AD_____

AWARD NUMBER:

W81XWH-08-2-0126

TITLE:

Developing Memory Reconsolidation Blockers as Novel PTSD Treatments

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CONTRACTING ORGANIZATION:
 The General Hospital Corporation
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REPORT DATE:

June 2010

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
          Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

    x Approved for public release; distribution unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)* 23/06-2010	2. REPORT TYPE Annual "	3. DATES COVERED *(From - To)* "" 3'LWP '422; '/'53'O C['4232

4. TITLE AND SUBTITLE
Developing Memory Reconsolidation Blockers as Novel PTSD Treatments

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-2-0126

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The General Hospital Corporation
Boston, MA, 02114

8. PERFORMING ORGANIZATION REPORT NUMBER 02

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
and Materiel Command
Fort Detrick, Maryland

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

So far this project has produced the original finding that the anti-progesterone and glucocorticoid receptor antagonist mifepristone, when administered systemically, reduces reconsolidation of a cue-conditioned fear response in rats. This has been demonstrated in two independent laboratories. We have produced the further original discovery that the beta-adrenergic blocker propranolol blocks this mifepristone effect. We have produced the further original discoveries that systemic administration of the synthetic cannabinoid nabilone, the posterior peptide hormone oxytocin, the anti-dopaminergic drug haloperidol, and the protein-synthesis inhibitor rapamycin all reduce reconsolidation of a cue-conditioned fear response in rats, although none of these is as powerful as mifepristone. We have produced the further original discovery that post-reactivation rapamycin reduces synaptic strength underlying auditory fear conditioning. We have discovered input timing-dependent plasticity in auditory fear conditioning. We have successfully launched a randomized, double-blind controlled study of six sessions of post-reactivation propranolol for the treatment of PTSD, and a pilot study of post-reactivation mifepristone's ability to reduce psychophysiologic responding during traumatic imagery in trauma-exposed human subjects. We conclude that animal and human studies show promise for the development of a novel treatment for PTSD based upon pharmacological blockade of memory reconsolidation. However, we are still a long way from demonstrating that any such treatment is efficacious.

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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	UU	19	USAMRMC 19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

The aim of this project is to develop post-reactivation (PR) pharmacologic interventions that may serve as novel treatments for posttraumatic stress disorder (PTSD). The underlying theory is that candidate drugs, when given following the reactivation of a conditioned fear response in animals, or a traumatic memory in humans, will reduce the strength of the conditioned response or traumatic memory. We plan to test such drugs, either alone or in combination, for their possible reconsolidation-blocking properties in a hierarchy of experiments. Drugs that show promise at a given stage of investigation will be advanced to the next stage. In Stage I, we will evaluate the ability of candidate drugs to reduce freezing in a Pavlovian cue-conditioned fear task in rats, as well as to reduce associated retrieval-induced activation of immediate early genes in the amygdala. In Stage II, we will evaluate the ability of candidate drugs to reverse fear conditioning-induced synaptic enhancement in rat amygdala slices using whole-cell electrophysiologic recording. In Stage III, we will test the ability of a single session of PR candidate drug to reduce subsequent psychophysiologic responding during script-driven imagery of the traumatic event in trauma-exposed human subjects. In Stage IV, we will test the ability of multiple PR candidate drug therapy sessions to reduce symptoms in PTSD patients.

The animal reconsolidation experiments will entail three phases: 1.) single-trial fear conditioning; 2.) presenting the conditioned stimulus (reactivation), followed by PR drug; and 3.) measuring the conditioned response in a test trial, followed by sacrificing the animal for immunohistochemical or electrophysiologic measurements. If the drug is an amnestic (i.e., reconsolidation-blocking) agent, the test conditioned response should be reduced in animals that previously received the drug. Because the (past) traumatic event itself represents the (phase 1) conditioning event, the human experiments will only have the last two stages: 2.) single or multiple sessions of traumatic memory reactivation followed by candidate drug; and 3.) measuring a.) psychophysiologic responses during script-driven imagery of the traumatic event, and/or b.) PTSD symptoms.

In order to rule out the possibility that nonspecific drug effects account for any findings, the experiments will incorporate non-reactivation (NR) drug control groups, as well as PR vehicle/placebo control groups

2. BODY
2.1. Animal work
2.1.1. Massachusetts General Hospital (MGH)
#### 2.1.1.1. Abstract of study submitted for publication.

2.1.1.1.1. <u>Background.</u> Reducing reconsolidation of reactivated traumatic memories may offer a novel pharmacological treatment for posttraumatic stress disorder (PTSD). Preclinical research is needed to identify candidate drugs. We evaluated the ability of post-reactivation mifepristone (RU38486, a glucocorticoid antagonist) and propranolol (a beta-adrenergic blocker), given systemically alone and in combination, to reduce cue-conditioned fear in rats.

2.1.1.1.2. <u>Methods.</u> On Day 1 a 30-sec. tone conditioned stimulus (CS) was paired with an electric shock unconditioned stimulus (US) (acquisition). On Day 2, the CS was presented without the US (reactivation), and the freezing conditioned response (CR) was measured. This was immediately followed by subcutaneous vehicle, mifepristone 30 mg/kg, propranolol 10 mg/kg, or both. On Days 3 and 10, the CR was again measured (tests). On Day 11, the US was presented alone (reinstatement). On Day 12 , the CR was again measured (test). A fifth group received mifepristone without the CS (non-reactivation) on Day 2. A sixth group was tested 4 hours after the Day 2 mifepristone injection to measure post-reactivation short-term memory.

2.1.1.1.3. <u>Results.</u> Post-reactivation, but not non-reactivation, mifepristone produced a robust decrement in the CR. Mifepristone did not show this effect when administered concurrently with propranolol. Propranolol alone had a nonsignificant effect. Post-reactivation mifepristone did not reduce short-term memory.

2.1.1.1.4. <u>Conclusions.</u> Systemic mifepristone blocks the reconsolidation of cue-conditioned fear in rats. Concurrent administration of propranolol prevents this effect. Post-reactivation mifepristone may be a promising treatment for PTSD, but not necessarily in combination with propranolol.

