Neural Signature of Reconsolidation Impairments by Propranolol in Humans

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Background: The retrieval of consolidated memories may result in their destabilization, requiring a restabilization process called reconsolidation. During reconsolidation, memories become sensitive to psychological and pharmacological modifications again, thus providing an opportunity to alter unwanted memories. Although such reconsolidation manipulations might open the door to novel treatment approaches for psychiatric disorders such as posttraumatic stress disorder, the brain mechanisms underlying reconsolidation processes in humans are completely unknown. Here, we asked whether a β-adrenergic receptor antagonist might interfere with the reconsolidation of emotional episodic memories and what brain mechanisms are involved in these effects.

Methods: Healthy participants were administered the β-adrenergic receptor antagonist propranolol or a placebo before they reactivated previously learned neutral and emotional material. Recognition memory was tested 24 hours later. Functional magnetic resonance images were collected during reactivation and recognition testing.

Results: Propranolol during reactivation specifically reduced the subsequent memory for emotional pictures; memory for neutral pictures remained unaffected. This emotional memory impairment was associated with significantly increased activity in the amygdala and the hippocampus for correctly recognized pictures at test. Most interestingly, the same structures were active (but not modulated by propranolol) during memory reactivation. Memory reactivation alone or propranolol without reactivation had no effect on subsequent memory.

Conclusions: Our results demonstrate how the consequences of memory reconsolidation processes are represented in the human brain, suggesting that the brain areas that are recruited during reactivation undergo changes in activity that are associated with subsequent memory recall.

Key Words: Amygdala, emotional memory, hippocampus, noradrenaline, propranolol, reconsolidation

Emotionally arousing experiences are usually better remembered than neutral experiences. Although generally adaptive to survival, this emotional memory enhancement may contribute to anxiety disorders such as posttraumatic stress disorder (PTSD) (1). Converging evidence suggests that the superior memory for emotional material is related to arousal-induced noradrenergic activity in the amygdala (2,3). In line with this view, administration of the β-adrenergic receptor antagonist propranolol during or shortly after learning abolishes the emotional enhancement of memory (4,5). First promising findings show that propranolol administered within a few hours after a traumatic event might reduce subsequent trauma memories and PTSD symptoms (6). However, the possibility to modulate the formation of trauma memories by propranolol is limited to a short time-window after the traumatic event (7), during which most individuals will not receive clinical treatment.

Accumulating evidence indicates that consolidated, apparently stable memories might re-enter an unstable state after their reactivation, thus requiring a process of restabilization that is known as reconsolidation (8–12). During reconsolidation, emotional memories become sensitive to amnesic agents, including blockade of β-adrenergic receptors by propranolol (13,14)—again, thus providing a second chance to modify unwanted memories. Despite the potential to reduce traumatic memories, which are a pathological hallmark of PTSD, during reconsolidation, the neural mechanism underlying reconsolidation processes and the impact of propranolol on the reconsolidation of emotional memories in particular is unknown in humans.

To the best of our knowledge, the present study is the first to directly investigate the brain processes associated with reconsolidation processes in humans. To examine the neural correlates of emotional memory reconsolidation impairments by propranolol, we collected functional magnetic resonance images while participants retrieved (i.e., reactivated) previously learned emotional and neutral information under propranolol as well as during a subsequent recognition memory test (Figure 1A). To rule out unspecific effects of memory reactivation or propranolol alone, we included control groups that reactivated memories under placebo or received propranolol without memory reactivation. We hypothesized that memory reactivation under propranolol would reduce subsequent memory for emotional material and that this reconsolidation impairment would be represented at the neural level by altered (i.e., enhanced or reduced) activity in the hippocampus and the amygdala, those brain areas that are crucial for emotional memory formation (15–17). In particular, the emotional memory modulation hypothesis suggests that the emotional memory enhancement is owing to noradrenergic activity in the amygdala, which then modulates memory in the hippocampus (3). Because noradrenergic activity is necessary for enhancing emotional memories but not for forming neutral memories (4,5), the reconsolidation of neutral memories should remain largely unaffected by β-adrenergic receptor blockade during reactivation.

Methods and Materials

Participants

Fifty-two healthy right-handed participants (18 to 30 years old; 26 men, 26 women) with normal or corrected-to-normal vision were randomly assigned to one of four experimental groups (n =

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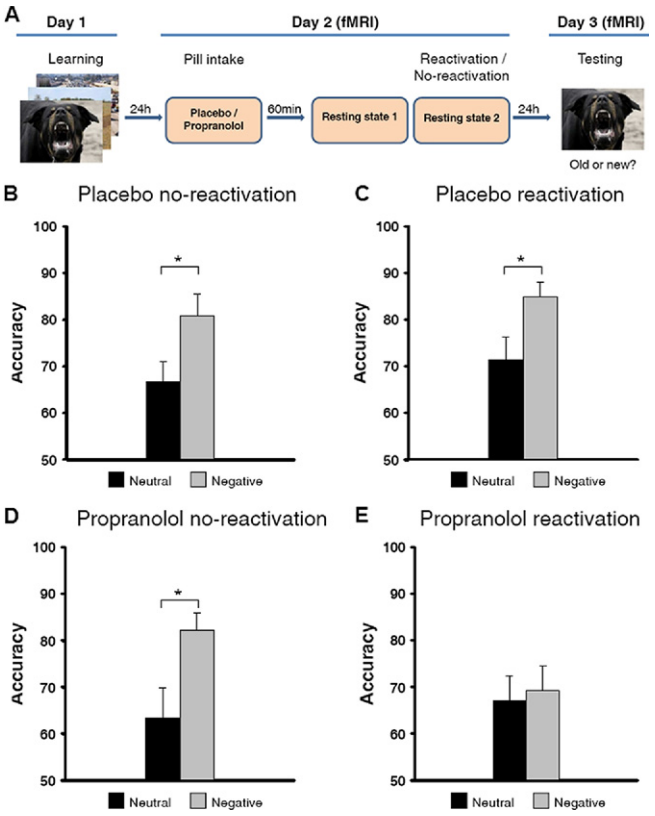


