Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Victor Taylor Warren April 14, 2013

An Analysis of Retrieval-Enhanced Extinction Paradigms Using Fear-Potentiated Startle and Cognitive Awareness Measures

by

Victor Taylor Warren

Dr. Seth D. Norrholm
Adviser

Department of Psychiatry

Dr. Seth D. Norrholm

Adviser

Dr. William Kelly

Committee Member

Dr. Barry Yedvobnick

Committee Member

2013

An Analysis of Retrieval-Enhanced Extinction Paradigms Using Fear-Potentiated Startle and Cognitive Awareness Measures

by

Victor Taylor Warren

Dr. Seth D. Norrholm

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Biology

2013

Abstract

An Analysis of Retrieval-Enhanced Extinction Paradigms Using Fear-Potentiated Startle and Cognitive Awareness Measures

By Victor Taylor Warren

Current research on fear conditioning and extinction has explored the role of fear memory retrieval and reconsolidation. Unlike traditional fear extinction studies, which display numerous fear recovery effects, recent experiments have demonstrated that the use of retrieval+extinction paradigms results in persistent dampening of the original conditioned fear. However, these studies have relied on skin conductance measures, which are widely known as a measure of general arousal as opposed to fear response. Here we attempt to translate the results of retrieval+extinction paradigms to our well established fear-potentiated startle protocol, which quantifies a reflexive response that is directly integrated into the fear learning circuitry of the mammalian brain. In addition, we attempt to analyze the cognitive effect of US-expectancy predictions on startle response via the use of a response pad. We provide evidence that our retrieval+extinction paradigm replicates the decreased fear recovery shown in other studies; however, recruitment of higher cortical brain regions via the response pad leads to enhanced fear learning and decreases the retrieval effects. These findings provide important information for future research endeavors, particularly those targeting treatment of anxiety disorders such as post-traumatic stress disorder.

An Analysis of Retrieval-Enhanced Extinction Paradigms Using Fear-Potentiated Startle and Cognitive Awareness Measures

by

Victor Taylor Warren

Dr. Seth D. Norrholm

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Biology

2013

Acknowledgements

I would like to thank Kemp Anderson, Cliffe Kwon, Lauren Bosshardt, and AJ McCarthy for assistance gathering and analyzing data. In addition, I would like to thank the Director of the Trauma Recovery Program Bekh Bradley and all affiliated investigators. I would also like to thank my committee members Bill Kelly, Barry Yedvobnick, and, in particular, my adviser Seth Norrholm for continuous support throughout not only this study, but my entire tenure in research at the Atlanta VA Hospital.

Table of Contents

Introduction:

Post-Traumatic Stress Disorder:

Post-traumatic stress disorder (PTSD) is a type of anxiety disorder resulting from exposure to a traumatic event that involved the threat of injury or death (American Psychiatric Association, 2000). Despite only relatively recently (1980) being formally classified in the Diagnostic and Statistical Manual for Mental Disorders (DSM), PTSD was recognized in numerous earlier military conflicts. Terms such as exhaustion, battle fatigue, and shell-shock were implemented to classify the unusual psychological symptoms of some soldiers who were exposed to trauma. Outside of combat, PTSD has also been observed in civilians traumatized by events including violent crimes, serious accidents, natural disasters, and terrorist attacks.

Symptoms of PTSD are divided into three diagnostic clusters: (1) reliving the traumatic event through flashbacks, nightmares, or uncomfortable physiological responses due to reminders of the event, (2) avoiding stimuli related to the trauma and detachment from others, often characterized by emotional numbness, and (3) hyper-arousal, often manifested as an enhanced startle reflex, and hyper-vigilance (APA, 2000; Norrholm and Jovanovic, 2010). Psychiatric diagnosis of PTSD, as opposed to Acute Stress Disorder (ASD), requires experiencing symptoms for longer than 30 days and significant impairment in basic function (APA, 2000; Norrholm and Jovanovic, 2010). PTSD is a heterogeneous psychiatric disorder and it is often co-morbid with major depressive symptoms, alcohol and/or drug abuse and dependence, as well as other anxiety disorders including panic disorder.

PTSD is the fourth most common psychiatric diagnosis, affecting approximately 10% of all men and 18% of all women (Breslau et al., 1998). Although incidence is not limited to the

military, traumatic combat experience is a major precursor to the disorder. According to a national study of readjustment for Vietnam veterans, 30.8% of males and 26.9% of females had suffered from PTSD at some point following their service (Kulka et al., 1990). Current reports on veterans from the Iraq and Afghanistan conflicts have found that 18% of these soldiers have experienced PTSD symptoms, a number greatly underestimated due to stigma against admitting to the condition (Hoge et al., 2004). The increase in worldwide combat zones and prevalence of violence in urban areas ensures that exposure to traumatic events will continue to occur in the future, necessitating increased attention to treatment of PTSD and other anxiety disorders (Norrholm and Jovanovic, 2010).

Fear Conditioning Model:

Several anxiety disorders are characterized by a failure to inhibit fear of a traumatic event, notably demonstrated by the re-experiencing symptoms associated with PTSD (Cannistraro and Rauch, 2003; Norrholm et al., 2010). From a Pavlovian classical fear conditioning perspective, the original traumatic event serves as an unconditioned stimulus (US) that produces an unconditioned response (e.g., fear, horror, helplessness). A conditioned fear response develops through the association of ambient cues present in the trauma environment with the event itself, resulting in several previously neutral sensory stimuli (cues) becoming conditioned stimuli (CS's; Norrholm et al., 2010). This can be modeled in a laboratory environment. For example, when a subject is presented with a neutral stimulus (e.g., colored shapes or lights) that is repeatedly paired with an aversive stimulus (e.g., air blast or electrical shock), the subject quickly learns that the neutral stimulus predicts an unpleasant event (Norrholm et al., 2010). CS's can later produce conditioned fear responses even in the absence of the original US due to

the previously formed US-CS association (Norrholm et al., 2010). This is termed the formation of a fear memory or trace. Due to the common neural circuits that mediate classical fear conditioning and anxiety disorder symptomatology, many clinical studies of anxiety disorders employ this model for investigations of anxiety disorder neurobiology, symptom severity, and treatment outcome.

Fear-Potentiated Startle:

Our group studies the fear-related symptoms of anxiety disorders using fear-potentiated startle methodologies. Fear-potentiated startle (FPS) is defined as the increase in the frequency or magnitude of the acoustic startle response in the presence of a previously neutral cue (CS) that has been repeatedly paired with an aversive unconditioned stimulus (US; Norrholm et al., 2011). The acoustic startle response is a reflexive behavior, present in all mammals, that is mediated by a simple, 3-synapse neural circuit such that the presentation of a sudden acoustic stimulus produces a brief contraction of the skeletal musculature (Norrholm et al., 2010). In humans, this response is best observed through electromyographic (EMG) recordings of the *orbicularis oculi* muscle that mediates the eyeblink response (Norrholm et al., 2008). The reflex can be activated by a brief burst of broadband noise between 95 and 112 decibels (dB) referred to as a startle probe (Norrholm et al., 2006). Fear-potentiated startle paradigms afford the investigator the opportunity to measure baseline startle as well as the acquisition, extinction, and return of conditioned fear. Recently, FPS has been successfully used to demonstrate impaired fear extinction in both combat and civilian populations with PTSD (see Norrholm et al., 2010; Norrholm and Jovanovic, 2011).

