Can Fearful Memories be Updated? Understanding the Boundary Conditions of Reconsolidation

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Abstract

Reconsolidation is the process where consolidated memories return to a malleable state and can be modified. The purpose of this dissertation was to examine factors that influence the reconsolidation of conditioned fear memories using a behavioural method, known as the post-retrieval extinction paradigm, across two separate studies. Fear potentiated startle (FPS) and skin conductance response (SCR) were used as measures of fear for both studies.

In Study 1, I investigated how the expectation for learning impacts the reconsolidation of conditioned fear memories. Testing took place over three consecutive days and expectation for learning was manipulated via verbal instructions prior to memory reactivation. On Day 1, participants underwent fear acquisition to two distinct spider images. On Day 2, participants were assigned to one of the following conditions: reactivation with expectation for learning condition (n = 16); a reactivation with no expectation for learning condition (n = 16); or a no reactivation condition (n = 16). All participants underwent extinction, and participants in the reactivation conditions had their memory for the conditioned stimulus (CS+) reactivated either with or without the verbal manipulation for the expectation for learning. On Day 3, fear was reinstated, and participants underwent re-extinction to examine if the manipulation on Day 2 affected the memory. Partially consistent with my predictions, I found limited evidence that the expectation for learning enhanced reconsolidation for FPS but not SCR.

In Study 2, I examined how the level of spider fear impacted the reconsolidation of conditioned fear memories. I also examined if the effects of reconsolidation generalized to similar stimuli (i.e., stimulus generalization). On Day 1, participants who were either high (n = 17) or low (n = 17) on spider fear underwent fear acquisition to a spider and a snake image, while a neutral image served as the CS-. On Day 2, participants viewed a single presentation of

the spider and the neutral images to reactivate their memories. Participants then underwent extinction. On Day 3, fear was reinstated, and participants underwent re-extinction to examine if the effect of reactivation on memory. Fear generalization was measured via presentation of similar but distinct spider and snake images. These images were presented prior to and post-acquisition (Day 1) and on Day 3 following reinstatement. On the same days (1 and 3), a behavioural approach test (BAT) was used as a second measure of fear generalization to assess approach behaviour to novel spider stimuli. Inconsistent with my predictions, for FPS, participants with high levels of spider fear displayed a return of fear to the non-reactivated snake image, but not to the reactivated spider image. Conversely, I found the opposite effect for the low spider fear group as they displayed a return of fear to the reactivated spider image but not the snake image. For SCR, participants in both groups displayed a return of fear to the reactivated spider image but not the snake image. I did not find any evidence that reconsolidation increased approach behaviour for either group during the BAT. These findings suggest that memories with high levels of distress may undergo reconsolidation when fear is measured with FPS but not SCR. The inconsistent findings between the groups suggest that conditioned and natural memories may undergo reconsolidation differently.

The findings from both studies have potential theoretical implications for the understanding of the boundary conditions of reconsolidation and have potential implications for the application of reconsolidation in clinical contexts. Limitations and future directions are also discussed.

Statement Regarding Contributions

I (J. Marinos) took the lead on the study design, data collection, data extraction and cleaning, data analysis, and manuscript writing. In addition to myself, the following people assisted with data collection: Elise Quint; Olivia Simioni; Ila Lennips; and Hannah Zhang. Gurvir Singh Rai and Olivia Simioni assisted with data extraction and cleaning. Dr. Andrea Ashbaugh took an advisory role and is an author on both manuscripts. Study 1 was submitted to PLOS ONE and is currently under revise and resubmit. Study 2 will be submitted to PLOS ONE in February 2021. The two studies were funded by a Natural Science and Engineering Research Council (NSERC) grant awarded to Dr. Andrea Ashbaugh.

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Chapter 1

General Introduction

Innovative neuroscience research has demonstrated that previously consolidated memories can return to a labile state and be updated with new information when reactivated (Nader, Schafe, Le Doux, 2000). This fundamental process of memory is called reconsolidation. The formation of new memories in contrast, is called consolidation and was previously thought to be permanent as well as stable once formed (McGaugh, 2000). The memory consolidation hypothesis was developed over 100 years ago and has been a central theme for research since. Consolidation and reconsolidation are processes related to long-term memory formation, the mechanisms of which are thought to be distinct from those involved in short-term memory formation. It has been found that pharmacological interventions can differentially block short or long-term memory suggesting that memory has at least two distinct processes and that protein synthesis is required only for the formation of long-term memory (Agranoff, Davis, & Brink, 1965). For example, protein synthesis inhibitors did not interrupt the learning of a new task (i.e., short-term memory) but did disrupt the recall of the training (i.e., long term memory). These findings suggest that different processes are involved in the formation of long and short-term memories.

Early models of memory conceptualized long-term memory as an exact and permanent representation of previously consolidated memories. However, a few researchers prior to 2000 found that long-term memory had the ability to change once consolidated (Lewis, 1979; Schneider & Sherman, 1968). This however was not the mainstream understanding of how long-term memory functioned and this research was not referenced often in the literature (Nader,

2013). Thus, prior to the year 2000, memory was mainly understood as static once it was acquired and formed.

Nader et al. (2000) changed the mainstream understanding of long-term memory (LTM) by demonstrating that memories can return to a labile state and be reconsolidated (e.g., updated). Nader et al. used a behavioural paradigm in which rats underwent fear conditioning where the conditioned stimulus (CS) was a tone and the unconditioned stimulus (US) was a footshock. They examined whether a 24-hour old conditioned fear response could be eliminated after injecting anisomycin, which blocks protein synthesis, into the amygdala. The injection was performed either 10-minutes or 6-hours after a presentation of the CS to "reactivate the memory." Reactivation is thought to cause the neural connections related to the fear-inducing CS to enter a labile state, allowing these connections to be disrupted or rewired. This technique is called pharmacological blockage.

Nader et al. (2000) tested memory for the conditioned fear 1 and 14 days following reactivation when the effects of anisomycin had worn off. The rats no longer exhibited fearful responses to the CS one and 14 days later in the condition where anisomycin was injected 10-minutes following reactivation. However, this effect was not observed if anisomycin was injected 6-hours following reactivation. These results suggest that neural connection patterns related to the CS enter a labile state following the reactivation induced by presentation of the fear-eliciting stimulus, and that this labile state lasts at least 10 minutes, but less that 6 hours. This study was the first to demonstrate that consolidated memories could be returned to a labile state and be updated within a 6-hour timeframe following reactivation. These results have been replicated in both rats using anisomycin (Debiec, LeDoux, & Nader, 2002; Kida et al., 2002; Lee, Everitt, Thomas, 2004) and in humans using an oral dosage of propranolol (Soeter & Kindt,

2011a; Kindt & Soeter, 2013; Soeter & Kindt, 2011b; Soeter & Kindt, 2013), discussed in detail below.

Reconsolidation illustrates the dynamic nature of memory. For example, when a memory undergoes reconsolidation the memory can be either updated (Schiller et al., 2010), strengthened (Lee, 2008), or blocked (Nader, Schafe, & LeDoux, 2000; Soeter & Kindt, 2011a). Reconsolidation is normally studied using a three-day procedure utilizing pharmacological or behavioural interventions (Agren, 2014). On Day 1, encoding of the memory takes place. On Day 2, the memory is reactivated rendering it labile and modifiable. The memory is typically reactivated by having the participant recall the previously encoded memory. In pharmacological blockade studies, a drug or a placebo is typically administered before or after reactivation. It is theorized that the drug administered inhibits protein synthesis and stops the memory from being reconsolidated. In behavioural studies, following memory reactivation, participants undergo extinction where the memory is updated with new non-fearful information. There is usually a control group that does not have their memory reactivated or receives the manipulation outside the reconsolidation window (e.g., greater than 6 hours following reactivation). On Day 3, changes to the memory are examined to determine whether reconsolidation has occurred by measuring the return of fear. The return of fear is measured by assessing spontaneous recovery (e.g., an increase in the fear response to the fear memory following the passage of time), renewal (e.g., a return of fear when the context is different from where extinction learning took place), and reinstatement (e.g., a return of fear following an unsignaled aversive event) (Rescorla & Heth, 1975). If a fear memory has been reconsolidated there is usually no return of fear as demonstrated by a lack of spontaneous recovery, reinstatement, or renewal because either reencoding has been blocked, in the case of pharmacological studies, or the fear memory has

been updated with new information, in the case of behavioural studies. However, the literature using behavioural methods to study reconsolidation has produced inconsistent results (Golkar, Bellander, Olsson, & Ohman, 2012; Kindt & Soeter, 2013; Schiller et al., 2010; Soeter & Kindt, 2011a; Steinfurth et al., 2014), whereas the studies using pharmacological interventions have consistently found that memories are dynamic and can be altered when reactivated (Kindt & Soeter, 2013; Soeter & Kindt, 2011b; Soeter & Kindt, 2013). The research employing each method will be reviewed in the following sections.

Reconsolidation and pharmacological blockage

Research examining the requirements that need to be met (i.e., boundary conditions) in order for reconsolidation to occur has primarily been conducted in animals (Schiller & Phelps, 2011). Pharmacological blockage is used in most animal research and involves injecting a chemical agent (e.g., anisomycin) into the brain (e.g., amygdala, hippocampus) to block protein synthesis dependent reconsolidation processes. Reconsolidation has been demonstrated in a variety of species, including crabs (Perez-Cuesta & Maldonado, 2009), rats (Nader, Schafe, LeDoux, 2000), mice (Suzuki et al., 2004), and honeybees (Stollhoff, Menzel, & Eisenhardt, 2005; Stollhoff, Menzel, & Eisenhardt, 2008), demonstrating that this is an effect seen across species.

Additionally, several researchers have examined factors that predict reconsolidation. For example, researchers have studied if the strength (i.e., level of distress) of a memory can impact reconsolidation processes (Bustos, Maldonado, & Molina, 2008; Suzuki et al., 2004; Wang, De Oliveira Alvares, & Nader, 2009). Suzuki et al. (2004) conducted several studies to examine the effect that the strength of a memory had on reconsolidation. Mice were placed in a chamber and received one or three footshocks to undergo contextual fear conditioning. It was assumed that

more shocks would create a stronger fear memory in mice. One day following conditioning, mice were then injected with either anisomycin or saline (i.e., to act as a control) and 30 minutes later placed back in the chamber for three minutes to reactivate the memory. Twenty-four hours later, the mice were placed back in the chamber for five minutes and freezing behaviour was measured. The study found that mice with stronger fear memories (i.e., conditioned with three footshocks) were resistant to memory disruption and did not undergo reconsolidation as they exhibited greater freezing behaviour when compared to the groups with weaker memories (e.g., conditioned with one footshock). These results are consistent with other studies that found that stronger fear memories were harder to reconsolidate (Bustos et al., 2008; Wang & Gorenstein, 2013). In a follow up study, Suzuki et al. (2004) predicted that greater time in the chamber during re-exposure would reactivate the memory, resulting in reconsolidation. To test this hypothesis, the same protocol as above was followed except during re-exposure mice were placed in the chamber for three, five, or 10 minutes. As predicted, these stronger memories only underwent reconsolidation when re-exposure lasted 10 minutes. Taken together, these studies demonstrate that stronger memories may be harder to reconsolidate and may require longer re-exposure time in order to reactivate the memory.

In addition to the strength of the memory, researchers have examined other factors that influence reconsolidation, such as the age of the memory. Utilizing pharmacological blockade methods, Debiec, LeDoux, and Nader (2002) examined if three and 45 day old fear memories could be reconsolidated in rats. Three and 45 days following conditioning, rats were placed in the same chamber to reactivate the memory and then infused with anisomycin in the hippocampus. The study found that rats in both age conditions who had their memory reactivated and then were infused with anisomycin exhibited significantly less freezing

behaviour compared to the control group. These results are inconsistent with previous findings that 8-week-old fear memories were resistant to reconsolidation (Suzuki et al., 2004).

Although animal research is informative, there are a number of difficulties replicating these findings in a human sample. The chemicals used in animal research, such as anisomycin, are toxic to humans and as a result present a barrier to studying the mechanisms involved in updating long-term human memories. Propranolol, however, is a beta-adrenergic receptor blocker, which partially blocks protein synthesis and has been used in examining reconsolidation blockage safely in humans (Brunet et al., 2011; Gelinas & Nguyen, 2005; Schiller & Phelps, 2011).

Kindt, Soeter, and Vervliet (2009) examined if it was possible to prevent the return of fear measured by fear potentiated startle (FPS) to a phobic image (i.e., spider image) by interfering with the reconsolidation process using an oral dosage of propranolol prior to reactivation. Following fear conditioning on Day 1, participants were assigned to one of three groups on Day 2; propranolol with reactivation, placebo with reactivation, and propranolol with no reactivation. Participants in the first two conditions took either propranolol or a placebo and then 90 minutes later had their memory reactivated by viewing the phobic image. Participants in the propranolol with no reactivation condition were administered propranolol but did not have their memory reactivated. This study found that fear (i.e., as measured by FPS response) did not return in the propranolol plus reactivation condition, whereas the other two conditions did display a fear response following reinstatement. The effect sizes from these analyses were in the large range (i.e., $\eta^2_p < .14$). These findings suggest that the fear memory could have been erased or that disrupting the reconsolidation process could have made the fear memory irretrievable. The authors hypothesized that propranolol disrupted protein synthesis of the fear memory in the

amygdala that resulted in the emotional (i.e., FPS) aspect of the memory being updated as non-fearful and the declarative (i.e., phobic image) memory remaining the same.

Updating emotional memories is important for the treatment of posttraumatic stress disorder (PTSD) and anxiety disorders because distressing memories are seen as a critical factor that maintain these disorders within an associative learning model (Mineka & Zinbarg, 2006). Kindt and colleagues' (2009) study found that reconsolidation was more effective in eliminating the return of fear than extinction, which is important because most treatments for anxiety disorders are based on extinction principles (Craske et al., 2014). Kindt's et al. (2009) findings have been replicated in a number of studies (Brunet et al., 2008; Brunet et al., 2011; Brunet et al., 2014; Kindt & Soeter, 2013; Soeter & Kindt, 2011a; Soeter & Kindt, 2013; Soeter and Kindt, 2015a; Soeter and Kindt, 2015b) with the exception of a few (Beckers & Kindt, 2014; Chalkia et al., 2019). Overall, these findings illustrate that reconsolidation is more resistant to the return of fear than standard extinction and thus treatments that incorporate these principles may help reduce relapse rates for anxiety disorders and PTSD.

The use of reconsolidation in the treatment of anxiety disorders seems promising but there is a need to conduct research examining how memories are reconsolidated with individuals experiencing high levels of anxiety symptoms. This need is due to the assumption that memories that maintain anxiety disorders may be stronger or older memories, which may be more resistant to reconsolidation (Suzuki et al., 2004; Wang et al., 2009). Soeter and Kindt (2013) examined if reconsolidation was less effective in individuals with high trait anxiety. The study used data from six previously published studies examining reconsolidation using pharmacological blockage. The researchers found that individuals with high levels of trait anxiety had a return of fear exhibited by increase in fear-potentiated startle following reinstatement, whereas individuals

with low levels of trait anxiety did not show a return of fear. These findings suggest that fear memories in individuals with higher levels of trait anxiety, as assessed by the self-reported trait anxiety, are more difficult to reconsolidate. Further research should examine if individuals with a predisposition to experience higher levels of anxiety need higher dosages of propranolol or different techniques to reactivate fear memories and undergo reconsolidation.

The above studies have examined reconsolidation using pharmacological blockage. However, there are a number of limitations to the use of propranolol to study reconsolidation. Propranolol is a beta-adrenergic receptor blocker that modulates protein synthesis and has similar effects to protein synthesis inhibitors (Gelinas & Nguyen, 2005; Schiller & Phelps, 2011). However, propranolol might only modify (rather than completely block) reconsolidation protein synthesis processes and as a result only indirectly target the mechanisms involved in reconsolidation (Gelinas & Nguyen, 2005; Schiller & Phelps, 2011). Also, individual differences (e.g., weight) may impact the effectiveness of propranolol because the dosage is not adjusted to account for these variances. Therefore, the drug will reach its peak effectiveness at different times in different people and as a result, hinder the drug's efficacy in yielding reconsolidation blockage. Despite propranolol being safe to use, it presents a number of limitations to studying reconsolidation in humans.

Reconsolidation and the post-retrieval extinction paradigm

As an alternative to pharmacological blockage methods, Monfils, Cowansage, Klann, and LeDoux (2009) designed a non-invasive behavioral technique to target reconsolidation of fear memories, which is based on extinction learning principles called the post-retrieval extinction paradigm. Extinction without reactivation is thought to produce a new memory and the two memories compete for expression (Bouton, 2000, 2002). As a result, following extinction, fear

returns because of time passing (i.e., spontaneous recovery; Pavlov, 1927), re-exposure to the stressful stimuli (i.e., reinstatement; Rescorla, & Heth, 1975), or a change in context from where the extinction training took place (i.e., renewal; Bouton & Bolles, 1979). However, Monfils et al. (2009) hypothesized that extinction that occurs during the reconsolidation window (i.e., less than 6 hours following reactivation) would update the original memory with new information and the fearful response would no longer be exhibited. Monfils et al. (2009) tested this hypothesis and found that rats in the conditions that had their memory reactivated and underwent extinction within the reconsolidation window illustrated significantly fewer freezing behaviours than the other conditions (e.g., extinction 6 hours after memory reactivation condition and no reactivation condition) and the effect was maintained 1 year later. Based on the study's findings, the technique appears promising at updating fearful memories without the use of drugs.

Building on Monfils's et al. (2009) work, Schiller et al. (2010) utilized the post retrieval extinction paradigm to examine if human fear memories could be updated with non-fearful information when reactivated during the reconsolidation window. Participants underwent fear conditioning to geometric shapes. Participants in the first two conditions had their memory reactivated followed by a 10-minute (i.e., within the reconsolidation window) or 6-hour break (i.e., outside the reconsolidation window). Participants then underwent extinction. The remaining participants did not receive the presentation of the CS+ (i.e., memory reactivation) and went directly to the 10-minute break followed by extinction. Schiller et al. (2010) found that participants who had their memory reactivated during the reconsolidation window did not display a fear response as measured by skin conductance following reinstatement. Participants who did not have their memory reactivated or had their memory reactivated and underwent extinction outside the reconsolidation window (i.e., greater than 6 hours) had a return of fear as

illustrated by increased skin conductance following reinstatement. These results suggest that consolidated fear memories were updated with non-fearful information when extinction learning took place after memory reactivation but within the reconsolidation window. This study was the first to demonstrate reconsolidation of fear memories in humans using a non-invasive learning paradigm. These findings have important implications for the treatment of fear-based disorders because current treatments are based on extinction, which is not permanent. Further research is needed to replicate the findings and to examine the boundary conditions of reconsolidation in a human sample to understand how it can be applied to the treatment of disorders.

The post retrieval extinction paradigm has been used in several studies to examine the reconsolidation of human fear memories (Golkar, Bellander, Olsson, & Ohman, 2012; Schiller, Kanen, LeDoux, Monfils, & Phelps, 2013; Steinfurth et al., 2014; Warren et al., 2014). One of the few studies to successfully replicate Schiller et al., (2010) was Steinfurth et al., (2014) who used the same procedures; however, reactivation plus extinction occurred either one or seven days later. The study found that fear memories up to 7 days old could be reconsolidated as all participants, regardless of the time since initial learning, who underwent reactivation plus extinction showed a reduction of fear following reinstatement. These findings build on previous animal research, which has inconsistently demonstrated that older fear memories are harder to reconsolidate (Debiec et al., 2002; Suzuki et al., 2004). The reconsolidation of older memories is of particular interest because often the events associated with the memories that maintain anxiety disorders or posttraumatic stress disorder will have occurred sometime in the past. Therefore, in order to understand how the reconsolidation process can be applied to clinical interventions, further research needs to examine what conditions need to be met in order for older memories to be reconsolidated.

Several other studies have failed to replicate Schiller's et al. (2010) findings that fearful memories can return to a labile state and be updated with non-fearful information (Golkar, Bellander, Olsson, & Ohman, 2012; Kindt & Soeter, 2013; Soeter & Kindt, 2011a). There are several possible reasons researchers have failed to replicate Schiller's et al. (2010) findings. One reason could be due to the methodological changes to the study design, such as the reinforcement schedule used. In Schiller et al. (2010) a 38% reinforcement schedule (e.g., the number of times the US and CS+ were paired together) was used whereas other studies have used 50-80% reinforcement schedules (Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013; Golkar, Bellander, Olsson, & Ohman, 2012; Kindt & Soeter, 2013; Soeter & Kindt, 2011a). The higher reinforcement schedule could have created a stronger memory or greater distress associated to the condition memory, and as a result the memory would be more difficult to reconsolidate. Also, fear relevant (e.g., spiders or angry male faces) instead of fear irrelevant stimuli (e.g., geometric shapes) have been used. Fear relevant stimuli are more resistant to extinction, which could have impaired the reconsolidation process as well (Auber et al., 2013; Mineka & Öhman, 2002). However, in all studies, the physiological measures showed that the fear response had been extinguished at the end of Day 2, regardless of the type of stimuli used (Golkar et al., 2012; Kindt & Soeter, 2013; Soeter & Kindt, 2011a). Therefore, given that the fear response was extinguished in all studies at the end of Day 2, one possible reason for the failure to replicate may be due to insufficient reactivation of the memory (Auber, Tedesco, Jones, Monfils, & Chiamulera, 2013). However, it is also possible that small sample sizes and underpowered analyses may have contributed to the replication issues in studies using behavioural methods. Schiller et al. (2010) did not report on effect size, nor did the study which replicated Schiller's et al. (2010) findings (Steinfurth et al., 2014). Furthermore, the studies that failed to replicate

Schiller's et al. (2010) findings were adequately powered for their planned analyses, but were not adequately powered to detect small effect sizes (Golkar, Bellander, Olsson, & Ohman, 2012; Kindt & Soeter, 2013; Soeter & Kindt, 2011a). Notably, as highlighted throughout this introduction when available, studies that have used propanrolol to study reconsolidation have more often reported on effect sizes and have found effects within the medium to large range (Brunet et al., 2008; Sevenster et al. (2013, 2012); Soeter & Kindt, 2015a,b). Taken together, given the inconsistent methodologies used across the studies noted above and the inability to replicate Schiller's et al. (2010) results, there is a need to understand what conditions need to be met in order for a memory to be rendered labile and consistently reconsolidated.

Expectation for learning and the impact on reconsolidation

The boundary conditions of when a memory is reactivated and returned to a labile state are not fully understood. It has been demonstrated that recalling a memory is not enough for the memory to return to a labile state and undergo reconsolidation (Forcato, Argibay, Pedreira, & Maldonado, 2009; Forcato, Rodríguez, Argibay, & Maldonado, 2010). Associative learning theorists suggest that prediction error is important for learning to occur. A prediction error occurs when there is a violation between expected and actual events signaling that something new can be learned on a given trial (Sevenster, Beckers, & Kindt, 2013). As a result, learning is maximized when there is a mismatch between what was previously learned and the new information. Pharmacological blockade studies have examined whether reactivation needs to signal that something new can be learned in order for a memory to be rendered labile (Sevenster, Beckers, & Kindt, 2012, 2013).

Sevenster et al. (2013) examined if a prediction error was needed in order for a memory to return to a labile state and undergo reconsolidation using standard pharmacological blockage

techniques in humans. In the negative prediction error group (n = 15), participants underwent 100% reinforced fear conditioning and on Day 2 had their memory reactivated with a single unreinforced presentation of the CS+. In the positive prediction error group (n = 15), participants underwent fear conditioning with partial reinforcement (e.g., 33%) and on Day 2 had their memory reactivated with a single reinforced presentation of the CS+. In both groups, this was done to create a mismatch between what was learned on Day 1 and what occurred during reactivation. In the no prediction error group (n = 15), there was no violation between learning on Day 1 and reactivation. The study found that fear did not return following reinstatement as demonstrated by an elimination of the startle response in the conditions where there was a prediction error. When there was no prediction error (e.g., reactivation presented the exact same information as the previous day) they found that fear did return following reinstatement as evidenced by elevated fear potentiated startle response. The effect sizes from these analyses were in the medium to large range ($\eta^2_p < .10$). Therefore, the study concluded that reconsolidation of human fear memories only occurs when the outcome of reactivation is not fully predictable.