Figure 1. Reconsolidation impairment by propranolol. **(A)** Procedure: volunteers learned a number of neutral and emotional pictures (example image shown is representative of International Affective Picture System images used for this study). Twenty-four hours later, they took a placebo or the β blocker propranolol (40 mg) before they underwent two resting state scans. During the second "resting state" scan, one-half of the participants reactivated the learned pictures. Again 24 h later, all participants completed a recognition memory test in the scanner. **(B)** Participants that received a placebo without memory reactivation had better memory for negative than for neutral pictures. **(C** and **D)** This emotional memory enhancement remained unchanged by memory reactivation under placebo or propranolol administration without memory reactivation. **(E)** Propranolol administered before memory reactivation abolished the emotional memory enhancement. Accuracy = hit rate – false alarm rate. Error bars show mean and SEM; $n = 51$, $^*p < .01$. fMRI, functional magnetic resonance imaging.

13/group): placebo no-reactivation, placebo reactivation, propranolol no-reactivation, and propranolol reactivation. The imaging data of one participant are missing due to technical problems. The Institutional Review Board of McGill University approved the study protocol, and written informed consent was obtained from all participants.

Stimuli

Stimuli consisted of 50 neutral and 50 negative pictures taken from the International Affective Picture System (18), on the basis of their standard scores for emotional arousal and valence. To ensure that pictures were indeed experienced as neutral and emotionally arousing, respectively, participants rated all pictures with respect to valence and arousal on 0–100 scales with the endpoints "very negative" versus "very positive" and "very calm" versus "very aroused," respectively. In retrospect, ratings of participants confirmed the classification of the pictures as neutral and negative, respectively: neutral pictures were rated as neutral (mean [M] = 52.6, SEM = .6), and negative pictures were rated as negative (M = 21.1, SEM = 1.1) [$F(1,48) = 799.79$, $p < .001$, $\eta^2 = .94$]. Negative pictures were experienced as significantly more arousing (M = 67.1, SEM = 1.4) than neutral pictures (M = 30.1, SEM = 2.2) [$F(1,48) = 221.63$, $p < .001$, $\eta^2 = .82$]. There were no significant differences between experimental groups in the valence and arousal ratings (all $p > .15$).

Pictures were subdivided into two sets, each consisting of 25 neutral and 25 negative pictures. Picture sets were matched according to the normative valence and arousal scores, complexity, and semantic categories (e.g., human/animal attack, mutilation, neutral faces, objects). The two picture sets used during learning and as new pictures in the recognition test were counterbalanced across participants.

Procedure

Participants were tested on 3 consecutive days: Day 1, learning outside the scanner; Day 2, pill intake and memory reactivation inside the scanner; Day 3, recognition testing inside the scanner (Figure 1A). On Day 1, participants saw 25 neutral and 25 negative pictures presented in randomized order and were asked to memorize these pictures. Each picture was presented for 2 sec. To control for possible group differences in encoding, an immediate free recall test was given after picture presentation. In this free recall test, participants described verbally all pictures they could remember in as much detail as possible, and the experimenter checked on a list the pictures that were remembered. If it was unclear to which pictures the participants were referring, the experimenter asked the participants for more details.

Twenty-four hours later, participants received a placebo or a propranolol pill (40 mg; Teva, Sellersville, Pennsylvania), depending on the experimental condition. To verify the action of the drug, heart rate measurements were taken immediately before as well as every 10 min in the hour after the drug intake (participants were not told about their heart rates). Sixty minutes after the drug intake, participants underwent two 10-min resting state scans during which they fixated on a cross presented at the center of a screen. After the first resting state scan, participants in the reactivation conditions were explicitly reminded of the learning session on Day 1. The experimenter asked them to remember the pictures they had seen on the previous day in as much detail as possible while they were fixating on the cross. We decided not to cue the memory of the learned pictures explicitly, because that would have complicated the interpretation of group differences in memory performance on Day 3 significantly. In particular, the presentation of the pictures from Day 1 in a recognition test or a cued-recall test would have represented another learning trial, which would have made comparisons between the reactivation and no-reactivation groups impossible. Although relearning processes might occur during retrieval also without external cuing (19), such relearning processes might have been more pronounced if the original learning material would have been presented again during reactivation. Furthermore, a free recall test was hardly possible in the scanner. However, in a brief interview after scanning, all participants in the reactivation conditions confirmed that they concentrated on the previously learned pictures and that they could remember many of them.

The 70-min interval between drug intake and reactivation was used, to be consistent with previous studies that have used propranolol to modify reconsolidation of fear conditioning in humans (13). This interval also coincides with the pharmacodynamics of propranolol (20) and ensured that peak propranolol levels were reached shortly after memory reactivation. Participants in the no-reactivation conditions received no reminder of the learned pictures; for them there was no difference between the resting state scans. Experimental day 2 took place in another building, in another

part of Montreal than the learning session, to avoid spontaneous memory reactivation by the spatial learning context (21).

On Day 3, participants completed a recognition test in the scanner during which they were presented the 50 pictures they had seen on Day 1 and 50 new pictures (25 neutral, 25 negative) in randomized order. Participants were instructed before scanning that they should decide, for each picture, whether they recognized the picture as having been presented during the learning session. The possible answers ("old" vs. "new") were shown below the pictures, and participants were instructed that they would receive a two-button response box and that they should press the left button for the left answer and the right button for the right answer. In each 5-sec trial, a picture was presented for 3 sec before participants indicated by button press whether the picture was "old" (i.e., presented on Day 1) or "new" (i.e., not presented on Day 1; 2 sec). Between trials, participants maintained fixation for 8 to 12 sec (random jitter 0 to 4 sec). After the recognition test, participants rated the valence and arousal of all pictures outside of the scanner.

