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14. ABSTRACT

Introduction: The β-adrenergic blocker propranolol has been shown to reduce reconsolidation of aversive memories in rodents. Administration of propranolol following reactivation of traumatic memories in male and female civilians with PTSD has been shown to reduce physiological responses during subsequent mental imagery the traumatic event. Aims: The present study aimed to examine whether the fear-weakening effect of propranolol may be due to non-specific actions of the drug. Here we investigated the effect of propranolol given with or without the reactivation otraumatic combat memories. Methods: Twenty-three male subjects with combat-related post-traumatic stress disorder received propranolol wit(n=12) or without (n=11) experimentally induced, concomitant traumatic memory retrieval (reactivation), randomized and double-blind. A week later, they engaged in script-driven mental imagery of their traumatic combevent while physiological responses were recorded. Results: The physiological responses during script-driven imagery of both the reactivation propranolol group and the non-reactivation propranolol group were below the normative cutoffs for PTSD. There were no significant between-group differences in physiological responses or in change in self-reported PTSD symptoms. Discussion: The lack of significant group differences fails to support the proposition that the putative fear-weakening effect of propranolol is mediated by a reconsolidation mechanism.

15. SUBJECT TERMS
Stress disorders, post-traumatic; reconsolidation; propranolol; imagery, psychotherapy; psychophysiology (all MeSH terms)

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BODY:

Animal research suggests that the retrieval (reactivation) of a consolidated memory returns it to a labile state from which it must be stabilized in order to persist [1]. This process, termed reconsolidation, involves mechanisms similar but not identical to those involved in consolidation [2]. Reconsolidation is largely demonstrated by its blockade, and derives support from experiments with a variety of species ranging from honeybees to humans [3;4]; in an assortment of experimental paradigms; and using a broad range of interventions, including systemic or localized drug administration [5], gene knockout [6], interference by new learning [4;7]; and manipulations of kinase activity [8;9]. The amnesia induced by blocking reconsolidation can be double-dissociated from extinction [10;11], and it is distinct from extinction in that it can be made to occur even when a reinforced trial is used to reactivate the memory [6;12], it does not show renewal after contextual shift [12], and it has a unique neurochemical signature [11]. Nader and Einarsson [13] provide a comprehensive review of the evidence for reconsolidation.

According to a translational model of the pathogenesis of post-traumatic stress disorder (PTSD), a psychologically traumatic event stimulates excessive production of endogenous stress hormones. These hormones facilitate strong consolidation of the memory of the event, leading to a powerful and persistent memory that is readily activated [14]. In this model of PTSD, there exists a narrow window of time following the traumatic event, on the order of minutes to hours [15], during which the traumatic memory is unstable and certain agents might interfere with its consolidation. Animal and human data indicate that the memory-modulating effects of stress hormones are mediated by noradrenergic activity in the amygdala and can be opposed by a β-adrenergic blocker such as propranolol [16]. Once the memory trace becomes stable, the window of opportunity is closed and β-adrenergic blockers can no longer exert their effects. Since most persons who develop PTSD are unlikely to receive clinical attention until long after this fleeting period of instability has elapsed, attempts to prevent PTSD by blocking consolidation of the traumatic memory have achieved limited success [17-20].

If the window of opportunity for weakening the traumatic memory could be reopened after a patient has already developed PTSD, this might present a more clinically feasible opportunity for intervention. Administering a suitable drug during reactivation-induced destabilization could reduce the strength of a traumatic memory by blocking its reconsolidation in a manner similar to attenuating consolidation immediately following the traumatic event. In animals, consolidated memories for aversive tasks have been shown to become sensitive to β-blockade following reactivation. Specifically, administration of propranolol at this time has been found to reduce inhibitory avoidance [21] and auditory fear conditioning [5].