Additionally, when there is a mismatch between old learning and reactivation, it signals that there is an opportunity for new learning to occur (Sevenster et al., 2012). When there is an opportunity for new learning, reconsolidation is activated. Sevenster et al. (2012) examined whether simply inducing the expectancy for learning would induce reconsolidation using standard pharmacological blockade technique in humans. SCR and FPS were used as measures of fear. On Day 2, prior to reconsolidation, in addition to a placebo group (n = 18), there were two reactivation plus propranolol groups: a shock expectancy (n = 18) and a no shock expectancy group (n = 19). In the shock expectancy group, participants were connected to the

shock apparatus prior to reactivation, thus when the CS+ was presented and there was no shock, a mismatch occurred, and the researchers predicted that reconsolidation processes should be activated. In contrast, participants in the no shock expectancy group were not connected to the shock apparatus, thus there was no expectation to receive a shock when the CS+ was presented. The researchers predicted that reconsolidation processes in this group should therefore not be activated. Partially consistent with predictions, Sevenster et al. (2012) found that only the group that expected to receive a shock demonstrated reconsolidation with FPS but not SCR as a measure of fear. The effect sizes from these analyses were in the large range ($\eta^2_p < .14$). To date, the role of expectancy and mismatch has been examined using only pharmacological blockage techniques. Particularly, since research is inconsistent with regards to the ability to demonstrate reconsolidation using purely learning techniques, it would be important to explore if expectancy and mismatch are also important using this paradigm.

How are extinction and reconsolidation different?

Many are left wondering how extinction and reconsolidation differ. Extinction does not erase or update the original fear memory, but rather inhibits the expression of the memory (Milad, Rauch, Pitman, & Quirk, 2006). During extinction, it is theorized that a new non-fearful memory is formed and therefore when recall of extinction is triggered it competes for expression with the original fear memory (Bouton, 2000, 2002). This process is different from reconsolidation, which updates the original memory with new information when reactivated (Schiller et al., 2010).

The distinction between reconsolidation and extinction is supported by research examining the neurological underpinnings of each. Animal research has found that the prefrontal cortex plays an important role in extinction learning (Milad et al., 2006; Mohammed & Gregory, 2002; Morgan, Romanski, & LeDoux, 1993). It has been demonstrated that when the CS is presented after fear

extinction, the infralimbic prefrontal cortex is activated and signals to the amygdala to activate inhibitory cells, which in turn inhibit fear responses (Sotres-Bayon & Quirk, 2010). Consistent with animal research, human studies have found that the prefrontal cortex and amygdala are important for extinction learning (Milad et al., 2007; Phelps, Delgado, Nearing, & LeDoux, 2004), activation of the ventromedial prefrontal cortex was linked to the retention of extinction learning (Phelps et al., 2004). Thus studies illustrate that the amygdala and prefrontal cortex play an important role in extinction learning.

In contrast, there has been limited research examining the neural mechanisms involved in reconsolidation with humans. Schiller, Kanen, Ledoux, Monfils, and Phelps (2013) examined how the engagement of the prefrontal cortex changed during reconsolidation compared to extinction. The study utilized the post retrieval extinction paradigm and measured blood-oxygenation levels (BOLD) during the three-day protocol with functional MRI (fMRI). The study found that reactivation followed by extinction does not engage the prefrontal cortex whereas extinction does (Schiller et al., 2013). The researchers found that the amygdala was activated during extinction consistently regardless of whether it took place following reactivation or not. Therefore, the researchers concluded that different areas in the brain were activated during reconsolidation compared to extinction, demonstrating that these two processes are distinct. Specifically, extinction appears to activate the prefrontal cortex and the amygdala whereas reconsolidation activates only the amygdala.

It remains unclear if or how reconsolidation and extinction processes differ in regard to the effects of stimulus generalization. Extinction learning is specific to the stimulus used during extinction (Hermans, Craske, Mineka, & Lovibond, 2006; Vervliet, Vansteenwegen, Baeyens, Hermans, & Eelen, 2005; Vervliet, Vansteenwegen, & Eelen, 2004). Therefore, the fear response

may return if an individual encounters a similar stimulus of the same sematic category for which they underwent extinction. For example, if someone underwent extinction learning to a small black spider, their fear may return if they encountered a large tarantula because they learned only that small black spiders were safe. Vervliet et al. (2005), using a differential fear-conditioning paradigm with geometric shapes as the conditioned stimulus, examined if participants were able to generalize what they learned during extinction when different geometric shapes (i.e., generalized stimulus) were used instead of the original conditioned stimulus from acquisition. They found that participants were not able to generalize what they learned during extinction training when they were presented with the CS+ used during fear conditioning following extinction. Participants displayed a return of fear (i.e., increased skin conductance) when they underwent extinction using generalized stimulus and then were presented with the CS+ used during fear conditioning. Overall, this study demonstrates that extinction learning does not generalize to other similar stimuli. In order for extinction processes to effectively treat fear-based disorders, the learned response must be able to generalize to different environments and similar objects (e.g., all birds, not just small blue birds).

It is important to investigate if the effects of reconsolidation generalize to other stimuli not used during learning or reactivation in order to understand the usefulness of incorporating reconsolidation in the treatment of emotional disorders, though only a few studies have done so (Bjôrkstrand et al., 2016; Soeter & Kindt, 2015a; Soeter & Kindt, 2015b). Soeter and Kindt (2015a), using pharmacological blockage, examined if an ambiguous cue, which they defined as the word “spider” rather than the image used in the conditioning procedure, was sufficient to reactivate a memory. Specifically, they examined if an ambiguous stimulus was sufficient to reactivate the fear memory or if the stimuli used to reactivate the memory had to be distinctly

associated with the original feared stimuli. The study compared one group (n = 15) that underwent standard fear conditioning using two distinct images of spiders to another group (n = 15) that underwent fear conditioning to an image of a spider and a snake. The study found that an ambiguous stimulus (i.e., the word "spider" instead of the image) triggered memory reconsolidation but only in the group that underwent fear conditioning to images of spiders and snakes. The generalized stimuli (i.e., the word "spider") was not distinct enough to signal to participants which image was to be recalled during memory reactivation in the group that underwent fear conditioning to spiders only. The analysis examining the differences between groups produced an effect size in the large range (η^2_p < .14). Soeter and Kindt, (2015a) concluded that generalized stimuli were able to reactivate a memory but were contingent on past learning (i.e., what images were used during original fear conditioning). These findings build on Soeter and Kindt's (2015b) other study, which found that the effect of reconsolidation can generalized to other spiders at a 1-year follow up.

Bjôrkstrand et al. (2016), using the post-retrieval extinction paradigm, examined if the effects of reconsolidation generalize to other spider images than the one used to reactivate the fear memory. Participants consisted of individuals with a reported high fear of spiders based on their score on the spider phobia questionnaire. To test spontaneous recovery 24 hours following memory reactivation, participants were shown the image used to reactivate their memory; to test if the effects generalize to other images, participants were shown images of spiders they had never seen before. Participants showed a reduced responding to the new spider images not used during reactivation or extinction learning as well as to the reactivated image. In contrast to the findings from Soeter & Kindt (2015a, 2015b), Bjôrkstrand's et al. (2016) results suggest that the effects of reconsolidation can generalize to other stimuli of the same sematic category. Clearly,

further research is needed using behavioural techniques to understand the effects of stimulus generalization on reconsolidation and the possibility of applying it to the treatment of emotional disorders.

Clinical implications

Anxiety disorders can result from biological or temperamental predispositions as well as learned associations to perceived fearful stimuli (Barlow, 2000). Within a learned association model, associative fear memories play a central role in the factors that maintain anxiety disorders (Mineka & Zinbarg, 2006). Anxiety disorders can result from individuals behaving as if a feared or ambivalent stimulus will later predict a negative outcome (Soeter & Kindt, 2015b). For example, an individual might associate an increased heart rate with a panic attack. Currently, extinction (i.e., exposure) based therapies are used in the treatment of anxiety disorders and have been proven to be effective (Vervliet, Craske, & Hermans, 2013). However, relapse rates can range from 19% to 62% (Craske & Mystkowski, 2006), indicating that extinction alone does not always have a long-term or permanent effect. One reason that might account for the high relapse rates is that extinction is thought to create a competing memory, which is susceptible to the return of fear (Monfils, Cowansage, Klann, and LeDoux, 2009). Another factor that may contribute to the high relapse rates is that individuals with anxiety disorders are more resistant to extinction learning which may prevent them from receiving the full benefits of treatment (Duits et al., 2015; Milad et al., 2006). Conversely, reconsolidation appears promising in the treatment of PTSD and anxiety disorders (e.g., specific phobias) given the updating effects. Specifically, reconsolidation may help in reducing relapse rates as current treatments are based on extinction learning.

The process of reconsolidation was applied to the treatment of posttraumatic stress disorder (Brunet et al., 2008; Brunet et al., 2011; Brunet et al., 2014; Vermes et al., 2020). In a double-blind randomized study, Brunet et al. (2008) examined if propranolol administered after the recall of a past traumatic event would reduce physiological responding (e.g., heart rate, skin conductance, and corrugator activity) to script-driven trauma imagery in individuals diagnosed with posttraumatic stress disorder. Participants listened to a description of their traumatic event and then received either propranolol (n = 9) or a placebo pill (n = 10). The study found that physiological responding to script driven imagery of their trauma was lower when propranolol was administered post recall of the traumatic event compared to the placebo group. Specifically, the propranolol group had significantly reduced heart rate and skin conductance responses compared to the placebo group one week after the intervention. The effect sizes from the analyses with heart rate and skin conductance response were in the large range ($\eta^2_p < .14$). Similarly using behavioural methods, Vermes et al. (2020) found that participants with a diagnosis of PTSD displayed a significant reduction in skin conductance response following the reactivation of their traumatic memory plus imaginal exposure. Conversely this reduction in physiological responding was not found in the control group which consisted of individuals with PTSD but had a neutral memory reactivated prior to imaginal exposure. Overall, these findings demonstrate the potential for the use of reconsolidation processes to reduce PTSD symptoms and have been replicated in open label studies (Brunet et al., 2011; Brunet et al., 2014; Vermes et al., 2020).

Reconsolidation has also been applied to the treatment of specific phobias (Elsey et al., 2020; Soeter and Kindt, 2015b; Telch et al., 2017; Telch et al., 2020). Given that stronger memories have been found to be resistant to reconsolidation, Soeter and Kindt (2015b) examined

if disrupting reconsolidation processes with propranolol would prevent the return of fear following reinstatement in individuals with high spider fear. The study found that participants with high spider fear (n = 15) who had their memory reactivated (i.e., by focusing on their fears) followed by the administration of propranolol were able to touch an actual spider during a behavioural approach test (BAT). Conversely, participants who received a placebo (n = 15) or did not have their memory reactivated (n = 15) were unable to touch the spider. These differences across groups remained 1-year later. Interestingly, all conditions scored the same on the Spider Phobia Questionnaire (i.e., above the cutoff indicating high spider fear) until the three-month follow-up, at which time participants in the reactivation plus propranolol condition no longer reported scores within a high phobic range and participants in the other two conditions still endorsed scores consistent with high spider fear. The effect sizes from all these analyses were in the large range ($\eta^2_p < .14$). These findings demonstrate that despite experiencing behavioural changes (i.e., being able to approach feared stimuli), cognitions related to spider fear took longer to change. One limitation of the study is that the sample consisted of sub-clinical participants with reported high spider fear.

Telch et al. (2017) also applied reconsolidation processes to the treatment of specific phobias using behavioural interventions. The study examined if memory reactivation would enhance *in vivo* exposure for individuals with spider and snake fears. Participants either had their memory reactivated 30 minutes before completing the *in vivo* exposure or right after the exposure. All participants completed a behavioural approach test one day and one month later. The study found that participants' performance on the BAT at the one-day follow up did not differ across groups. However, participants that had their memory reactivated 30 minutes before the *in vivo* exposure exhibited decreased fearful behaviour at the one-month follow up compared

to the group who had their memory reactivated after the exposure task. Overall, these results demonstrate that reconsolidation based interventions and standard exposure are equally effective at the initial reduction of fear but reconsolidation is more resistent to the return of fear overtime. These findings are consistent with Soeter and Kindt (2015b) who observed decreased fearful behaviour up to one year following reconsolidation based expsoure combined with propranolol. Interestingly, Elsey et al., (2020) found anxiety about public speaking decreased following a reconsolidation based exposure in participants who were administered either propranolol or placebo. Elsey et al. (2020) attributed the decreases in public speaking anxiety measures and self-report measures of distress to placebo or practice effects as there were no differences between groups. Taken together, applying reconsolidation processes to the treatment of anxiety disorders appears promising, but it remains unclear how reconsolidation can be consistently applied to treatments.

Present studies

The boundary conditions of reconsolidation are still unclear. Further research is needed to understand what renders a memory labile and how reconsolidation can be effectively applied to the treatment of anxiety disorders. The aim of the studies in this dissertation was to explore three important boundary conditions using the post retrieval extinction paradigm: 1) how the expectation for learning impacts reconsolidation; 2) how the level of distress associated with the conditioned fear memory influences the reconsolidation process; and 3) if the effect of reconsolidation is specific to the stimulus shown during reactivation or if it generalizes to other similar stimuli.

Study 1 examined whether the expectation for learning (i.e., prediction error) is needed to render a memory labile and undergo reconsolidation in an undergraduate sample. More

specifically, the study examined if creating a prediction error enhanced reactivation to more effectively allow for memory updating to occur. To my knowledge, this study was the first to use the post retrieval extinction paradigm to examine how the expectation for learning influences reconsolidation. Understanding these boundary conditions are important because studies have failed to replicate Schiller et al.'s (2010) findings, and one possible reason for the failure to replicate may be due to insufficient reactivation of the memory in order to render it labile (Schiller & Phelps, 2011). In pharmacological blockage studies, reconsolidation appears to occur only when reactivation signals that something new will be learned as demonstrated through a mismatch between previous learning and information presented during reactivation (Sevenster et al., 2012). Researchers have yet to examine how the expectation for learning influences reconsolidation using behavioural methods.

Study 2 looked at how the level of distress associated with the conditioned fear memory impacts the reconsolidation process by comparing individuals with high and low spider fear. Examining how reconsolidation occurs with individuals with high spider fear is important because it is believed that memories that maintain anxiety disorders are stronger and may be more resistant to being updated (Soeter & Kindt, 2013). Study 2 also examined if the effects of reconsolidation generalize to other stimuli of the same semantic category (i.e., eliminate physiological fear response to other spiders or increase approach behaviour during a BAT).

In order for reconsolidation processes to be applied to the treatment of emotional disorders it is important to examine if the effects are specific only to the stimuli presented during reactivation or if it generalizes to stimuli of the same semantic category. This is of significance because multiple cues may act as triggers for traumatic events which produce a fear response and because anxiety disorders can be formed when fear is generalized to multiple situations (Schiller

et al., 2010). Clarifying these boundary conditions are essential to identify which requirements need to be satisfied for a memory to be modified and for reconsolidation processes to be incorporated in the treatments for anxiety disorders.

Chapter 2

Study 1

Impact of the expectation on memory reconsolidation using a post retrieval extinction paradigm

Marinos, J. & Ashbaugh. A.R. (2020). *PLoS ONE* (Status: Revise and resubmit)

Abstract

Objective: The present study examined if the expectation for learning enhances reconsolidation of conditioned fear memories using the post-retrieval extinction paradigm in an undergraduate sample (n = 48). **Methods:** The study took place over three consecutive days. The expectation for learning was manipulated through oral instructions prior to memory reactivation. On Day 1, participants underwent differential fear conditioning to two spider images (CS+ and CS-). On Day 2, participants were assigned to either a reactivation with expectation for learning condition, a reactivation with no expectation for learning condition, or a no reactivation condition. On Day 3, return of fear in response to the CS+ spider image was measured following reinstatement (i.e., four shocks). Fear potentiated startle (FPS) and skin conductance response (SCR) were taken as measures of fear. **Results:** The study found evidence that the expectation for learning may enhance reconsolidation with FPS as a measure of fear as it was only the expectation for learning group in which FPS to the CS+ remained stable following reinstatement, however this effect was small and non-robust. In contrast, no evidence of reconsolidation was observed for SCR, as all participants exhibited a return of fear following reinstatement. **Implications:** These findings suggest that a verbal manipulation of the expectation for learning may not be salient enough to induce reconsolidation as measured by SCR but may be sufficient as measured by FPS. Additionally, given the inconsistent findings between SCR and FPS, the study's results bring into question whether the post-retrieval extinction paradigm is appropriate to investigate reconsolidation using these physiological measures concurrently.

Introduction

Reconsolidation is the process where a long-term memory, once reactivated, returns to a malleable state and can be updated (Schiller et al., 2010), strengthened (Lee, 2008), or blocked (Nader, Schafe, Le Doux, 2000; Soeter & Kindt, 2011). Memory reconsolidation has been observed in a number of species (Debiec, LeDoux, & Nader, 2002; Finnie & Nader, 2012), including humans (Soeter & Kindt, 2011).

Studies in humans have consistently demonstrated that a conditioned fear response, as measured by fear potentiated startle (FPS), can be eliminated if reactivation is paired with oral administration of propranolol (Kindt et al., 2009; Kindt & Soeter, 2013; Soeter & Kindt, 2011; Soeter & Kindt, 2015), though a few studies have failed to replicate this effect (Beckers & Kindt, 2014; Chalkia, et al., 2019). These findings have also been replicated for traumatic memories in individuals diagnosed with posttraumatic stress disorder (Brunet et al., 2008; Brunet et al., 2011; Brunet et al., 2014). Brunet et al. (2008) demonstrated that physiological responding (i.e., heart rate and skin conductance responses (SCR)) to personalized script driven trauma imagery was reduced one week after the administration of propranolol post recall of the traumatic event compared to a placebo group. These findings were replicated in open-label studies (Brunet et al., 2011; Brunet et al., 2014).

Additionally, reconsolidation has also been incorporated in treatment of the specific phobia of spiders using propranolol. Soeter and Kindt (2015) examined if blocking reconsolidation in individuals with high levels of spider fear would inhibit the return of fear following reinstatement using a behavioural approach test (BAT) as a measure of fear. Participants who had their memory reactivated (i.e., by focusing on their fears) followed by an oral dose of propranolol displayed greater approach behaviour during a BAT than participants

who received a placebo or did not have their memory reactivated. Differences between these groups were exhibited at all follow-up periods (i.e., 16 days, three months, and 1 year later) demonstrating that the reduction of fear following reconsolidation persisted long-term. These studies illustrate that reactivated memories following the administration of propranolol, can be modified and this process might be beneficial in the treatment of anxiety based psychological disorders.

However, there are a number of limitations to the use of propranolol to study memory reconsolidation. First, propranolol is a drug that modulates protein synthesis and as a result, may only indirectly target the mechanisms involved in reconsolidation (Gelinas & Nguyen, 2005; Schiller & Phelps, 2011). Second, research has found that individuals have a preference for behavioural interventions over drug therapy for the treatment of anxiety disorders (McHugh et al., 2013). It is important to consider treatment preferences when considering the translational impact reconsolidation research can have for the treatment of anxiety disorders and posttraumatic stress disorder. As a result, there is a need to examine memory reconsolidation using behavioural methods.

Schiller et al. (2010) examined memory reconsolidation in a human sample using a behavioural design (i.e., Post-reterival extinction paradigm). The study found that participants who had their memory reactivated and then underwent extinction after a 10-minute delay period, did not display a conditioned fear response as measured by skin conductance following reinstatement. Conversely, participants who only underwent extinction or who had their memory reactivated and then underwent extinction outside the reconsolidation window (i.e., greater than 6 hours) exhibited a return of conditioned fear as measured by increased skin conductance following reinstatement 24 hours later. Though these results have been replicated (Schiller et al.,

2013; Steinfurth et al., 2014), several studies have failed to reproduce these results (Golkar et al., 2012; Kindt & Soeter, 2013; Kindt et a., 2009; Soeter & Kindt, 2011). The inconsistencies in replicating these findings suggest that recalling a memory is not always sufficient to reactivate a memory. It is therefore important to better understand the conditions under which reconsolidation occurs.

Pharmacological blockade studies have examined whether reactivation needs to signal that something new can be learned (i.e., prediction error) for a memory to be rendered labile (Sevenster, et al., 2012, 2013). Prediction error occurs when there is a violation between expectation and actual events and is believed to signal that something new can be learned (Sevenster et al., 2013). Prediction error has been found to strengthen initial learning, as well as extinction learning (Rescorla, & Wagner, 1972), and prediction error may be important to facilitate memory reconsolidation (Fernandez, et al., 2016).

Using propanrolol, Sevenster et al. (2013) examined if a prediction error was needed in order for a memory to return to a labile state and undergo reconsolidation. When there was a prediction error (i.e., reactivation signaled new information) the conditioned fear response did not return following reinstatement, as demonstrated by an elimination of the startle response. In contrast, when there was no prediction error (e.g., reactivation presented the exact same information as the previous day) fear did return following reinstatement as demonstrated by an elevated fear potentiated startle response following reinstatement. These findings suggest that when reactivation signals that something new can be learned (i.e., prediction error) the conditioned fear memory is more likely to be rendered labile and undergo reconsolidation.

Sevenster et al. (2012) examined whether inducing the expectancy for learning can trigger reconsolidation by manipulating participants' expectation to receive a shock prior to reactivation

using propanrolol in humans. In the shock expectancy group, participants were connected to the shock equipment during reactivation but did not receive a shock as expected. Therefore, a mismatch occurred when the CS+ was presented in the absence of a shock. In the no shock expectancy group, there was no expectation to receive a shock when the CS+ was presented because participants were not connected to the shock equipment during reactivation. As expected, Sevenster et al. (2012), found that the group that expected to receive a shock demonstrated reconsolidation, whereas the group that had no expectation for receiving the shock did not demonstrate reconsolidation. These studies illustrate that the expectation for learning during reactivation appears to be critical to the reconsolidation of conditioned fear memories using pharmacological methods.

Overall, research has demonstrated that recalling a memory is not sufficient for a memory to undergo reconsolidation (Golkar et al., 2012; Kindt & Soeter, 2013; Kindt et al., 2009; Soeter & Kindt, 2011). Pharmacological blockade studies have found that reactivation should indicate that something new can be learned in order for a memory to return to a malleable state and undergo reconsolidation (Sevenster, et al., 2012, 2013). However, studies utilizing behavioural experiments (i.e., post-retrieval extinction paradigm) have yet to investigate how the expectation for learning impacts reconsolidation. The expectation for learning during reactivation could be a boundary condition of memory reconsolidation. Difficulty inducing an expectation for learning during reactivation could be one explanation for why some studies have not found evidence of reconsolidation using purely behavioral methods (Golkar et al., 2012; Kindt & Soeter, 2013; Kindt et al., 2009; Soeter & Kindt, 2011).

The purpose of the current study was to examine if the expectation for learning prior to memory reactivation impacted the reconsolidation process using the post-retrieval extinction

paradigm. Participants were randomly assigned to a no-reactivation, a expectation for learning, or a no expectation for learning condition. The level of expectancy for learning was manipulated by providing each group with different instructions prior to reactivation. We predicted that participants that had their memory reactivated and expected to learn something new would not display a return of fear following reinstatement on Day 3 (i.e., there would be no change in participants' SCR or FPS response from the end of extinction on Day 2 to the beginning of re-extinction following reinstatement on Day 3). Conversely, we predicted that the participants who did not expect to learn something new and participants that did not receive reactivation would demonstrate a return of fear on Day 3 following reinstatement as demonstrated by increased SCR and FPS.