Image Acquisition and Analysis

Scanning was conducted at the Montreal Neurological Institute on a 1.5-T Siemens SonataVision scanner (Siemens, Malvern, Pennsylvania). For each participant, one anatomical scan was acquired on Day 2 and on Day 3 (slice thickness, 1 mm isotropic; repetition time, 22 msec; echo time, 9.2 msec). The two resting state scans on Day 2 (205 volumes each) and the functional scan on Day 3 (688 volumes) were acquired transversely along the direction of anterior commissure–posterior commissure line minus 30° (slice thickness, 4 mm isotropic; 34 slices; repetition time, 2.88 sec; echo time, 50 msec). Imaging data were analyzed with SPM8 (Wellcome Trust Center for Neuroimaging, University College London, London, United Kingdom), including standard preprocessing procedures (slice timing correct, spatial realignment, coregistration, segmentation, spatial normalization, and smoothing) and modeling the data by general linear models. We used explorative whole brain analyses as well as region of interest (ROI) analyses. A priori ROIs included the amygdala and the hippocampus, which were identified in previous neuroimaging studies on emotional memory (15,16). For the explorative whole brain analyses, the significance threshold was set to $p < .05$ (family-wise error [FWE] corrected). The ROI analyses were performed with the small volume correction options of SPM8 ($p < .05$). For details on the behavioral and imaging analysis, please see Supplement 1.

Results

Impairment of Emotional Memory Reconsolidation by Propranolol

Performance of participants in the free recall test immediately after learning on experimental day 1 showed an emotional memory enhancement: Participants remembered significantly more negative (M = 14.9, SEM = .5) than neutral pictures (M = 10.5, SEM = .4) [$F(1,48) = 110.75$, $p < .001$, $\eta^2 = .70$]. There were no differences between the experimental groups or any interaction effects in the immediate free recall test, thus ruling out group differences in picture encoding (all $p > .20$) (Table S1 in Supplement 1).

Twenty-four hours after learning, participants were administered propranolol (40 mg; $n = 26$) or a placebo ($n = 26$) before they underwent two 10-min resting state scans. Changes in heart rate after pill intake on Day 2 verified the action of propranolol. Before the first resting state scan, participants that were administered propranolol had a significantly lower heart rate than those that had received a placebo [$F(1,48) = 14.64$, $p < .001$, $\eta^2 = .23$], whereas there were no group differences before ($p = .32$) and shortly after the drug intake [$p = .12$; drug × time point of measurement interaction: $F(6,288) = 5.08$, $p < .01$, $\eta^2 = .10$] (Table S2 in Supplement 1).

Another twenty-four hours later, participants completed a recognition memory test in the scanner. There were no group differences in heart rate before memory testing on Day 3, indicating that propranolol was no longer active during recognition testing ($p = .36$; Table S2 in Supplement 1). The pattern of results in the recognition memory test on Day 3 showed that propranolol impaired the reconsolidation of emotional memories (Figures 1B–1E) (for hit and false alarm rates, see Table S3 in Supplement 1). Participants that had received a placebo and did not reactivate the previously learned pictures had significantly better memory (expressed as hit rate – false alarm rate) for negative than for neutral pictures [$t(12) = 4.35$, $p < .01$]. This emotional memory enhancement disappeared in those participants who had been administered propranolol before memory reactivation [$t(12) = .57$, $p = .58$]. Reactivation without propranolol or propranolol without reactivation was not sufficient to abolish the emotional enhancement of memory, which was still present in the placebo reactivation and propranolol no-reactivation groups [both $t(12) > 3.99$, both $p < .01$]. An analysis of variance revealed that propranolol selectively affected the reconsolidation of emotional memories [emotionality × drug × reactivation effect: $F(1,48) = 4.39$, $p < .05$, $\eta^2 = .09$]. For emotional pictures, there was an interactive influence of drug and reactivation [$F(1,48) = 3.88$, $p = .05$, $\eta^2 = .08$] that led to significantly impaired memory in the propranolol reactivation group (vs. all other groups: all $p < .05$, Least Significant Difference post hoc tests). For neutral pictures, there was no such interaction effect [$F(1,48) = .01$, $p = .94$, $\eta^2 < .01$].

Neural Correlates of Memory Reactivation Under Placebo and Propranolol

Next, we sought to identify the brain structures involved in the reconsolidation impairment by propranolol. A priori ROIs included the hippocampus and the amygdala, which are consistently implicated in emotional memory processes (15,16). First, we examined the neural correlates of memory reactivation under placebo and propranolol. We contrasted the brain activity during the second "resting state" scan (i.e., when one-half of the participants were asked to reactivate the learned pictures) with the one during the first resting state scan and submitted this contrast to a full factorial model with the factors drug (placebo vs. propranolol) and reactivation (no-reactivation vs. reactivation). This analysis yielded a significant main effect of reactivation in the right hippocampus and the right amygdala (both $p < .05$, FWE corrected), indicating that these areas were significantly more active in the reactivation condition than in the no-reactivation condition (Figure 2; and Table S4 in Supplement 1). There was no main effect of drug and no drug × reactivation interaction. Furthermore, there were no differences between the placebo reactivation and propranolol reactivation groups, indicating that propranolol did not change brain activation during memory reactivation.