Fear-potentiated startle studies possess several benefits. First, FPS provides an objective measure of fear, generates a non-zero baseline, offers cross species generalization, and is mediated by a well-characterized neuronal system (Davis, 1997). In addition, using FPS also establishes a model for assessing the effects the psycho-pharmacological agents on the manifestation of fear related disorders in humans (Walker et al., 2002; Ledgerwood et al., 2003). Complementary psychophysiological studies have used skin conductance responses (SCR) and reaction time as measurements of fear; however, these tools are best described as indices of general arousal and cognitive resource allocation, respectively. Based on our previous work, the acoustic startle response is advantageous with respect to the latter measures in that it provides an intrinsic measure of emotional valence. Emotional valence can be exhibited as differential responding to the reinforced stimulus (CS+) and the non-reinforced stimulus (CS-; Norrholm et al., 2006). In other words, startle magnitude is sensitive to opposite stimuli (negative images increase startle response relative to positive images; Norrholm et al., 2006). In contrast, SCR magnitude does not vary with emotional valence (Lang, 1995; Vansteenwegen et al., 1998).

In light of the many advantages associated with fear-potentiated startle, there are some perceived limitations to using the acoustic startle response as an outcome measure, including the idea that a startle probe may be considered aversive and serve as a secondary US. However, this idea has been disproven through previous studies which have shown that startle response to the CS- decreases over fear acquisition even though the stimuli are followed by startle probes (Norrholm et al., 2006). High startle to the CS- at the beginning of acquisition is believed to be due to initial generalization between the CS's, often seen in similar discrimination paradigms (e.g., Norrholm et al., 2006).

Fear Extinction and Recovery Effects:

As described above, fear acquisition procedures result in the formation of a CS-US association that can be termed the original fear memory or trace. Fear extinction is a form of learning in which the frequency and/or intensity of a conditioned response is reduced through repeated presentation of the conditioned stimulus without the unconditioned stimulus (Myers and Davis, 2002). This process can be termed extinction training and involves the formation of a second memory trace that competes with the original fear memory. Extinction occurs in a variety of organisms and response systems and in both aversive and appetitive Pavlovian conditioning paradigms (Norrholm et al., 2008).

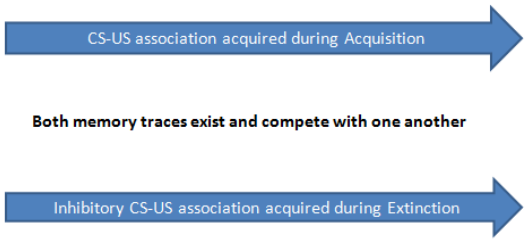

Figure 1: Competing Memory Traces: Following acquisition, one fear memory trace exists due to the CS-US pairing. After extinction, a parallel inhibitory memory is formed, which competes with the original fear memory.

Inhibitory fear learning (most notably extinction) is believed to leave previous learning intact, rather than erasing or unlearning the association between CS and US (Myers and Davis, 2002). This is supported by the observation that extinguished conditioned responses can return with the passage of time (spontaneous recovery; Pavlov, 1927), a change in context (renewal; Bouton and Bolles, 1979) or after unsignaled presentations of the US (reinstatement; Bouton and Bolles, 1979). Therefore, the initial fear is not erased following extinction, but instead suppressed by a competing parallel inhibitory process (Myers and Davis, 2002).

Animal studies have suggested that spontaneous recovery, or the return of a conditioned fear response with time, can be abolished if extinction occurs within minutes of acquisition. In rats, extinction is resistant to all three recovery effects when initiated 10 min after acquisition rather than 72 hours after acquisition (Myers et al., 2006). In another study, spontaneous recovery of fear was observed in rats only when extinction training occurred 1 hr after acquisition (Quirk, 2002). Therefore, a species specific critical time period for disrupting fear return in rats appears to occur between 10 min and 1 hr (Quirk, 2002). Extinction during this critical time period may erase the fear memory by interfering with ongoing fear memory consolidation (Norrholm et al., 2008). Despite the results of immediate extinction animal studies, this "unlearning" hypothesis due to short term extinction has not been observed in humans. For example, Alvarez et al. found significant renewal when extinction occurred immediately after acquisition, contrary to the animal results of Myers (Alvarez et al., 2007). This may be because, humans, unlike rats, have the capacity to anticipate an expansive range of outcomes and contingencies with the presentation of each experimental session (extinction immediately after acquisition as another acquisition session where CS-US is reversed; Norrholm et al., 2008).

Conditioned fear can also return after extinction training through the process of reinstatement (Rescorla and Heth, 1975; Bouton and Swartzentruber, 1991). In this paradigm, subjects undergo acquisition and extinction training, followed by a small number of US's which are disassociated from the CS. It has been demonstrated that the conditioned fear response will return following representation of the original CS (Rescorla and Heth, 1975) as long as the test occurs in the same context as the unsignaled US's (Westbrook et al. 2002). In essence, unsignaled US presentations are believed to reinstate conditioned responses. Conditioned fear extinction and reinstatement was first demonstrated in animals by observing fear responses

including freezing, avoidance, and fear-potentiated startle (as referenced by Norrholm et al., 2006). Following these initial studies, reinstatement had been observed in humans using verbal ratings of fear and US expectancy (Hermans et al., 2005), reaction time task performance (Dirikx et al., 2004) and skin conductance (Labar and Phelps, 2005). In the first study of its kind, our group demonstrated reinstatement using fear-potentiated startle in humans as well (Norrholm et al., 2006). These studies provide links between animal models of associative learning and show the potential for clinical applications (Norrholm et al., 2006). Importantly, the reinstatement paradigm may underlie observed symptom elevation in anxiety disorder patients as a result of re-exposure to trauma-related life events (Steketee, 1993).

Fear Memory Formation and Disruption:

Given the frequent recovery of fearful memories (spontaneous recovery, renewal, reinstatement) even after extinction, a key question concerning this research is how to persistently weaken aversive CS-US associations, or dampen traumatic memories in psychopathological cases (Monfils et al., 2009). Several modifications have been analyzed in studies concerning fear extinction and recovery of fear. Altering the presentation timing of non-reinforced CSs during extinction and the use of US predictions in particular are addressed in the current research.

The formation of fearful memories is important to understand prior to modifications of this process. Fearful memories are initially labile, or capable of disruption, but become progressively consolidated via synthesis of new proteins (Squire and Davis 1981; McGaugh, 2000). The reconsolidation hypothesis suggests that memories can be consolidated each time they are transiently retrieved to a labile state via a CS-alone trial (Misanin et al., 1968). Retrieval is a protein synthesis-dependent mechanism that is necessary for the transition between stable

and labile memory states (Duvarci and Nader, 2004). In addition, motor and declarative memory studies in humans suggest that new information can interfere by impairing or modifying older memories (Walker et al., 2003). Based on this information, reconsolidation serves as an evolutionary mechanism for allowing new information acquired at the time of retrieval to be integrated into the initial memory, emphasizing that memory formation is a dynamic process (Schiller et al., 2010).