Methods

All procedures were approved by the University of Ottawa's Research Ethics Board and all participants provided informed consent.

Participants

Exclusion criteria included: a self-reported heart condition (e.g., heart transplant, artificial cardiac pacemakers; cardiac arrhythmias, uncontrolled hypo- or hypertension, myocardial infarction); or reported current use of a beta-blocker. We collected data until we had enough participants with usable physiological data. Ninety-nine undergraduate students from the University of Ottawa were recruited through an online participant pool run by the School of Psychology. Of the 99 participants, the following were excluded: 33 participants dropped out; six individuals were not invited back after Day 1 because they could not identify which image was paired with the shock; and 12 were excluded because none of their physiological data was readable. The final sample consisted of 48 participants (see power analysis section below).

Participants were compensated with partial course credit for participation on Day 1 and Day 2 and received $5 for their participation on Day 3.

Materials

Conditioned stimuli. The conditioned stimuli (CS) consisted of two different images of spiders selected from the International Affective Picture System (IAPS images 1200 and 1201; Lang et al., 2008). The standardized conditioned stimuli were designed to study emotions and the selected IAPS images have been demonstrated to be emotionally arousing in a student population (Lang et al., 2008). During fear conditioning on Day 1, one of the images was sometimes paired with the shock (CS+) and the other image was never paired with the shock (CS-). The image associated with CS+ and CS- was counterbalanced across participants.

Unconditioned stimulus (US). The US consisted of an electric shock. The shock was delivered by a Grass SD9 Square Pulse Stimulator via two disposable (3.81 x 2.54cm) Ag/AgCl sensors (pre-applied with 0% chloride wet gel) attached to the wrist of the dominant hand. Two 2-meter touchproof snap leads were attached to the sensors and the leads were plugged into the Grass SD9 Square Pulse Stimulator. Before testing, participants determined the level of shock used throughout the three days. The shock was administered starting at 10 volts and increased by 2.5 volts until the participant determined the shock was uncomfortable but not painful up to 60 volts. The same voltage was used on all three days of testing.

Measures

The Spider Phobia Questionnaire (SPQ; Klorman et al., 1974). The SPQ is a 31-item self-report questionnaire measuring fear of spiders. Participants endorsed either true or false to indicate if each item applies to them. The SPQ has demonstrated acceptable test-retest reliability and discriminant validity in a student sample and can differentiate between participants with and

without a specific phobia (Muris & Merckelbach, 1996). This measure was used to ensure level of spider fear was similar across all three groups. Cronbach's alpha was $\alpha = .91$ for the current sample.

Spielberger State Trait Anxiety Inventory (STAI; Spielberger et al., 1983). The STAI consists of two 20-item self-report questionnaires that assess trait (i.e., STAI-T) and state (i.e., STAI-S) anxiety. The STAI-T asks participants to rate how anxious they generally feel on a 4-point scale ranging from *almost never* (1) to *almost always* (4). The STAI-S asks participants to rate how anxious they feel right now on a 4-point scale ranging from not at all (1) to very much so (4). Both scales have demonstrated adequate convergent validity and excellent test-retest reliability (Creamer et al., 1995). This measure was used to assess if there were differences in trait and state anxiety levels between groups because high levels of anxiety have been demonstrated to impair reconsolidation (Soeter & Kindt, 2013). Cronbach's alpha for the STAI-S was $\alpha = .92$ and the STAI-T was $\alpha = .92$ for the current sample.

Manipulation check. Participants were asked on Day 3, before debriefing, to rate how much they were expecting to receive a shock at the beginning of Day 2 on a scale of 0 (not at all) to 5 (very much) to determine if we were successful in manipulating the expectation for learning among the different conditions.

Physiological measures

Skin conductance response. SCR was used as a measure of fear and emotional arousal (Öhman & Mineka, 2001). SCR is primarily considered a measure of anxiety and is a direct measure of sympathetic activity, which is influenced by the stimulation of the behavioral inhibition system (Cacioppo, Tassinary, & Berntson, 2007). SCR is recommended as a measure of anxiety in situations where stimuli cannot be actively avoided.

SCR was measured throughout the study and was recorded with BioLab Acquisition Software 3.1.13 from MindWare Technologies Ltd. SCR was measured in micro-siemens and sampled constantly at 1000 Hz. The leads were connected to a 16-Channel Electrode Box and the signal was amplified with a Galvanic Skin Conductance Amplifier from Mindware Technologies Ltd. The gain was set to 50 and the low pass was at 5Hz. Participants had two disposable (3.81 x 2.54cm) Ag/AgCl sensors (pre-applied with 0% chloride wet gel) placed on the palm of their non-dominant hand on the thenar eminence and hypothenar eminence and two 2-meter touchproof snap leads were connected to the sensors. Leads were taped on the skin with hypoallergenic surgical tape to reduce movement which can interfere with the recording of the data. All physiological measures were recorded on a different computer than the visual stimuli to ensure the data is recorded with minimal impediment as the computer can overload when both applications are run and can create additional noise in the physiological data.

Fear potentiated startle. Fear potentiated startle was measured in accordance with the recommendations from the Committee report: Guidelines for human startle eyeblink electromyographic studies (Blumenthal et al., 2005). FPS measures the response to the conditioned stimuli by EMG surface measurement of the orbicularis oculi (i.e., muscle located under the lower eyelid that closes the eye during a blink). This measure is used to assess the startle response of the participant as neurologically it represents the connections from the amygdala to the startle-reflex pathway in the brainstem (Davis and Whalen, 2000). A loud white noise (40 msec; 104dB) was presented at each presentation of the CS through headphones (model AKG K92 closed back studio) to participants to elicit a startle response. EMG was measured throughout the study and was recorded with BioLab Acquisition Software 3.1.13 from MindWare Technologies Ltd. The leads were connected to a 16-Channel Electrode Box, after

the skin surface was cleaned with alcohol. To assess the activity of the orbicularis oculi muscle, one electrode (diameter of 5mm Ag/Ag-Cl unshielded electrode filled with Signa Gel) was placed below the lower eyelid on the left side in line with the pupil when looking straight. A second electrode was placed about 1-2cm laterally from the first electrode. A third electrode acted as the isolated ground electrode and was placed on the forehead.

Procedure

Testing took place on three consecutive days each 24 hours apart in the Integrated Neurocognitive and Social Psychophysiology Interdisciplinary Research Environment (INSPIRE) laboratory (https://socialsciences.uottawa.ca/inspire/). Participants were connected to the skin conductance, EMG, and shock electrodes at all time points throughout the study. On all days, testing sessions began with a 5- minute baseline of physiological measures followed by 10 habituation startle probes (i.e., loud white noise; 40 msec; 104dB).

Day1. Informed consent was obtained, and participants completed all self-report measures. In all conditions, participants were connected to SCR, EMG, and shock electrodes. Following baseline and habituation, participants underwent fear conditioning. They received the following instructions prior to the start of fear conditioning: *"We are going to start. There will be two images presented. The shock will only be paired with one image. Monitor the relationship between the image you are seeing and when a shock is received. Please keep your eyes on the screen at all times."* Participants underwent fear conditioning, which consisted of two presentations of the CS+, eight presentations of the CS-, and six presentations of the CS+ paired with the US in a pseudo random order. At the end of Day 1, participants were asked which image they learned was paired with the shock to ensure participants learned that the shock was only paired with one image. Participants were not invited back for the other two days of

testing if they were not able to identify which image was paired with the shock (n = 6).

Day 2. Participants were randomly assigned to one of three groups: expectation for Learning (n=16), no expectation for learning (n=16), or no reactivation (n=16). The website research randomizer (https://www.randomizer.org) was used to randomly assign participants to each condition. In all conditions, participants were connected to SCR, EMG, and shock electrodes throughout testing. Baseline and FPS habituation trials were completed prior to randomization.

The expectation for learning and no expectation for learning conditions both underwent reactivation. Prior to reactivation, these two groups were given separate instructions on the screen and verbally by the experimenter to manipulate the expectation for learning. Participants in the expectation for learning condition were told: *"We are going to start. Shortly you will see the images you saw yesterday. The relationship between the shock and the images has changed. Please observe how it has changed. Please keep your eyes on the screen at all times."* Participants in the no expectation for learning condition were provided with the following instructions prior to reactivation: *"We are going to start. You will see the same images you saw yesterday. However, today you <u>WILL NEVER</u> receive a shock at any point during the experiment. Please keep your eyes on the screen at all times."* Participants were provided with these instructions so they knew what to expect throughout testing and as a result minimize the expectation that something new could be learned. Participants in these two groups then had their memory reactivated via a single presentation of the CS+. Participants in these two groups then took a 10-minute break where they watched a TV show. This break allows for the activation of the neural mechanisms needed for reconsolidation to take place (Schiller et al., 2010). Participants in the no reactivation condition were not exposed to the single presentation of the

CS+ (i.e., their condition fear memory was not reactivated) and instead proceeded straight to the 10-minute break.

Following the 10-minute break, all participants underwent extinction where the CS+ and the CS- were presented without the US. Before extinction, participants in all three conditions were provided with the following instructions: *"We are going to start. Please monitor the relationship between the image and when a shock is received. Please keep your eyes on the screen at all times. Are you ready?"* The number of presentations of the CS+ and CS- were equivalent across groups during extinction and for this reason, the no reactivation condition received one additional CS+ during extinction. All groups received the same instructions prior to extinction.

Day 3. In all conditions, participants were connected to SCR, EMG, and shock electrodes. Baseline and FPS habituation trials were completed, and participants were presented with the following instructions prior to reinstatement: *We are going to start. Please monitor the relationship between the image and when a shock is received. Please keep your eyes on the screen at all times. Are you ready?* Reinstatement consisted of four unpaired presentations of the US. Then participants took a 10-minute break and watched another clip of the TV show. Once the break was completed, participants were presented with the same instructions as they were prior to reinstatement and then underwent re-extinction, which consisted of presentations of the CS+ and the CS- without the US. Participants were then disconnected from the electrodes, debriefed, and compensated for their time.

Prior to debriefing, participants in the expectation for learning and no expectation for learning conditions were administered a manipulation check to determine if the level of expectancy to receive a shock prior to reactivation on Day 2 was successfully manipulated.

Participants rated how much they expected to receive a shock at the beginning of Day 2 (0 = not at all to 5 = very much). Participants in the no reactivation condition were not administered the question because the level of expectancy to receive a shock was not manipulated on Day 2.

Power Analysis

G* power 3.1.9.2 was used to estimate the sample size (Faul, Erdfelder, Lang, & Buchner, 2007). The power analysis was based on the most complex analysis of the study, a mixed ANOVA with Condition (no-reactivation vs. expectation for learning vs. no expectation for learning) as the between-participant factor and Time (last trial of extinction on Day 2 vs. first trial of re-extinction on Day 3) and Stimulus (CS+ vs. CS-) as the within-participant factors. A medium effect was selected for the power analysis because researchers who have examined reconsolidation and prediction error have found medium to large effects (e.g., η^2_p = .10 - .21; Sevenster et al., 2012, 2013). An alpha level of .05 was selected because previous research using this learning paradigm examined reconsolidation using an alpha level of .05 (Schiller et al., 2010; Steinfurth et al., 2014). The correlation among the repeated measures (e.g., skin conductance response and fear potentiated startle) has not been reported on in previous studies. In theory, it would be expected that physiological measures at Time 1 and Time 2 would be highly correlated regardless of condition, however, there are no studies that have reported on this information. As such, an r = . 5 was used to assist in calculating power. According to G*power a sample size of 42 would be needed to achieve a power of .80 with an alpha of .05 and effect size set to f=.25 (medium effect; Cohen, 1988). Assuming 5% attrition, a total sample of 44 would be needed.

Statistical Analysis

Statistical analysis was performed using SPSS software version 23 (IBM corp, 2016).

Statistical assumptions were violated and as a result, data was square root transformed to correct for violations of normality.

The early acquisition phase for SCR and FPS was calculated by taking the averages of trials two and three on Day 1. The first trial for both the CS+ and the CS- were disregarded to help reduce the impact of an orientating effect. The late acquisition phase for both SCR and FPS were calculated by taking the averages of trials seven and eight on Day 1.

For extinction, the early phase of extinction was calculated by taking the averages of trials one and two on Day 2. The late phase of extinction was calculated by taking the averages of trials 10 and 11 on Day 2. The late and early phase for extinction were calculated in the same way for SCR and FPS.

Initial analyses were conducted separately to examine if acquisition and extinction were successful. Separate ANOVAs and follow-up planned comparisons were computed for SCR and FPS for acquisition and extinction with Condition (expectation for Learning vs. no expectation for learning vs. no reactivation) as the between-participant factor, and Stimulus (CS+ vs. CS-), and Time (phase) as within participant factors.

To test our main analysis of interest, the effect of expectation of learning on reconsolidation, two mixed ANOVAs (i.e., for SCR and FPS) were calculated with Condition (no-reactivation vs. expectation for learning vs. no expectation for learning) as the between-participant factor and Time (last trial of extinction on Day 2 vs. first trial of re-extinction on Day 3) and Stimulus (CS+ vs. CS-) as the within-participant factors. It was expected that there would be a Stimulus x Time x Condition interaction whereby participants in the expectation for learning condition would not show a return of fear, whereas the other two groups would exhibit a return of fear as demonstrated by an increase in SCR and FPS from the last trial of extinction to the first

trial of re-extinction.

Results

Participant Characteristics

The final sample consisted of 48 participants with a mean age of 19.28 years (SD = 1.86), 62% female. A one-way ANOVA revealed no differences between the conditions with regards to age, F (2, 44) = 1.20, p = .31. A chi-squared analysis revealed no differences with regards to gender, χ^2 (3) = .67, p = .72. Table 1 provides a summary of participants' scores on the anxiety and spider fear measures, as well as the mean voltage selected by participants. One-way ANOVAs found no differences between conditions with regards to the SPQ, F (2, 44) = .14, p = .87, η^2_p = .006, STAIT-T, F (2, 44) = .04, p = .96, η^2_p = .002 or for the STAI-S, F (2, 44) = 2.29, p = .11, η^2_p = .094. Although the p-value for the measure of state anxiety is not below traditional statistically significant thresholds (i.e., .05), the effect size is in the medium range which suggests that there may be meaningful differences between conditions on this measure. Table 1 shows that the no expectation for learning condition had a higher mean on the state anxiety measure on Day 1 before testing than the other two conditions.

Manipulation Check

An independent t-test was computed to determine if there were differences in ratings taken at the end of the study between the expectation for learning and no expectation for learning conditions with regards to how much they expected to receive a shock on Day 2. We predicted that participants in the expectation for learning condition would report a greater expectation to receive shock on Day 2. Participants in the expectation for learning condition did not differ (M = 4, SD = 1.48, min = 0 and max = 5), from the no expectation for learning condition (M = 3.81,

SD = 1.38, min = 0 and max = 5), t (25) = .34, p = .74, d = .13, with regards to the degree to which they expected to receive a shock on Day 2.

Skin Conductance

Acquisition. To establish that participants across the three groups underwent successful acquisition on Day 1, we conducted a Stimulus (CS+ vs. CS-) x Time (start vs. end) x Condition (expectation for learning vs. no expectation for learning vs. no reactivation) mixed ANOVA. Figure 1 displays the mean of each trial during acquisition. We found main effects for Stimulus, F (1, 43) = 6.44, p = .02, η^2_p = .13, and Time, F (1, 43) = 15.18, p < .001, η^2_p = .26, but no effect for Condition, F (2, 43) = 1.27, p = .29, η^2_p = .06, but the effect size was in the medium range. As expected, the main effects were moderated by a Stimulus x Time interaction, F (1, 43) = 6.58, p = .01, η^2_p = .13. There were no other two- or three-way interactions, *Fs* (1, 43) < .74, *p*s > .48, η^2_p < .03. As seen in Figure 1, there was no difference in the CS+ versus CS- at the start of acquisition, t (45) = - .31, p = .76, d = .05, but by the end of acquisition participants had higher SCRs in response to the CS+ than the CS-, t (45) = 3.13, p = .003, d = .48.

Extinction. To assess if participants underwent successful extinction on Day 2, we conducted a Stimulus (CS+ vs. CS-) x Time (Day 2: start vs. end) x Condition (expectation for learning vs. no expectation for learning vs. no reactivation) mixed ANOVA. Figure 1 displays the mean of each trial during extinction. As expected, we found main effects of Stimulus, F (1, 43) = 6.32, p = .02, η^2_p = .13, and Time, F (1, 43) = 52.06, p <.001, η^2_p = .55 but no main effect for Condition, F (2, 43) = .35, p = .71, η^2_p = .02. None of the two-way or the three-way interactions were meaningful, *Fs* (1, 43) < 1.14, *p*s > .23, η^2_p < .03. Given our a priori hypothesis (i.e., we predicted that participants would have a greater response to the CS+ than to the CS- at the start of extinction and no difference in their response to the CS+ and CS- at the

end), follow-up *t*-tests were conducted to compare the mean SCR response to the CS+ and the CS- at the start of extinction and at the end of extinction. As seen in Figure 1, participants had a greater SCR response to the CS+ than to the CS- at the start of extinction, t (45) = 2.29, p = .03, d = .25, whereas there were no differences in response to the CS+ and CS- at the end of extinction, t (45) = .48, p = .63, d = .07.

Reconsolidation. To examine whether the expectation for learning prior to memory reactivation prevents reinstatement of the conditioned fear response, a Stimulus (CS+ vs. CS-) x Time (last trial of extinction on Day 2 vs. first trial of re-extinction on Day 3) x Condition (expectation for learning vs. no expectation for learning vs. no reactivation) mixed ANOVA was conducted. There were no main effects of Stimulus, F (1, 43) = .03, p = .87, η^2_p < .01, or Condition, F (2, 43) = .44, p = .65, η^2_p = .02, but there was a main effect of Time, F (1, 43) = 42.18, p < .001, η^2_p = .50. Contrary to predictions, all the two- and three-way interactions were greater than p = .05, Fs (2, 43) < .95, ps >.40, η^2_p < .04, though the Time x Condition interaction, F (2, 43) = 1.39, p = .26, η^2_p = .06, had an effect in the medium range. Given the main effect of time and our a priori hypothesis, separate follow-up *t*-tests were conducted to compare the return of fear in each of the Conditions (i.e., The last trial of extinction on Day 2 compared to the first trial of re-extinction on Day 3). As seen in Figure 2, inconsistent with our predictions, participants in the expectation for learning condition showed an increase in their SCR to both the CS+, t (14) = -3.27, p = .01, d = -.89, and CS-, t (14) = - 3.82, p = 002, d = -1.32, demonstrating a return of fear following reinstatement. This effect was also seen in the no expectation for learning condition, as participants showed a significant increase in their SCR to the CS+, t (14) = -2.56, p = .02, d = -.91, and the CS-, t (14) = -4.13 p = .001, d = -.90. The no reactivation condition showed an increase in their SCRs to the CS+, t (15) = -2.47, p = .03, d = -.84, but not

to the CS-, t (15) = -1.86, p = .08, d = -.58, from the end of extinction on Day 2 to the beginning of re-extinction on Day 3. Thus, participants, regardless of condition, showed a return of fear on Day 3. That is, we observed no evidence that reconsolidation took place, as measured by SCR.

Fear Potentiated Startle

The same statistical analyses outlined above for SCR were computed for FPS.

Acquisition. Figure 3 depicts participants' mean FPS response to each trial during acquisition. We found a main effect for Time, F (1, 45) = 25.77, p = < .001, η^2_p = .36, and Stimulus, F (1, 45) = 5.56, p = .02, η^2_p = .11, but no main effect for Condition, F (2, 45) = 4.05, p = .02, η^2_p = .15. None of the two-way interactions had p-values below .05, Fs (2, 45) < .52, ps > .60, η^2_p < .02, but the Time x Condition interaction, F (2, 45) = 2.59, p = .09, η^2_p = .10, had an effect size within the large range. The Stimulus x Time x Condition interaction was meaningful, F (2, 45) = 4.05, p = .02, η^2_p = .15.

Follow up analyses were conducted given the three-way interaction. For the expectation for learning condition, there were no differences in the CS+ compared to the CS- at the start, t (16) = .26, p = .80, d = .03, or the end of acquisition, t (15) = .72, p = .48, d = .11. For the no expectation for learning condition, there was no difference in FPS to the CS+ compared to the CS- at the start of acquisition, t (15) = .49, p = .63, d = .06, but FPS was greater for CS+ than the CS- at the end of acquisition, t (15) = 2.37, p = .03, d = .4. For the no reactivation condition, participants showed greater FPS to the CS+ than the CS-, t (15) = 2.65, p = .02, d = .33, at the start of acquisition, but did not by the end, t (15) = -.19, p = .85, d = -.03. Thus, only the no expectation for learning condition displayed successful fear acquisition.

Extinction. Figure 3 shows participants' mean FPS to each trial during extinction. As expected, we found main effects of Stimulus, F (1, 45) = 5.26, p = .03, η^2_p = .11, and Time, F (1,

45) = 33.61, p = < .001, η^2_p = .43, but no main effect for Condition, F (2, 45) = .001, p = .99, η^2_p = < .001. None of the two- or three-way interactions were meaningful, Fs (2, 45) < 1.04, ps > .36, η^2_p < .04. Although the p-values were not meaningful, the effect size was in the medium range for the Stimulus x Condition interaction, F (1, 45) = 1.84, p = .17, η^2_p = .08. We calculated follow-up t-tests comparing the mean FPS to the CS+ and the CS- at the start of extinction and at the end of extinction. Consistent with our expectations, participants had a larger FPS to the CS+ than the CS- at the start of extinction, t (47) = 2.13, p = .04, d = .16, but by the end of extinction there was no difference between the CS+ and CS-, t (47) = 1.09, p = .28, d = .07. These results suggest that extinction occurred in all conditions, but the magnitude of this effect was not large.

Reconsolidation. Our 2 (Stimulus) x 2 (Time) x 3 (Group) ANOVA showed no main effects for Condition, F (2, 45) = .22, p = .80, η^2_p = .01, or Stimulus, F (1, 45) = 14.25, p = < .001, η^2_p = .24 but the effect size for Stimulus was in the large range. There was a main effect of Time F (1, 45) = 14.25, p < .001, η^2_p = .24. There was also a Stimulus x Condition interaction, F (2, 45) = 3.57, p = .04, η^2_p = .14. Inconsistent with our predictions, the Time x Stimulus interaction was not meaningful, F (1, 45) = .23, p = .67, η^2_p = .01. Although the p-values were not below the traditional statistically significant thresholds, the effect size was in the medium range for the Time x Condition interaction, F (2, 45) = 3.57, p = .26, η^2_p = .06, and the Time x Stimulus x Condition interaction, F (2, 45) = 1.60, p = .21, η^2_p = .07.

Given the main effect of Time, that the three-way interaction with the effect size in the medium range, and our a priori hypothesis, follow-up t-tests were conducted. As seen in Figure 4, participants in the expectation for learning condition showed no difference in their FPS response to the CS+, t (15) = -1.20, p = .25, d = -.29 or CS-, t (15) = -.93, p = .37, d = -.25, from

the last trial of extinction on Day 2 to the first trial of re-extinction on Day 3, suggesting that there was no return of fear. Conversely, in the no expectation for learning condition participants had an increase in FPS response to the CS+, t (15) = -2.72, p = .02, d = -.75, and the CS-, t (15) = -3.27 p = .01, d = -.99, from the end of extinction of Day 2 to the beginning of re-extinction following reinstatement on Day 3, suggesting that they did experience a return of fear. The no reactivation condition (i.e., extinction alone) also showed an increase in FPS from the end of extinction on Day 2 to the beginning of re-extinction on Day 3 to the CS+, t (15) = -3.76, p = .01, d = -.54, but not the CS-, t (15) = -1.59, p = .13, d = -.28, again suggesting that they also experienced a return of fear.