Recent preclinical studies examining reconsolidation blockade of conditioned fear memory in humans offer promise for the clinical application of such an intervention. Kindt and colleagues employed a three-session (across three days) Pavlovian differential fear conditioning and reactivation procedure using images of spiders as conditioned stimuli [22]. These investigators found that administering propranolol along with memory reactivation blocked return of the

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conditioned response, as measured by fear-potentiated startle, whereas the declarative memory of the fear association remained intact. Neither reactivation with placebo nor propranolol without reactivation produced this effect. Soeter and Kindt replicated these findings for potentiated startle, but not for skin conductance, as the dependent fear measure [23]. In a differential conditioning study, Schiller and colleagues showed that immediate, but not delayed, extinction following reconsolidation resulted in reduced skin conductance responses that did not show spontaneous recovery or reinstatement [24]. Although this study did not employ a pharmacological manipulation, the findings of Schiller *et al.* support the interpretation that a single presentation of the conditioned fear stimulus opens a window of lability during which fear memories may be altered through a reconsolidation-like mechanism.

In a preliminary, placebo-controlled investigation of reconsolidation blockade in PTSD, Brunet and colleagues employed a validated psychophysiological script-driven imagery technique in 19 subjects with PTSD resulting from various traumatic events [25]. Physiological responses during traumatic imagery have been shown to reliably discriminate PTSD from non-PTSD trauma-exposed individuals [26]. Subjects underwent a script preparation procedure that entailed describing their traumatic event, which served to reactivate its memory. Immediately thereafter they received propranolol or placebo. A week later they engaged in script-driven mental imagery of their traumatic event while heart rate, skin conductance, and left corrugator electromyogram were measured. In comparison to the placebo subjects, overall physiological responding during mental imagery of the traumatic event was significantly smaller in the subjects who had received post-reactivation propranolol a week earlier, suggesting that the traumatic memory had been weakened. These results are consistent with reconsolidation blockade. However, a limitation of this study was that it did not include a non-reactivation propranolol group; consequently, the possibility that non-specific actions of propranolol were responsible for the effect cannot be ruled out.

The first aim of the present study was to investigate whether propranolol administered with memory reactivation weakens traumatic memories associated with combat-related PTSD. The second aim was to rule out the possibility that such an effect, if found, is due to non-specific actions of this drug. We hypothesized that subjects who underwent combat-script preparation accompanied by propranolol (reactivation propranolol, RP) would show smaller psychophysiological responses during script-driven imagery testing a week later compared to those who received propranolol in the absence of the script preparation procedure (non-reactivation propranolol, NRP).

METHODS

Subjects

Recruitment and inclusion criteria. Research subject candidates were male veterans ages 24 to 64 who had received a clinical diagnosis of combat-related PTSD. They were drawn from referrals from the VA Medical Centers in Bedford, MA and Manchester, NH, as well as from advertisements in the media [27].

<u>Exclusion criteria.</u> Prior to enrollment, subject candidates were clinically screened and excluded if they had a current psychotic, bipolar I, or melancholic disorder; a current substance dependence disorder (however four subjects turned out to have positive urines, as described below); a medical condition that contraindicated the administration of propranolol, *e.g.*, congestive heart failure, diabetes, chronic bronchitis, or emphysema; a history of an asthmatic attack within the past ten years, a history of an asthmatic attack precipitated by a β-adrenergic blocker at any time, or currently being treated for asthma regardless of when last attack occurred; previous adverse reaction to, or non-compliance with, a β-adrenergic blocker; pregnant or breast feeding; initiation of, or change in, psychotropic medication within one month prior to recruitment; current use of a medication that may have dangerous interactions with propranolol, *e.g.*, other β-adrenergic blockers, antiarrhythmics, and calcium channel blockers; and resting heart rate <60 beats per minute or resting systolic blood pressure <100 mmHg.

<u>Ethical approval and informed consent.</u> After a full explanation of the study procedures, which were approved by the Partners Human Research Committee, the Manchester/Bedford VA Medical Centers Human Studies Subcommittee, and the U.S. Army Medical Research and Materiel Command Human Research Protection Office, subjects gave written informed consent.

Study medication

Propranolol hydrochloride is a non-selective synthetic β_1- and β_2-adrenoreceptor antagonist that crosses the blood brain barrier. On Day 0 and Day 2 (see below), we administered a first dose of 0.67 mg/kg short-acting (SA) oral propranolol or placebo (rounded to the nearest 10 mg). If the SA dose was well-tolerated (which it was in all subjects), and if systolic blood pressure had not decreased by more than 10 mm Hg to below a level of 100 mmHg (which did not happen in any subject), 90 minutes later 1 mg/kg of long-acting (LA) oral propranolol or placebo (rounded to the nearest 20 mg) was administered. As in Kindt *et al.* [22] and Soeter and Kindt [23], subjects were given the drug 90 minutes prior to memory retrieval to allow propranolol to reach peak plasma concentration before reactivation [28]. The study medication was well-tolerated by subjects.

