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Supplementary Materials

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Materials and Methods
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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.

Anxiety 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 hyperresponsive across the anxiety disorders (*3*). Pharmacological and behavioral treatments of anxiety reduce symptomatology and amygdala activity (*4*) but have limited success because relapses 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

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(Fig. 1B, right panel). 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).

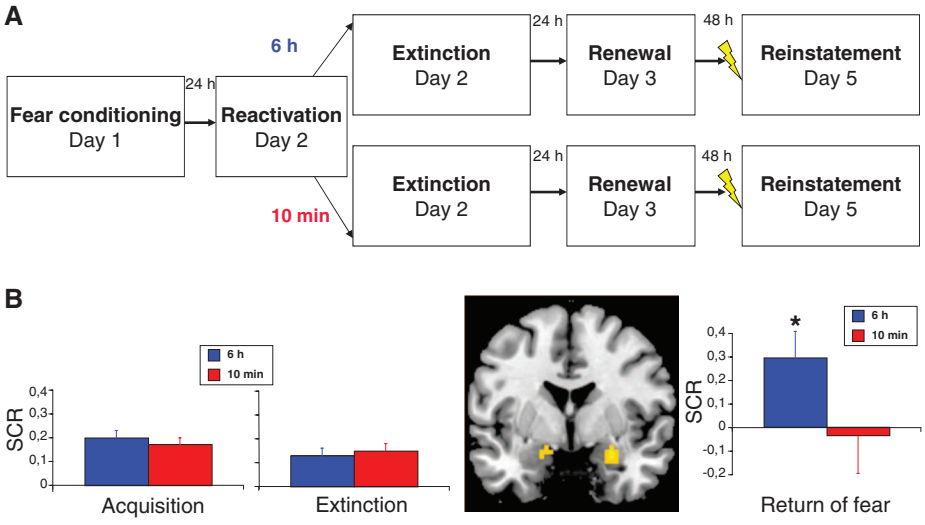

Fig. 1. Extinction during reconsolidation blocks reinstatement of fear and abolishes a memory trace in the amygdala. (**A**) After fear conditioning on day 1, when 16 shocks were paired with a visual cue, a memory reminder was given on day 2, and extinction was performed after 10 min or 6 hours, by exposure 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, return of fear was evoked by presenting unpaired shocks before CSs were again presented. (**B**) Groups were equivalent in acquisition [$t(20) = 0.66$, $P = 0.51$] and extinction [$t(20) = 1.03$, $P = 0.31$]. Return of fear was confirmed in the 6 hours group [blue bar; $t(10) = 2.72$, $P = 0.02$] but not in the 10 min group [red bar; $t(8) = 0.23$, $P = 0.82$]. fMRI demonstrated a remaining fear memory representation in the amygdala after reactivation and normal reconsolidation but not after reactivation followed by disrupted reconsolidation. The voxels reflecting the bilateral memory trace, encompassing the basolateral amygdala, indicate superior memory representation in the 6 hours as compared with the 10 min group (brain coordinates: $x, y, z = 27, 5, -17$; Z-score = 2.46; $P = 0.007$; 378 mm^3; $x, y, z = -15, -1, -14$; $Z = 2.22$; $P = 0.013$; 162 mm^3). 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.