Neural Correlates of Reconsolidation Impairment by Propranolol

To examine changes in brain activity that were associated with the effect of propranolol and reactivation on subsequent memory performance, we classified responses of participants in the recognition test as "correct" (including hits and correct rejections) or "incorrect" (including false alarms and misses) and compared brain activations associated with correct versus incorrect responses, separately for neutral and

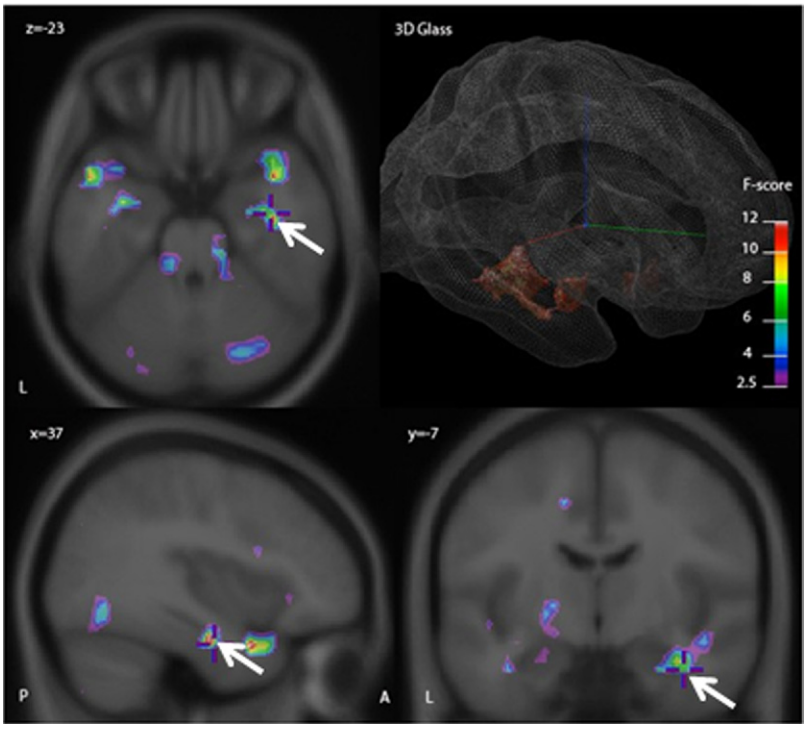
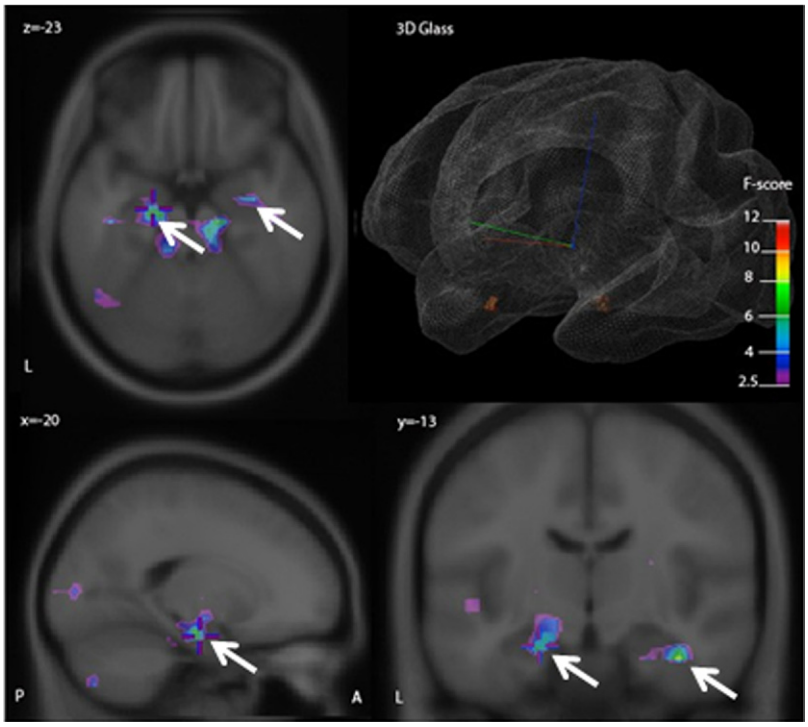


Figure 2. Activity of the amygdala and the hippocampus associated with memory reactivation. Regions of the amygdala (30, −4, −18) and the hippocampus (Montreal Neurological Institute coordinates of peak voxel: 34, −24, −14) showing significantly increased activation in the second "resting state" (i.e., when participants were instructed to remember the previously learned pictures) when contrasted against the first resting state scan. Shown are representative views from coronal, horizontal, and sagittal cuts and a glass brain with the extent of the significant activations within hippocampus and amygdala whose x, y, and z orientation is shown in red, green, and blue, respectively. The arrows point to the amygdala and the hippocampus, respectively. A, anterior; 3D, three-dimensional; L, lateral, P, posterior.

negative pictures. These contrasts were again submitted to a full factorial model with the factors drug and reactivation. In line with our finding that neutral memories were not affected by reactivation and propranolol, there were no significant main or interaction effects for neutral pictures. For negative pictures, however, we obtained a significant drug × reactivation effect in the left amygdala and the right hippocampus (both $p < .05$, FWE corrected) (Figure 3; and Table S5 in Supplement 1). To pursue this analysis, we assessed the impact of propranolol on the brain activations for the contrast correct-negative minus incorrect-negative in the no-reactivation and reactivation conditions separately. In line with the concept of memory reconsolidation (8,9), propranolol had no effect on brain activity when there was no memory reactivation. In the reactivation condition, however, the propranolol and placebo groups differed significantly. As shown in Figure 4, the left amygdala ($p < .05$, FWE corrected) and the bilateral hippocampus (both $p < .01$, FWE corrected) were significantly more active in the propranolol reactivation group than in the placebo reactivation group (Table S5 in Supplement 1).

Figure 3. Neural correlates of reconsolidation impairment by propranolol. Interactive influence of reactivation and drug in regions of the amygdala (−28, −4, −28) and the hippocampus (Montreal Neurological Institute coordinates of peak voxel: 34, −8, −22) when correct responses for negative pictures were compared with incorrect responses for negative pictures. Shown are representative views from coronal, horizontal, and sagittal cuts and a glass brain with the extent of the significant activations within hippocampus and amygdala whose x, y, and z orientation is shown in red, green, and blue, respectively. The arrows point to the amygdala and the hippocampus, respectively. Abbreviations as in Figure 2.