2.1.1.1.5. <u>Figures.</u>
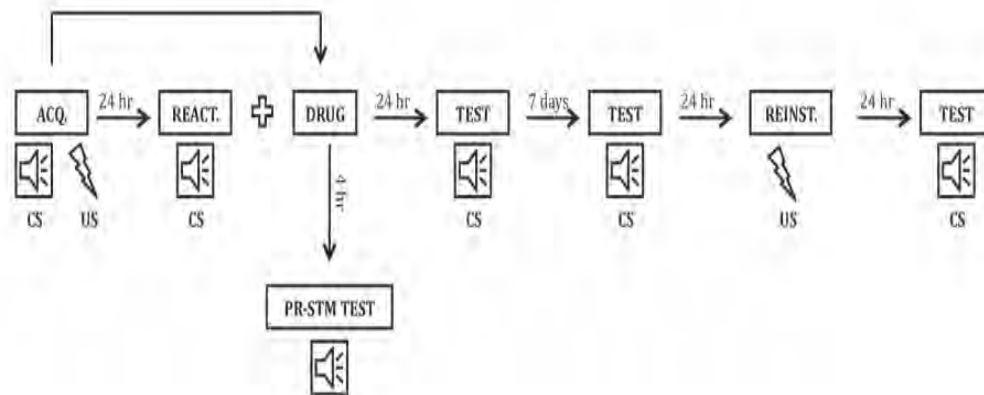

 <u>Figure 1.</u> Overview of experimental procedure. CS=Conditioned stimulus (speaker icon), US=unconditioned stimulus (lightning bolt icon), ACQ.=Acquisition, REACT.=Reactivation, NR-MIF=non-reactivation mifepristone, PR-STM=post-reactivation short-term memory, REINST.=Reinstatement, TEST=long-term memory test.
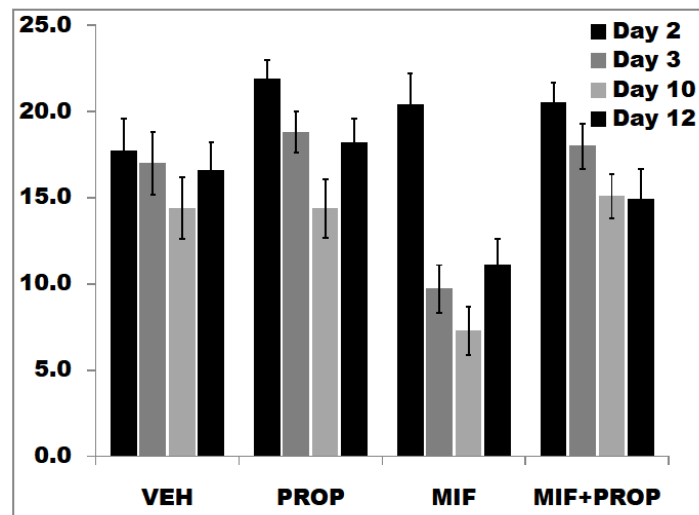
<u>Figure 2.</u> Group mean seconds of freezing to the tone (i.e., conditioned fear response) on Day 2 (following acquisition but prior to post-reactivation drug), Days 3 and 10 (test days following Day 2 post-reactivation drug), and Day 12 (test day following Day 11 reinstatement). VEH=vehicle, PROP=propranolol 10 mg/kg, MIF=mifepristone 30 mg/kg, MIF+PROP=both mifepristone and propranolol. Bars=standard error.
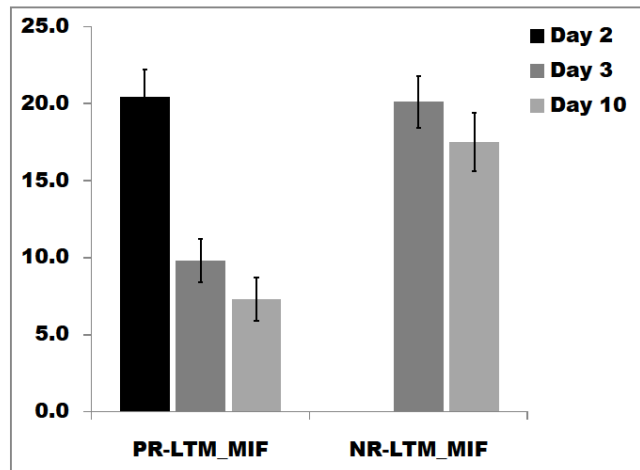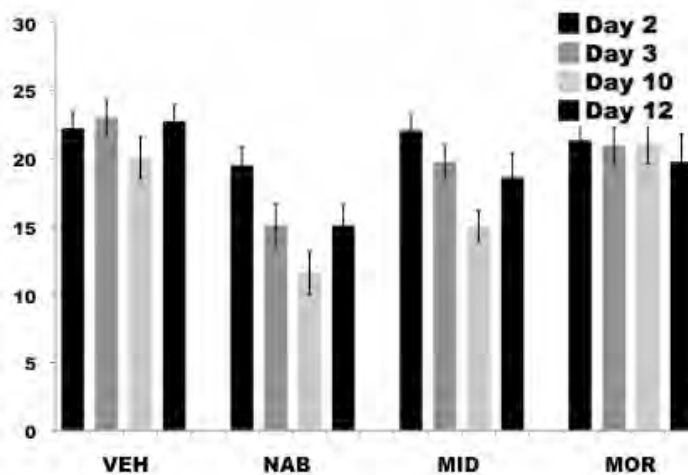
Figure 3. Group mean seconds of freezing (i.e., conditioned fear response) on Day 2 (following acquisition but prior to mifepristone, and Days 3 and 10 (test days following either Day 2 post-reactivation or non-reactivation mifepristone). PR-LTM_MIF=post-reactivation long-term memory with mifepristone; NR-LTM_MIF=non-reactivation long-term memory with mifepristone. No Day 2 data are shown for the NR-LTM_MIF group because the conditioned stimulus was not presented to this group on that day. Bars=standard error.
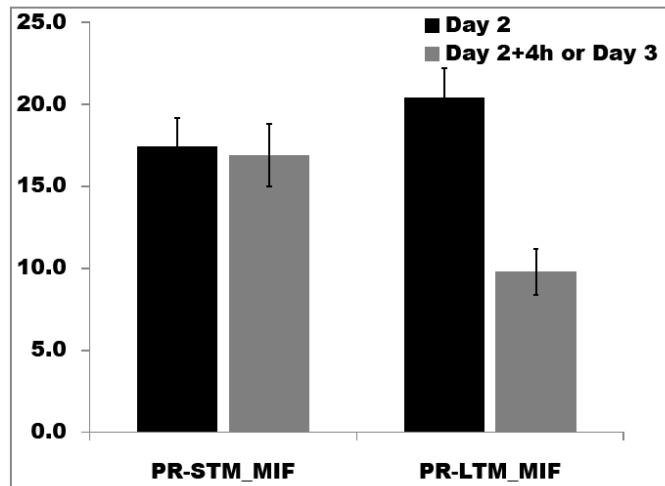
Figure 4. Group mean seconds of freezing (i.e., conditioned fear response) on Day 2 (following acquisition but prior to mifepristone, black bars), and again either 4 or 24 hours following post-reactivation mifepristone (Day 2+4h or Day 3, gray bars) . PR-STM_MIF=post-reactivation short-term memory with mifepristone; PR-LTM_MIF=post-reactivation long-term memory with mifepristone. Bars=standard error.