Equipment and physiological measures

A Coulbourn Lablinc V Human Measurement System (Coulbourn Instruments, Allentown, Pennsylvania) was used to record physiologic analog signals, including heart rate (HR), skin conductance (SC), and electromyogram (EMG) of the left corrugator and left frontalis facial muscles. Interbeat interval was recorded via standard limb electrocardiogram leads connected to a High Gain Bioamplifier (V75-04) and converted to HR. SC was measured by a Coulbourn Isolated Skin Conductance coupler (V71-23) using a constant 0.5 V through 9 mm (sensor diameter) Invivo Metric Ag/AgCl electrodes placed on the hypothenar surface of the subject's non-dominant hand in accordance with published guidelines [29]. The SC electrodes were separated by 14 mm, as determined by the width of the adhesive collar. For EMG recordings, the

skin was lightly abraded, and 4 mm (sensor diameter) Invivo Metric Ag/AgCl electrodes filled with electrolyte paste were placed over the corrugator and frontalis muscle sites according to published specifications [30]. The EMG was amplified by a Coulbourn High Gain Bioamplifier (V75-04), filtered so as to retain the 90 to 1000 Hz frequency range, and integrated by a Coulbourn Contour Following Integrator (V76-23A) with a 200 ms time constant. Physiologic analog signals were digitized by a Coulbourn analog to digital converter (V19-16). A Cobalt notebook computer (IBM-compatible) with custom-designed software was used to sample and store the digitized physiological signals.

Procedures

On Day 0, subjects randomized to the NRP group received propranolol, whereas subjects randomized to the RP group received matching placebo capsules. All subjects then viewed an emotionally neutral movie. By design, subjects were not permitted to discuss their combat events or PTSD symptoms on Day 0 to reduce the chances of traumatic memory reactivation.

On Day 2, subjects randomized to the RP group received propranolol, whereas subjects randomized to the NRP group received placebo. All subjects then underwent a "script preparation" session [31] in which an investigator elicited five discrete personal memories, including two traumatic combat experiences, a stressful non-combat experience, a positive experience, and a neutral one. Subjects were asked to describe each experience in writing on a standard form. The subject then selected from a list of bodily responses those that accompanied the experience. The investigator later composed a script approximately 30 seconds in duration portraying each experience in the second person present tense and incorporating up to five of the selected bodily responses. Subjects also completed a baseline Impact of Event Scale-Revised (IES-R; [32]) for each of their five personal events separately. A psychologist administered the Clinician-Administered PTSD Scale: Current and Lifetime Diagnosis Version (CAPS-DX; [33]) to verify the presence of current, combat-related PTSD, and the Structured Clinical Interview for DSM-IV (SCID; [34]) to evaluate the presence of any other Axis I comorbidity. The CAPS-DX and SCID were not administered prior to subject enrollment on Day 0 so as not to reactive the traumatic memory prematurely.

Subjects returned on Day 8 for the script-driven imagery testing session [31]. Prior to this testing, urine samples were collected and then sent for analysis of substances of abuse. The psychophysiological testing occurred in a sound-attenuated laboratory. After the subject was seated in a comfortable armchair and recording electrodes were attached, he listened to 2 minutes of relaxation instruction. The subject then sat quietly for 5 minutes. He then listened to eleven stimulus scripts presented sequentially in pseudorandom order, which consisted of the five personal scripts prepared on Day 2 and six standard scripts. Standard scripts included two neutral experiences, two hypothetical fear experiences (public speaking and locked in a sauna), a positive experience, and an action experience. Each script presentation consisted of four sequential 30-second periods: baseline, listening, imagery, and recovery. The subject was instructed to listen carefully during the playing of each script as it was presented (listening period) and at the end of the script to continue imagining the event portrayed from beginning to

end, as if it were happening again (imagery period) until he heard a tone. He was further instructed to stop imagining the script at the tone and to relax (recovery period) until a second tone was heard. The baseline period for the next script began after a rest period of 90 seconds or when the subject's heart rate (HR) had returned to within 5% of its value during the previous baseline period, whichever was longer. The rest period did not exceed 3 minutes following any script in any subject. Following the script-driven imagery procedure, subjects again completed IES-R ratings for each of the five personal events that they narrated on Day 2.