Post hoc analysis. Given that not all conditions displayed physiological fear acquisition with FPS as the measure of fear, we ran a post hoc analysis and included only participants who displayed greater FPS to the CS+ than the CS- during the late phase acquisition (Total n = 20; expectation for learning condition n = 5, no expectation for learning condition n = 6, no reactivation condition n = 9). We calculated a Stimulus (CS+ vs. CS-) x Time (last trial of extinction on Day 2 vs. first trial of re-extinction on Day 3) x Condition (expectation for learning vs. no expectation for learning vs. no reactivation) mixed ANOVA to examine whether the expectation for learning prior to memory reactivation prevents reinstatement of the conditioned fear response.

We found no meaningful effects for Stimulus, F (1, 17) = 2.00, p = .18, η^2_p = .11, or Time, F (1, 17) = 1.20, p = .29, η^2_p = .07, but the effect sizes were in the medium to large range. There was no effect of Condition, F (2, 17) = .06, p = .94, η^2_p = .01. The Time x Condition, F (2, 17) = .43, p = .65, η^2_p = .05, the Stimulus x Condition, F (2, 17) = 2.71, p = .10, η^2_p = .24, and the Time x Stimulus, F (1, 17) = 1.90, p = .19, η^2_p = .10, did not have meaningful p-values,

however, the effect sizes ranged from medium to large. The Time x Stimulus x Condition, F (2, 17) = .56, p = .58, η^2_p = .06, did not have a meaningful p-value but the effect size was in the medium range. The pattern of response was similar to that found in the entire sample. FPS response remained stable in the expectation for learning and the no expectation for learning conditions from the end of extinction to the beginning of reinstatement. The no reactivation condition displayed an increase in their FPS response following reinstatement.

Discussion

The current study examined if the expectation for learning impacts the reconsolidation of conditioned fear memories using the post-retrieval extinction paradigm in an undergraduate sample using both SCR and FPS as measures of the fear response. We found a dissociation between SCR and FPS. Specifically, for SCR, there was no evidence of reconsolidation as participants across all three conditions (i.e., the expectation for learning, the no expectation for learning, and the no reactivation conditions) exhibited a return of fear following reinstatement on Day 3 of testing. In contrast, with FPS, we found evidence that increasing the expectation for learning enhanced reconsolidation, as it was only the expectation for learning condition in which FPS to the CS+ remained stable following reinstatement. Participants in the no expectation for learning condition showed a generalized response as they displayed a return of fear to both the CS+ and CS-. The no reactivation condition showed an increase in FPS to the CS+, suggesting that fear returned in this condition as well. However, it should be emphasized that this effect was small and non-robust. Furthermore, when we examined this effect only in participants that showed evidence of fear acquisition as measured by FPS, the pattern of responses was similar to that found in the entire sample.

The dissociation we found between the SCR and FPS is consistent with previous research (Sevenster et al., 2012; Soeter et al., 2011). Researchers have suggested that SCR reflects a cognitive representation of arousal and is more difficult to observe evidence of reconsolidation (Kindt & Soeter, 2013; Kindt et al., 2009; Soeter & Kindt, 2011). In contrast, FPS is believed to represent an automatic emotional fear response and memory reconsolidation has been more successfully demonstrated with FPS (Kindt et al., 2009; Kindt & Soeter, 2013; Soeter & Kindt, 2011; Soeter & Kindt, 2015). It is notable that in studies where both SCR and FPS are measured, evidence of reconsolidation was found for FPS but not for SCR (Sevenster et al., 2012; Soeter & Kindt, 2011). Conversely, studies which only measure SCR have been more successful in demonstrating reconsolidation using SCR as a measure of fear (Agren, et al., 2017; Schiller, et al., 2010; Steinfurth et al, 2014).

FPS is frequently measured by using a loud startle probe and it is possible that this may interfere with the measurement of SCR. Previous research has found that shocks, unpleasant sounds, and a loud tone, similar to the one used in the current study to induce the startle response, are equally effective USs to produce fear acquisition in a classical conditioning paradigm using SCR as a measure of fear (Neumann & Waters, 2006). Most studies that have examined reconsolidation using FPS as a measure of fear have utilized a loud tone to initiate startle response. It is possible that the loud tone used in the current study may be too aversive and as a result, may interfere with the measurement of the SCR because participants are anticipating the delivery of the loud tone. Future researchers may consider using different methods to induce a FPS response to limit the potential interference with SCR.

The limitations of the study restrict the generalization and interpretation of the findings. First, participants did not exhibit successful physiological fear acquisition on Day 1 with FPS as

the measure of fear, as defined by greater FPS to the CS+ than the CS-. However, it should be noted that differences between the CS+ and CS- were in the expected direction, albeit small. Furthermore, by the start of Day 2, participants across all three groups did display a greater response to the CS+ than the CS-. This suggest that differential fear learning took place, but the effects were not apparent until Day 2.

Additionally, the results from the manipulation check found that the groups did not differ (i.e., the expectation for learning and the no expectation for learning conditions) in their expectation to receive a shock on Day 2. Thus, it is possible that we did not successfully manipulate the expectancy to learn something new during reactivation. However, upon reflection, the question asked may not have accurately measured participants' expectancy to learn something new but simply measured whether they expected to receive a shock during reactivation. This was further supported with anecdotal evidence that the no expectation for learning condition anticipated to receive a shock during memory reactivation. Participants in this condition often asked the experimenter during the transition to the 10-minute break whether shocker was working because they did not receive a shock. Additionally, as the question was asked on Day 3 (to ensure that the manipulation check did not interfere with the actual experiment), it is possible that participants' responses might not accurately represent their expectation to receive a shock on Day 2.

It is also possible that the verbal manipulation of expectancy for learning was not salient enough to differentiate the groups. In addition to verbal instructions, Sevenster et al. (2012) maximized differences across conditions by not connecting participants in the no expectation for learning condition to the shock equipment to ensure that they absolutely could not expect to learn something new during reactivation. With the rationale of keeping conditions similar in all

respects, except for the expectation for learning, we chose to connect participants in the no expectation for learning condition to the shock equipment and only provided verbal instructions that they would not receive a shock. It is possible that manipulating the expectation for learning needs to be more salient, and the variations in methods could explain the differences in results across these two studies. In addition to the possibility that the expectation for learning may not influence reconsolidation, research should also explore whether the salience of the expectation for learning needs to be stronger to influence reconsolidation using behavioural methods.

Although an a priori power analysis indicated that the present study was sufficiently powered for a medium effect, several of the interactions in the present study had *p*-values above .05 but had effect sizes in the medium range. There is little research on how the expectation for learning impacts reconsolidation using behavioural methods. Pharmacological studies have found that the expectation for learning is a potential boundary condition for memory reconsolidation (Sevenster et al., 2012, 2013). It may be beneficial to replicate the current study with a larger sample size to minimize a Type II error given the medium effect sizes found in the present study and the inconsistent findings using behavioural methods to study reconsolidation (Golkar, Bellander, Olsson, & Ohman, 2012; Kindt & Soeter, 2013; Soeter & Kindt, 2011a; Schiller et al., 2010; Steinfurth et al., 2014).

Despite these limitations, the findings from the current study have important implications. The present study demonstrated that a verbal manipulation of the expectation for learning is not sufficient to induce reconsolidation with SCR, but we found limited evidence that it may be for FPS as a measure of fear. Furthermore, the inconsistent results between SCR and FPS in the current study as well as in previous studies (Sevenster et al., 2012; Soeter et al., 2011) raise questions about measuring SCR and FPS concurrently. Future researchers should consider

using alternative methods to induce FPS when measuring SCR concurrently or consider having separate conditions for each measure. Overall, the findings demonstrate how nuanced memory reconsolidation is and raise the critical question about whether the post-retrieval extinction paradigm is appropriate to study reconsolidation using physiological measures concurrently.

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Table 1

Participants' means on the anxiety and spider fear measures

Variable	Expectation for learning group M (SD)	No expectation for learning group M (SD)	No reactivation M (SD)
Voltage	36.50 (1.06)	36. 03 (10.97)	40.50 (10.00)
SPQ	9.05 (5.63)	8.12 (6.36)	7.80 (8.06)
STAI-T	41.21 (9.83)	41.82 (9.03)	40.80 (11.03)
STAI-S	40.53 (9.27)	48.04 (10.96)	42.53 (10.61)

Note. M; Mean; SD; Standard deviation; SPQ; Spider Phobia Questionnaire (Klorman, Weerts, Hastings, Melamed, & Lang, 1974); STAIT; Spielberger Trait Anxiety Inventory (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983); STAIS; Spielberger State Anxiety Inventory (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983).

Mean SCR during acquisition and extinction

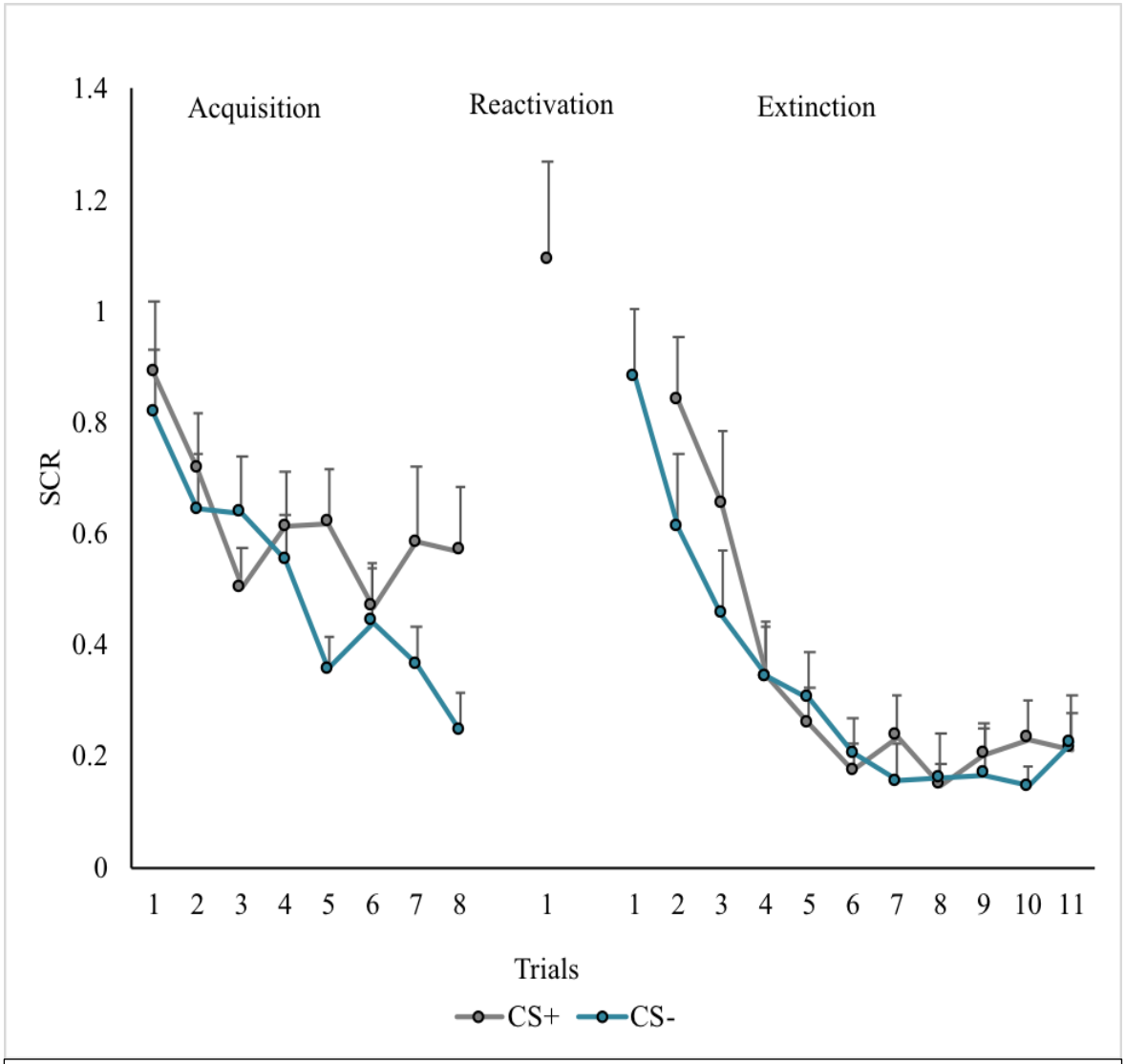

Figure 1. *Note.* Mean skin conductance response (SCR) across trials collapsed across groups. Acquisition consisted of presentations of the CSa+ that was sometimes paired with the US and the CS- that was never paired with the US. Extinction consisted of the presentation of the CSa+ and the CS- without the US. Measures of SCR were taken at every presentation of a stimuli.

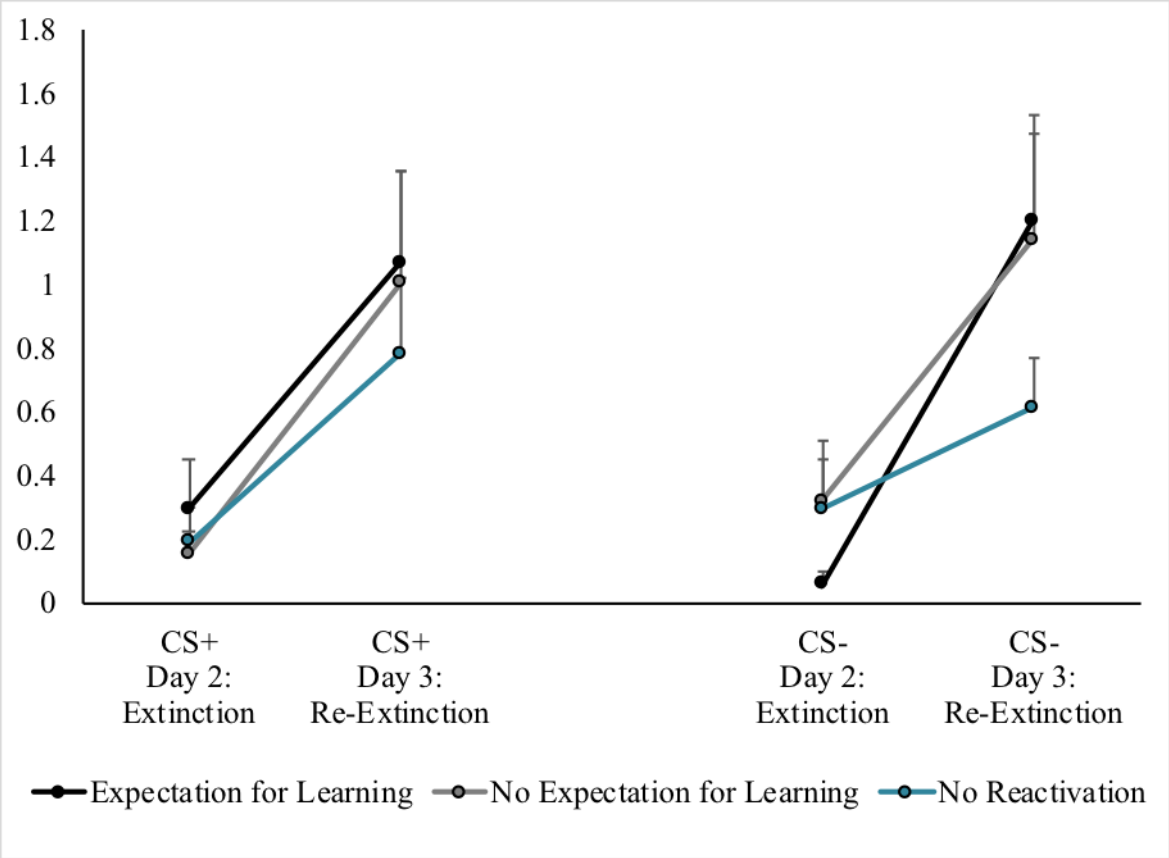

Figure 2. *Note.* Results for skin conductance (SCR) for reconsolidation. Day 2 is a measure of fear following extinction (i.e., last trial of extinction). Day 3 is a measure of fear following reinstatement of fear (i.e., first trial of re-extinction). Measures of SCR were taken at every presentation of a stimuli.

Mean FPS for acquisition and extinction

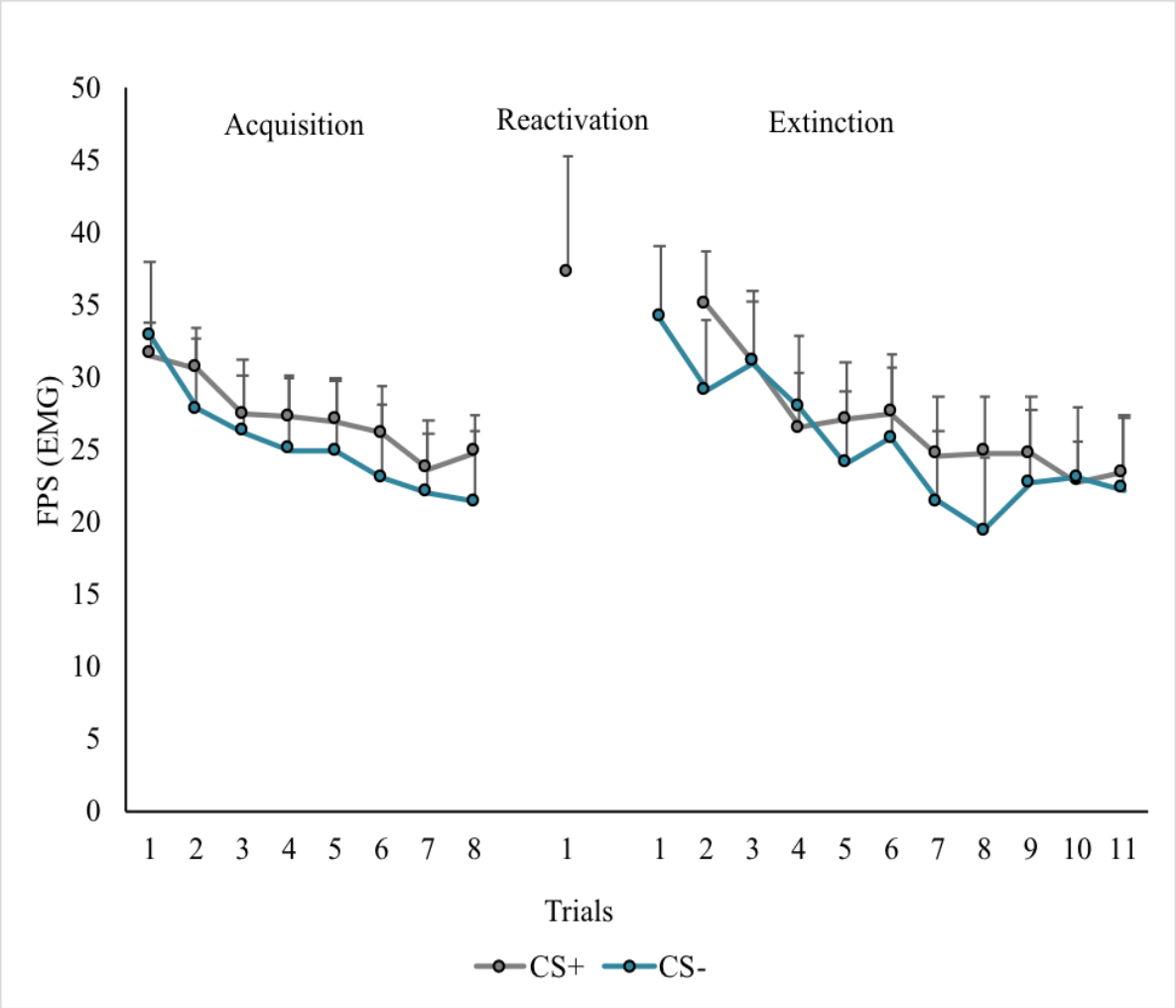

Figure 3. *Note.* Mean fear potentiated startle (FPS) across trials collapsed across groups. Acquisition consisted of presentations of the CSa+ that was sometimes paired with the US and the CS- that was never paired with the US. Extinction consisted of the presentation of the CSa+ and the CS- without the US. The error bars represent standard error. Measures of FPS were taken at every presentation of a stimuli.

Return of fear following reinstatement as measured by FPS

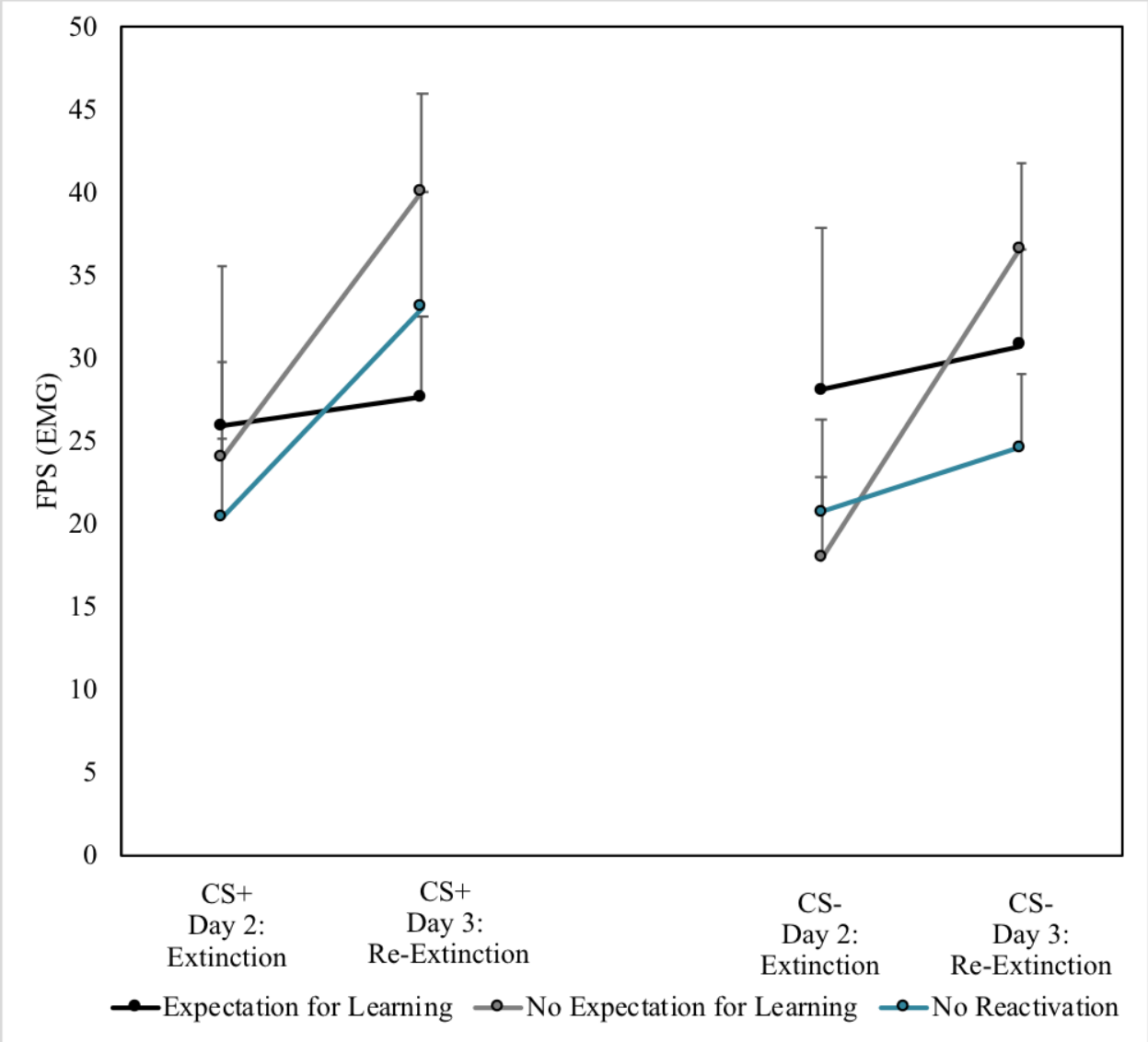

Figure 4. Note. Results for fear potentiated startle (FPS). Day 2 is a measure of fear following extinction (i.e., last trial of extinction). Day 3 is a measure of fear following reinstatement of fear (i.e., first trial of re-extinction). The error bars represent standard error. Measures of FPS were taken at every presentation of a stimuli.