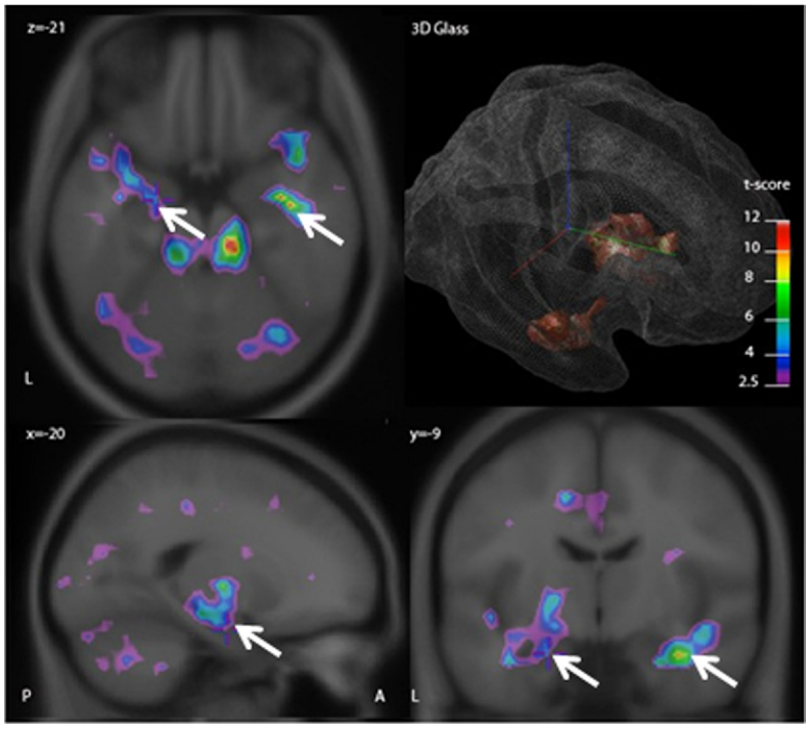


Figure 4. Brain activity associated with the subsequent recall of memories reactivated under propranolol. Increased activity in the amygdala (Montreal Neurological Institute coordinates of peak voxel: −22, −8, −18) and the hippocampus (left: −22, −18, −22; right: 34, −8, −28) for correct responses to negative pictures versus incorrect responses to negative pictures in participants that received propranolol before memory reactivation compared with those that received a placebo before reactivation. Shown are representative views from coronal, horizontal, and sagittal cuts and a glass brain with the extent of the significant activations within hippocampus and amygdala whose x, y, and z orientation is shown in red, green, and blue, respectively. The arrows point to the amygdala and the hippocampus, respectively. Abbreviations as in Figure 2.

To rule out the possibility that these group differences in brain activity are simply due to differences in the number of correct and incorrect responses, we analyzed our data with an analysis of covariance in which performance was entered as a covariate. This analysis showed that our pattern of results remained when we controlled for differences in performance (Table S6 in Supplement 1), thus ruling out the possibility that group differences in the number of correct and incorrect responses could account for the obtained differences in brain activity.

In addition to the aforementioned ROIs, exploratory whole brain analyses revealed several activations—mainly in frontal and temporal areas—that became only active, however, at the more lenient threshold of $p < .003$, uncorrected (Table S7 in Supplement 1). The opposite contrast incorrect minus correct revealed no significant effects of drug or reactivation on brain activity. Furthermore, we obtained no significant correlations between brain activity and memory performance.

Discussion

Over the past decade, reconsolidation effects have been shown across treatments and species (13,22–27). The underlying brain processes, however, remained unknown. This study is the first to shed light on the neural correlates of the consequences of reconsolidation manipulations in humans. One previous study showed that the β-adrenergic receptor antagonist propranolol might disrupt the reconsolidation of a conditioned fear in humans (13). Here, we show for the first time an effect of propranolol on the reconsolidation of episodic emotional memories. The administration of the β-adrenergic receptor antagonist propranolol during memory reactivation abolished the emotional enhancement of memory and made emotional memories comparable to neutral memories. Because the emotional memory enhancement was not influenced by memory reactivation alone or by propranolol without memory reactivation, this finding suggests that propranolol indeed impaired the reconsolidation of emotional memories. Our brain imaging data shed light on the neural correlates of reconsolidation impairments by propranolol in the human brain.

The reactivation of the learned pictures recruited the amygdala and the hippocampus, structures that are commonly associated with the successful retrieval of emotional and neutral material (28–30). In the absence of behavioral parameters of memory reactivation (which cannot be collected in the scanner without confounding the interpretation of the memory test on Day 3), this finding provides neural evidence that participants indeed reactivated the previously learned pictures. Importantly, propranolol did not affect the brain activity during memory reactivation, which might suggest that the β blocker did not modulate the reactivation itself but processes occurring within a few hours after reactivation (i.e., in the reconsolidation window) (24).

The influence of the β blocker during memory reactivation was reflected in impaired emotional memory performance in the recognition memory test 24 hours later, when the drug was no longer active. This behavioral effect was paralleled by reactivation-dependent propranolol effects on subsequent brain activity during recognition testing. We obtained an interaction of propranolol and reactivation for correct responses to emotional pictures in the same structures that were activated during memory reactivation (i.e., the amygdala and the hippocampus). Both the amygdala and the hippocampus were more active during correct responses to emotional pictures in the propranolol reactivation group than in the placebo reactivation group. This suggests that, in those participants that received propranolol before memory reactivation, greater hippocampus and amygdala activity might have been required at test to successfully remember the learned material. Blockade of β-adrenergic receptors after memory reactivation might have reduced the strength of the (emotional) memory trace in the hippocampus or the interplay between hippocampus and amygdala that is needed to (re)build emotional memories (15–17). To compensate for this, stronger activation of these structures might have been necessary for successful retrieval during recognition testing (31–33).