Data reduction

Response scores for each physiological measure for each script were calculated by subtracting the 30-second baseline period mean from the 30-second imagery period mean. Responses to the two traumatic scripts were averaged and square-root transformed prior to analysis. An *a priori* discriminant function derived from the HR, SC, and corrugator EMG responses during personal traumatic imagery of reference samples of previously studied individuals with and without PTSD using the same technique was used to calculate each subject's posterior probability of being classified with PTSD. This posterior probability served as a composite measure of overall physiological responding during script-driven traumatic imagery, eliminating the need for multivariate analyses of physiological responses in the small samples studied. (In cases in which one of these three physiological measures was missing due to technical failure, the physiological probability was calculated on the basis of the remaining two.) Additionally, discriminant function analyses of the references samples yielded optimal PTSD cut-offs for each response variable [35]. Each subject's baseline and outcome IES-R ratings were also averaged for their two traumatic combat scripts. IES-R change scores were calculated by subtracting the Day 8 IES-R total score from the Day 2 IES-R total score.

Statistical analysis

Between-group Student's t-tests were performed for all outcome measures. For our measure of primary interest, namely overall physiological responding (posterior probability), the threshold for statistical significance was $p < 0.05$. We selected *a priori* a group difference of 0.20 as clinically meaningful. Based upon prior work, we estimate the standard deviation of posterior probability in PTSD subjects to be 0.21. Therefore, a between-group effect size of $d=0.95$ for posterior probability was considered meaningful. For each univariate physiological response measure, the Bonferroni-corrected significance threshold was 0.0125. Finally for our secondary outcome measure, namely change in Impact of Event Scale Score, the threshold for statistical significance was also $p < 0.05$.

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RESULTS

Randomization, adverse events, drop-outs, and subjects excluded from data analysis

Twelve subjects were randomized to the RP group, and eleven to the NRP group. One subject in the RP group was withdrawn from the study following his relapse into opioid abuse following his Day 2 participation. This relapse was attributed to the stress of narrating his traumatic event, although the subject had a prior history of numerous alternating episodes of abstinence and relapse. Following this incident, any substance dependence within a year prior to study participation was added as an exclusion criterion. One RP subject and one NRP subject dropped out following their Day 2 participation; these drop-outs were also was attributed to the stress of narrating their traumatic events. Two subjects in the NRP group did not meet PTSD diagnostic criteria as determined by the CAPS on Day 2. Data from all these subjects were excluded from the analysis.

Subject characteristics

As shown in the top part of Table 1, there were no significant differences in age, baseline IES-R rating, or CAPS score between the two groups. Current comorbid mental disorders according to the SCID included: RP group: major depressive disorder (MDD, n=2), panic disorder (n=2), simple phobia (n=2), social phobia (n=2), bipolar II (n=1), generalized anxiety disorder (n=1); NRP group: MDD (n=4), panic disorder (n=1), social phobia (n=1), obsessive-compulsive disorder (n=1).

Outcome measures

The correlation between Day 2 total CAPS score and Day 8 physiological probability score across both groups was r=0.45, n=17; p=0.07. The group difference in Day 8 physiological PTSD probability score, a measure of overall arousal during script-driven imagery, was not significant. With the successful recruitment of only 10 usable subjects in the reactivation propranolol group and only 8 usable subjects in the non-reactivation propranolol group, our study conferred 61% power to test this group difference. According to the upper 95% confidence limit shown in Table 1, the posterior probability of PTSD patients treated with reactivation propranolol could be no more than 0.06 lower than the posterior probability in PTSD patients treated with non-reactivation propranolol. Given our *a priori* choice of 0.20 as the minimum clinically significant group difference in posterior probabilities, we can conclude that RP is not superior to NRP in this design. In contrast, the lower confidence limit of -0.31 means that we cannot reject the possibility that NRP is superior to RP, even though we neither predicted nor proved (p=0.17) this. There were no significant group differences on any univariate physiological response measure.