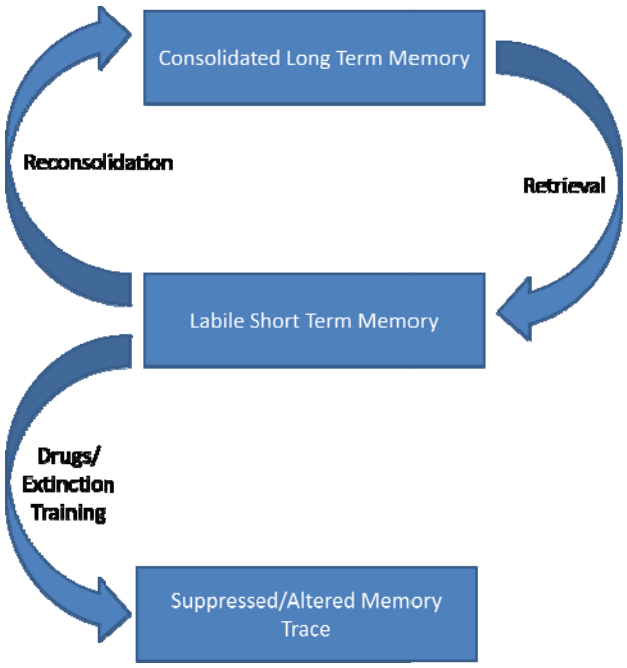

Figure 2: Fear Memory Consolidation and Disruption: Consolidated memories can be returned to a labile state following retrieval (presentation of isolated CS trial). Upon entering the labile state, the memory is subject to reconsolidation of the original fear or disruption via drugs (suppresses memory) or extinction training (alters memory).

The ability to impair maladaptive emotional memories has important implications for anxiety disorders linked to traumatic memories. Interestingly, consolidated fear memories that are

retrieved at a later time can be modified in one of two ways (Monfils et al., 2009). Once retrieved, the fearful memory becomes sensitive to enhancement (via reconsolidation) or disruption (via drugs/extinction) during the brief reconsolidation window (several hours after retrieval depending on the species; Duvarci and Nader, 2004). Reactivated memories have been shown to be modifiable by pharmacologically blocking the updating process following retrieval within the reconsolidation window (Monfils et al., 2009). Pharmacological manipulations permanently suppress targeted memories; however, these drugs are toxic to humans (protein synthesis inhibitors; Schiller et al., 2010).

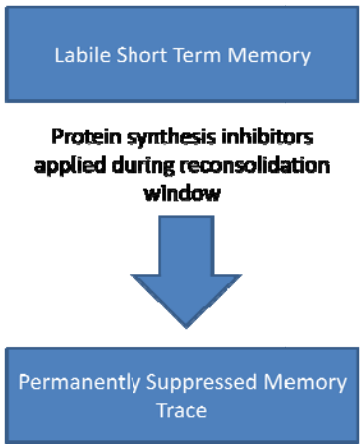

Figure 3: Protein Synthesis Inhibitors Following Retrieval: The use of toxic protein synthesis inhibitors following retrieval will permanently suppress the original fear memory.

Extinction training, as described above, has been found to be non-permanent due to its role in suppression rather than modification of the initial memory. The efficacy of extinction is questioned due to the common reemergence of previously extinguished fear through renewal, reinstatement and spontaneous recovery. Exposure therapy (founded on the principles of extinction-based experiments), the repeated presentation of the fear evoking CS in the absence of

aversive consequences, is currently the most effective treatment for anxiety disorders (Foa, 2000). However, based on observations of fear recovery effects, current anxiety disorder treatment through extinction based exposure therapy does not work in all clinical cases.

Retrieval+Extinction Paradigms:

The work of Monfils and colleagues addresses the two problematic paradigms that have been previously used to reduce fear: disruption of retrieval induced reconsolidation with toxic drugs and potentially non-permanent extinction. To tackle these issues, an experiment was devised with an isolated retrieval trial presented before extinction to destabilize and reinterpret the fear memory (no drugs required; Monfils et al., 2009). The extinction phase was applied to the reconsolidation window (after activation with the brief retrieval session) in order to store a new nonthreatening memory of the CS (Monfils et al., 2009). For aversive memories, the process weakens the emotional impact of the previously fear inducing stimulus by altering the original memory trace (Monfils et al., 2009). This resulted in more enduring reduction of fear compared to extinction outside of the reconsolidation window (extinction as usual resulted in spontaneous recovery; Monfils et al., 2009). Even after reinstatement, a potent fear recovery mechanism, fear remained extinguished up to at least a year (Schiller et al., 2010). Updating older fear memories with non-fearful ones during the reconsolidation window provides a viable and non-invasive technique for rewriting emotional memories in humans (Schiller et al., 2010).

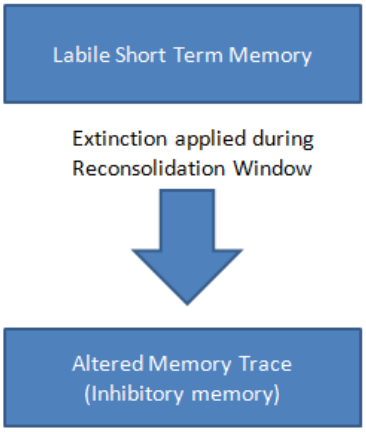

Figure 4: Role of Extinction Following Retrieval: The use of extinction training following retrieval will result in an altered memory trace (inhibitory memory).

Because a traumatic event can be associated with multiple cues, each could potentially trigger the fear response (Schiller et al., 2010). In order to examine the effect of reconsolidation of one cue on another associated cue, Monfils and colleagues used two CS+'s that were paired with the US. Responses to both CS+'s were successfully extinguished, but while the non-reminded CS+ was reinstated, the reminded CS+ was not (Schiller et al., 2010). Therefore, extinction applied during reconsolidation only affected the reactivated memory, emphasizing the impact of the isolated retrieval trial.

Current Research:

The work of Monfils and others suggests that our memory reflects the information acquired at last retrieval rather than an exact account of the original memory (Schiller et al., 2010). Cross-species similarities are believed to be due to an evolutionary preserved adaptive mechanism, where fear memory can be altered through time-dependent molecular processes (Schiller et al.,

2010). The main distinguishing characteristic between the experimental groups was the different time interval between the first and second CS presentation based on the timing of retrieval and extinction phases. Although not yet verified, this time period might be necessary to destabilize the memory by engaging different molecular mechanisms in the amygdala (Monfils et al., 2009). A key feature explored in this study was the isolation of the initial CS immediately before extinction and the sensitivity of the process to subtle manipulations.

Retrieval-based fear extinction, as originally tested in animals, used auditory CS's and resulted in permanently attenuated fear memory without the use of potentially toxic drugs (e.g., Monfils et al., 2009). Upon translation of this experiment to humans, Monfils and colleagues used visual CS's. Our group has shown that both auditory and visual CS's demonstrate enhanced fear-potentiated startle to the CS+ (US reinforced), discrimination between the CS+ and CS- (non-reinforced), extinction to the previously reinforced CS+, and marked spontaneous recovery in humans (Norrholm et al., 2011). The success of this translational study emphasizes the validity of fear-potentiated startle as a measure of fear in retrieval-based extinction experiments.

Our current experiment expands on the work of Monfils and Schiller, who discovered that altering the timing of CS presentations before extinction results in more persistent attenuation of fear in rats and humans. Monfils and Schiller's work in humans was based on skin conductance responses (Monfils et al., 2009; Schiller et al., 2010). We are now exploring the findings reported by Monfils and Schiller using our established human fear-potentiated startle paradigm. This will enable us to better understand this putative learning and memory process as it relates to humans, and to investigate whether these findings translate to startle-based fear learning paradigms. If we were to replicate their findings in humans using fear-potentiated startle, this could afford the opportunity to potentially refine extinction-based exposure therapies

for PTSD. In other words, the use of retrieval+extinction paradigms may rescue PTSD-related impairments.