2.1.1.2. As yet unpublished work

2.1.1.2.1. <u>Midazolam, morphine, nabilone.</u> Since the successful results with 30mg/kg mifepristone, we have explored the potential of several other drugs and combinations to be similarly implemented for reconsolidation blockade. As noted in the initial application, we are only interested in drugs that have been approved for human use and can be administered systemically. Therefore, as far as possible we select the dosage we use in rodents to reflect an appropriate human dosage. Our next experiment following mifepristone examined midazolam, a rapid-acting benzodiazepine, which has well recognized anterograde amnestic properties. We found midazolam to have little reconsolidation-blocking capacity at a dose of 1.5mg/kg. Rats that received this drug showed only a 10% reduction in freezing from Day 2 to Day 3 compared to over 50% in the mifepristone group. Similarly, a group of 24 rats that received 1.0mg/kg of morphine, which inhibits locus ceruleus activity and noradrenergic activities in areas to which it projects, including amygdale, showed virtually no decline in freezing (2%) on Day 3. In contrast, nabilone, a synthetic cannabinoid, at a dose of 1.0mg/kg, showed significant reconsolidation-blocking effects by reducing freezing by 23% on Day 3. Our experiments in control groups indicate that, like mifepristone, nabilone does not appear to reduce post-reactivation short-term memory. Further experiments are now underway to investigate nabilone's effect on non-reactivated controls. Freezing behavior data for nabilone, morphine, and midazolam are shown in Figure 5.

Figure 5. Group mean seconds of freezing to the tone (i.e., conditioned fear response) on Day 2 (following acquisition but prior to post-reactivation drug), Days 3 and 10 (test days following Day 2 post-reactivation drug), and Day 12 (test day following Day 11 reinstatement). VEH=vehicle, NAB=Nabilone 1.0 mg/kg, MID=midazolam 1.5 mg/kg, MOR=morphine 1 mg/kg, Bars=standard error.

2.1.1.2.2.<u>Oxytocin.</u> We then explored oxytocin, a posterior pituitary hormone with known amnestic properties, in three doses. At, 0.05mg/kg, oxytocin-treated rats showed no decline in freezing behavior from Day 2 to Day 3. At 1.25mg/kg, however, rats showed a 27% decline in

freezing on Day 3, and at 10.0mg/kg, freezing was reduced by 23%. Because raising the dose from 1.25 to 10.0mg/kg showed no increase in effectiveness, we selected 1.25mg/kg and sought to determine whether combining oxytocin with mifepristone would have an additive effect. Rats that received mifepristone plus oxytocin showed a modest decline in freezing on day 3 at 22%, which does not represent an improvement over mifepristone alone. Freezing behavior data for oxytocin at all doses, and in combination with mifepristone, are shown in the Figure 6.
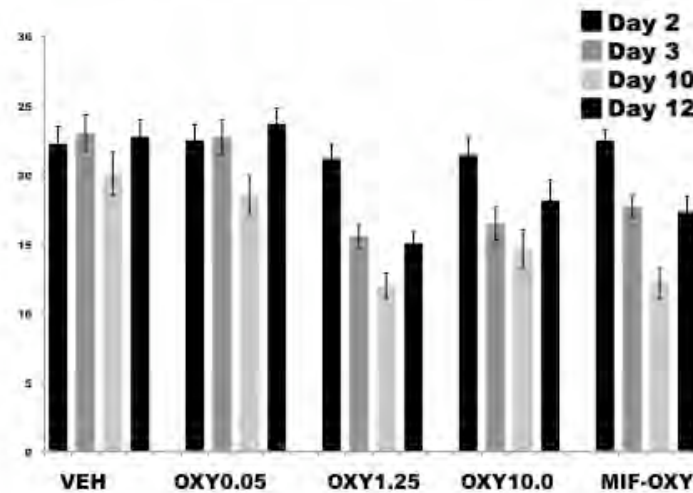

Figure 6. Group mean seconds of freezing to the tone (i.e., conditioned fear response) on Day 2 (following acquisition but prior to post-reactivation drug), Days 3 and 10 (test days following Day 2 post-reactivation drug), and Day 12 (test day following Day 11 reinstatement). VEH=vehicle, OXY=Oxytocin 0.05, 1.25 mg/kg, and 10.0 mg/kg. Bars=standard error.

2.1.2. McGill University

2.1.2.1. Replication of mifepristone effect in independent laboratory. Last year, our colleagues at the MGH were able to show reconsolidation blockade using a 30 mg/kg dose of the anti-progesterone and glucocorticoid receptor antagonist mifepristone. However, in our hands, mifepristone failed to block reconsolidation. After comparing the fear conditioning protocols, we identified two differences – the rat supplier and the conditioning chambers. We were finally able to replicate the results obtained by the MGH group using the same rat supplier and a similar set-up of conditioning chambers. We obtained a significant impairment of the CR in the mifepristone-treated animals compared to the controls, hence, confirming mifepristone's effectiveness as a reconsolidation blocker (figure 1).

2.1.2.2. Double post-reactivation mifepristone. To examine further mifepristone's ability to disrupt memory reconsolidation, we trained rats and reactivated them twice, administering mifepristone (30mg/kg) after each reactivation session. We hypothesized that two reactivation sessions paired with mifepristone injections might lead to a stronger impairment of the CR. The results showed that the maximum impairment is obtained after the first reactivation plus injection

session, confirming that the second session does not induce a larger impairment (figure 2). Consequently, we did not further pursue the use of multiple reactivation/treatment sessions.

2.1.2.3. Scopolamine. We have also investigated the reconsolidation blockade of auditory fear memories using various doses (0.5, 1.0 and 2.0 mg/kg) of the muscarinic-acetylcholine receptor antagonist, scopolamine, injected immediately after the reactivation session. Our results showed the inability of scopolamine to block reconsolidation under the parameters tested (figure 3). We concluded that scopolamine might not be effective in the specific task and parameters we used in this study.

