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

Thomas Agren,¹ Jonas Engman,¹ Andreas Frick,¹ Johannes Björkstrand,¹ Elna-Marie Larsson,² Tomas Furmark,³ Mats Fredrikson¹

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 31. F. Unrein, R. Massana, L. Alonso-Sáez, J. M. Gasol, Limnol. Oceanogr. 52, 456 (2007).

Acknowledgments: D. Bottjer and M. Hogan provided advice for Figs. 1 to 3. Water samples were collected with the help of S. Curless, M. Church, S. Wilson, S. Tozzi, and the captain and crew of the research vessel Kilo Moana. On-board flow cytometry was made possible by K. Doggett and D. Karl. Funding was provided by the Gordon and Betty Moore Foundation (J.P.Z.) and the NSF Center for Microbial Oceanography: Research and Education (C-MORE). The Max Planck Society sponsored the HISH-SIMS analysis. We thank G. Lasky (Max Planck Institute, Bremen) for advice and suggestions for data analysis.

REPORTS

J. Waterbury provided the scientific name for UCYN-A. D.V. was supported by PHYMTEMAGTE (JST-CNRS), METAPICO (Genoscope), and Micro B3 (funded by the European Union, contract 287549). BIOSOPE metagenome sequencing was performed at Genoscope (French National Sequencing Center) by J. Poulain. We thank H. Claustr, A. Sciaandria, D. Marie, and all other BIOSOPE cruise participants. GenBank accession nos.: JX291679 to JX291804 and JX291547 to JX291678 (see table S4 for detail).

Supplementary Materials
www.sciencemag.org/cgi/content/full/337/6101/1546/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S6
References (J4–46)
2 April 2012; accepted 20 July 2012
10.1126/science.1222700

¹Department of Psychology, Uppsala University, SE-751 42 Uppsala, Sweden. ²Department of Radiology, Oncology and Radiation Science, Uppsala University, SE-751 42 Uppsala, Sweden.
³To whom correspondence should be addressed. E-mail: thomas.agren@psyk.uu.se

1550 21 SEPTEMBER 2012 VOL 337 SCIENCE www.sciencemag.org
Groups were equivalent in acquisition shocks before CSs were again presented. (Fig. 1B). 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; \( P = 0.007; 378 \text{ mm}^3 \)).

**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 \( (x, y, z = 21, -1, -17; Z = 2.06; P = 0.002; 999 \text{ mm}^3; x, y, z = -21, -4, -14; Z = 2.38; P = 0.009; 1107 \text{ mm}^3) \). In the 10 min group (bottom), an area in the right temporal claustrum extending into the amygdala was also related to SCR \( (x, y, z = 33, 2, -23; Z = 2.49; P = 0.006; 324 \text{ mm}^3) \). 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 \( (x, y, z = 24, -1, -20; Z = 2.35; P = 0.009; 378 \text{ mm}^3; x, y, z = -15, 4, -17; Z = 2.27; P = 0.012; 189 \text{ mm}^3) \). No covariation existed in the 10 min group (bottom).

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.
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 co-localized to areas involved in fear memory recall during extinction (Fig. 3B). Finally, all these areas overlapped with each other 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, 13) 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 undisturbed 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.

Acknowledgments: We thank the Swedish Research Council, Heumanska stiftelsen, and the Swedish Council for Working Life and Social Research for support.

References and Notes


Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6101/1550/DC1

Materials and Methods

Supplementary Text

Fig. S1

References (24–28)

5 April 2012; accepted 26 July 2012

10.1126/science.1223006