The IES-R change score was in the positive direction (decrease in score) for the NRP group and in the negative direction (increase in score) for the RP group, but neither of these within-group changes was significant (p=0.18 and p=0.34, respectively). Moreover, the group difference in IES-R change score was not significant. These findings are summarized in Table 1. Inspection of

Table 1. Group Means (standard deviations) and Statistical Contrasts for Baseline and Outcome Measures

Baseline Measures	**NR Propranolol** n = 8	**R Propranolol** n = 10	df, t, p	Difference in Means	95% Confidence Interval
Age	33.3 (11.5)	38.7 (14.9)	16, -0.85, 0.41	-5.4	-19.0 to 8.1
Day 2 IES-R Score	43.3 (14.2)	45.0 (18.3)	16, -0.22, 0.83	-1.7	-18.5 to 15.0
Clinician Admin PTSD Scale	58.6 (14.8)	62.7 (13.7)	16, -0.61, 0.55	-4.1	-18.4 to 10.2
Outcome Measures					
Day 8 IES-R Score	34.3 (15.8)	51.8 (39.2)	13, -2.06, 0.06	-17.5	-35.9 to 0.9
Change in Impact of Event Scale	8.2 (13.0)	-4.5 (13.2)	13, 1.83, 0.09	12.7	-2.3 to 27.6
Physiological PTSD probability score	0.32 (.22)	0.45 (.30)	15, -1.45, 0.17	-12.7	-0.31 to 0.06
Heart rate response (BPM)*	0.82 (-0.18)	0.76 (-0.52)	15, 0.08, 0.93	0.06	-1.6 to 1.7
Skin conductance response (µS)*	0.19 (0.85)	0.43 (0.64)	15, -0.65, 0.53	-0.24	-1.0 to 0.53
Frontalis EMG response (µV)*	0.01 (0.66)	0.65 (0.77)	15, -1.79, 0.09	-0.64	-1.4 to 0.1
Corrugator EMG response (µV)*	0.54 (0.64)	0.78 (1.37)	15, -.043, 0.67	-0.24	-1.3 to 0.8

n=sample size; df=degrees of freedom (where lower than expected according to sample sizes, this is due to missing data); t=Student's t; p=statistical probability level;

NR=Non-reactivation, R=reactivation; PTSD=Posttraumatic stress disorder; Physiological PTSD probability score=posterior probability of classification in PTSD reference group, which is an *a priori* measure of overall physiological responding during script-driven traumatic imagery (see text for explanation);

BPM=beats per minute; µS=microsiemens; µV=microvolts

Note: All physiological responses are square-root transformed

*Empirical cut-offs for PTSD are: Heart rate response 1.85 BPM; Skin conductance response 0.50 µS; Frontalis EMG response 0.83 µV; Corrugator EMG response 1.48 µV

the confidence intervals suggests the same conclusions as for physiological posterior probability, *i.e.*, that we can reject the possibility that RP is superior to NRP in producing symptom improvement because a group difference in IES-R change of 2.3 would not be regarded as clinically meaningful. However, we cannot reject the possibility that NRP is superior to RP because a group difference of 27.6 would be meaningful.

According to urine testing on Day 8, four subjects were found to be taking one or more potentially confounding substances, including opiates, barbiturates, and methadone, at the time of the script-driven imagery procedure. When the analyses were repeated excluding these subjects, the group means for posterior probability score became nearly identical: NRP n=6, M=0.32, SD=0.12; RP n=7, M=0.32, 0.04; group difference M=-0.00, SD=0.08, 95% confidence interval=-0.12 to 0.12, p=0.98. In contrast, the group difference in IES-R change scores widened: NRP n=5, M=10.1, SD=13.5; RP n=6, M=-11.4, SD=9.8; group difference M=21.5, SD=11.6, 95% confidence interval=5.6 to 37.4, p=0.01; however this latter significant result is qualified by the small sample sizes and unpredicted direction of the group difference.