Chapter 3

Bridge Between Studies

The overarching purpose of my dissertation was to understand factors that impact the reconsolidation of conditioned fear memories. Study 1 examined how the expectation for learning influenced the reconsolidation of conditioned fear memories using the post-retrieval extinction paradigm. I found limited evidence that the expectation for learning facilitated reconsolidation with fear potentiated startle as the measure of fear. Study 1 and Study 2 were planned concurrently prior to the start of data collection for Study 1. Due to the complexity of these studies and time constraints, I proceeded with Study 2 without fully analyzing the data from Study 1. I decided to include the expectation for learning instructions prior to reactivation because if any effects were detected in Study 1 it would maximize the effect in Study 2 and was unlikely to adversely influence results if the effect was not present.

The purpose of Study 2 was to investigate other possible boundary conditions of memory reconsolidation. The purpose of Study 2 was to examine if the level of spider fear influenced the reconsolidation of conditioned fear memories and to understand if the effects of reconsolidation could generalize to other similar stimuli. Specifically, Study 2 compared the reconsolidation of conditioned fear memories in individuals with high and low spider fear using the post retrieval extinction paradigm. Participants were presented with distinct but similar stimuli following reinstatement to understand if the effect of reconsolidation could generalize to stimuli of the same sematic category. I also examined if there were differences in performance on a behavioural approach test between groups as another measure of stimulus generalization. The collective studies aimed to further understand which factors impact reconsolidation of conditioned fear memories which is critical in order to identify which requirements need to occur

for a memory to be consistently updated. These studies will potentially help to clarify how reconsolidation processes can be applied to treatments for anxiety and other fear-based disorders.

Chapter 4

Study 2

Examining the effects of memory reconsolidation in individuals with high and low spider fear

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Abstract

Objective: The present study examined if the level of spider fear impacted the reconsolidation of conditioned fear memories using the post-retrieval extinction paradigm in an undergraduate sample. The study also investigated if the effects of reconsolidation generalize to other similar stimuli. **Methods:** Participants were undergraduate students with high (HPF; *n* = 17) or low (LSF; *n* = 17) levels of spider fear. The study took place over three consecutive days. On Day 1, participants underwent fear conditioning where the CSa+ (spider image) and the CSb+ (snake image) were sometimes paired with the US (shock), and the CS- (clock image) was never paired with the US. On Day 2, participants were presented with an unpaired presentation of the CSa+ and the CS- to reactivate the memory and then underwent extinction to all three CSs. On Day 3, return of fear was assessed following reinstatement (four unpaired shocks). Fear potentiated startle (FPS) and skin conductance response (SCR) were taken as measures of fear. Participants completed a behavioural approach test as another measure of fear and stimulus generalization. **Results**: We found limited evidence of reconsolidation in the HSF group as FPS to the CSa+ remained stable from the end of extinction to post reinstatement. This effect was not found for SCR in the HSF group. Participants in the LSF group exhibited a return of fear as measured by FPS and SCR. The study did not find any evidence that reconsolidation increased approach behaviour for either group during the BAT. **Implications:** We found limited evidence that memories associated with high distress in participants with HSF can be reconsolidated. However, the results were non-robust and should be interpreted with caution. We conclude that further research is needed to understand how memories linked to high levels of fear can undergo reconsolidation.

Introduction

Exposure therapy is an evidence-based treatment for anxiety disorders, which is based on extinction principles (Vervliet, Craske, & Hermans, 2013). However, relapse rates for individuals with anxiety disorders following exposure therapy can range from 19% to 62% (Craske & Mystkowski, 2006). Furthermore, although exposure therapy is one of the evidence-based treatments for anxiety disorders, a meta-analysis found that extinction learning can be inhibited in individuals with anxiety disorders (Duits et al., 2015). Extinction learning is theorized to create a new non-fearful memory that competes for expression with the original fear memory after extinction (Milad, Rauch, Pitman, & Quirk, 2006). Importantly, extinction is believed to inhibit the expression of the original fear memory but does not modify or erase the memory (Bouton, 2000, 2002). Thus, individuals with anxiety disorders potentially have a reduced ability to learn during extinction and may not be able to obtain the full benefits of extinction learning.

Recent advances in our understanding of memory suggests that under certain circumstances old memories can be updated or modified with new information, a process known as reconsolidation (Nader, Schafe, Le Doux, 2000). When reconsolidation processes are active, memories can be updated (Schiller et al., 2010), strengthened (Lee, 2008), or blocked (Nader, Schafe, Le Doux, 2000; Soeter & Kindt, 2011). Since reconsolidation appears to directly change the original memory, incorporating reconsolidation into the treatment of anxiety disorders (e.g., specific phobias) may produce long-lasting or permanent fear reduction. Pharmacological blockage studies have consistently demonstrated that reconsolidation is more resistant to the return of fear than is standard extinction (Kindt et al., 2009; Kindt & Soeter, 2013; Soeter & Kindt, 2011; Soeter & Kindt, 2015), though a few studies have failed to find this effect (Beckers

& Kindt, 2014; Chalkia et al., 2019). Reconsolidation principles have successfully been applied to treat fear of snakes and spiders using behavioural interventions (Telch et al., 2017; Lancaster, Monfils, and Telch, 2020). Reconsolidation principles have also been applied to the treatment of spider (Soeter & Kindt, 2015; Telch et al., 2017) and mouse (Elsey & Kindt, 2017) phobias, as well as posttraumatic stress disorders using pharmacological blockade (Brunet et al., 2008; Brunet et al., 2011; Brunet et al., 2014).

Telch et al. (2017) examined if the incorporation of memory reactivation would enhance an *in vivo* exposure intervention for individuals with naturally developed spider and snake fears. Half of the participants had their memory reactivated and then 30 minutes later completed an *in vivo* exposure. The other half completed the *in vivo* exposure and then had their memory reactivated outside the reconsolidation window. Both groups completed a behavioural approach test (BAT) one day and one month later. The researchers found that participants' performance on the BAT did not differ between groups one day following reactivation plus *in vivo* exposure. However, participants who had their memory reactivated before the exposure task displayed decreased fearful behaviour at the one month follow up compared to the group who had their memory reactivated following exposure. These results suggest that reconsolidation is equally effective as standard exposure at initially reducing fear but more resistant to the return of fear over time. These results are also consistent with another study that found that the effects of their reconsolidation-based exposure intervention combined with propranolol lasted up to one year post treatment (Soeter & Kindt, 2015). In contrast, a follow up study by Lancaster et al. (2020) found that reactivtion prior to exposure reduced the duration of exposure treatment needed to achieve clincially meaningful fear readuction.

However, not all findings support the notion that reconsolidation can be applied to the treatment of anxiety disorders or high levels of trait anxiety (Elsey et al., 2020; Soeter and Kindt, 2013). Reconsolidation principles were applied and tested as a treatment for fear of public speaking using propranolol without success (Elsey et al., 2020). The study found that although there were reductions in self report measures of distress and public speaking, there were no meaningful differences between the propranolol and placebo conditions. Similar to studies that found extinction learning is inhibited in people with anxiety disorders (Duits et al., 2015), Soeter and Kindt (2013) found that reconsolidation was inhibited in individuals with high levels of self-report trait anxiety using fear relevant stimuli. Although the various methodologies may explain the differences in results, these studies highlight the inconsistent findings on whether a memory associated with high levels of distress can undergo reconsolidation. Therefore, there is a need to further examine if reconsolidation is inhibited by high levels of distress before it can be consistently applied to interventions.

The long term effect of reconsolidation appears promising but the generalization of reconsolidation needs to be examined when considering the translational impact for the treatment of anxiety disorders. However, limited research has examined if the effects of reconsolidation generalize to other stimuli not used during fear conditioning or reactivation (Bjôrkstrand et al., 2016; Soeter & Kindt, 2015a; Soeter & Kindt, 2015b). Soeter and Kindt (2015a) using pharmacological blockage, examined if a semantic cue (e.g., words instead of images) not used during original fear conditioning, was sufficient to reactivate a memory. Specifically, the study examined if a semantic stimulus was sufficient to reactivate the fear memory or if the stimuli used to reactive the memory had to be distinctly associated with the original feared stimuli. The study compared one group that underwent standard fear conditioning using two distinct images

of spiders to another group that underwent fear conditioning to an image of a spider and a snake. The researchers found that semantic stimulus (i.e., the word "spider" instead of the image) triggered memory reconsolidation but only in the group that underwent fear conditioning to images of spiders and snakes. The generalized stimuli (i.e., the word "spider") was not distinct enough to signal to participants which image was to be recalled during memory reactivation in the group that underwent fear conditioning to spiders only. These findings suggest that generalized stimuli can be sufficient to reactivate a memory but are contingent on past learning (i.e., what images were used during original fear conditioning). These findings build on Soeter and Kindt's (2015b) other study, which found that the effect of reconsolidation could generalize to other spiders at a 1 year follow up.

Björkstrand et al., (2016) used a behavioural reactivation plus exposure paradigm to examine if the effects of reconsolidation generalize to spider images other than the one used to reactivate the memory. Participants with high levels of spider fear were presented with a spider image to reactivate their memory, followed by exposure either 10 minutes or 6 hours following reactivation. Participants were presented with new spider images (e.g., that were not used during reactivation or exposure) to test if the effects of reconsolidation generalize to other spider images following spontaneous recovery. Participants who completed the exposure task 10 minutes following reactivation displayed reduced amygdala activity to the reactivated image and to the novel spider images. Conversely, individuals who completed the exposure task 6 hours following reactivation displayed increase amygdala activity to all images. These findings suggest that the effects of reconsolidation can generalize to other stimuli of the same sematic category. However, further research is needed to understand if stimulus generalization occurs in the context of reconsolidation given the inconsistent findings using behavioural techniques.

In summary it remains unclear if high levels of fear impact reconsolidation and if reconsolidation can consistently generalize its effects to other related stimuli. Understanding both of these aspects of reconsolidation is important to establish if reconsolidation can be applied to the treatment of fear-based disorders. Therefore, the first goal of this study was to test if people's level of spider fear impacts the reconsolidation of conditioned fear memories by comparing individuals with high and low spider fear using the post retrieval extinction paradigm. Specifically, the study examined if the reduction of fear following extinction or reactivation plus extinction remains stable for both individuals with high and low spider fear following reinstatement. The second goal of this study was to examine if the effects of reconsolidation generalize to other stimuli from the same category (i.e., reduce the fear response to all spiders or increased approach behaviour during a behavioural approach test).

On Day 1, participants with high and low levels of spider fear underwent fear conditioning to a spider (CSa+), a snake (CSb+), and a neutral image of a clock (CS-). Following fear conditioning, participants were presented with a new spider image (GSa) and a new image of a snake (GSb) in order to test stimulus generalization. On Day 2, the CSa+ and the CS- were presented to reactivate the memory. Participants then underwent extinction to all CSs. On Day 3, following fear reinstatement (e.g., four unpaired shocks), participants completed post reinstatement physiological measures of fear followed by a behavioural approach test, and then underwent re-extinction. Fear potentiated startle and skin conductance response were used as measures of fear. Participants completed a behavioural approach test as an additional measure of fear and stimulus generalization.

We predicted that individuals with low levels of spider fear would demonstrate successful memory reconsolidation (i.e., no return of fear response following post-reinstatement).

Conversely, we predicted that high levels of spider fear would inhibit reconsolidation but not prevent it completely. Consistent with previous research mentioned above on the return of fear and extinction, we expected that participants in both groups would demonstrate a return of fear to the image that underwent standard extinction (snake image). We also predicted that there would be stimulus generalization (i.e., reduction of the fear response to all spider images) following reconsolidation, but the effect would be greater for individuals with low spider fear. Lastly, we predicted that if reconsolidation was successful, approach behaviour during the BAT would increase from predictions on Day 1 to actual behaviours following reinstatement on Day 3 of testing for both groups.

Method

All procedures were approved by the University of Ottawa's Research Ethics Board and all participants provided informed consent.

Participants

Exclusion criteria were the following: self-reported heart condition or current diagnosis of an anxiety disorder, and current use of a beta-blocker. Participants were assigned to the high spider fear group if they: a) endorsed 5 or more on two pre-screening questions that asked participants to rate their fear and avoidance of spiders on a 7 point scale (higher scores represent greater fear and avoidance); b) obtained a 3 or higher on the clinician administered specific phobia section of the Anxiety and Related Disorders Interview Schedule for DSM-5 (ADIS-5; Brown & Barlow, 2014); *and* c) scored 17 or higher on the Spider Phobia Questionnaire (Klorman, Weerts, Hastings, Melamed, & Lang, 1974). Participants were assigned to the low spider condition if they: a) endorsed 4 or below on the pre-screening questions; b) obtained a 2 or below on the clinician administered specific phobia section of the ADIS-5 (Brown & Barlow,

2014); *and* c) scored 16 or below on the Spider Phobia Questionnaire (Klorman, Weerts, Hastings, Melamed, & Lang, 1974).

A total of seventy-nine participants were recruited via an undergraduate participant pool at the University of Ottawa and via online advertisement. A total of 45 participants were excluded for the following reasons: 15 dropped out; 11 were unable to identify which images were paired with the shock (this was a requirement to test that successful acquisition took place); 12 had technical difficulties/unreadable physiological measures; and seven reported inconsistent levels of spider fear on measures used to assign participants to high and low spider fear groups. The final sample consisted of 34 participants (High Spider Fear n = 17; Low Spider Fear n = 17). Participants were given partial course credits in addition to $15 for their time.

Materials

Conditioned stimuli. The conditioned stimuli (CS; spider images: IAPS# 1200; 1201; snake images: IAPS# 1050;1052; clock image: IAPS# 721) consisted of several different images selected from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2008). The CSa+ was a spider image, the CSb+ was a snake image, and the CS- was an image of a clock. In order to test the generalizing effect of reconsolidation and extinction, the generalized stimulus (GSa) was another unique spider image, and the GSb was a unique snake image. Images were counter balanced between the CS and GS for each category of stimuli.

Unconditioned stimulus. The US was an electric shock delivered by a Grass SD9 Square Pulse Stimulator via two disposable (3.81 x 2.54cm) Ag/AgCl sensors attached to the wrist of the dominant hand. Two 2-meter touchproof snap leads were attached to the sensors and the leads were plugged into the Grass SD9 Square Pulse Stimulator. On the first day of testing, participants selected a shock level that was uncomfortable but not painful, starting at 10 volts and

increasing by 2.5 volts (up to 60 volts). This level was used across all three days of testing.

Measures

Pre-screening questions. The following pre-screening questions were used to categorize participants into low and high spider fear: 1) On a scale of 1 (*not at all*) to 7 (*extremely*) to what degree do you fear spiders? 2) On a scale of 1 (*not at all*) to 7 (*extremely*) to what degree do you avoid spiders? Participants scoring 5 or more on both questions were assigned to the high spider fear condition and participants scoring 4 or less were assigned to the low spider condition.

Anxiety and Related Disorders Interview Schedule for DSM-5 (ADIS-5; Brown & Barlow, 2014). The ADIS-5 is a semi structured clinical interview and was administered by a clinical psychology graduate student (JM) under the supervision of a licensed clinical psychologist (ARA). The ADIS-5 was used to assess participants' level of spider fear and to assign participants to either high (i.e., scored 3 or more) or low spider fear (i.e., scored 2 or below) groups. A score of 3 which means that the symptoms reported by an individual do not meet clinical thresholds for a diagnosis (Brown & Barlow, 2014), was used to categorize individuals with high spider fear. A previous version of the ADIS has shown good to excellent reliability in an outpatient sample (Brown, Di Nardo, Lehman, & Campbell, 2001).

Spider Phobia Questionnaire (Klorman, Weerts, Hastings, Melamed, & Lang, 1974). The SPQ is a 31-item self-report measure which assesses the level of spider fear using a dichotomous true or false response for each item. The SPQ has demonstrated acceptable test-retest reliability and discriminant validity in a student sample and can differentiate between participants with and without a specific spider phobia (Muris & Merckelbach, 1996). Cronbach's alpha was $\alpha = .93$ for the current sample.

Behavioural Measure

Behavioural Approach Test (BAT). A perceived-threat behavioural approach test (PT-BAT) adapted from Cochrane, Barnes-Holmes, and Barnes-Holmes (2008), was used to assess participants' degree of fear and willingness to approach spiders on Days 1 and 3. No real spiders were used during the PT-BAT. Deception was used and participants were informed that there were different probabilities of spiders being in each jar used in the PT-BAT. Participants were debriefed at the end of the study at which time they were informed that no real spiders were used in the PT-BAT.

The PT-BAT consisted of eight opaque black jars, labeled with the probability of a house spider being in each of the first 7 jars that gradually increased (e.g., Jar 1 is empty, jar 3 has a 20% chance of having a house spider). Participants were informed that there was a 100% chance that a tarantula was in the 8th jar. There were in fact no spiders in any of the jars. On Day 1 of testing, participants rated how willing they would be to place their hand in each jar on Day 3 of testing (0 = willing to 100 = very unwilling). They received a score of one for each jar they predicted they would place their hand in on Day 3 of testing (maximum score = 8). Predictions were taken on Day 1 to preserve the believability of PT-BAT for Day 3. On Day 3, participants were asked to place their hand in each jar for 30 seconds. On Day 3, they received a score of one for every jar they actually placed their hand in (maximum score = 8). During debriefing, all participants stated they believed there were real spiders in the jars except one person in the low spider fear group, suggesting that the task was believable. Analysis using the BAT scores were conducted with and without this participant and the results were not affected by whether or not the participant was included.

Psychophysiological Measures

Skin conductance response (SCR). SCR was used as a measure of anxiety and emotional arousal (Öhman & Mineka, 2001). SCR is considered a measure of sympathetic activity which is impacted by the stimulation of the behavioral inhibition system and is recommended as a measure of anxiety in circumstances where stimuli cannot be avoided (Cacioppo, Tassinary, & Berntson, 2007).

SCR was measured throughout all three days of the study and was recorded with BioLab Acquisition Software 3.1.13 from MindWare Technologies Ltd. SCR was measured in micro-siemens and sampled constantly at 1000 Hz. The gain was set to 50 and the low pass was at 5Hz. The leads were connected to a 16-Channel Electrode Box and the signal was amplified with a Galvanic Skin Conductance Amplifier from Mindware Technologies Ltd. Participants had two disposable (3.81 x 2.54cm) Ag/AgCl sensors (pre-applied with 0% chloride wet gel) placed on the palm of their non-dominant hand on the thenar eminence and hypothenar eminence and two 2-meter touchproof snap leads were connected to the sensors. To reduce movement, which can create artificial noise while recording the data, the leads were taped on the skin with hypoallergenic surgical tape. All physiological measures were recorded on a different computer than the visual stimuli to reduce artificial noise which can occur when both programs are run on the same computer.

Fear potentiated startle. Fear potentiated startle (FPS) was measured in accordance with the recommendations from the Committee report: Guidelines for human startle eyeblink electromyographic studies (Blumenthal et al., 2005). FPS measures the response to the conditioned stimuli by EMG surface measurement of the orbicularis oculi (i.e., muscle located under the lower eyelid that closes the eye during a blink). This measure is used to the startle response as neurologically it represents the connections from the amygdala to the startle-reflex

pathway in the brainstem (Davis and Whalen, 2001). A loud white noise (40 msec; 104dB) was presented at each presentation of the CS through headphones (model AKG K92 closed back studio) to elicit a startle response. EMG was measured throughout the study and was recorded with BioLab Acquisition Software 3.1.13 from MindWare Technologies Ltd. The leads were connected to a 16-Channel Electrode Box, after the skin surface was cleaned with alcohol. To assess the activity of the orbicularis oculi muscle, one electrode (diameter of 5mm Ag/Ag-Cl unshielded electrode filled with Signa Gel) was placed below the lower eyelid on the left side in line with the pupil when looking straight. A second electrode was placed about 1-2cm laterally from the first electrode. A third electrode acted as the isolated ground electrode and was placed on the forehead.

Procedure

The post-retrieval extinction paradigm was used (Schiller et al., 2010) and all testing took place on three consecutive days twenty-four hours apart in the Integrated Neurocognitive and Social Psychophysiology Interdisciplinary Research Environment (ISPIRE) laboratory (https://socialsciences.uottawa.ca/inspire/). All testing sessions began with a 5-minute baseline during which physiological measures were collected, followed by 10 habituation startle probes (i.e., loud white noise; 40 msec; 104dB).

Day 1. At the start of testing on Day 1, participants' spider fear was assessed with the ADIS-5. Participants then made predictions of whether they would place their hand in each jar of the PT-BAT on Day 3 of testing. Participants then completed the SPQ online, and then the electrodes were attached. The physiological baseline and habituation trials were administered, and participants were presented with a single presentation of the CSa+ (i.e., spider image), CSb+

(i.e., snake image), CS- (i.e., clock image), GSa (i.e., another distinct spider image), and GSb (i.e., another distinct snake image) to measure baseline response to each image.

Then participants received the following instructions prior to undergoing fear acquisition: "*We are going to start. Monitor the relationship between the image you are seeing and when a shock is received. Please keep your eyes on the screen at all times*." Participants underwent fear acquisition, where the CSa+ and CSb+ were sometimes paired with the US and the CS- was never paired with the US. The order in which the conditioned stimuli were presented were randomized. Each stimulus was presented for 8 seconds with an inter-trial interval between 10 to 12 seconds during which time participants viewed a black screen with a white fixation cross between stimuli (i.e., between images).

Following acquisition, participants were presented with one GSa and one GSb to test stimulus generalization (i.e., to examine if there was an increase in the fear response to the GSs from prior to the end of fear acquisition). At the end of Day 1, participants were asked which image they learned was paired with the shock to verify that fear conditioning was successful.

Day 2. On Day 2, participants were connected to the electrodes and a physiological baseline was acquired. Participants had their memory reactivated by viewing a single presentation of the CSa+ and the CS- but not the CSb+. Participants were provided with the following instructions prior to memory reactivation to increase their level of expectation for learning which has been found to facilitate memory reactivation (Marinos & Ashbaugh, 2020; Sevenster, Beckers, Kindt, 2012): *"We are going to start. Shortly you will see the images you saw yesterday. The relationship between the shock and the images has changed. Please observe how it has changed. Please keep your eyes on the screen at all times."* Participants then took a 10-minute break during which they watched a clip from an episode of *The Simpsons*. After the

break, participants were presented with 10 FPS habituation trials, and then underwent extinction where the CSa+, CSb+, and the CS- were randomly presented without the US.

Day 3. On Day 3, participants were connected to the electrodes and a physiological baseline was acquired, followed by the 10 habituation trials. Participants then underwent reinstatement, which consisted of four unpaired presentations of the US. Participants then took a 10-minute break and watched another clip from an episode of *The Simpsons*. Following the break, participants viewed a single image of the CSa+, CSb+, CS-, GSa, and GSb, to assess return of fear to the CSs and GSs following reinstatement. Participants then completed the PT-BAT and underwent re-extinction during which the CSa+, CSb+, and the CS- were presented without the US. The order in which the conditioned stimuli were presented was randomized. Participants were then debriefed and compensated for their time.