Alternatively, it is tempting to speculate that only those memories "survived" the reconsolidation impairment by propranolol that were strongly encoded (i.e., associated with greater hippocampal and amygdala activity at encoding). In line with this view, there is evidence that brain activity at encoding predicts subsequent memory performance (34,35) and that strong training makes memories less susceptible to reconsolidation manipulations (36).

Previous evidence showed that propranolol before the reactivation of a conditioned fear memory erases the behavioral expression of the fear 24 hours later without changing the declarative fear memory (13). Although this finding might, at first glance, seem to be in conflict with the impairment in emotional episodic memories that is reported here, it is well in line with the present results. Emotionally arousing experiences are associated with increased noradrenergic activation in the amygdala (37). According to the memory modulation hypothesis, the superior memory of emotional relative to neutral material is mediated by arousal-induced noradrenergic activation in the amygdala that then modulates memory processes in other brain areas such as the hippocampus (3,5). Noradrenergic activation of the amygdala strengthens memory through β-adrenergic receptors that stimulate the cyclic adenosine monophosphate-dependent protein kinase pathway (38). Like Kindt *et al.* (13), we assume that propranolol after reactivation exerted its effects via the blockade of β-adrenergic receptors in the amygdala. This might have prevented the stimulation of the cyclic adenosine monophosphate-dependent signaling cascade and the modulatory influence of the amygdala on the reconsolidation of the emotional pictures, which were then reconsolidated (most likely in the hippocampus) more similarly to neutral pictures. In other words, when propranolol was administered before reactivation, emotional memories might have been treated like neutral memories during reconsolidation, thus requiring greater amygdala and hippocampus activity for correct responses in the recognition test. Interestingly, the amygdala and the hippocampus were exactly those structures that were active during reactivation.

A key question in the pharmacological manipulation of reconsolidation processes is the timing of the drug administration. When should the drug be administered to make sure that it affects reconsolidation processes? Peak levels of propranolol can be expected in humans at approximately 90 min after (oral) administration (20). If propranolol is administered before reactivation it might affect the reactivation itself. If propranolol is administered after reactivation it might be that peak levels of drug activity occur outside of the reconsolidation window, which is limited to a short period after reactivation (8). In one previous study propranolol was administered after reactivation (39), whereas in another study (13) propranolol was—as in the present study—administered before reactivation. Because the general pattern of results (subsequent emotional memory impairment) was similar in these studies, one might assume that propranolol affects the reconsolidation of emotional memories, irrespective of the exact timing of the drug administration. This conclusion, however, might be premature. How propranolol altered subsequent memory might depend on the timing of the drug administration. In particular, if propranolol is administered before reactivation, as in the present study, effects on the reactivation itself can hardly be ruled out. For example, a recent study that administered propranolol before the reactivation of previously learned neutral and emotional items reported impaired emotional memory in a memory test 24 hours after reactivation but also during reactivation (40). Because we did not assess memory during reactivation, we cannot rule out similar effects in the present study, although there were no propranolol effects on brain activation on Day 2. One might argue that, even if the reactivation was reduced by propranolol, this should not impair subsequent memory because memory was also not impaired in the no-reactivation groups. However, propranolol might have increased the likelihood of unsuccessful retrieval during reactivation, which might have impaired subsequent memory. Future studies on reconsolidation need to include behavioral measures of reactivation to control for such effects.

Moreover, the sample size of this first neuroimaging study on the consequences of reconsolidation impairments by propranolol was rather moderate. Future studies on this topic would benefit from larger sample sizes that provide more statistical power (e.g., for correlational analyses) and allow for additional analyses. For example, there is accumulating evidence for gender differences in memory processes and their underlying neural circuits (41), and it would be important to examine whether the effects of reconsolidation manipulations are similar for men and women. Furthermore, it would be interesting to see whether a higher dose of propranolol (e.g., 100 mg) (39) would result in more severe reconsolidation impairments and whether the propranolol effects are limited to the reconsolidation of negative memories or whether propranolol might also affect the reconsolidation of positive memories (which should be the case if these memories are emotionally arousing).

To conclude, administration of the β blocker propranolol during memory reactivation (i.e., during reconsolidation) provides a promising opportunity to change unwanted memories in disorders such as PTSD or drug addiction (39,42). The current study highlights for the first time how the consequences of the reconsolidation impairment by propranolol might be represented in the human brain, suggesting that propranolol alters the activity in the amygdala and the hippocampus, those structures that were recruited during memory reactivation.

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Supplementary material cited in this article is available online.

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Neural Signature of Reconsolidation Impairments by Propranolol in Humans

Supplemental Information

Supplemental Methods and Materials

Behavioral Data Analysis

In order to assess the emotional memory enhancement, accuracy (hit rate – false alarm rate) was compared for neutral and negative pictures by means of paired *t*-tests in each of the experimental groups, separately. Group differences in memory performance were analyzed by a mixed-design analysis of variance (ANOVA) with emotionality (neutral vs. negative pictures) as within-subjects factor and drug (placebo vs. propranolol) and reactivation (no reactivation vs. reactivation on day 2) as between-subjects factors. A significant three-way interaction was pursued by separate drug × reactivation ANOVAs for neutral and negative pictures, which were followed by least significant difference post-hoc tests if indicated. All reported *p*-values are two-tailed; we used Cohen's η^2 as a measure of effect size.

Image Acquisition and Analysis

Scanning was conducted at the Montreal Neurological Institute (MNI) on a 1.5 T Siemens SonataVision scanner. For each participant, one anatomical scan was acquired on day 2 and on day 3 (slice thickness, 1 mm isotropic; 176 sagittal slices; repetition time (TR), 22 ms; echo time (TE), 9.2 ms; flip angle, 30°; field of view (FOV), 256 mm). The two resting state scans on day 2 (205 volumes each) and the functional scan on day 3 (688 volumes) were acquired transversely along the direction of anterior commissure to posterior commissure line minus 30° (slice thickness, 4 mm isotropic; 34 slices; TR, 2.88 s; TE, 50 ms; flip angle, 90°, matrix, 64 × 64; FOV, 256 mm). For resting state and functional scans, the first 3 volumes were discarded for signal stabilization.