2.1.2.4. Haloperidol. Next we decided to explore another candidate drug, the dopaminergic antagonist haloperidol. We tested the ability of haloperidol (1mg/kg) to block reconsolidation, either alone or in combination with propranolol (10 mg/kg) in male and female rats. We hypothesized that by blocking both dopamine and beta-adrenergic receptors we could achieve a stronger memory impairment on day 3 than with propranolol or haloperidol alone. So far, our initial results are consistent with our hypothesis (figure 4). As observed before, propranolol slightly reduced the CR; however, the fear response of these animals was not significantly different from the vehicle-injected controls. On the other hand, haloperidol and the drug combination were effective at reducing the fear-related memory compared to the controls (both $p<0.05$). Overall, the results show a significant main effect of treatment ($p<0.001$) and day ($p<0.0001$), but not a significant treatment x day interaction. We are already in the process of replicating these results with additional animals. Nonetheless, the current data set is encouraging.

2.1.2.5. Pre-reactivation D-cycloserine plus post-reactivation mifepristone. Most recently, we explored the effect of mifepristone (30 mg/kg) in combination with a pre-reactivation injection of D-cycloserine (DCS) (15mg/kg), a partial NMDA agonist. This choice was motivated by a recent publication from Bustos et al. (2010), in which they show that DCS can enhance memory lability and make a resistant memory more susceptible to disruption by amnestic agents. We evaluated the combination of DCS and mifepristone using a stronger training protocol (three shocks instead of one) to make the memory more resistant to disruption, as it could be the case in post-traumatic stress disorders. We injected DCS 30 minutes prior and mifepristone immediately after reactivation, and then tested for memory retention the next day and a week later. We hypothesized that this drug combination would induce a