Power Analysis

G* power 3.1.9.2 was used to estimate the sample size (Faul, Erdfelder, Lang, & Buchner, 2007). The power analysis was based on the most complex analysis of the study, a mixed ANOVA with Group, (low vs. high spider fear) as between-participant factor, and Time (Day 2: last trial of extinction vs. Day 3: first trial of post-reinstatement) and Stimulus (CSa+ vs. CSb+ vs. CS-), as the within-participant factors. An alpha level of .05 was selected because previous research has used an alpha level of .05 (Schiller et al., 2010; Steinfurth et al., 2014). Previous researchers who have examined reconsolidation with individuals with high spider fear and high trait anxiety have found large effects in studies that have used propranolol to study reconsolidation (e.g., η^2_p = .31 - .69; Soeter, & Kindt et al., 2013, 2015a). Researchers who have used behavioural methods generally have not reported on effect size (Schiller et al., 2010; Steinfurth et al., 2014), and have difficulty replicating results (Golkar, Bellander, Olsson, &

Ohman, 2012; Kindt & Soeter, 2013; Soeter & Kindt, 2011a). As a result, a medium effect size (Cohen, 1988) was used. There are no studies that have reported on the correlation between physiological measures (e.g., skin conductance response and fear potentiated startle) at Time 1 and Time 2, however, it would be expected that these time points would be highly correctly. Therefore, an r = . 5 was used to assist in calculating power. According to G*power a total sample size of 34 would be needed to achieve a power of .80 with an alpha of .05 and effect size set to f=.25.

Statistical analysis

Statistical analysis was performed using SPSS software version 23 (IBM corp, 2016). Assumptions for parametric statistical analysis were violated and therefore data were square root transformed to correct for violations of normality. Outliers were defined as being greater than three standard deviations from the mean. There was one participant removed from the FPS measure as readings were greater than three standard deviations away from the mean.

Acquisition and extinction. Participants were considered to show successful fear acquisition and were included in analyses if they correctly identified which images were paired with the shock following acquisition on Day 1 of testing (n = 34). Separate ANOVAs and follow-up planned comparisons were run for SCR and FPS for acquisition and extinction with Group (low vs. high spider fear) as the between-participant factor, and Stimulus (CSa+ vs. CSb+ vs. CS-) and Time (phase) as within participant factors.

The early acquisition phase for SCR and FPS was calculated by taking the averages of trials two and three to account for an orienting effect to the first presentation of the CSs. The late acquisition phase for both SCR and FPS were calculated by taking the averages of trials seven and eight.

For extinction, the early phase was calculated by taking the averages of trials one and two. The late phase of extinction was calculated by taking the averages of trials 10 and 11. The late and early phase for extinction were calculated in the same way for both SCR and FPS.

Spider fear. To examine whether the level of spider fear impairs reconsolidation following reinstatement, separate mixed ANOVAs were calculated for SCR and FPS with Group (low vs. high spider fear) as between-participant factor, and Time (Day 2: last trial of extinction vs. Day 3: first trial of post-reinstatement) and Stimulus (CSa+ vs. CSb+ vs. CS-) as the within-participant factors.

Stimulus generalization. To examine the second main question, whether fear generalized to other similar stimuli following acquisition, a mixed ANOVA was calculated with Group (low vs. high spider fear) as the between participant factor, and Stimulus (Day 1: GSa+ vs. GSb+) and Time (baseline before acquisition vs. baseline after acquisition) as the within participant factors for SCR and FPS separately.

To examine if reconsolidation has a stimulus generalization effect following reinstatement, a mixed ANOVA was calculated with Group (low vs. high spider fear) as the between participant factor, and Stimulus (Day 3: GSa+ vs. GSb+) as the within participant factor. To examine whether memory reconsolidation impacted participant's approach behavior to a novel spider, a mixed ANOVA was calculated with Group (low vs. high spider fear) as the between participant factor, and Time (Day 1 predictions vs. Day 3 behaviours) as the within participant factor.

Results

Participant Characteristics

The mean age for the current sample was 19 (*SD* = 2.40) years. See Table 1 for a summary of participant characteristics. There were no differences with regards to gender distribution, χ^2 (1) = .57, *p* = .45. Additionally, a one-way ANOVA revealed there were no differences between the low and high spider fear groups with regard to the voltage participants selected, *F* (1, 34) = .001, *p* = .98, η^2_p = <.001. As expected, participants in the high spider fear group reported higher scores on the SPQ than the low spider fear group, *F* (1, 33) = 147.08, *p* = <.001, η^2_p = .82, but there were no differences with regards to the STAI-T, *F* (1, 33) = .17, *p* = .68, η^2_p = .01, across groups.

Acquisition

Skin Conductance Response. To test whether participants across the two groups underwent successful physiological acquisition on Day 1, we conducted a Stimulus (CSa+ vs. CSb+ vs. CS-) x Time (early vs. late phase) x Group (low vs. high spider fear) mixed ANOVA. If acquisition was successful, we expected that participants would display greater SCR to the CSa+ and CSb+ than the CS- by the end of acquisition. Figures 1 and 2 show the means of each trial during acquisition. There were main effects for Stimulus, *F* (1, 32) = 7.49, *p* = .001, η^2_p = .20, and Time, *F* (1, 32) = 16.18, *p* = < .001, η^2_p = .34, but no main effect for Group, *F* (1, 32) = .01, *p* = .92, η^2_p = < .001. None of the two-way interactions were meaningful, *Fs* (1, 32) < .40, *p*s > .68, η^2_p < .01. Though the Time x Stimulus x Spider Fear interaction was not statistically meaningful, *F* (1, 32) = 2.94, *p* = .06, η^2_p = .08, the effect size was medium (Cohen, 1988).

As seen in Figure 1, at the start of acquisition, participants in the low spider fear group displayed higher SCRs to CSa+ than the CS-, *t* (16) = 2.30, *p* = .04, *d* = .53, and the CSb+, *t* (16) = 2.22, *p* = .04, *d* = .44, whereas the CSb+ was not different from the CS-, *t* (16) = .56, *p* = .58, *d* = .12. At the end of acquisition, participants with low spider fear had higher SCRs to the CSa+, *t*

(16) = 2.57, p = .02, d = .52, and the CSb+, t (16) = 3.45, p = .003, d = .71, compared to the CS-. By the end of acquisition, there was no difference between the CSa+ and the CSb+, t (16) = -1.00, p = .33, d = -.21. These results suggest that fear conditioning was successful in the low spider fear group.

As seen in Figure 2, participants in the high spider fear group displayed higher SCRs to CSa+ than the CS-, t (16) = 2.07, p = .06, d = .59, however the CSb+ was not different from the CSa+, t (16) = .32, p = .76, d = .12, or CS-, t (16) = 1.64, p = .12, d = .40, at the start of acquisition. At the end of acquisition, participants with high spider fear had higher SCRs to the CSa+ compared to the CS-, t (16) = 2.35, p = .03, d = .50. By the end of acquisition, there was no difference between the CSb+ compared to the CSa+, t (16) = 1.28, p = .22, d = .26, and the CS-, t (16) = 1.17, p = .26, d = .27. This demonstrates that fear acquisition was not successful as fear response remained stable.

Fear potentiated startle. Figures 3 and 4 illustrate the FPS during acquisition. We found main effects for Stimulus, F (1, 32) = 6.90, p = .002, η^2_p = .18, Time, F (1, 32) = 19.94, p = < .000, η^2_p = .28, and Group, F (1, 32) = 4.31, p = .05, η^2_p = .12. There was no Time x Stimulus interaction, F (1, 32) = 2.60, p = .08, η^2_p = .08, but there was a medium effect. There were no other two-way or the three-way interactions, Fs (1, 32) < .87, ps > .42, η^2_p < .03.

As seen in Figure 3, participants in the low spider fear group displayed no difference in their FPS to the CSa+ compared to the CS-, t (16) = 1.04, p = .31, d = .13, and compared to the CSb+, t (16) = .32, p = .75, d = .04, and no difference between the CSb+ compared to the CS-, t (16) = 1.09, p = .29, d = .10, at the start of acquisition. At the end of acquisition, there was no difference between the CSa+ and CS-, t (16) = 1.73, p = .10, d = .24, or the CSa+ and CSb+, t (16) = -.76, p = .46, d = - .09, in the low spider fear group. FPS was greater for the CSb+

compared to the CS-, t (16) = 2.23, p = .04, d = .32, at the end of acquisition for individuals with low spider fear. This demonstrates that participants only displayed successful physiological fear acquisition as measured by FPS to the CSb+ but not to the CSa+.

As seen in Figure 4, participants in the high spider fear group displayed no difference in the FPS to the CSa+ compared to the CS-, t (16) = 1.39, p = .18, d = .10, and CSb+, t (16) = 1.10, p = .29, d = .10, and the CSb+ was not different from the CS-, t (16) = -.04, p = .97, d = -.003, at the start of acquisition. At the end of acquisition, participants with high spider fear had higher FPSs to the CSa+ compared to the CS-, t (16) = 2.59, p = .02, d = .35. There was no difference between the CSb+ compared to the CSa+, t (16) = 1.16, p = .26, d = .10. Participants with high spider fear had higher (though not statistically so) FPS to the CSb+ than the CS-, t (16) = 2.02, p = .06, d = .27 at the end of acquisition. This suggests that participants in the high spider fear group displayed successful physiological fear acquisition as measured by FPS.

Extinction

Skin conductance. To determine if participants underwent successful extinction on Day 2, we conducted a Stimulus (CSa+ vs. CSb+ vs. CS-) x Time (Day 2: start vs. end) x Group (low vs. high spider fear) mixed ANOVA. We expected that at the start of extinction participants would exhibit greater SCR to the CSa+ and CSb+ than the CS-, whereas there would be no difference in participants' SCR across the three stimuli (i.e., CSa+, CSb+, and CS-) by the end of extinction. Figures 1 and 2 display the mean of each trial during extinction. As expected, we found main effects of Stimulus, F (1, 32) = 6.00, p = .004, η^2_p = .16, and Time, F (1, 32) = 20.28, p = <.001, η^2_p = .39, but no main effect for Group, F (1, 32) = .62, p = .44, η^2_p = .02. None of the two-way interactions were meaningful, Fs (1, 32) < 1.21, ps > .31, η^2_p < .04.

Although the *p*-value was not in the traditional statistically significant range, the Time x Stimulus x Group interaction had a medium effect, F (1, 32) = 2.02, p = .14, η^2_p = .06.

For participants with low spider fear, at the start of extinction there was no difference in SCR to the CSa+ compared to the CSb+, t (16) = -.64, p = .53, d = -.12, or the CS-, t (16) = 1.64, p = .12, d = .40, but there was a difference between the CSb+ compared to the CS-, t (16) = 3.09, p = .01, d = .47. In participants with low spider fear there was no difference in SCR to the CSa+ compared to the CS-, t (16) = .31, p = .76, d = .06, and CSb+, t (16) = -.23, p = .09, d = -.06, or between the CSb+ and the CS-, t (16) = .57, p = .58, d = .13, at the end of extinction.

For participants with high spider fear, at the start of extinction there was no difference in SCR to the CSa+ compared to the CSb+, t (16) = .28, p = .78, d = .04, or the CS-, t (16) = 1.73, p = .10, d = .24, and there was no difference between the CSb+ compared to the CS-, t (16) = .93, p = .37, d = .20. In participants with high spider fear, at the end of extinction there was no difference in SCR to the CSa+ compared to the CS-, t (16) = .59, p = .56, d = .32. Participants showed a smaller SCR to the CSa+ compared to the CSb+, t (16) = -2.44, p = .03, d = -.86, and greater SCR to the CSb+ compared to the CS-, t (16) = 2.44, p = .03, d = .96, at the end of extinction.

Fear potentiated startle. Figures 3 and 4 illustrate the average FPSs during extinction. There was no main effect of Stimulus, F (1, 32) = .30, p = .74, η^2_p = .01, or Group, F (1, 32) = .08, p = .78, η^2_p = .002 but there was a main effect for Time, F (1, 32) = 39.17, p <.001, η^2_p = .55. There was a Stimulus x Group interaction, F (1, 32) = 3.11, p = .05 η^2_p = .09. None of the other two-way interactions were meaningful, Fs (1, 32) < 1.44, ps > .24, η^2_p < .04. There was no Stimulus x Time x Group interaction, F (1, 32) = 2.37, p = .10 η^2_p = .09, however the effect size was in the medium range.

For participants with low spider fear, at the start of extinction there was no difference in FPS to the CSa+ compared to the CSb+, t (16) = -.32, p = .76, d = -.02, or the CS-, t (16) = 1.15, p = .27, d = .41, or between the CSb+ compared to the CS-, t (16) = 1.57, p = .14, d = .13 at the beginning of extinction. In participants with low spider fear there was no difference in FPS to the CSa+ compared to the CS-, t (16) = -2.06, p = .06, d = -.24, and CSb+, t (16) = -1.81, p = .09, d = -.18, or between CSb+ and the CS-, t (16) = -.48, p = .64, d = -.06, at the end of extinction.

For participants with high spider fear, at the start of extinction there was no difference in FPS to the CSa+ compared to the CSb+, t (16) = 1.78, p = .10, d = .13 and CS-, t (16) = .78, p = .45, d = .08, nor for the CSb+ compared to the CS-, t (16) = -.45, p = .66, d = -.05. At the end of extinction participants with high spider fear showed no difference in their FPS to the CSa+ compared to the CS-, t (16) = 1.40, p = .18, d = .18, and CSb+, t (16) = -1.82, p = .09, d = .28. There was no difference in FPS to the CSb+ compared to the CS-, t (16) = -1.05, p = .31, d = -.08, at the end of extinction.

Reconsolidation.

Skin conductance. To examine whether the level of spider fear inhibits the reconsolidation of a conditioned fear memory differently for high and low spider fearful participants, a Stimulus (CSa+ vs. CSb+ vs. CS-) x Time (Day 2: last trial of extinction vs. Day 3: first trial of post reinstatement) x Group (low vs. high spider fear) mixed ANOVA was calculated. If reconsolidation was successful, we expected that SCR would remain stable in response to the CSa+ from the end of Day 2 to the beginning of Day 3, but would increase to the CSb+. There were no main effects for Stimulus, F (1, 32) = .56, p = .58, η^2_p = .02, but there was a main effect of Time, F (1, 32) = 10.14, p = .003, η^2_p = .24. The p-value was not within the traditional significant threshold for the main effect of Group, F (1, 32) = 2.81, p = .10, η^2_p = .08

the effect size was in the medium range. There was no Time x Stimulus interaction, F (1, 32) = 2.39, ps = .10, η^2_p = .07, but the effect size was medium. Inconsistent with our predictions, none of the two-way or the three-way interactions were meaningful, Fs (1, 32) < .70, ps >.41, η^2_p < .02.

Given the main effect of Time, the medium effect size of Group and Time x Stimulus interaction, and our a priori hypothesis (i.e., we expected that reconsolidation would be inhibited in the high spider group), separate follow-up t-tests were computed to compare the return of fear across each of the groups. As seen in Figure 5, inconsistent with our predictions, there was an increase in the SCR for the reactivated CSa+ from the end of Day 2 to start of Day 3 in both the low, t (16) = -3.15, p = .01, d = -1.07, and high spider fear groups, t (16) = -2.77, p = .01, d = -.93. For the non-reactivated CSb+, SCR remained stable from end of Day 2 to start of Day 3 in both the low, t (16) = -.67, p = .51, d = -.26, and the high spider fear groups, t (16) = -1.20, p = .25, d = -.30. For the CS-, the low spider group showed an increase in SCR from the end of Day 2 to the start of Day 3, t (16) = -1.99, p = .06, d = -.73, whereas the high spider group showed no change in SCR, t (16) = -2.11 p = .04, d = -.73. These results suggest that reactivation prior to extinction did not prevent the return of fear in any of the groups with SCR as the measure of fear.

Fear potentiated startle. Figure 6 illustrates the average FPSs of the last trial of extinction and the first trial of post-reinstatement. There was no main effect for Stimulus, F (1, 32) = 2.73, p = .07, η^2_p = .08, but the effect was medium. There was a main effect of Time, F (1, 32) = 8.30, p = .01, η^2_p = .21, but not for Group, F (1, 32) = .29, p = .59, η^2_p = .01. There was no Time x Stimulus, F (1, 32) = 1.94, p = .15, η^2_p = .06, or Time x Stimulus x Group, F (1, 32) = 1.88, p = .16, η^2_p = .06, interactions but the effect sizes were medium. None of the other two-way or the three-way interactions were meaningful, Fs (1, 32) < 1.35, ps >.27, η^2_p < .04.

Given the main effects, the three-way interaction with an effect size in the medium range, and our a priori hypothesis, follow-up *t*-tests were computed to compare the return of fear across the low and high spider fear groups. There was an increase in FPS to the reactivated CSa+ from the end of Day 2 to start of Day 3 in the low spider fear group, t (16) = -2.17, p = .05, d = -.42, but not in the high spider fear group, t (16) = -.53, p = .61, d = -.15. For the unreactivated CSb+, there was an increase in FPS to the CSb+ from the end of Day 2 to start of Day 3 in the high spider fear group, t (16) = -2.15, p = .05, d = -.51, but not in the low spider fear group, t (16) = -.16, p = .89, d = -.03. For the reactivated CS-, there was an increase in FPS from the end of Day 2 to start of Day 3 in the low, t (16) = -3.00, p = .01, d = -.38, and high spider fear groups, t (16) = -2.38, p = .03, d = -.67.

Stimulus Generalization

Skin conductance. To assess if fear generalized following acquisition on Day 1 of testing, a Time (baseline before acquisition vs. baseline after acquisition) x Stimulus (Day 1: GSa+ vs. GSb+) x Group (low vs. high spider fear) mixed ANOVA was calculated. If stimulus generalization occurred, we expected that all participants would show increased SCR to the GSa and GSb from baseline to the end of acquisition. Figure 7 displays the average SCR for trails of GSa and GSb. We found no main effects for Stimulus, F (1, 32) = .24, p = .63, η^2_p = .01, Time, F (1, 32) = 1.24, p = .27, η^2_p = .04, or Group, F (1, 32) = .09, p = .77, η^2_p = .003. There were no two or three-way interactions with p-values below .05, Fs (1, 32) < 1.81, ps > .19, η^2_p < .05. These findings suggest that the effect of fear acquisition did not generalize to other similar stimuli with SCR as the measure of fear response. Therefore, we did not examine if reconsolidation generalizes to other stimuli on Day 3 of testing following reinstatement.

Fear potentiated startle. Following fear acquisition on Day 1, there was a main effect for Stimulus, F (1, 32) = 12.65, p = 001, η^2_p = .28, but not for Time, F (1, 32) = 1.78, p = .19, η^2_p = .05, or Group, F (1, 32) = 3.12, p = .09, η^2_p = .09, but the effect size for Group was in the medium range. There were no two- or three-way interactions, Fs (1, 32) < 1.65, ps > .21, η^2_p < .05. As seen in Figure 8, post hoc pairwise comparison found that all participants displayed a higher FPS to the snake images than the spider images (p = .001). Additionally, participants in the high spider fear group tended to have higher FPS to all stimuli than the low spider fear participants (p = .010). There was no evidence of stimulus generalization.

Behavioural Approach Test. To examine whether memory reconsolidation impacted participants' approach behavior to a potential spider, a Time (Day 1: predictions vs. Day 3: behaviours) x Group (low vs. high spider fear) a mixed ANOVA was conducted. There was no main effect of Time, F (1, 33) = 2.62, p = .11, η^2_p = .07, but the effect size was in the medium range. There was a main effect for Group, F (1, 33) = 41.24, p < .001, η^2_p = .55, and the effect size was large. There was no Time x Group interaction, F (1, 33) = .16, p = .69, η^2_p = .01. Figure 9 illustrates participants' response to the BAT on Day 1 and Day 3. Participants in the low spider group predicted on Day 1 that they would place their hand in more jars on Day 3 than the high spider fear group. This is consistent with actual behaviour on Day 3. Participants in the low spider group placed their hands in more jars on Day 3 than participants in the high spider fear group. There were no differences between predictions and actual behaviour for the low, t (17) = .72, p = .48, d = .23, or high spider fear groups, t (16) = 2.00, p = .07, d = .32. Specifically, both groups placed their hands in almost the same number of jars on Day 3 as they predicted they would on Day 1.

Discussion

There were two goals to the current study. First, this study examined if the level of spider fear influenced the reconsolidation of conditioned fear memories. The post-retrieval extinction paradigm was used in the current study with SCR, FPS, and a BAT as measures of fear. The current study found a dissociation between SCR and FPS, which is consistent with our previous study (Marinos & Ashbaugh, 2020). For SCR, participants in both the low and high spider fear group exhibited an increase in SCR to the reactivated CSa+ from the end of Day 2 compared to post-reinstatement on Day 3 (i.e., return of fear), whereas there was no change in SCR to the non-reactivated CSb+ in either group. This finding is directly opposite to what we hypothesized would happen if reconsolidation occurred following reactivation. For FPS, we found different responses across the spider fear groups. FPS increased from the end of extinction to post-reinstatement to the CSa+ in the low spider fear group but remained stable across time in the high spider fear group. Overall the high spider fear group was the only group to show possible evidence of reconsolidation, but only when FPS was used to measure fear. However, it is important to note that this effect was small, non-robust, and it is unclear whether participants successfully acquired a fear response, as there was no difference in FPS to the CSs and CS- in the high spider fear group at the end of acquisition.

The study also examined if the effects of reconsolidation generalized to other similar stimuli, however we did not find evidence of stimulus generalization following fear acquisition in either SCR and FPS. As result, we did not examine if the effects of reconsolidation generalize to other similar stimuli post reinstatement. Unexpectedly, we found that participants in both groups exhibited higher FPS to novel snake images (i.e., GSb) than spider images (i.e., GSa).

Lastly, the study assessed if the effects of reconsolidation increased approach behaviour during a BAT post reinstatement as another way to measure fear and stimulus generalization.

The study found no meaningful differences between predictions on Day 1 of testing and actual behaviours during the BAT on Day 3 post-reinstatement across both groups. Additionally, participants in the low spider fear group predicted and actually placed their hand in more jars on Day 3 than participants with high spider fear. These findings are consistent with previous research which has found that reactivation prior to exposure did not produce greater approach behaviour 24-hours after the intervention (Lancaster et al., 2020; Telch et al., 2017) or that reactivation plus propranolol did not produce meaningful differences between the placebo condition on self-report measure of distress and public speaking at one and three months follow-ups (Elsey et al., 2020). However, Telch et al. (2017) did find that reactivation plus exposure increased approach behaviour one month later, and Lancaster et al. (2020) found that memory reactivation prior to exposure reduced the amount of exposure treatment needed to produce clinically meaningful fear reduction than exposure alone. Based on the above, it appears that the differences between reconsolidation plus exposure versus exposure alone on behaviour are not immediate but may appear over time or that reconsolidation reduces the dose of exposure needed to attain clinically meaningful fear reduction. Overall, the present study was not able to explicitly demonstrate reconsolidation and it remains unclear how reconsolidation consistently effects approach behaviour.

Interestingly, contrary to our predictions, we found some evidence that reconsolidation was observed with FPS as a measure of fear in individuals with high levels of spider fear but not in individuals with low levels of spider fear. This is inconsistent with previous research which found that reconsolidation was inhibited in individuals with high levels of trait anxiety (Soeter, & Kindt, 2013). It is important to note that despite the differences between our groups on measures of spider fear which was evident by both self-report measures and their performance on

the BAT, there were no differences on the measure of trait anxiety. This means that our sample was highly fearful to specific fear relevant stimulus (e.g., spiders), but they were not generally more fearful or anxious. Mosig et al. (2014) found that individuals with high levels of spider fear exhibited an increased ability to discriminate between fear relevant and fear irrelevant cues. It may be that a general tendency to feel anxious inhibits memory reconsolidation, whereas elevated fear to a specific stimulus does not inhibit memory reconsolidation and could facilitate it.