In addition to measurements of fear potentiated startle, our study aims to analyze the human cognitive processing of fear learning (e.g., transformation of a neutral signal to a danger signal and return of a danger signal to a neutral/safety signal) through the use of a US-expectancy response pad. Fear ratings and psychophysiological measures are not always consistent, and dissociation between subject expectancy and fear-potentiated startle measures has been demonstrated in previous studies (Hermans et al., 2005; Norrholm et al., 2006). Currently there is no evidence to indicate that the act of recording one's US-expectancy rating can affect startle responses, but this process will be further analyzed in the current study. One could argue that there is the potential for interference as a result of resource allocation (mediated, in part, by the prefrontal cortex) during expectancy affecting the physiological startle response. However, there is also the possibility that fear and/or extinction learning could be enhanced through the recruitment of higher cortical brain regions.

Methods:

Participants:

42 subjects (13 males/29 females) with a mean age of 20.55 years old participated in the study after signing an informed consent form approved by Emory University Institutional Review Board and the Atlanta Veterans Administration Medical Center Research and Development Committee. Healthy civilians were recruited from the Emory University community, and

consisted predominantly of undergraduate and graduate students. Requirements for participation included no significant visual impairment (corrected 20/20 vision) and tone detection at 30 dB of frequencies ranging from 250-4000 Hz (assessed with a Grason-Stadler Model GS1710 pure threshold audiometer). Subjects were screened for current or past psychiatric illness and/or mood disorders through self reported measures and a brief semi-structured interview (assessed by various mood/medical history surveys and the Structured Clinical Interview for DSM-IV Axis I Disorders, SCID-1). Participants were also confirmed to have no loss of consciousness lasting longer than 5 minutes, no HIV/AIDS (as the virus can enter the central nervous system and, in turn, produce cognitive/behavioral alterations), and no current drug or alcohol abuse or dependency (urine toxicology sample taken).

Experimental Setup:

The aforementioned startle probe was a 108 dB, 40 msec burst of broadband noise with near instantaneous rise time delivered binaurally through headphones and was also referred to as the noise alone trial (NA). The aversive stimulus (US) was a 250 msec air blast at 140 psi directed to the larynx (uncomfortable but not painful). It was delivered from a compressed air tank via plastic tubing attached to a CamelBak. Acoustic startle response magnitude was recorded via electromyography (EMG) readings of the right *orbicularis oculi* muscle. Two 5 mm Ag/AgCl electrodes filled with electrolyte gel were placed 1 cm below the pupil and 1 cm below the lateral canthus. EMG signals were amplified and digitalized with the BIOPAC MP150 monitoring system (Biopac Systems, Inc., Aero Camino, CA). Impedances through these electrodes were less than 6 kΩ. In addition to recording eye blink response using EMG readings, respiration rate was determined using a respiration belt, electrical activity of the heart was determined using

electrocardiogram (ECG) readings, and electrical skin conductance was determined using galvanic skin response (GSR) electrodes on the ring and middle fingers. All recorded waveforms were analyzed with AcqKnowledge software.

Figure 5: Experimental Setup: Sample participant set up with electrodes in the startle booth

Startle Booth Sessions:

Subjects were seated in a sound-attenuated startle booth for the experimental sessions. An elevated computer monitor was used to present conditioned stimuli within the booth. Subjects were randomly assigned to one of four experimental groups: Retrieval/Keypad (n=8), No Retrieval/Keypad (n=7), No Retrieval/No Keypad (n=7), or Retrieval/No Keypad (n=20). Booth sessions consisted of initial acquisition on the first day, reminder (retrieval; depending on the experimental group) after 24 hours, extinction 10 minutes later, test for spontaneous recovery after 24 hours, re-extinction 10 minutes later, and reinstatement 10 minutes following re-extinction. Each session began with an acclimation period followed by a habituation phase in which the noise alone trials were presented in order to determine a baseline acoustic startle response. Each group experienced acquisition on the first day in order to condition particular

stimuli as fearful. Three colored shapes were initially presented as non-reinforced neutral stimuli on the computer screen followed by the startle probe. Two of the colored shapes (blue square and orange circle) were conditioned as the CSa+ and CSb+, respectively, by following them with the aversive air blast (US). The third shape (purple triangle) remained unassociated from the US and became the CS-. The inter-trial interval was randomized between 9 and 22 seconds.

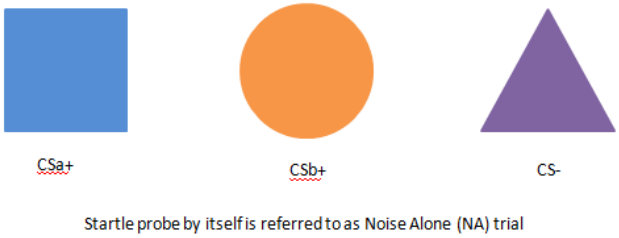

Figure 6: Experimental Stimuli

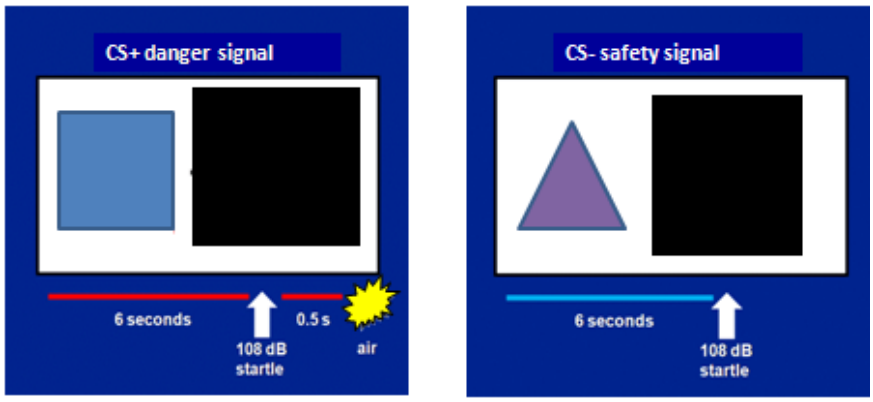

Figure 7: Differential Fear Conditioning Timing: The order of presentation of stimuli during fear conditioning is CS→Startle Probe→Presence of Air/Absence of Air. The order of presentation is important in order to measure fear-potentiated startle.

One day later, the designated groups went through a brief reminder session in which one CSa+ trial was presented without the US. The CS- trial was also presented one time in order to

replicate the study design of Schiller. All groups received the first extinction session 10 minutes later in which all CS's were presented without the aversive air blast in order to extinguish the previously conditioned fear. On the third day, groups were tested for extinction retention or spontaneous recovery by presenting only non-reinforced shapes. Re-extinction followed 10 minutes later and a test for reinstatement followed 10 minutes after that. In reinstatement, four unsignaled US's were presented followed by unreinforced trials of the three colored shapes.

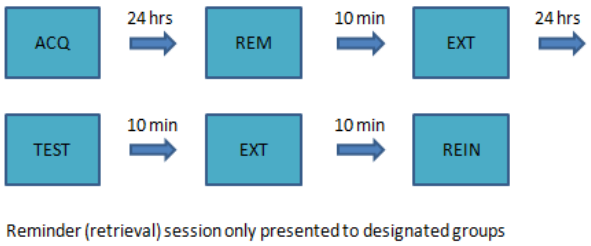

Figure 8: Session Schematic: Order of presentation of experimental sessions

Startle and Expectancy Measures:

Each session was divided into blocks which consisted of several trials of the NA, CSa+, CSb+ and CS-. Within each block, the mean startle magnitudes for each of the trials were used to calculate the Difference Score using the formula: Difference Score = [Mean startle magnitude to probe in presence of CS] – [Mean startle magnitude to startle probe alone (NA)]. Startle magnitude was determined as the peak amplitude of the EMG contraction 20-200 ms following the acoustic stimulus.