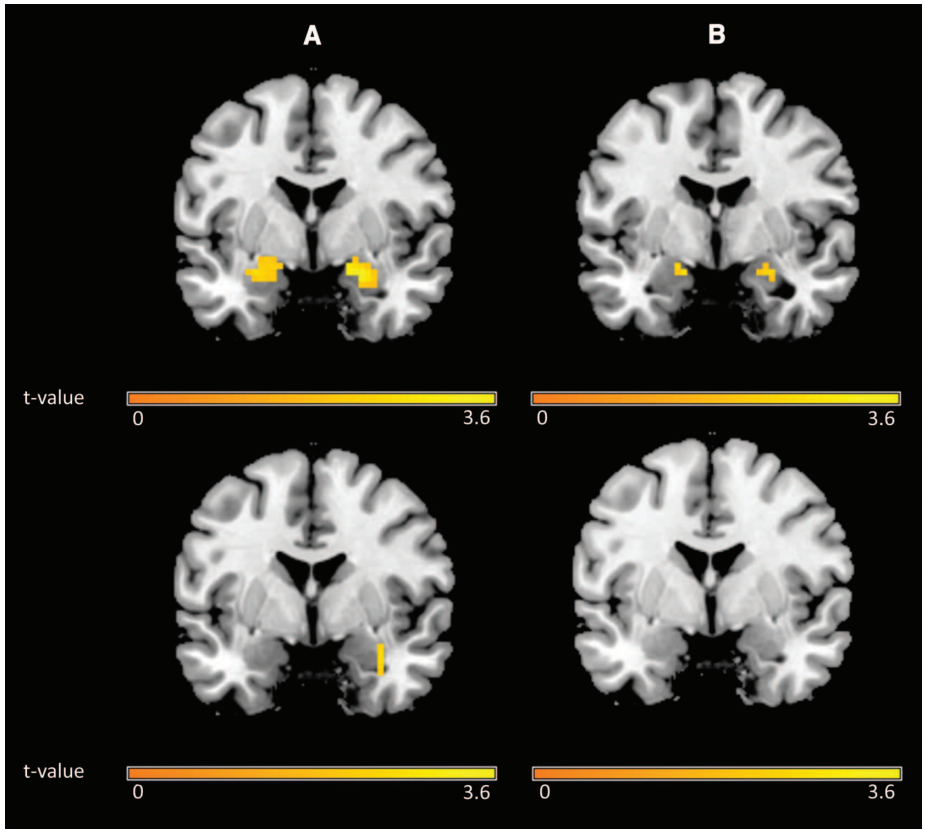

Fig. 2. Amygdala activity predicts return of fear and correlates with recall of fear. (**A**) In the 6 hours group (top), activity bilaterally in the basolateral amygdala predicted return of fear 2 days later ($x, y, z = 21, -1, -17$; $Z = 2.06$; $P = 0.002$; 999 m^3; $x, y, z = -21, -4, -14$; $Z = 2.38$; $P = 0.009$; 1107 m^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).

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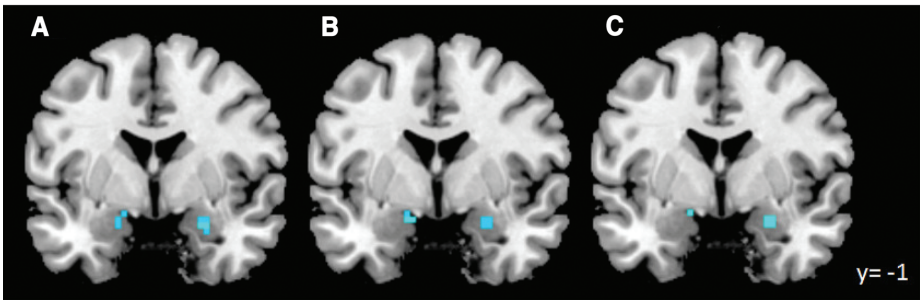

Fig. 3. Areas in the amygdala that correlate with recall and return of fear are colocalized with the memory trace. (**A**) The amygdala areas reflecting the memory trace (Fig. 1) overlapped with the areas that predicted return of fear in the 6 hours group (x, y, z = 24, 2, −17; 189 mm^3; x, y, z = −15, −1, −14; 108 mm^3), illustrated in Fig. 2A (top). (**B**) The localization of the memory trace also overlapped with areas involved in recall during extinction (x, y, z = 24, 2, −20; 81 mm^3; x, y, z = −15, −4, −17; 135 mm^3) illustrated in Fig. 2B (top), in the 6 hours group only. (**C**) These three areas overlapped with each other (x, y, z = 24, 2, −20; 135 mm^3; x, y, z = −15, −1, −14; 27 mm^3).

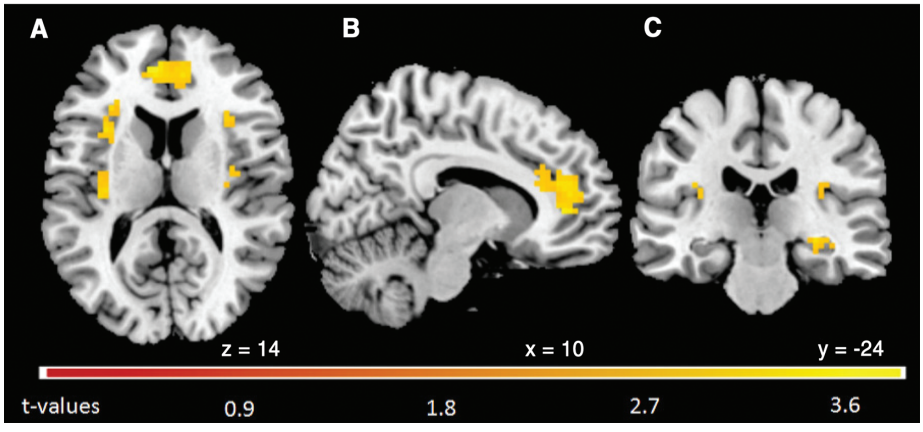

Fig. 4. Amygdala memory trace activity is functionally coupled to the fear network in the brain after normal memory reconsolidation but not after disrupted reconsolidation. The connectivity analysis, using BOLD activity in the amygdala from areas representing memory trace activity in the 6 hours group as a seed of interest, demonstrated stronger functional couplings in the 6 hours than in the 10 min group in structures forming the fear-circuit of the brain, including the midline anterior cingulate cortex (**A** and **B**) (x, y, z = −9, 47, 13; Z = 3.14; 5994 mm^3), bilateral insula (**A** and **C**) (x, y, z = 33, 20, 7; Z = 3.28; 1890 mm^3; x, y, z = −27, 29, 10; Z = 2.95; 4077 mm^3), and bilateral hippocampus (C), although only the right hippocampus is illustrated (x, y, z = −27, −13, −14; Z = 2.51; 297 mm^3; x, y, z = 30, −25, −8; Z = 2.51; total 675 mm^3).

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.

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Supplementary Materials

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Fig. S1
References (*24*–*28*)

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