It is also possible that differences in the age of fear memories might explain our finding that memory reconsolidation occurred in the high spider fear group but not the low spider fear group. Scully, Napper, and Hupbach, (2016) found in a meta-analysis that reconsolidation was more pronounced in older fear memories which are often associated with a strong network of linked memories compared to newly formed memories. Walsh et al. (2018) found in a meta-analysis that reconsolidation was inhibited with older conditioned fear memories in animal experiments, but this effect was not found in naturally acquired human memories which were formed outside the laboratory and in the context of everyday life. Reconsolidation may have been observed in the high spider fear group with FPS as a measure of fear in the present study because we are targeting a long-standing network of fear memories and not just the conditioned fear memory created during the study. Scully, Napper, and Hupbach, (2016) noted that 24-48 hours may not be enough time for a fear memory to fully consolidate. It is possible that in the low spider fear group the conditioned fear memory was not fully consolidated and as a result, might explain why reconsolidation was not observed. It is also possible that our groups are not true comparisons because the high spider fear group may have a strong long-standing network of naturally developed spider fear memories whereas the conditioned spider fear memory was

created during the experimental in the low spider fear group. Thus, studying only conditioned fear memories may limit the understanding of how memories associated with high distress undergo reconsolidation, as natural and conditioned memories appear to reconsolidate differently (Scully, Napper, & Hupbach, 2016).

Unexpectedly, physiological fear acquisition was inconsistently observed in this study despite all participants identifying which images were paired with the shock. This is surprising as SCR but not FPS conditioning has been shown to be consistent with contingency awareness (Sevenster, Beckers, & Kindt, 2014). Thus, we expected to find SCR differential learning because all participants were aware of the relationship between the shock and the images. Researchers have found that individuals with anxiety disorders or high trait anxiety display increased fear response to the CS- during fear acquisition and thus exhibit a general fear response to all images during acquisition (Duits et al., 2015; Gazendam, Kamphuis, and Kindt, 2013). However, the current study was unable to successfully observe fear acquisition in both the low and high spider groups, thus it is unlikely that the inhibited differentiation between the CSs was due to high levels of anxiety only. Furthermore, it is important to note that upon visual inspection of the graphs, by Day 2 of the study fear differential learning did occur across the groups.

The present study has a number of limitations that hinder the generalizability and interpretation of the results. First, we did not measure self-reported fear of snakes, which is important because there is high comorbidity (i.e., 47%) between spider and snake phobias among university students (Seim & Spates, 2009). Notably in the present study, participants displayed greater fear responses to the generalized snake image than the generalized spider image on Day 1 of testing. Therefore, it would have been beneficial to have a measure of snake fear.

Additionally, several of the interactions in the present study had *p*-values above .05 but had medium effects. Although the present study was sufficiently powered for a medium effect, it is possible that the large variability in responding among participants may have reduced the ability to detect differences using standard null hypothesis testing. Therefore, given the medium effects and the limited research on reconsolidation using behavioural methods with individuals with high spider fear, it could be beneficial to replicate the study with a larger sample size to minimize Type II error.

Overall, despite the limitations of the study, the surprising findings are interesting and have important implications. We found limited evidence that memories associated with high distress in participants with high levels of spider fear may undergo reconsolidation with FPS as a measure of fear but not with SCR. The findings also highlight the importance of examining naturally developed memories independent of conditioned fear memories as they may undergo reconsolidation differently. Future research should examine how naturally developed memories with high distress undergo reconsolidation in order to be applied to treatments of fear- and anxiety- related mental health disorders.

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Table 1

Summary of descriptive means for high and low spider fear participants

Variable	Low Spider Fear	High Spider Fear
Voltage *M* (SD)	38.97 (8.01)	39.12 (9.72)
SPQ *M* (SD)	7.00 (4.01)	21.41 (2.69)
STAI-T *M* (SD)	37.71 (8.65)	39.11 (12.52)

Note. SPQ; Spider Phobia Questionnaire (Klorman, Weerts, Hastings, Melamed, & Lang, 1974); STAIT; Spielberger Trait Anxiety Inventory (Sielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983); STAI-T; Spielberger State Trait Anxiety Inventory (Sielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983).

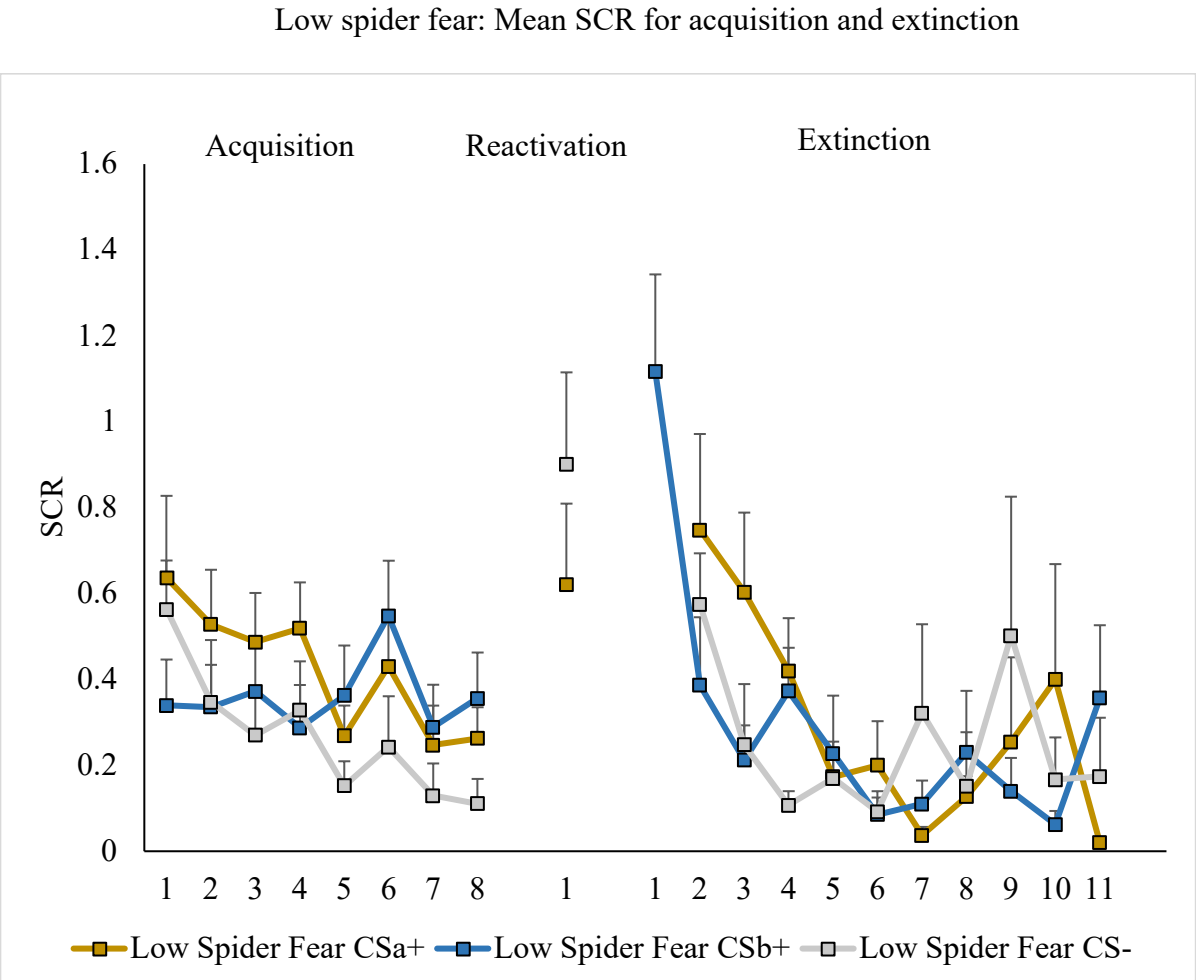

Figure 1. *Note.* Mean skin conductance response (SCR) across trials. Acquisition consisted of presentations of the CSa+ and CSb+ that were sometimes paired with the US and the CS- that was never paired with the US. Extinction consisted of the presentation of the CSa+, CSb+ and the CS- without the US. The error bars represent standard error. Measures of SCR were taken at every presentation of a stimuli.

High spider fear: Mean SCR for acquisition and extinction

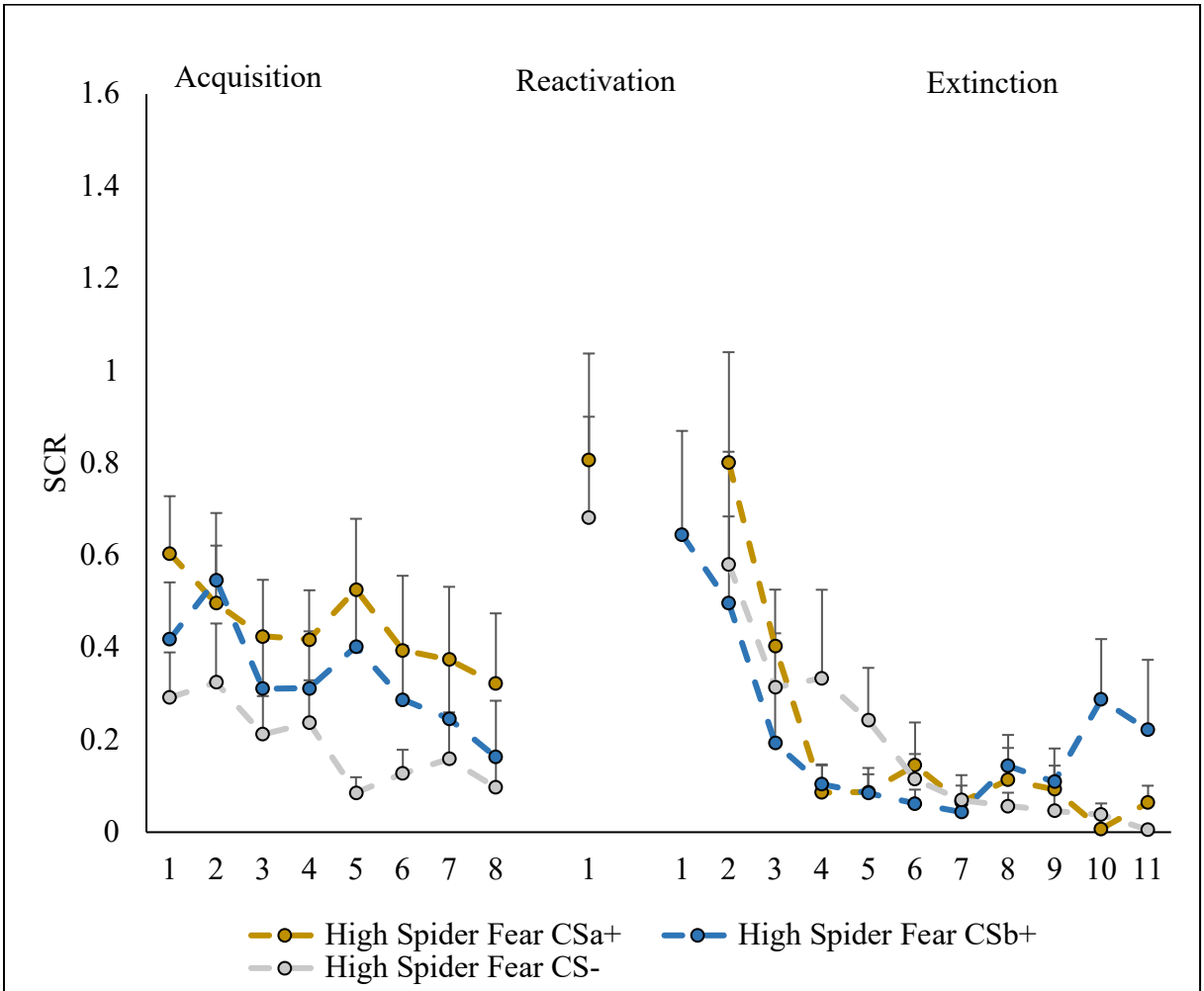

Figure 2. *Note.* Mean skin conductance response (SCR) across trials. Acquisition consisted of presentations of the CSa+ and CSb+ that were sometimes paired with the US and the CS- that was never paired with the US. Extinction consisted of the presentation of the CSa+, CSb+ and the CS- without the US. The error bars represent standard error. Measures of SCR were taken at every presentation of a stimuli.

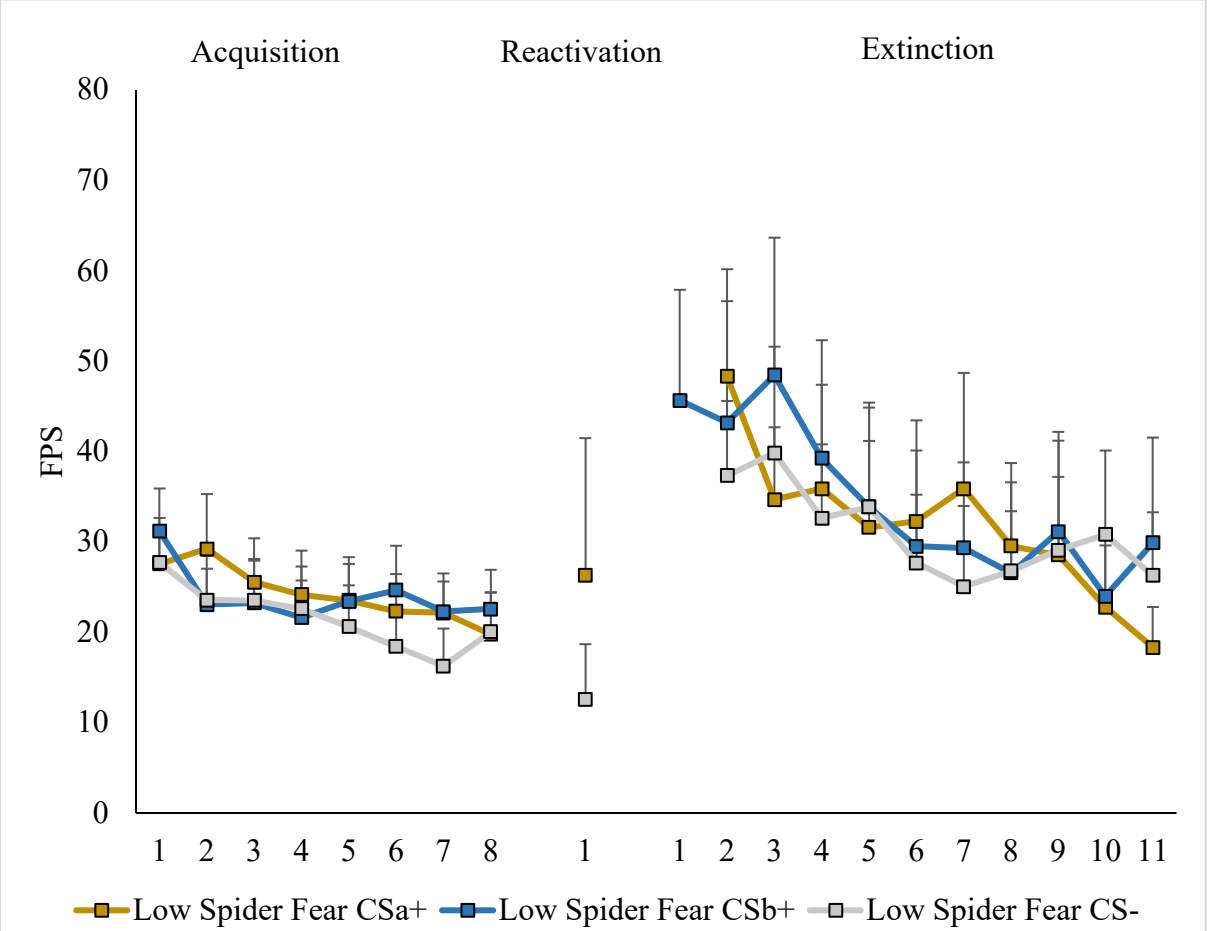

Figure 3. *Note.* Mean fear potentiated startle (FPS) across trials. Acquisition consisted of presentations of the CSa+ and CSb+ that were sometimes paired with the US and the CS- was never paired with the US. Extinction consisted of the presentation of the CSa+, CSb+ and the CS- without the US. The error bars represent standard error. Measures of FPS were taken at every presentation of a stimuli.

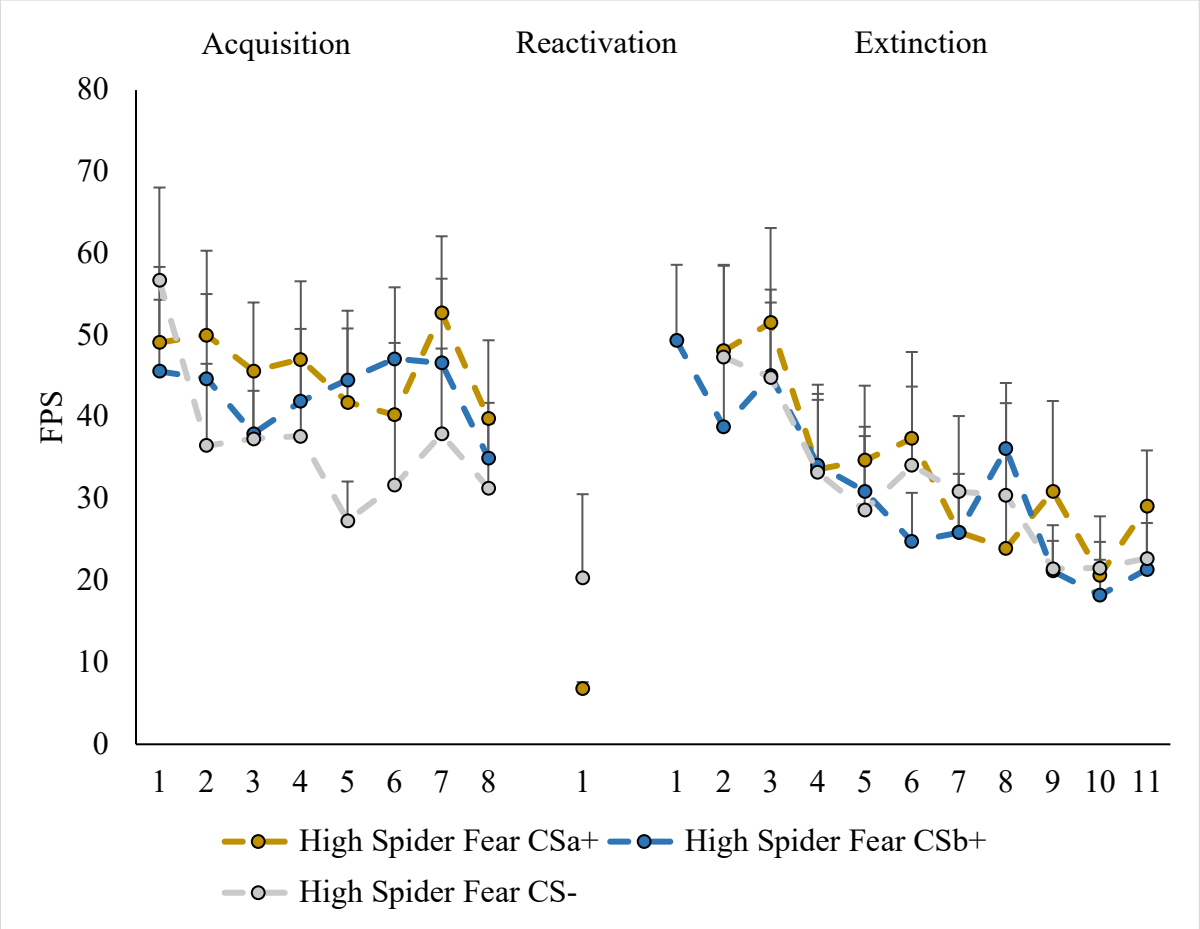

Figure 4. *Note.* Mean fear potentiated startle (FPS) across trials. Acquisition consisted of presentations of the CSa+ and CSb+ that were sometimes paired with the US and the CS- was never paired with the US. Extinction consisted of the presentation of the CSa+, CSb+ and the CS- without the US. The error bars represent standard error. Measures of FPS were taken at every presentation of a stimuli.

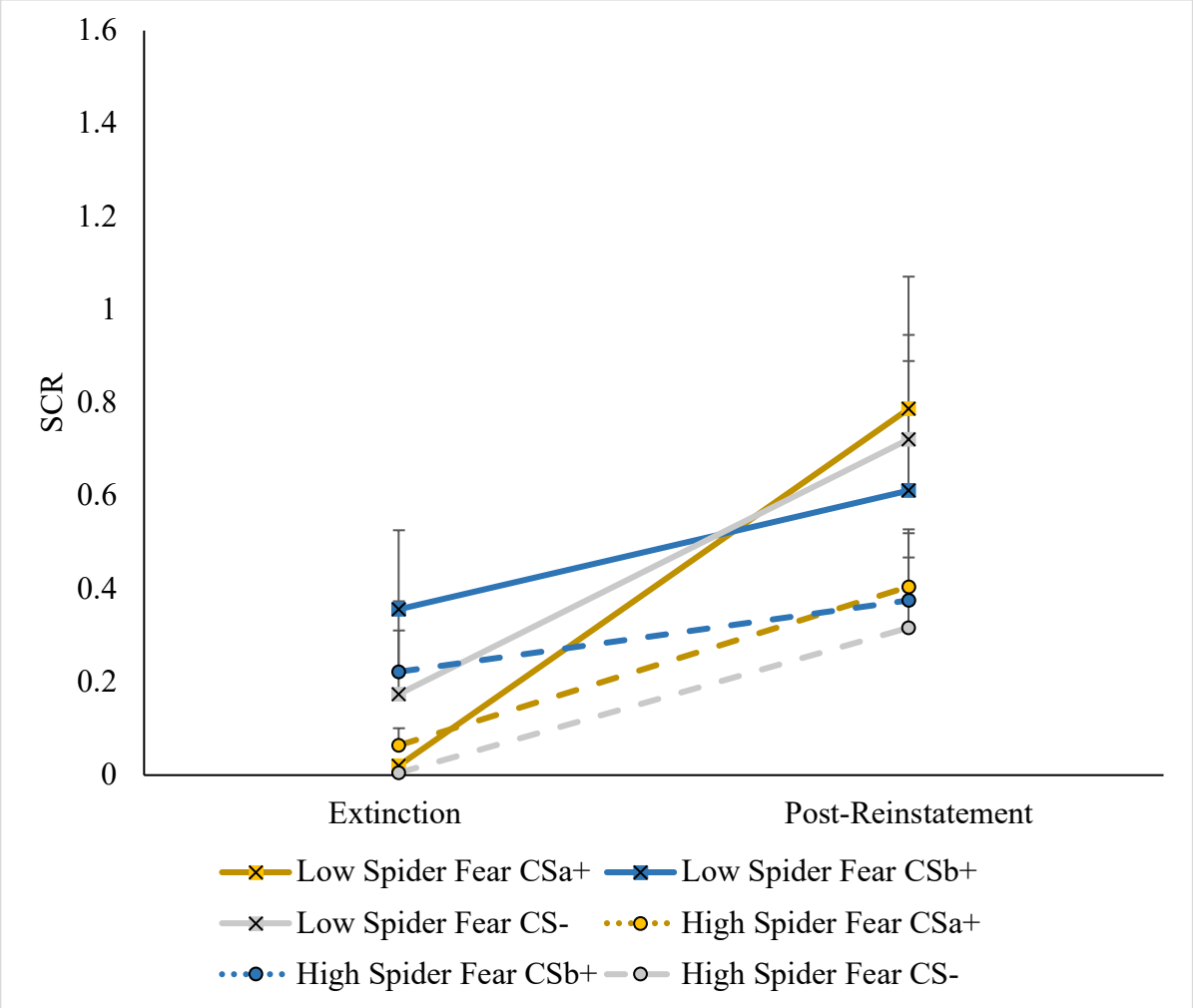

Figure 5. Note. Results for skin conductance response (SCR) for reconsolidation. Day 2 is a measure of fear following extinction (i.e., last trial of extinction). Day 3 is a measure of fear following reinstatement of fear (i.e., first trial of post-reinstatement). The error bars represent standard error. Measures of SCR were taken at every presentation of a stimuli.