Depending on experimental group, some of the participants received a response keypad (Superlab, Cedrus Corp., San Pedro, CA) to record their expectancy of the US on each CS trial. The three button response pad was used to collect trial by trial ratings of US-expectancy.

Participants received verbal instructions prior to each session on how to use the keypad. A response of "+" signaled that the subject expected the shape to be followed by the US, a response of "-" signaled that the subject expected the shape to not be followed by the US, and a response of "0" signaled that the subject was uncertain. They were instructed to respond as soon as the shape appeared on the screen in order to record an accurate prediction. US expectancy ratings were also analyzed over blocks for each of the booth sessions.

Results:

Fear Acquisition

Acoustic Startle Measures

Fear-potentiated Startle

All participants exhibited fear-potentiated startle to the reinforced CS's (termed CS+a and CS+b; CS+a vs. Noise Alone (NA): Repeated Measures ANOVA, Main Effect of Trial Type, $F(1,40) = 25.97$, $p < 0.001$; CS+b vs. NA: Repeated Measures ANOVA, Main Effect of Trial Type, $F(1,40) = 42.74$, $p < 0.001$). There were no significant group differences between the groups with and without a response pad.

CS+/CS- Discrimination

All participants displayed significant discrimination between the reinforced CS+'s and the non-reinforced CS- (CS+a vs. CS-: Repeated Measures ANOVA, significant Main Effect of Trial Type, $F(1,40) = 15.71$, $p < 0.001$; CS+b vs. CS-: Repeated Measures ANOVA, significant Main

Effect of Trial Type, F(1,40) = 8.69, p = 0.005). There were no significant group differences between the groups with and without a response pad.

Response Pad Measures

CS+/CS- Discrimination

All participants reporting US expectancy on the response keypad displayed significant discrimination between the reinforced CS+'s and the non-reinforced CS- (CS+a vs. CS-: Repeated Measures ANOVA, significant Trial x Trial Type interaction, F(1,13) = 473, p < 0.001; CS+b vs. CS-: Repeated Measures ANOVA, significant Trial x Trial Type interaction, F(1,13) = 618, p < 0.001).

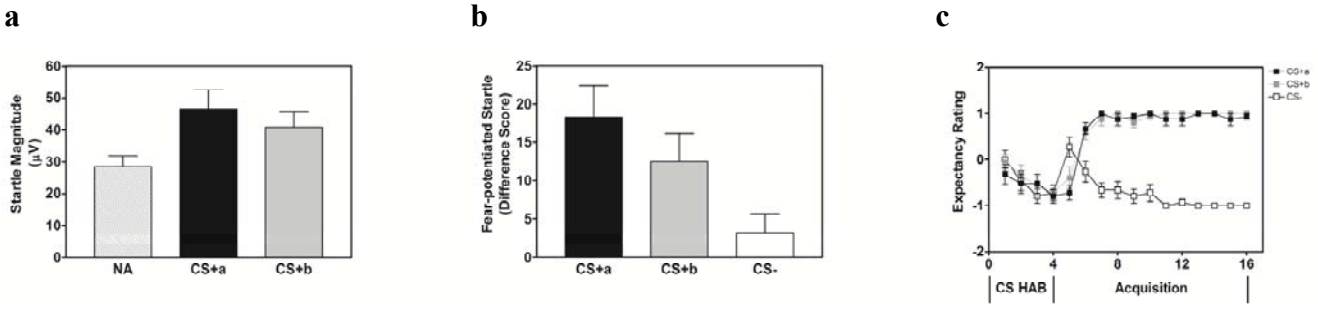

Figure 9: Fear Acquisition: Data from each group were pooled as there were no significant differences between them during Acquisition. **a,** Startle magnitude to the CS+'s. **b,** FPS to the CS+'s and the CS-. **c,** Expectancy ratings to the CS+'s and the CS-.

Fear Extinction

Acoustic Startle Measures

An analysis of extinction block, trial type (CS+a, CS+b), presence or absence of response keypad, and presence or absence of the reminder cue (CS+a), indicated a four-way interaction (Repeated Measures ANOVA, Block x Keypad x Reminder interaction, $F(5,180) = 2.43$, $p < 0.05$). The four-way interaction was followed up by examining the effect of extinction block and trial type within each of the four groups (No Keypad/No Reminder, No Keypad/Reminder, Keypad/No Reminder, Keypad/Reminder). There was a significant effect of extinction block in all four groups, $F(5,30)=3.06$, $p < 0.05$, $F(5,95)=2.74$, $p < 0.05$, $F(5,20)=3.18$, $p < 0.05$, $F(5,35)=18.61$, $p < 0.001$, respectively. The No Keypad/Reminder group was the only one that also displayed an interaction effect of Block X Trial Type, $F(5,95)=2.71$, $p <0.05$. Although both CS+a and CS+b show extinction, in this group, fear-potentiated startle was significantly lower to CS+a (the reminded cue) compared to CS+b in the first block of extinction (Repeated Measures ANOVA, Trial Type $F(1,19)=5.75$, $p<0.05$).

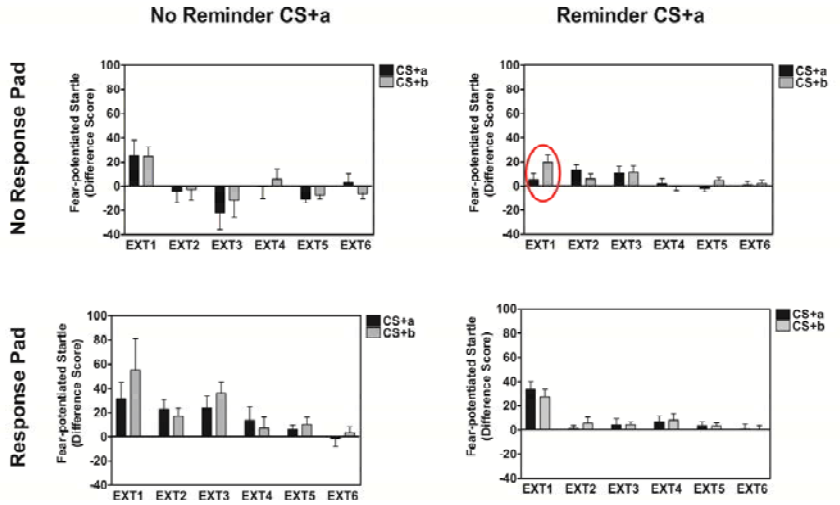

Figure 10: Fear Extinction: FPS to the CS+'s

Response Pad Measures

All participants reporting US expectancy on the response keypad displayed significant reduction of expectancy ratings upon presentation of the previously reinforced CS+'s during extinction (Repeated Measures ANOVA, significant Main Effect of Trial, $F(1,13) = 30.1$, $p < 0.001$). There was no significant effect of trial type nor were there any significant differences between the Reminder and No Reminder groups.

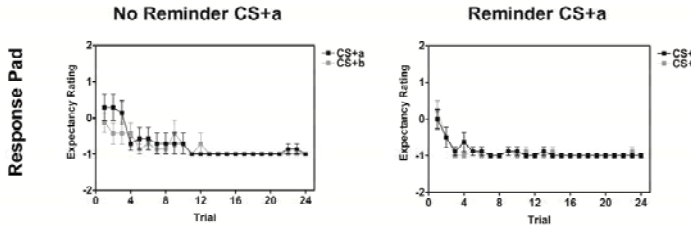

Figure 11: Fear Extinction: Expectancy ratings to the CS+'s

Extinction Test, Re-Extinction, and Reinstatement

Acoustic Startle Measures

Extinction Test: We first tested recall of extinction 24 hours after extinction training. There were no significant effects of group or trial type, however, there was a significant main effect of response keypad ($F(1,37)=5.35$, $p<0.05$).