more pronounced decrease of the fear-related memory response than mifepristone alone. So far, our results showed a tendency of the drug combination to reduce the CR more than mifepristone alone, 1 week after receiving the treatment (figure 5). On the other hand, DCS does not seem to have any effect on its own at both time points compared to the control group. Overall, the results show a significant main effect of day ($p<0.0001$) and treatment x day interaction ($p<0.01$), but not a significant main effect of treatment. We are planning on testing more animals in order to achieve statistical significance. However, the data obtained thus far are promising.
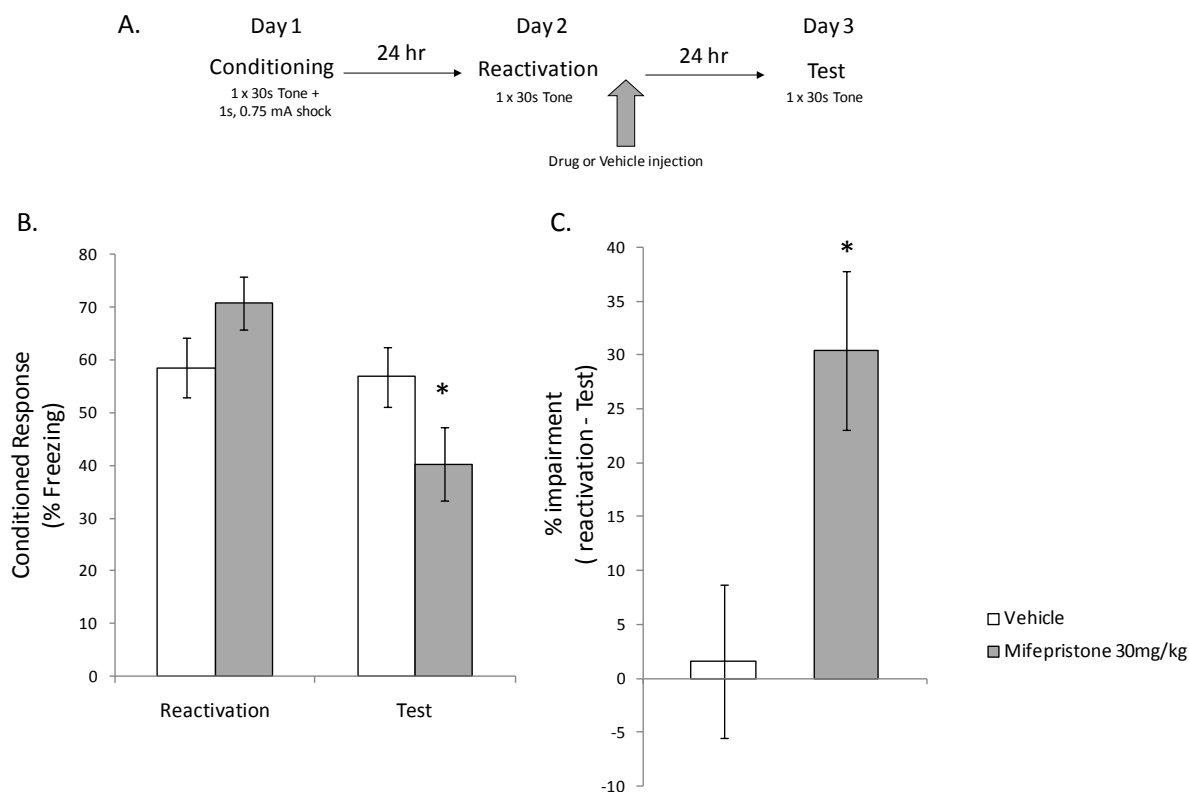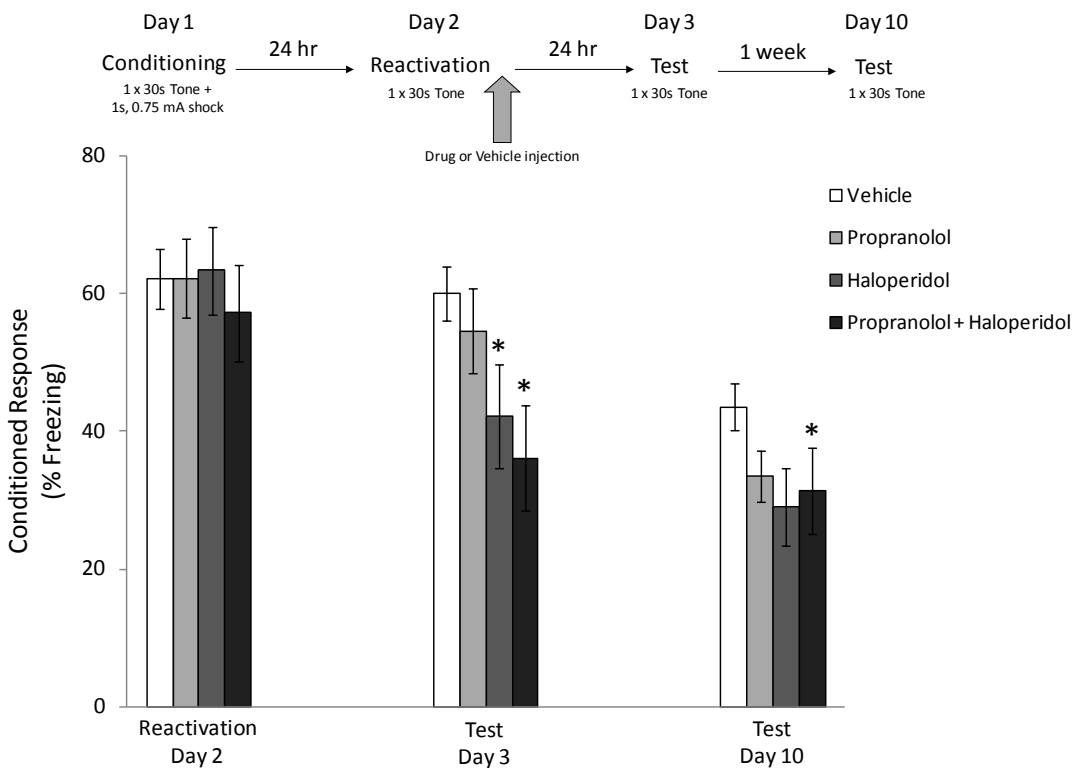
Figure 1. Mifepristone blocks reconsolidation of auditory fear memories. A) Experimental protocol. B) Mifepristone 30 mg/kg (n=14) injected immediately after reactivation significantly reduced the conditioned response compared to the vehicle-injected group (n=13) when tested on day 3. C) Mifepristone showed a significantly higher memory impairment than the vehicle-treated controls. *p<0.05
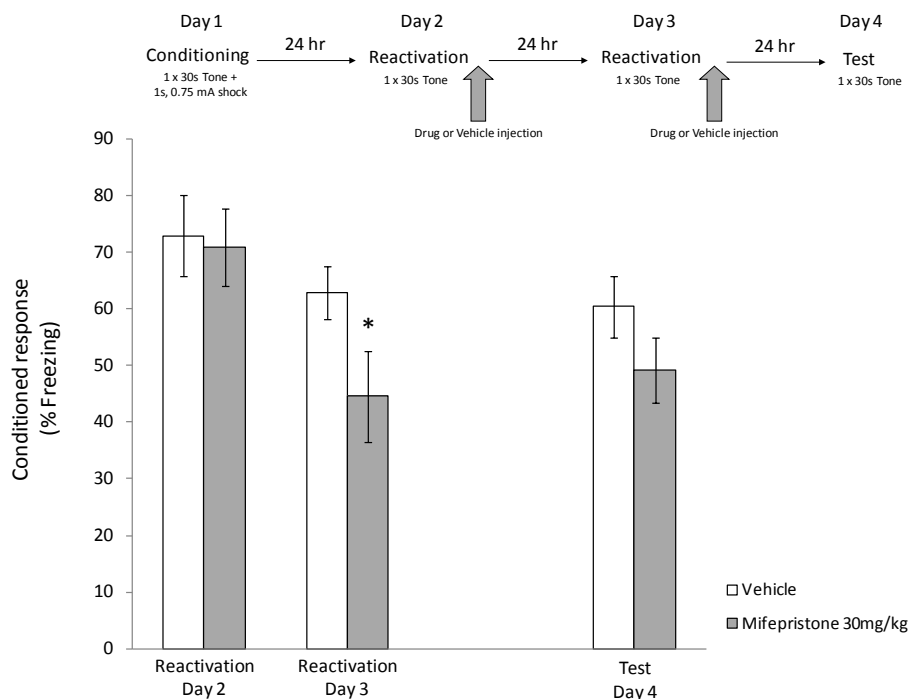
Figure 2. Two reactivation sessions paired with mifepristone injections do not lead to a stronger impairment of the conditioned response. Above: experimental protocol. Mifepristone 30 mg/kg (n=8) blocks reconsolidation of auditory fear memories after the first reactivation session compared to the vehicle-treated animals (n=8). A second reactivation plus mifepristone treatment does not induce further reduction in the conditioned response when tested on day 4. *p<0.05
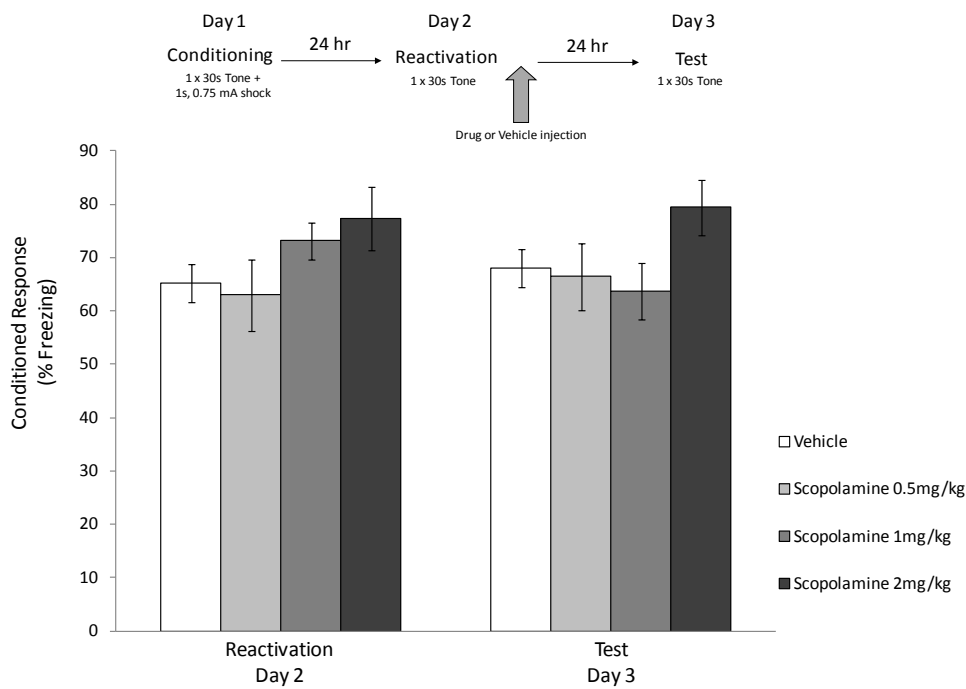
Figure 3. Scopolamine does not disrupt reconsolidation of auditory fear memories. Above: experimental protocol. At the doses of 0.5, 1.0 or 2.0mg/kg (respectively n=9, n=25, n=9), scopolamine did not significantly impair reconsolidation when injected immediately after the reactivation session compared to the vehicle group (n=20).