Image analysis was performed using SPM8 (Wellcome Trust Center for Neuroimaging, University College London). Functional data were corrected for slice-timing and head motion. Structural images were segmented into gray matter, white matter, and

cerebrospinal fluid. Gray matter images were normalized to the MNI template image. Normalized gray matter images were used for normalization of the structural and functional images. Finally, data were spatially smoothed using an 8 mm full-width half-maximum Gaussian kernel.

General linear models (GLMs) were estimated separately for the resting state scans on day 2 and the functional scan on day 3. We implemented seed-based functional connectivity analysis to investigate the correlation between activity in our a priori regions-of-interest (ROIs), hippocampus and amygdala, with activity in all other voxels in the brain. This is one of the classical techniques for investigating resting-state fMRI data (1). The a priori selection of the seed regions in the hippocampus and amygdala was guided by our primary hypotheses associated with these regions. To perform the analysis, we first generated a model time-series from the ROIs, and then quantified the similarity between the model time-series from the first scan with that of the second. In this form, the correlation of the ROI time-series between scans provided us with information about the similarity in activation between the different scans. For the functional scan on day 3, the following regressors were included: correct_neutral, incorrect_neutral, correct_negative, incorrect_negative, fixation, button press, and the six movement regressors. Regressors of interest were constructed by a stick function convolved by a hemodynamic response function (HRF). The data were filtered in the temporal domain using a nonlinear high-pass filter with a 128 s cut-off. Contrast estimates were calculated for rest2 - rest1, correct_neutral - incorrect_neutral, and correct_negative - incorrect_negative.

For all GLMs, linear contrasts were used to obtain subject-specific estimates for each effect of interest, which were then entered into a second-level (group) analysis, treating subject as a random effect and using a full factorial model with the factors drug (placebo vs. propranolol) and reactivation (reactivation vs. no-reactivation). We used explorative whole brain analyses as well as ROI analyses. For the explorative whole brain analyses, the

significance threshold was set to $p < 0.05$ on voxel-level, corrected for multiple testing (family-wise error (FWE) correction). ROI analyses were performed using the small volume correction options of SPM8 ($p < 0.05$). A priori ROIs included the amygdala and the hippocampus which were identified in previous neuroimaging studies on emotional memory (2, 3). The respective ROI masks were taken from the MNI brain atlases available from their servers.

Table S1. Number of pictures recalled in the free recall test on day 1.

	Neutral	**Negative**
Placebo no-reactivation	9.38 ± 0.68	15.77 ± 0.98*
Placebo reactivation	10.92 ± 0.94	15.17 ± 0.81*
Propranolol no-reactivation	10.23 ± 0.97	14.39 ± 0.91*
Propranolol reactivation	11.00 ± 0.93	15.08 ± 1.05*

Data represent means ± SEM.
*Significantly higher than the number of remembered neutral pictures ($p < 0.01$, two-tailed; paired *t*-test).

Table S2. Heart rate (in beats per minute) across the experiment.

	Baseline	10 min after drug intake	20 min after drug intake	30 min after drug intake	40 min after drug intake	50 min after drug intake	60 min after drug intake	Day 3
Placebo no-reactivation	78.0 ± 2.7	76.0 ± 2.5	73.3 ± 2.2	72.0 ± 2.0	72.3 ± 1.7	72.5 ± 1.3	72.6 ± 2.0	75.2 ± 2.0
Placebo reactivation	78.9 ± 4.3	76.0 ± 3.2	74.2 ± 2.8	73.2 ± 2.6	70.3 ± 2.7	68.4 ± 2.5	70.1 ± 3.1	79.6 ± 4.8
Propranolol no-reactivation	72.1 ± 3.4	68.8 ± 2.9	66.7 ± 2.4	65.2 ± 2.1*	62.6 ± 2.7*	60.0 ± 2.6*	60.9 ± 2.5*	71.9 ± 3.9
Propranolol reactivation	77.6 ± 3.5	73.8 ± 3.1	68.9 ± 2.7	64.3 ± 3.0*	63.4 ± 3.0†	62.5 ± 3.4¶	61.2 ± 3.0*	76.6 ± 3.1

Data represent means ± SEM.
* Significantly different from the placebo groups (LSD post-hoc tests $p < 0.05$).
† Significantly different from the placebo no-reactivation group (LSD post-hoc tests $p < 0.05$) and different from the placebo reactivation group at trend level (LSD post hoc tests $p < 0.10$).
¶ Significantly different from the placebo no-reactivation group (LSD post-hoc tests $p < 0.05$).
LSD, least significant difference.

Table S3. Hits rates, false alarm rates, and accuracies for neutral and negative pictures on day 3.

	Hit rate neutral	Hit rate negative	False alarm rate neutral	False alarm rate negative	Accuracy neutral	Accuracy negative
Placebo no-reactivation	80.6 ± 3.3	92.0 ± 2.1	13.8 ± 3.2	11.0 ± 3.0	66.8 ± 4.1	81.0 ± 4.7
Placebo reactivation	80.0 ± 4.4	90.7 ± 2.1	8.7 ± 2.0[§]	5.7 ± 2.0	71.3 ± 5.0	85.0 ± 3.0
Propranolol no-reactivation	82.2 ± 4.5	92.3 ± 2.1	18.8 ± 3.1	10.1 ± 2.4	63.4 ± 6.5	82.2 ± 3.8
Propranolol reactivation	83.1 ± 3.1	85.0 ± 3.2[#]	16.0 ± 3.7	16.6 ± 2.8[+]	67.1 ± 5.4	68.4 ± 5.3*

Memory accuracy was calculated as hit rate – false alarm rate. Data represent means ± SEM.
* $p < .05$ compared to the other three groups.
[#] $p \leq .10$ compared to the other three groups.
[+] $p \leq .15$ compared to the other three groups.
[§] $p < .15$ compared to the two propranolol groups.