Re-extinction: In order to test re-extinction, we included the test block as the initial extinction block. We found a significant effect of block and interaction effect of block and response keypad (Repeated Measures ANOVA, Block, $F(6,204) = 4.02$, $p = 0.001$; Repeated Measures ANOVA, Block x Keypad, $F(6,204) = 4.12$, $p = 0.001$). In the absence of a response keypad, there was no

significant Main Effect of Block ($F(1,22) = 1.41$, $p = 0.25$). In the groups with a response keypad during re-extinction, there was a significant Main Effect of Block ($F(1,13) = 13.26$, $p = 0.003$).

Reinstatement: After the 6 blocks of re-extinction, we delivered four unpaired US's in order to examine reinstatement of the CS's. We then compared fear-potentiated startle during the last block of re-extinction to the reinstatement block. The results showed a significant Main Effect of trial type (Repeated Measures ANOVA, $F(1,35) = 6.73$, $p = 0.01$) and a three-way Interaction Effect (Repeated Measures ANOVA, Block x Reminder x Keypad, $F(1,35) = 11.02$, $p = 0.002$). We followed up the interaction within each of the response pad groups (with/without) and found that the group without the response keypad showed an Interaction Effect (Repeated Measures ANOVA, Block x Reminder, $F(1,23) = 8.56$, $p = 0.008$), with the group without the reminder showing a bigger decrease in fear-potentiated startle than the group with the reminder. In the group with the response keypad, we found an Interaction Effect of Block and Trial Type (Repeated Measures ANOVA, $F(1,12) = 5.45$, $p = 0.04$) with CS+a (reminded cue), showing a bigger increase in fear-potentiated startle than the CS+b.

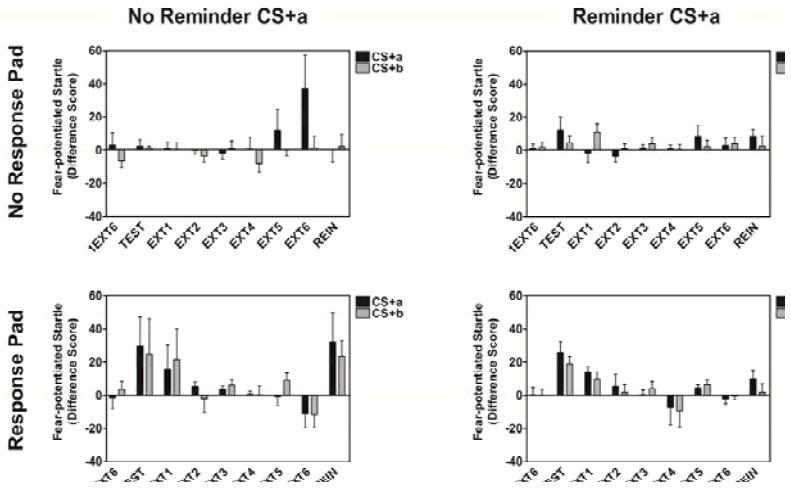

Figure 12: Test for Spontaneous Recovery, Re-Extinction, and Reinstatement: FPS to the CS+'s

Response Pad Measures

Extinction Test: We first tested recall of extinction 24 hours after extinction training. There was a significant Group x Trial x Trial Type interaction, $F(1,13) = 4.82$, $p < 0.05$. The Reminder group showed an initial increase in US expectancy rating that immediately returned to extinguished levels whereas the No Reminder group showed a persistent increase in expectancy ratings across the extinction test.

Re-extinction: In order to test re-extinction, we included the test block as the initial extinction block. We found a significant effect of trial (Repeated Measures ANOVA, Block, $F(1,13) = 10.57$, $p = 0.006$). There was no significant effect of trial type nor were there any significant differences between the Reminder and No Reminder groups.

Reinstatement: After the 6 blocks of re-extinction, we delivered four unpaired US's in order to examine reinstatement of the CSs. We then compared US expectancy ratings during the last block of re-extinction to the reinstatement block. There was a significant Group x Trial x Trial Type interaction, $F(7,91) = 3.22$, $p = 0.004$. The No Reminder group displayed a greater increase in responding to the CS+b (non-reminded cue) as compared to the Reminder group.

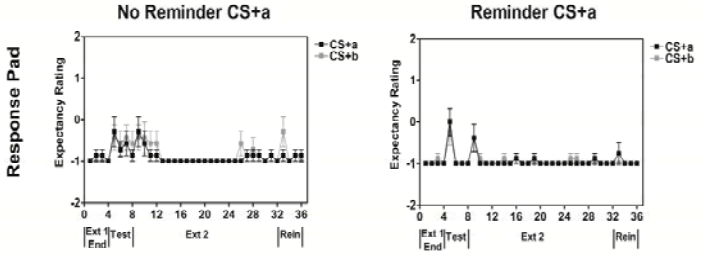

Figure 13: Test for Spontaneous Recovery, Re-Extinction, and Reinstatement: Expectancy ratings to the CS+'s

Discussion:

Summary of Results:

By the end of acquisition all groups demonstrated enhanced fear-potentiated startle to both reinforced CS+'s. In other words, there was a significant increase in startle responses to the CS+'s as compared to NA in all experimental groups. In addition, all groups showed discrimination between the two reinforced CS+'s and the one non-reinforced CS-. By demonstrating discrimination with the use of two CS+'s, this study goes one step further than previous fear-potentiated startle studies in which only one reinforced CS+ was discriminated from a non-reinforced CS-. Reflecting the work of Schiller, the use of two CS+'s mimics a traumatic event in which multiple environmental factors can serve as triggers.

Regardless of the presence of a reminder or the response pad, all groups showed significant extinction to both CS+'s by the end of the first extinction session, as demonstrated by a linear decrement in fear-potentiated startle responses to the previously reinforced CS+'s. In the No keypad/Reminder group, the CSa+ produced less startle compared to the CSb+ in the first extinction block (EXT1). This group specific discrepancy in early extinction may be due to the impact of the response keypad during disruption of the original fear memory to CS+a (reminded cue). The reminder effect found by Monfils, and demonstrated in this group, may be overcome by the presence of the keypad, which facilitates learning through engagement of additional neural circuitry such as prefrontal cortex or hippocampus. When these regions of the brain are not engaged during extinction training, by virtue of no response pad, there was less fear-potentiated startle during extinction on the second day. Two possible explanations for less startle

are the groups have less memory of the original acquisition or better inhibition occurred in the absence of the keypad.

Using the response pad to measure the cognitive processing of fear, we found that the CS+'s were successfully conditioned as danger cues and the CS- was conditioned as a safety cue. During the first extinction session, all groups extinguished the conditioned danger cues almost immediately by reporting the two CS+'s as safe. Given these extinction results, there was a lag between cognitive awareness and the reflex of a startle response. The difference in time of extinction between our two measures is consistent with the fact that startle is much more sensitive to fear circuitry in the amygdala, while the response pad involves higher cognitive behavior making it easier to have an accurate expectation than to inhibit a reflex fear response.

Similar to the first extinction session, all groups demonstrated complete extinction to both CS+'s by the end of the second extinction session. However, during re-extinction there was a significant effect of the presence of the response keypad. Those in this condition displayed significant spontaneous recovery; an effect that may be due to enhanced retrieval of the original fear memory via recruitment of higher brain regions such as hippocampus and frontal cortices. In addition, groups with the response pad showed a significant main effect of block leading to a typical fear extinction curve, while those without the response pad did not. In other words, since the groups without the response pad did not display spontaneous recovery (due to a weaker association between the CS+ and US), there was no elevated startle response to extinguish during re-extinction.