Figure 4. Haloperidol, alone or in combination with propranolol, effectively disrupts reconsolidation of auditory fear memories. Above: experimental protocol. Propranolol 10 mg/kg (n=12) injected immediately after reactivation did not significantly reduced the conditioned response compared to the vehicle-injected group (n=12) when tested the next day. However, haloperidol 1mg/kg (n=12) and the haloperidol and propranolol combination (n=12) significantly impaired the conditioned response on the day 3 compared to the vehicle group. On day 10, all drug groups showed a non statistically different reduced conditioned response illustrating a tendency for a long lasting effect on reconsolidation. *p<0.05 compared to vehicles.
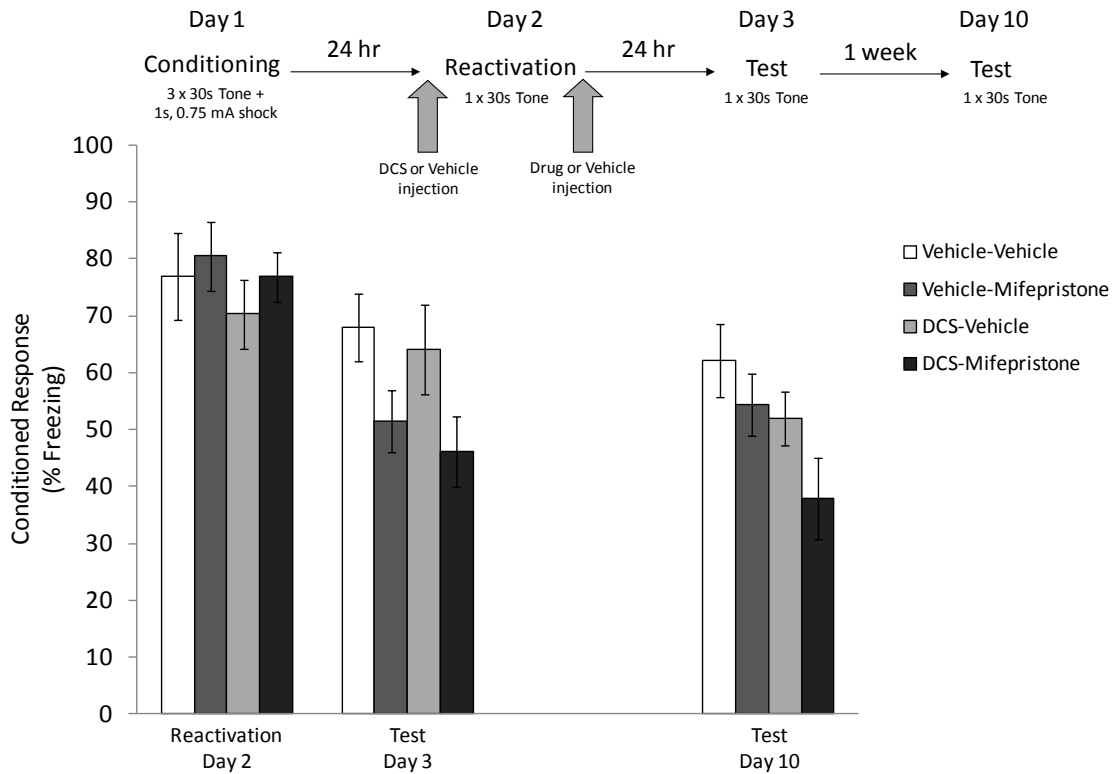
Figure 5. A combination of D-cycloserine and mifepristone disrupts reconsolidation of auditory fear memories. Above: experimental protocol. D-cycloserine (DCS) 15mg/kg injected 30 minutes prior reactivation in combination with mifepristone 30 mg/kg (n=12) injected immediately after reactivation reduced the rats' conditioned response compared to the vehicle-injected control group (n=12) when tested the next day or a week later. Animals treated only with mifepristone 30mg/kg (n=12) showed a decreased fear response at day 3 but not at day 10 compared to the vehicle group. When injected alone, DCS (n=12) did not impaired the conditioned response on day 3 and day 10.

2.1.3. <u>McLean Hospital.</u> In our experiments, we are training rats in the auditory fear-conditioning paradigm and then relating changes in synaptic transmission in afferent inputs to the amygdala to fear memory following fear conditioning and fear memory reconsolidation. We are testing the ability of different compounds, blocking fear memory reconsolidation, to prevent changes in synaptic transmission in inputs to the amygdala associated with fear memory recall. Spague-Dawley rats (250-300 g) were trained in a single-trial fear-conditioning paradigm. The rats were conditioned on the training day and tested at 24 h post-training in the second context. One hour later, the rats were used for electrophysiological recordings. In these experiments, we confirmed that synaptic strength in thalamic input to the LA, as assessed by input-output curves for AMPA receptor-mediated EPSCs, is significantly increased in slices from fear-conditioned rats compared to control animals. The fear learning-associated increases in synaptic function at thalamo-LA synapses were not accompanied by changes in membrane excitability of neurons in the LA. These findings are consistent with the notion that the acquisition of fear memory to auditory conditioned stimuli (CS) is associated with synaptic strengthening in the CS pathways.

