SCRs was used as a regressor of interest in SPM 8. Coordinates are in the MNI space. For anatomical referencing we used the Mai, Assheur and Paxinos medial temporal lobe atlas (28) with MNI coordinates transformed into Talairach space.
Correlation analyses

Correlation analyses determined the strength of the linear dependence between BOLD signal changes reflecting memory activation during renewal on day 3 and return of fear on day 5. Also, BOLD activity during renewal was correlated with extinction-induced memory activation day 2. For these analyses the first trial was included because renewal and reinstatement processes in this (6, 7) and similar (17, 19) protocols are most salient during the first few trials. This approach, using the first trial, increases comparability across studies (6, 17). SCR s and BOLD signal changes were treated identically. Data in the statistical analyses were range corrected to minimize error variance (25) in order to maximize the impact of the experimental conditions. In correlation analyses, data were not range corrected in order to maximize between-subject variability.

Results
Fig. S1. Extinction during reconsolidation prevents reinstatement of extinguished fear. Mean range corrected SCRs during acquisition, extinction and reinstatement for CS+ and CS-. Late and early responses are means of the first and last halves of the 16 acquisition trials day 1, 8 extinction trials day 2 and 4 reinstatement trials day 5 (after unpaired shock presentations). The blue color illustrates the 6 hrs group and the red color the 10 min group. Filled symbols illustrate responses to CS+ paired with shock and open symbols responses to the CS- never paired with the shock. Error bars represent standard errors.
References