Reinstatement did not occur in the groups without the response pad, once again due to weaker CS+-US association. In contrast, use of the response pad led to enhanced fear learning and discrimination between CSa+ and CSb+ during reinstatement. Between the groups with the

response pad, there was less reinstatement in the group with the reminder compared to the group without the reminder. In this sense, the response pad groups showed Monfils-type reminder effects. However, these groups also showed increased startle to the CSa+ (reminded cue) compared to the CSb+, which conflicts with previous experimental results for two reinforced stimuli. These reinstatement results should not be over-interpreted due to the added complexity of the response pad. Since a response pad was not present in the Monfils studies, it is difficult to speculate on the interaction effect of the reminder and the response pad.

The combination of the response pad and the reminder session produced the most pronounced US-expectancy data for the test, re-extinction, and reinstatement sessions. The presence of both the response pad and the reminder led to a distinct return to uncertainty (response of "0") during the test, emphasizing a combined effect of the response pad and reminder in fear learning. In contrast, there was a persistent increase in expectancy in the no reminder group. The return to uncertainty found in both groups at the beginning of re-extinction is consistent with previous startle experiment findings (Norrholm et al., 2006). US-expectancy results during reinstatement in the no reminder group showed increased responding to the CS+b compared to the CS+a. Similar to reinstatement startle results above, these results should not be over-interpreted due to the unknown interaction effect of the reminder and the response pad.

Discussion of Results:

The first goal of this study was to investigate the results of Monfils and colleagues via measurements of startle instead of measurements of skin conductance. The second goal of this study was to analyze the role of the cognitive expectancy process in fear learning. Through the implementation of a response pad, the subjective experience of the participant, a key difference

between animal and human studies, can be measured and compared to the physiological fear response. Interestingly, Monfils-type effects were found using this paradigm, however these effects were mostly overcome by using the response pad to engage higher level brain activity and enhance fear learning.

Duplication of Monfils results using fear-potentiated startle is important for future anxiety disorder treatment methods using fear conditioning, due to current brain circuitry knowledge. The fear-circuitry involved in Pavlovian fear conditioning is directly integrated in the amygdala: a dense collection of neurons within the temporal lobes of the brain (Davis, 2000). PTSD is one disorder that is associated abnormal amygdala regulation. Through brain imaging studies, PTSD has been associated with amygdala over-reactivity and deficient inhibition of the amygdala by the medial prefrontal cortex and the hippocampus (Shin et al., 2006). Fear conditioning models that address both of these abnormalities are necessary for sufficient study of PTSD.

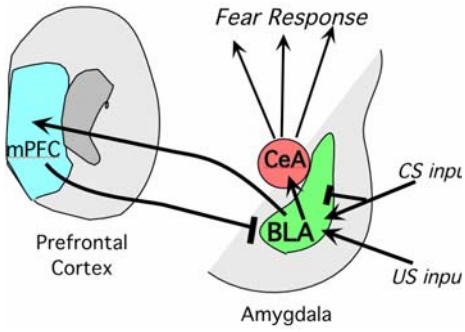

Figure 14: Neurobiology of Fear Circuitry: During fear conditioning, CS and US sensory inputs are processed in the amygdala, followed by the expression of a fear response. The prefrontal cortex is associated with inhibition of the fear response in the amygdala.

Monfils and colleagues utilized skin conductance, a measure of electrical conductivity of the skin due to sweat gland activity, which is an index of sympathetic nervous system activation and, therefore, a measure of arousal (Glover et al., 2011). There are several disadvantages to using skin conductance. SCR will increase in presence of a CS that was previously paired with a US (Ohman and Soares, 1993); however, studies on emotional valence suggest that SCR is related to arousal (regardless of positive or negative valence) rather than fear (Lang et al., 1998). SCR studies in humans have no translational animal models and the neural mechanism is regulated by a diffuse and complex neural network, which makes it difficult to correlate fear-related behaviors to specific brain regions when using SCR (Glover et al., 2011).

On the other hand, fear-potentiated startle measures the acoustic startle response: an integrative motor reflex with a simple and short neural pathway directly connected to the amygdala (Davis, 2000). Startle reflex has been shown to increase during aversive CS presentations and has been used in many animal studies emphasizing its role as a translational tool (Glover et al., 2011). Translation to animal models allows for investigation of neural background of fear expression and fear inhibition, which can later be used to modify human research.

The act of reporting expectancy ratings during sessions was another important factor in the results of fear acquisition and extinction. In the absence of the response keypad, the reminder group displayed some Monfils reminder effects in early extinction, but it was difficult for the subjects to maintain the original fear memory over the course of multiple days. This finding is evident in the loss of a conditioned response to the previously reinforced CS+'s at the outset of re-extinction on day three. In other words, groups without the response pad did not display a typical fear extinction curve due to poor learning or loss of the original fear memory. In contrast,

by engaging higher cortical brain regions, the response pad both enhanced fear learning and eliminated the few Monfils reminder effects seen in groups without the response pad. Importantly, the response pad had the unique effect of ensuring attention during lengthy experimental sessions. There was no significant difference in acquisition results between groups, most likely due to frequent airblasts maintaining the engagement of groups lacking the response pad. However, during extinction sessions the response pad was crucial to observe within session extinction because, as humans, it appears as though we need greater focus/attention during learning.

It was previously believed that reporting expectancy ratings on a response pad has little to no effect on startle response, but very few studies have looked at this interaction. Our study shows that, if anything, the response pad allocates more attention to the task, with uncertainty measurements leading to better discrimination between reinforced and non-reinforced stimuli. However, as a result of enhanced engagement in the task and recruitment of higher cortical brain regions, the reminder effect discovered by Monfils is overcome and the original fear memory is preserved. These findings present a quandary in which a response pad is needed to maintain attention, yet in doing so, it brings in higher learning, which eliminates Monfils effects. Our novel study is a launching point for further investigation of this dilemma. The role of the reminder phase and cognitive processing of fear learning should be further examined in future studies. In particular, application of this methodology to PTSD subjects would be an excellent way to analyze fear learning and modify existing treatment strategies.

Experimental Shortcomings:

A major improvement in our study design would involve the use of a gradient response tool, perhaps a dial ranging from -100 to +100, instead of the three button response pad. Based on our current study design, fewer choices in the cognitive processing of fear may lead to a more rapid perception of extinction or may be a reflection of uncertainty rather than fear extinction.

In the original design of our pilot study, data was only gathered in the no response pad/reminder group in order to be consistent with the designs of Monfils and Schiller. We have learned recently from the literature that the prefrontal cortex may play a role in fear memory formation, so the study was expanded to four unique experimental groups. These groups were used to analyze the interplay between the reminder phase and the response pad. Therefore, there are considerably more participants in the original pilot group (n=20) compared to the other three groups. As a result, a shortcoming in the experimental methods is the low n value for three of the four experimental groups. This was a consequence of the three consecutive day length of the study and the relatively short period of time to gather results. We intend to increase the group N values in the coming months to gather more definitive data, particularly concerning the interaction effect between the response pad and reminder.

References:

American Psychiatric Association. (2000). Diagnostic and Statistical Manual for Mental Disorders. Fourth Edition, Text Revision. Washington, D.C.