2.1.3.1. <u>Rapamycin.</u> We tested the effects of systemically-delivered rapamycin (serolimus), which is a blocker of mTOR, on post-retrieval reconsolidation of fear memory and LTP in thalamic input to the LA in slices from the same rats which were used in behavioral studies. mTOR is a protein kinase that regulates protein synthesis at the translational level. Following reactivation, rats received one injection of rapamycin (20 mg/kg, I.P.) shortly after the fear memory test. Fear memory was re-tested at 24 h post-retrieval. The US intensity in these experiments was 0.6 mA (2-s duration). This US was reliably producing fear memory while conditioned fear responses did not reach the saturation level. We found that systemically delivered rapamycin significantly suppressed fear memory reconsolidation after retrieval (t test, $P < 0.05$). Immediately after the last behavioral test, we prepared brain slices for electrophysiological analysis. In blind experiments, we found that pairing-induced LTP in projections from the auditory thalamus (thalamic input) to the LA was significantly increased in slices from rats which received the injection of rapamycin after retrieval of fear memory, compared to the vehicle-injected rats (Figure 1; t test, $P < 0.01$ between groups). As the acquisition of fear memory was shown previously to potentiate auditory synaptic inputs to the LA and occlude LTP in slices (induced by electrical stimulation), our results suggest that the injection of rapamycin shortly after fear memory retrieval resulted in the decreased synaptic strength at thalamo-LA projections, which manifested itself as increased LTP (because LTP was no longer occluded). This is an interesting finding, as it suggests that inhibition of mTOR shortly after fear memory retrieval could abolish synaptic facilitation in the CS pathways produced by the acquisition of fear memory. We plan to continue these studies, extending our analyses of LTP mechanisms after fear memory recall to cortical input to the LA, because projections from the auditory cortex to the LA also play an essential role in fear conditioning. As systemically-injected mifepristone also has an effect on reconsolidation of fear memory after retrieval, we plan to repeat the above-described experiments with mifepristone.
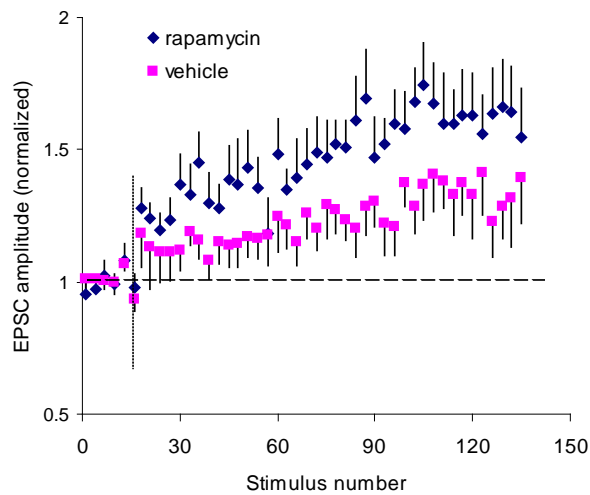
Figure 1. LTP in slices from post-reactivated rapamycin- or vehicle-injected rats. LTP of the EPSCs in thalamic input to the LA was induced (at a dashed vertical line) by pairing of postsynaptic depolarization to +30 mV and low-frequency (2-Hz) presynaptic stimulation in slices from vehicle-injected (n = 10 neurons from 6 rats) or rapamycin-injected rats (n = 10 neurons from 6 rats; $P < 0.01$ for the magnitude of LTP in rapamycin-injected versus vehicle-injected animals). Synaptic responses were normalized to the pre-LTP baseline. Following reactivation, rats received one injection of either rapamycin (20 mg/kg, I.P.) or vehicle. Error bars are SEM.

2.1.3.2. <u>Input timing-dependent plasticity.</u> As an interesting development of this project, we found that continuous paired stimulation of thalamic and cortical auditory inputs to the lateral nucleus of the amygdala with the interstimulus delay mimicking a temporal pattern of their synaptic activation in behaving animals during auditory fear conditioning resulted in persistent potentiation of synaptic transmission in cortico-amygdala pathway. This novel form of input timing-dependent plasticity (ITDP) in cortical input depended on $InsP_3$-sensitive Ca^{2+} release from the internal stores and postsynaptic Ca^{2+} influx through calcium permeable kainate receptors during its induction. ITDP in the CS pathways, determined by characteristics of presynaptic activity patterns, may contribute to the encoding of the complex auditory CS. To explore whether ITDP could play a role in fear conditioning, we tested ITDP in slices from conditioned rats. In these experiments, conditioned fear was produced by a single pairing of the acoustic CS with an electric footshock (US). Memory of fear was assessed by measuring an increase in the freezing response to the tone (CS) following fear conditioning (Figure 2). Shortly after the fear memory test, we performed whole-cell recordings from neurons in slices from conditioned or control rats. We found that virtually no potentiation could be observed in cortical input to the LA in slices from conditioned rats (CS-US group) at 35-40 min after the delivery of the ITDP induction protocol (t test, p = 0.18 versus baseline). However, normal ITDP was observed in slices from behaviorally naïve rats (p < 0.05 versus ITDP in slices from the CS-US group) or rats which received the CS only (p < 0.05 versus ITDP in slices from the CS-US group). Thus ITDP in cortical input to the LA is occluded following the acquisition of fear memory to the auditory CS, suggesting that ITDP mechanisms may contribute to encoding the fear memory trace. These findings are important because different forms of synaptic plasticity could be differentially recruited during fear conditioning and reconsolidation of fear memory after retrieval. It was necessary to characterize in detail this newly discovered form of synaptic plasticity in fear conditioning pathways, as we found that it might be recruited during fear

conditioning (Fig. 2). In our future studies, we plan to differentiate between the effects of reconsolidation on different forms of LTP (including the newly discovered ITDP), thus establishing what kind of cellular substrates of memories are the best candidates for us to attempt to influence. The manuscripts describing these studies are presently under review in Neuron and Nature Neuroscience.
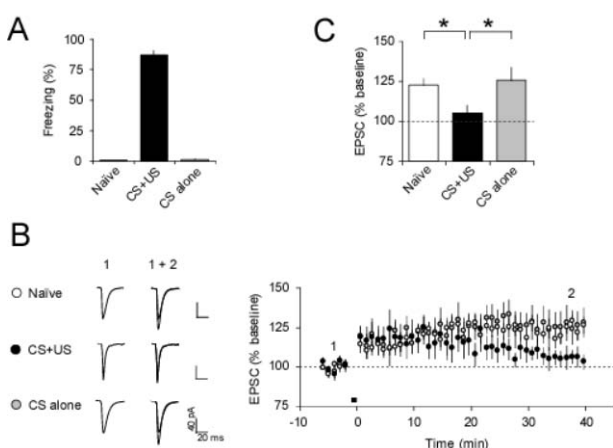

Figure 2. ITDP in cortico-LA pathway is occluded in slices from fear-conditioned rats. (A) Freezing responses in different groups of rats. (B) Left, cortico-LA EPSCs recorded before (1) and after (1 + 2) the delivery of the ITDP protocol in slices from all experimental groups. Right, ITDP at the cortico-LA synapses was occluded in slices from fear-conditioned rats (n = 12 neurons from 8 rats; t test, p = 0.18), while significant ITDP was observed in naïve rats (n = 14 neurons from 9 rats; t test, p < 0.05) or the "CS-only" rats (n = 7 neurons from 4 rats; t test, p < 0.001). (C) Summary of the EPSC amplitude changes.

2.4. Human work

2.4.1 <u>MGH.</u> On the basis of the animal results reported in §2.1.1.1 and §2.1.2.1 above, we decided to perform a pilot study of post-reactivation mifepristone's ability to reduce psychophysiologic responding during traumatic imagery in trauma-exposed human subjects. At the time of the last annual report, we had succeeded in obtaining an investigational new drug (IND) approval from the FDA for this novel post-marketing application of mifepristone. Since then, we obtained all necessary IRB approvals for this study. We also completed the difficult task of negotiating a contract between Danco Laboratories and MGH to provide the drug at cost. Finally, after an enormous amount of administrative paperwork and many months elapsed, we began this study two months ago. To date, we have completed running three subjects. One subject is in the middle of participation. Eight more subjects are scheduled to be studied over the next two months. We have not yet looked at any data.