Table S4. Significant ROI activations for the contrast rest2 minus rest1.

Effect	Brain area	x	y	z	*Z*-score	$P_{corrected}$
Drug × reactivation interaction	No significant activations					
Main effect reactivation	L Amygdala	-28	-4	-22	2.21	0.121
	R Amygdala	30	-4	-18	2.90	0.024
	R Hippocampus	34	-24	-14	3.24	0.030
Main effect drug						
Placebo reactivation > Propranolol reactivation	No significant activations					
Propranolol reactivation > Placebo reactivation						

All *p*-values are family-wise error corrected for the respective region of interest (ROI). Coordinates are given in Montreal Neurological Institute space.
L, left; R, right.

Table S5. Significant ROI activations for the contrast correct_negative minus incorrect_negative.

Effect	Brain area	x	y	z	*Z*-score	$P_{corrected}$
Drug × reactivation interaction	L Amygdala	-28	-4	-28	2.81	0.042
	R Hippocampus	34	-8	-22	3.45	0.015
Placebo reactivation > Propranolol reactivation	No significant activations					
Propranolol reactivation > Placebo reactivation	L Amygdala	-22	-8	-18	2.18	0.036
	L Hippocampus	-22	-18	-22	3.88	0.009
	R Hippocampus	34	-8	-28	4.10	0.001
Placebo no-reactivation > Propranolol no-reactivation	No significant activations					
Propranolol no-reactivation > Placebo no-reactivation						

All *p*-values are family-wise error corrected for the respective region of interest (ROI). Coordinates are given in Montreal Neurological Institute space.
L, left; R, right.

Table S6. Significant ROI activations for the contrast correct_negative minus incorrect_negative when memory performance was entered as a covariate.

Effect	Brain area	x	y	z	*Z*-score	$P_{corrected}$
Drug × reactivation interaction	L Amygdala	-26	-4	-26	2.43	0.083
	R Hippocampus	34	-8	-22	3.23	0.033
Placebo reactivation > Propranolol reactivation	No significant activations					
Propranolol reactivation > Placebo reactivation	L Amygdala	-22	-8	-18	2.57	0.057
	L Hippocampus	-22	-16	-22	3.47	0.019
	R Hippocampus	34	-8	-26	3.84	0.003
Placebo no-reactivation > Propranolol no-reactivation	No significant activations					
Propranolol no-reactivation > Placebo no-reactivation						

All *p*-values are family-wise error corrected for the respective region of interest (ROI). Coordinates are given in Montreal Neurological Institute space.
L, left; R, right.

Table S7. Results of the exploratory whole-brain analyses.

Effect	Brain area	x	y	z	*Z*-score	*P*
DAY 2: rest2 – rest1						
Drug × reactivation interaction	L inferior frontal gyrus	-54	4	14	3.36	< 0.001
	L supplementary motor area	-6	-4	62	3.28	< 0.001
	L middle frontal gyrus	-22	12	50	3.03	0.001
Main effect reactivation	L fusiform gyrus	-38	-40	-18	3.76	< 0.001
	R parahippocampal gyrus	34	-24	-14	3.19	< 0.001
	R superior frontal gyrus	26	0	66	3.41	< 0.001
	L superior frontal gyrus	-22	-4	62	3.17	0.001
	R inferior temporal gyrus	54	-56	-14	2.95	0.002
	R superior occipital gyrus	22	-92	22	2.77	0.003
Main effect drug	L postcentral gyrus	-58	-16	42	3.04	0.001
DAY 3: correct_negative – incorrect_negative						
Drug × reactivation interaction	L middle temporal gyrus	-50	-60	14	4.20	< 0.001
	R medial temporal pole	38	8	-26	3.57	< 0.001
	L medial temporal pole	-50	12	-30	3.56	< 0.001
	L cerebellum	-26	-48	-42	3.38	< 0.001
	L fusiform gyrus	-34	-72	-10	2.97	0.002
Propranolol_reactivation > Placebo_reactivation	L middle temporal gyrus	-62	-20	-2	4.00	< 0.001
	L superior temporal gyrus	-54	-20	2	3.85	< 0.001
	R inferior frontal gyrus	34	8	34	3.61	< 0.001
	R middle occipital gyrus	42	-76	30	3.55	< 0.001
DAY 3: correct_negative – incorrect_negative						
Propranolol_reactivation > Placebo_reactivation	L medial temporal pole	-50	8	-30	3.32	< 0.001
	L cerebellum	-14	-36	-22	3.30	< 0.001
Placebo_reactivation > Propranolol_reactivation	No suprathreshold activations					
Main effect reactivation	L cerebellum	-42	-72	-14	3.82	< 0.001
	R thalamus	18	-24	-2	3.75	< 0.001
	R fusiform gyrus	34	-64	-6	3.32	< 0.001
	L lingual gyrus	-26	-68	-2	3.18	0.001
	R middle occipital gyrus	34	-92	6	3.13	0.001
	L middle occipital gyrus	-30	-88	18	3.13	0.001

	R supplementary motor area	6	12	62	3.07	0.001
Main effect drug	L parahippocampal gyrus	-18	-16	-18	3.20	0.001
DAY 3: correct_neutral – incorrect_neutral						
Drug × reactivation interaction	R medial orbital gyrus	14	52	-2	3.90	< 0.001
Main effect reactivation	No suprathreshold activations					
Main effect drug	R superior frontal gyrus	18	4	54	3.57	< 0.001
	L precuneus	-2	-68	54	3.32	< 0.001
	R middle cingulate cortex	10	12	34	3.23	0.001
	R superior frontal gyrus	14	32	42	3.17	0.001
	L precentral gyrus	-22	-20	58	3.08	0.001

All *p*-values are thresholded at $p < 0.003$ (uncorrected). Coordinates are given in Montreal Neurological Institute space.
L, left; R, right.

Supplemental References

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