DISCUSSION

This study aimed to replicate and extend earlier findings that propranolol accompanying traumatic memory reactivation weakens psychophysiological responding during subsequent mental imagery of the traumatic event [25]. In an earlier study, subjects were randomized to receive either post-reactivation propranolol or post-reactivation placebo; the investigators did not include a control group that received propranolol in the absence of memory reactivation. This left open the possibility that the observed fear-weakening effect of propranolol might have been due to non-specific actions of the drug. The present study addressed this question by using a control group that received propranolol in the absence of traumatic memory activation and contrasting it with a group that combined propranolol with memory reactivation. The results indicated no significant differences between these two groups, either with respect to subsequent psychophysiological responses during script-driven traumatic imagery or change in PTSD symptoms. In fact, the results trended in the direction opposite to predicted. Inspection of confidence intervals allowed us to rule out Type II error and conclude that reactivation propranolol is not superior to non-reactivation propranolol in either producing lower physiological responses during traumatic imagery or in reducing self-reported PTSD symptoms. However, we could not rule out the possibility that non-reactivation propranolol is superior to reactivation propranolol in both regards, although why the former should be superior to the latter is unclear.

The univariate psychophysiological responses of the reactivation propranolol group in the present study were only slightly higher than those exhibited by the post-reactivation propranolol group in the previous study [25], and they were below the normative cutoffs for PTSD as noted in Table 1.

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However, in contrast to the previous study that employed a reactivation placebo group, the univariate psychophysiological responses of the present non-reactivation propranolol control group were also below the PTSD cutoffs. In other words, both groups showed small psychophysiological responses. This pattern of findings suggests that the putative fear-weakening effect of propranolol may not be mediated by a reconsolidation mechanism.

The reliability of above inference is limited not only by the small sample sizes but also by several possible interpretations of the results. If the subjects believed that merely receiving propranolol would alleviate their symptoms, this expectancy effect may have been reflected in the low psychophysiological reactivity in the non-reactivation propranolol group. Importantly, the present results might also be explained if we happened to have recruited non-reactive subjects in both groups, which if true would not have provided a suitable test of the hypothesis. A second control group in which subjects received placebo on both non-reactivation Day 0 and reactivation Day 2 would have controlled for these two possibilities. Inclusion of such a group would be important in future studies. Assessing baseline psychophysiological reactivity prior to Day 0 might also have ruled out the latter explanation. However, baseline psychophysiological reactivity was not assessed so as to avoid both reactivating the traumatic memory in the control group, and habituating the subject to the script-driven imagery procedure. Another possible interpretation of the results is that, although the non-reactivation propranolol subjects watched an emotionally neutral movie on Day 0, they nevertheless may have recalled (reactivated) their traumatic events on that day merely by coming to participate in a PTSD research project, or in anticipation of narrating them on Day 2. The study was designed to minimize this possibility by not permitting any discussion of traumatic events or PTSD symptoms on Day 0.

Cohort demographics also differed between the previous and present studies. Specifically, the present study recruited only male subjects whereas the previous study included both men and women [25]. A *post-hoc* analysis of the earlier study's data revealed that women given post-reactivation propranolol tended to show lower posterior probabilities than men given propranolol (A. Brunet, personal communication, 2010). Female sex hormones have been shown to affect activity in the basolateral amygdala, a region implicated in conditional fear learning [36-39]. These considerations suggest that the effect of propranolol on reconsolidation may differ between men and women, which is a question that warrants further exploration.

The timing of propranolol administration in relation to memory reactivation also differed between the previous and present studies. In the earlier study, propranolol was administered following memory retrieval [25]. In the present study, a short-acting dose was given 90 minutes prior to retrieval to ensure that drug plasma levels would peak during reactivation. Propranolol administered 90 minutes prior to reactivation of a fear memory has been shown to successfully eliminate the conditioned fear response in normal humans while preserving the declarative memory for the contingency between the conditioned and unconditioned stimulus [22;23]. However, by suppressing the conditioned fear response, early administration of propranolol may have precluded subsequent reconsolidation blockade of this component of the fear memory.

It should be noted that, according to the pre-and post-intervention IES-R data, reactivation propranolol conferred no clinical benefit in this study despite both groups showing relatively low psychophysiological reactivity to the combat trauma scripts. The present results illustrate that translating reconsolidation blockade into clinical applications will not be simple or straightforward. More research is needed to search for potent pharmacological agents and administration paradigms that confer lasting clinical benefit by disrupting the fear component of the memory trace. Even if such efforts are successful, it is important that studies incorporate sufficient controls and sample sizes to support the inference that reconsolidation blockade is the mechanism underlying those effects.

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REPORTABLE OUTCOMES:

A manuscript reporting the above results is currently under review.

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