2.4.2. McGill University/Douglas Mental Health University Institute
2.4.2.1 <u>Background for current study.</u> Despite the negative results obtain with propranolol in the animal work described above, we decided to proceed with a double-blind, randomized, placebo-controlled trial of multiple sessions of post-reactivation propranolol for the treatment of PTSD. Several considerations motivated this decision. First, an influential article published in early 2009 succeeded in demonstrating that propranolol blocked the reconsolidation of a conditioned fear memory in normal humans (Kindt al, 2009), in a sense bypassing the need for further confirmatory rat studies. Second, in previously published work, we succeeded in demonstrating that a single session of propranolol following reactivation of the traumatic memory in PTSD patients significantly reduced a biological PTSD marker, viz., physiologic responding during subsequent script-driven imagery of the event (Brunet et al, 2008). Third, an analysis of a previously collected data set from an open label, six session, post-reactivation propranolol case series in 32 PTSD patients yielded promising results. Results from that work serve as the basis for

the double-blind, randomized, placebo-controlled trial that is now underway. The study is looking at the therapeutic effects of six weekly treatment sessions consisting of reactivating the trauma memory while under the influence of either propranolol or placebo. The therapeutic effects are measured in two ways: (1) PTSD symptoms before, during and up to four months after the treatment, and psychophysiologic responding to script-driven imagery depicting the person's traumatic event (post-treatment and at follow-up).

2.4.2.2. Initiation of study. During the early portion of the 02 year, we succeeded in finally overcoming various obstacles to beginning recruitment.

2.4.2.2.1. Human subject certification. One obstacle preventing the launching of the project was the human subject certification which was required for all study personnel. After an extensive search, a recognized Canadian organization was finally found to provide such training, as none of the team was certified (this certification is not common practice in Canada). The team members were trained by the FRSQ (Fonds de la Recherche en Santé du Quebec) and successfully met the requirements for Basic Training in Research Ethics involving human subjects. The FRSQ is recognized by the Research Tri-council of Canada as a certified provider of such training. The tri-council of Canada is recognized by the DoD as an organization outside of the U.S. that has the capacity to certify providers of Human subject certification. The certification was obtained on October 14, 2009. The certificates were immediately sent to the DoD officials.

2.4.2.2.2. IRB approvals. An obstacle to DoD IRB approval was the necessity of getting the Partners (MGH) Human Research Committee to sign an agreement stating that MGH may rely on the Douglas Mental Health University Institute IRB for review and continuing oversight of its human subject research pertaining to the project. This seemed superfluous, given that no subjects in this study were to be studied at MGH, and it was obvious from the beginning that the Douglas IRB should oversee the human subjects research study being done at Douglas. Unfortunately, this seemingly pointless administrative obstacle took several months to overcome. Finally, we received official authorization to start recruiting human subjects in February 2010.

2.4.2.3. Progress to date. Thirty-six PTSD patients have been screened. Sixteen patients have come to the first encounter. One patient was immediately excluded for medical reasons (asthma). Of the remaining 15, 3 did not complete the treatment protocol for the following reasons: not wanting to talk about the event, did not have the time, unspecified reasons. Nine patients have completed the treatment protocol, and 3 more are in progress. Collapsed across drug groups, the nine completed patients show a mean of 48% improvement on the PTSD Checklist and 30% improvement on the Clinician-Administered PTSD Scale. Four out of the nine no longer qualified for the PTSD diagnosis. We have not broken the blind.

3. KEY RESEARCH ACCOMPLISHMENTS

3.1. Original discovery and replication in an independent laboratory that the anti-progesterone and glucocorticoid receptor antagonist mifepristone, when administered systemically, reduce reconsolidation of a cue-conditioned fear response in rats. Further original discovery that the beta-adrenergic blocker propranolol blocks this mifepristone effect.

3.2. Original discovery that the synthetic cannabinoid nabilone reduces reconsolidation of a cue-conditioned fear response in rats.

3.3. Original discovery that the posterior peptide hormone oxytocin reduces reconsolidation of a cue-conditioned fear response in rats.

3.4. Original discovery that the dopamine blocker, alone and in combination with propranolol, reduces reconsolidation of a cue-conditioned fear response in rats.

3.5. Original discovery that the protein-synthesis inhibitor rapamycin reduces reconsolidation of a cue-conditioned fear response in rats. Further original discovery that post-reactivation rapamycin reduces synaptic strength underlying auditory fear conditioning.

3.6. Original discovery of Input timing-dependent plasticity in auditory fear conditioning.

3.7. Successful launch of a randomized, double-blind controlled study of six sessions of post-reactivation propranolol for the treatment of PTSD.

3.8 Successful launch of a pilot study of post-reactivation mifepristone's ability to reduce psychophysiologic responding during traumatic imagery in trauma-exposed human subjects.

4. REPORTABLE OUTCOMES

The following manuscripts reporting work supported by this grant have been submitted to date for publication.

Cho JH, Bayazitov IT, Meloni EG, Zakharenko SS, Bolshakov VY. (2010). Time-locked sequential activation of thalamic and cortical pathways induces input timing-dependent plasticity in the lateral amygdala. Submitted to *Neuron*.

Pitman RK, Milad MR, Igoe SA, Vangel MG. Orr SP, Gamache K, Nader K. Systemic mifepristone blocks reconsolidation of cue-conditioned fear in rats; concomitant propranolol prevents this effect. Submitted to *Neuropsychopharmacology.*

Shin RM, Tully K., Li Y., Cho JH, Bolshakov VY (2010). Hierarchical order of coexisting pre- and postsynaptic forms of LTP at synapses in amygdala. Submitted to *Nature Neuroscience.*

5. CONCLUSION

Animal and human studies show promise for the development of a novel treatment for PTSD based upon pharmacological blockade of memory reconsolidation. However, we are still a long way from demonstrating that any such treatment is efficacious.

6. REFERENCES

Brunet A, Orr SP, Tremblay J, Robertson K, Nader K, Pitman RK (2008). Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *J Psychiatr Res* **42**: 503-506.

Bustos SG, Giachero M, Maldonado H, Molina VA (2010). Previous stress attenuates the susceptibility to Midazolam's disruptive effect on fear memory reconsolidation: influence of pre-reactivation D-cycloserine administration. *Neuropsychopharmacology* **35**: 1097-1108.

Kindt M, Soeter M, Vervliet B (2009). Beyond extinction: erasing human fear responses and preventing the return of fear. *Nat Neurosci* **12**: 256-258.

7. APPENDICES/SUPPORTING DATA

None