Breslau N, Kessler RC, Chilcoat HD, Schultz LR, Davis GC, Andreski P. Trauma and posttraumatic stress disorder in the community: the 1996 Detroit Area Survey of Trauma. *Arch Gen Pyschiatry.* 1998;55:626-632.

Kulka RA, Schlenger WE, Fairbank JA, et al. *Trauma and the Vietnam War Generation: Report of Findings from the National Vietnam Veterans Readjustment Study.* New York, New York: Brunner/Mazel;1990.

Hoge CW, Castro CA, Messer SC, McGurk D, Cotting DI, Koffman RL. Combat Duty in Iraq and Afghanistan, mental health problems and barriers to care. *N Engl J Med.* 2004;351:13-22.

Norrholm SD & Jovanovic T. Tailoring therapeutic strategies for treating posttraumatic stress disorder symptom clusters. *Neuropsyc Disease and Treatment*. 2010;6:517-532.

Cannistraro PA & Rauch SL. Neural circuitry of anxiety: Evidence from structural and functional neuroimaging studies. *Psychopharmacol. Bull*. 2003;37:8-25.

Norrholm SD, Jovanovic T, Olin IW, Sands LA, Karapanou I, Bradley B, Ressler KJ. Fear extinction in traumatized civilians with posttraumatic stress disorder: relation to symptom severity. *Biological Psychiatry*. 2010;69(6):556-63.

Norrholm SD, Anderson KM, Olin IW, Jovanovic T, Kwon C, Warren VT, McCarthy AJ, Bosshardt L, Sabree J, Duncan EJ, Rothbaum BO, Bradley B. Versatility of fear-potentiated startle paradigms for assessing human conditioned fear extinction and return of fear. *Frontiers in Behav Neurosci*. 2011;5:77.

Norrholm SD, Vervliet B, Jovanovic T, Boshoven W, Myers KM, Davis M, et al. Timing of extinction relative to acquisition: A parametric analysis of fear extinction in humans. *Behav Neurosci.* 2008;122:1016-1030.

Norrholm SD, Jovanovic T, Vervliet B, Myers KM, Davis M, Rothbaum BO, Duncan EJ. Conditioned fear extinction and reinstatement in a human fear-potentiated startle paradigm. *Learn Mem.* 2006;13:681-685.

Norrholm SD & Jovanovic T. Translational fear inhibition models as indices of trauma-related psychopathology. *Current Psychiatry Reviews*. 2011;7(3):194-204.

Davis M. The neurophysiological basis of acoustic startle modulation: Research on fear motivation and sensory gating. *Attention and orienting: Sensory and motivational processes.* 1997:69-96.

Walker DL, Ressler KJ, Lu KT, Davis M. Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of d-cycloserine as assessed with fear-potentiated startle in rats. *J Neurosci.* 2002;22:2343-2351.

Ledgerwood L, Richardson R, Cranney J. Effects of d-cycloserine on extinction of conditioned freezing. *Behav Neurosci.* 2003;117:341-349.

Lang PJ. The emotional probe: Studies of motivation and attention. *American Pyschology.* 1995;50:372-385.

Vansteenwegen D, Crombez G, Baeyens F, Eelen P. Extinction in fear conditioning: effects on startle modulation and evaluative self-reports. *Pyschophysiology.* 1998;35:729-736.

Myers KM & Davis M. Behavioral and neural analysis of extinction. *Neuron.* 2002;36:567-584.

Pavlov IP. *Conditioned Reflexes*. Oxford, England: Oxford University Press; 1927.

Bouton ME & Bolles RC. Role of conditioned contextual stimuli in reinstatement of extinguished fear. *J Exp Pysch: Animal Behav Processes.* 1979;5:368-378.

Myers KM, Ressler RJ, Davis M. Different mechanism of fear extinction dependent on length of time since fear acquisition. *Learning and Memory.* 2006;13:216-223.

Quirk GJ. Memory for extinction of conditioned fear is long lasting and persists following spontaneous recovery. *Learning and Memory.* 2002;9:402-407.

Alvarez RP, Johnson L, Grillon C. Contextual-specificity of short-delay extinction in humans: Renewal of fear-potentiated startle in a virtual environment. *Learning and Memory.* 2007;14:247-253.

Rescorla RA & Heth CD. Reinstatement of fear to an extinguished conditioned stimulus. *J Exp Pysch: Animal Behav Processes.* 1975;104:88-96.

Bouton ME & Swartzentruber D. Sources of relapse in Pavlovian and instrumental learning. *Clin Pyschol Review*. 1991;11:123-140.

Westbrook RF, Iordanova M, McNalley G, Richardson R, Harris JA. Reinstatement of fear to an extinguished conditioned stimulus: Two roles for context. *J Exp Pysch: Animal Behav Processes.* 2002;28:97-110.

Hermans D, Dirikx T, Vansteenwegen D, Baeyens F, Van den Bergh O, Eelen P. Reinstatement of fear responses in human aversive conditioning. *Behav Res Ther*. 2005;43:533-551.

Dirikx T, Hermans D, Vansteenwegen D, Baeyens F, & Eelen P. Reinstatement of extinguished conditioned responses and negative stimulus valence as a pathway to return of fear in humans. *Learning and Memory.* 2004;11:549-554.

Labar KS & Phelps EA. Reinstatement of conditioned fear in humans is context dependent and impaired in amnesia. *Behav Neurosci.* 2005;119:677-686.

Steketee G. Social support and treatment outcome of obsessive compulsive disorder at 9-month follow-up. *Behav Psychother.* 1993;21:81-95.

Monfils MH, Cowansage KK, Klann E, LeDoux JE. Extinction-Reconsolidation Boundaries: Key to Persistent Attenuation of Fear Memories. *Science*. 2009;324:951-955.

Squire LR & Davis HP. The pharmacology of memory: a neurobiological perspective. *Annu Rev Pharmacol Toxicol.* 1981;21:323-356

McGaugh JL. Memory- a Century of Consolidation. *Science.* 2000;287:248-251.

Misanin JR, Miller RR, Lewis DJ. Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science.* 1968;160:554-555.

Duvarci S & Nader K. Characterization of fear memory reconsolidation. *J Neurosci.* 2004;24(42):9269-9275.

Walker MP, Brakefield T, Hobson JA, Stickgold R. Dissociable stages of human memory consolidation and reconsolidation. *Nature.* 2003;425:616-620.

Schiller D, Monfils MH, Raio CM, Johnson DC, LeDoux JE, Phelps EA. Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature.* 2010;463:49-53.

Foa EB. Pyschosocial treatment of posttraumatic stress disorder. *J Clin Psychiatry.* 2000;61:43-48.

Davis M. The role of the amygdala in conditioned and unconditioned fear and anxiety. In: Aggleton JP, editor. *The Amygdala Vol. 2*. Oxford, England: Oxford University Press; 2000:213-287.

Shin LM, Rauch SL, Pitman RK. Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Ann NY Acad Sci*. 2006;1071:67-79.

Glover EM, Phifer JE, Crain DF, Norrholm SD, Davis M, Bradley B, Ressler KJ, Jovanovic T. Tools for translational neuroscience: PTSD is associated with heightened fear responses using acoustic startle but not skin conductance measures. *Depression and Anxiety.* 2011;28:1058-1066.

Ohman A & Soares JJF. On the automatic nature of phobic fear: conditioned electrodermal responses to masked fear-relevant stimuli. *J Abnorm Psychol.* 1993;102:121-132

Lang PJ, Bradley MM, Cuthbert BN. Emotion motivation and anxiety: brain mechanisms and psychophysiology. *Biol Psychiatry.* 1998;44:1248-1263.