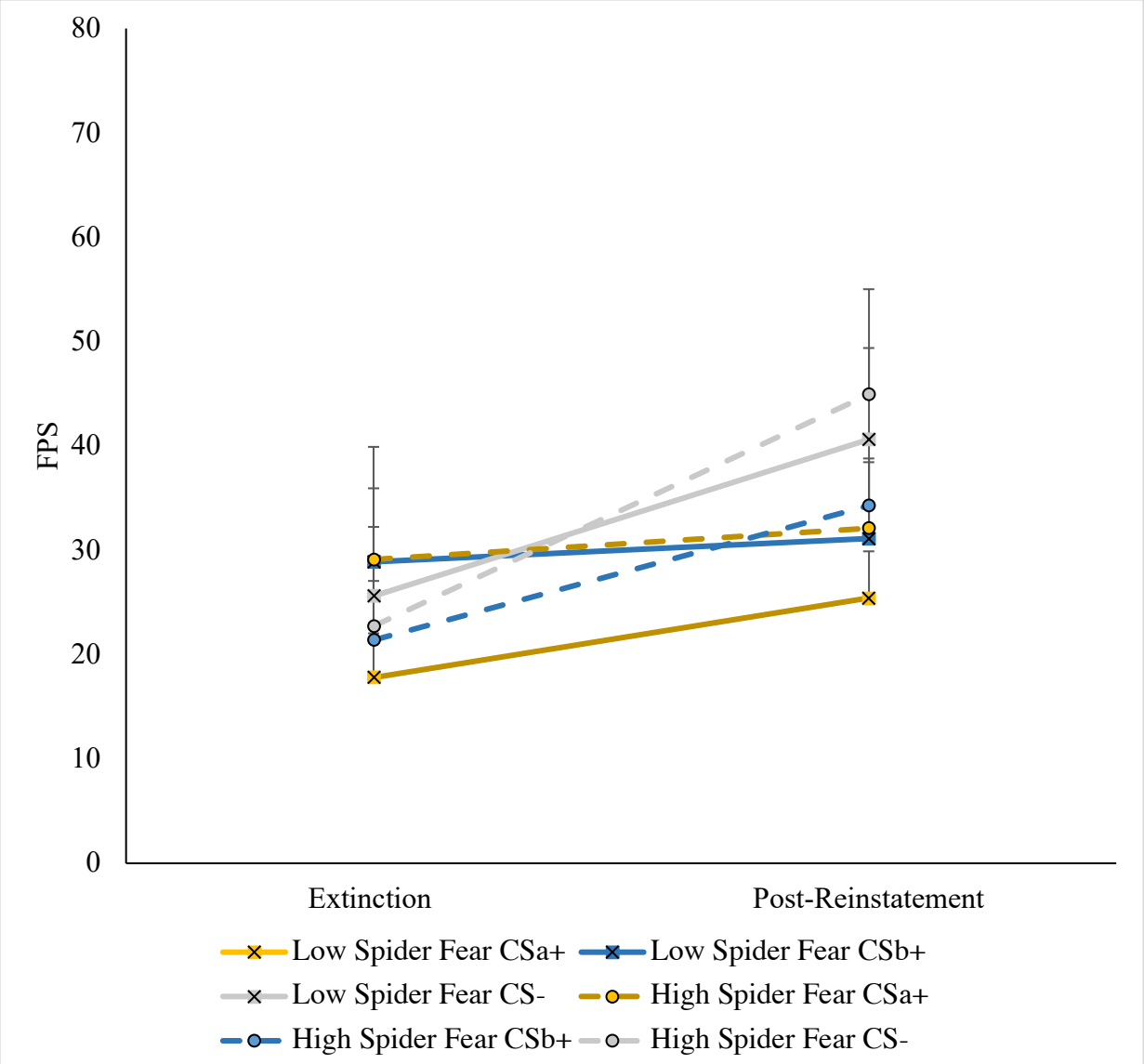

Figure 6. Note. Results for fear potentiated startle (FPS). Day 2 is a measure of fear following extinction (i.e., last trial of extinction). Day 3 is a measure of fear following reinstatement of fear (i.e., first trial of post-reinstatement). The error bars represent standard error. Measures of FPS were taken at every presentation of a stimuli.

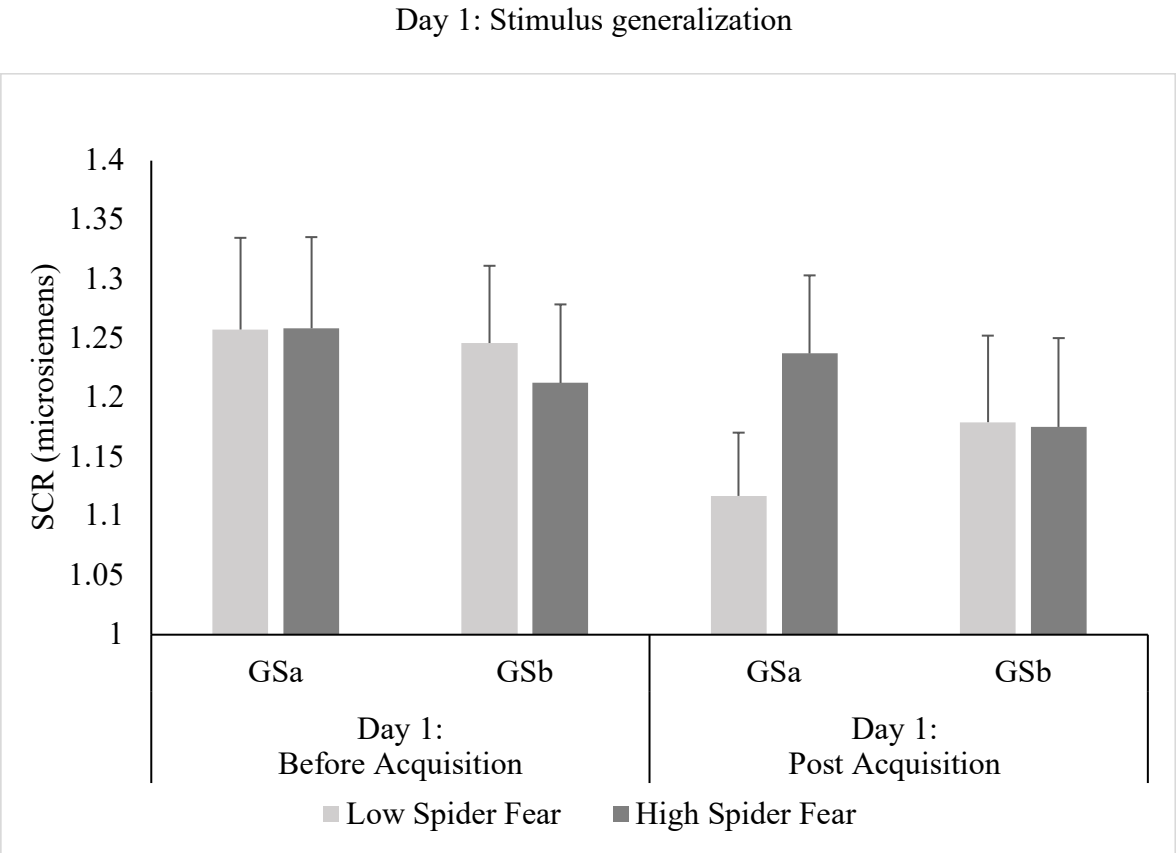

Figure 7. Note. Results for skin conductance response (SCR). Measures of SCR are taken at every presentation of a stimuli. GSa+ is a spider image and GSb+ is a snake image.

Day 1: Stimulus generalization

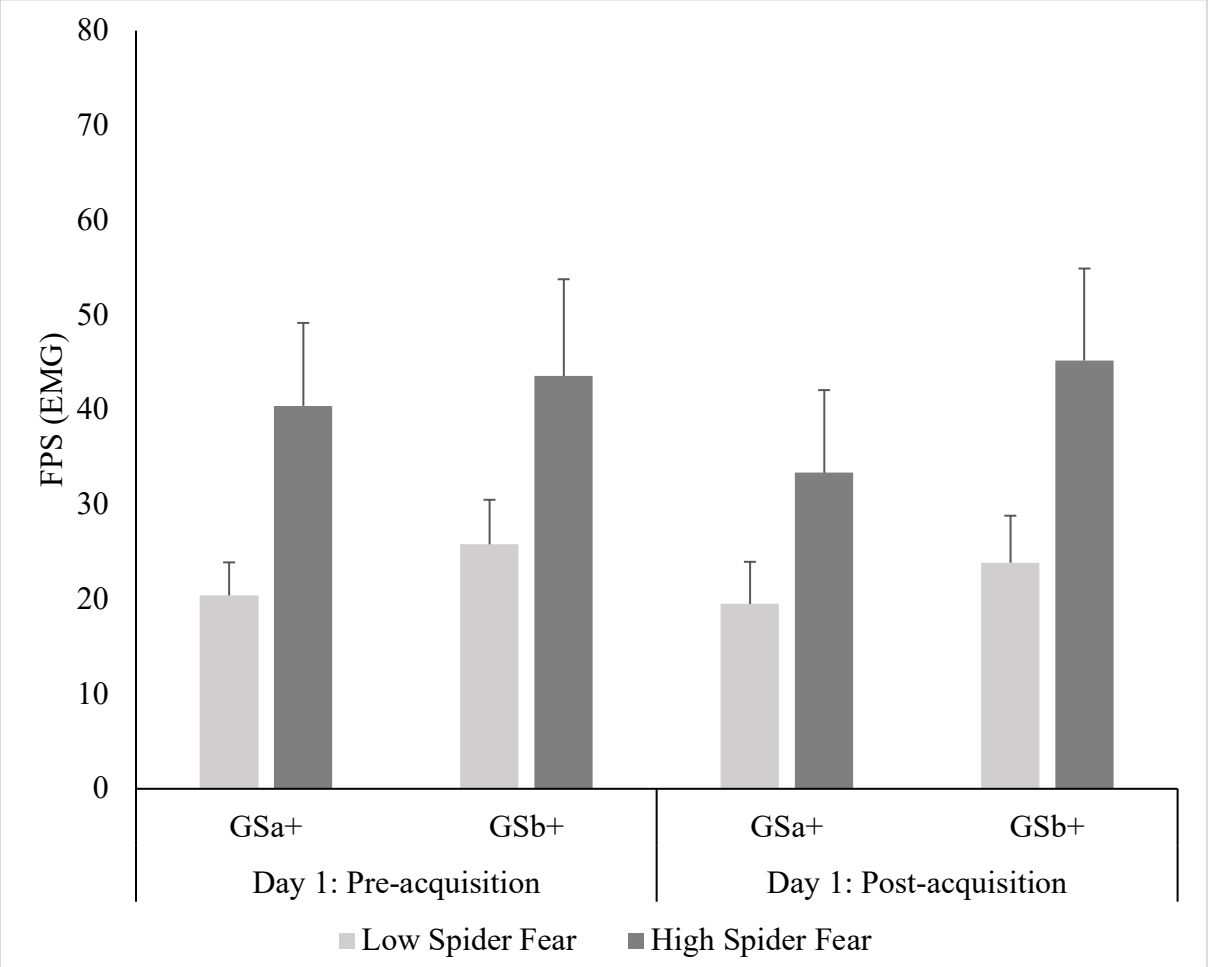

Figure 8. Note. Results for fear potentiated startle (EMG). Measures of fear potentiated startle are taken at every presentation of a stimuli. GSa+ is a spider image and GSb+ is a snake image.

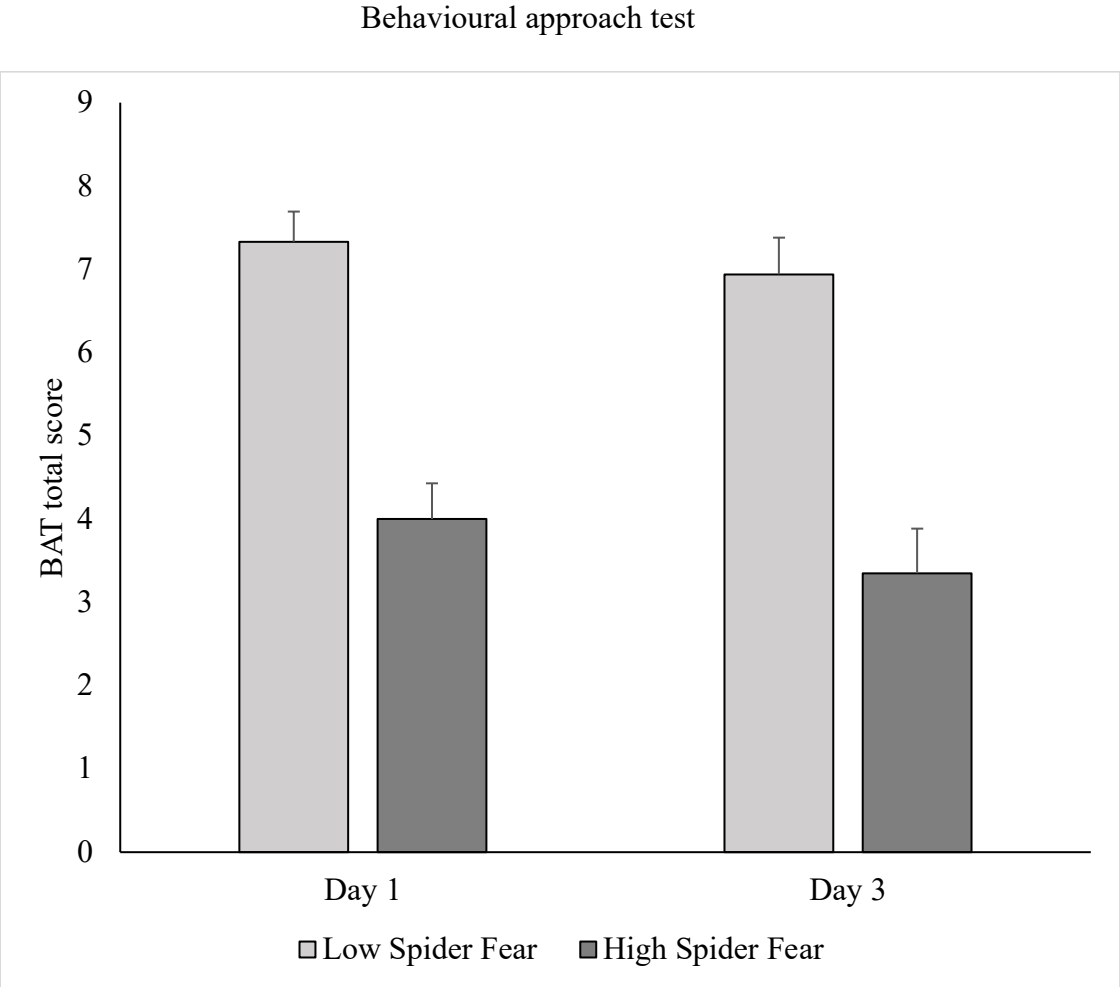

Figure 9. Note. Day 1 is an average of participant's predictions for completing the behavioural approach test (BAT) on Day 3. Day 3 is the average of participants total score on the BAT (i.e., high score means they placed hands in more jars).

Chapter 5

General Discussion

The purpose of this dissertation was to examine several possible boundary conditions of reconsolidation. Research on reconsolidation using pharmacological blockade methods has demonstrated that when a memory is activated, it returns to a malleable state and is updated with new information (Nader et al., 2000). However, behavioural methods used to examine reconsolidation have produced inconsistent results (Golkar, Bellander, Olsson, & Ohman, 2012; Kindt & Soeter, 2013; Soeter & Kindt, 2011a). One reason for these inconsistent results is that the conditions which need to occur for a memory to consistently undergo reconsolidation are unclear. Pharmacological studies have found that reconsolidation appears to occur only when reactivation signals that something new will be learned (Sevenster et al., 2012) and that memories associated with high levels of distress inhibit reconsolidation (Soeter & Kindt, 2013). However, these effects have not been tested using behavioural methods. Therefore, this dissertation examined three potential boundary conditions of reconsolidation: 1) how the expectation for learning affects the reconsolidation of conditioned fear memories; 2) how the level of spider fear influences the reconsolidation process; and 3) if the effect of reconsolidation is specific to the stimulus shown during reactivation or if it generalizes to other similar stimuli.

Study 1 examined how the expectation for learning impacted memory reconsolidation using the post retrieval extinction paradigm. The expectation for learning was manipulated through verbal instructions prior to memory reactivation. Study 1's results were partially consistent with my predictions. The study found some evidence that the expectation for learning enhanced reconsolidation with FPS as a measure of fear but not with SCR. This was evident as FPS to the CS+ remained stable from the end of extinction to post reinstatement only for

participants in the expectation for learning group. However, it is important to note that these findings should be interpreted with caution as the results were small and non-robust.

The second study had two main goals. The first was to examine how the level of spider fear impacted reconsolidation using the post retrieval extinction paradigm. The second goal was to examine if the effect of reconsolidation (i.e., reduction of physiological response and increased approach behaviour) generalized to similar stimuli, which is known as stimulus generalization.

To examine the first goal, participants were categorized to either the high or low spider fear groups based on their responses to psychometrically valid measures of spider fear and a semi-structured interview. SCR, FPS, and a behavioural approach test were used as measures of fear. Consistent with Study 1, Study 2 found a disassociation between SCR and FPS. Participants in the low and high spider fear groups displayed an increase in their SCR to the reactivated CSa+ from the end of extinction to the beginning of post-reinstatement (i.e., there was a return of fear). Conversely, there was no return of fear for either group to the unreactivated CSb+ for SCR as it remined stable across both days. FPS increased from the end of extinction to post reinstatement for the low but not the high spider fear group. Therefore, it was only the high spider fear group that exhibited some evidence of reconsolidation as their FPS remained stable across both days. These results are the direct opposite of our predictions. Similar to Study 1, it is important to interpret these results with caution. In addition to the effects being small and non-robust, it was unclear if participants successfully acquired physiological fear conditioning. Despite successfully identifying which image was paired with the US, there were no differences in FPS to the CS+ and the CS- at the end of acquisition.

Study 2 did not find evidence of fear generalization following fear acquisition on Day 1.

As a result, the effects of stimulus generalization following reconsolidation were not examined. Interestingly, participants in both the low and high spider fear groups displayed greater FPS to the snake image (GSb) than the spider image (GSa) in general on Day 1.

As another way to assess stimulus generalization, Study 2 examined if the effects of reconsolidation increased approach behaviour during a BAT following reinstatement. Inconsistent with our predictions, there were no meaningful differences between participants' predictions on Day 1 of testing compared to their actual behaviours during the BAT on Day 3 following reinstatement. With regards to differences between groups, participants in the low spider fear group predicted on Day 1 and actually placed their hand in more jars on Day 3 than the high spider fear group.

Overall, the complicated and inconsistent findings across both studies have implications for our understanding of reconsolidation and the potential application of reconsolidation principles in clinical contexts. The implications of the findings on understanding the boundary conditions of reconsolidation and the possible issues of using learning paradigms with physiological measures are discussed. The limitations and possible direction for future research are outlined below.

Theoretical Implications

Expectation for learning. Study 1's findings suggest that the verbal manipulation of the expectancy for learning was not sufficient to differentiate the groups on both physiological measures. However, it is important to note that results from the manipulation check in Study 1 found that groups did not differ (i.e., the expectation for learning and the no expectation for learning groups) in their expectation to receive a shock prior to reactivation. It is therefore possible that we did not successfully manipulate the expectancy to learn something new through

verbal instructions. Study 1's results are inconsistent with Sevenster et al. (2012) which examined how the expectation for learning impacted reconsolidation using a pharmacological intervention and FPS as a measure of fear. Sevenster and colleagues (2012) found that only the participants who were connected to the shock electrodes and expected to learn something new demonstrated reconsolidation. Conversely, participants who were not connected to the shock electrodes and were instructed that they would not receive any shocks exhibited a return of FPS following reinstatement. In contrast, in Study 1 both groups were connected to the shock electrodes and the expectancy to learn something new was manipulated only through verbal instructions prior to reactivation. Participants who expected to learn something new did not display a return of fear as measured by FPS but did show a return of fear using SCR. Participants in the no expectation for learning group displayed a return of fear when measured by both FPS and SCR. Overall, although the evidence was weaker than I predicted, the results suggest that the expectation for learning may facilitate memory reconsolidation when FPS is used as a measure of fear but when not SCR is used as a measure of fear.

One possible explanation for the weak evidence that the expectation for learning facilitates memory reconsolidation is that the method of inducing expectation for learning was not strong enough. New research found that behavioural tagging, which is a process that utilizes a tagging and capture procedure, mediates the reconsolidation of memories in rats (Orlandi et al., 2020). Orlandi et al. (2020) found that reactivation needs to be novel to induce a specific level of protein synthesis in order to create a learning tag and reactivate a memory. Additionally, animal studies have also found that the stimuli used to reactivate a conditioned fear memory cannot just be novel, but that a prediction error needs to occur during reactivation (Junjiao et al.,

2019). Therefore, it is possible that reactivation during Study 1 did not produce a strong enough prediction error to generate the needed levels of protein synthesis to reactivate the fear memory.

Interestingly, other studies examining expectancy in the context of extinction have found that purely verbal manipulations of expectancy are adequate. Similar to the current study, Sevenster, Beckers, and Kindt (2012) connected participants to SCR and FPS electrodes and half of the participants were instructed that they would not receive a shock and the other half were not given any instructions prior to extinction learning. Fear response returned for FPS but not SCR following reinstatement for individuals who underwent standard extinction. Participants who received instructions prior to extinction exhibited an immediate elimination of SCR which did not return following reinstatement. Conversely, this effect was not found with FPS. These findings regarding instructed extinction contrast with findings from Study 1. It is possible that the differences in findings between the current study that examined reconsolidation and Sevenster et al.'s (2012) study on extinction may provide further evidence that reconsolidation and standard extinction are separate processes. It appears based on these results that reconsolidation requires more salient expectation violation from previous learning (i.e., prediction errors; behavioural + verbal manipulations), whereas extinction learning may require less salient violation. Given the small number of studies and methodological differences, further research is needed to fully understand the relationship between the expectation for learning and reconsolidation.

Reconsolidation and memories associated with high levels of distress. Study 2 found some evidence of reconsolidation in individuals with high levels of spider fear (only as measured by FPS) but not in individuals with low levels of spider fear. These findings are inconsistent with predictions and with previous research, which found that reconsolidation was inhibited in

individuals with high levels of trait anxiety (Soeter & Kindt, 2013). The differences in reported levels of trait anxiety across studies may explain the inconsistent results between these studies. In Study 2, though participants in the high spider fear group had higher levels of self-reported spider fear and exhibited more avoidance behaviour during the BAT compared to participants with low spider fear, there were no differences between groups on a measure of trait anxiety (i.e., STAI-T). Thus, our sample of participants were highly fearful of spiders, but they were not generally anxious. This lack of elevated trait anxiety in the high spider fear group may explain why Study 2 did not find evidence of impaired reconsolidation and Soeter & Kindt, (2013) did. Consistent with this explanation, research suggests that there may be distinctions between individuals with high trait anxiety and individuals with a specific fear. For example, research has found that individuals with high levels of spider fear displayed an increased ability to discriminate between fear relevant and fear irrelevant cues compared to non-spider fearful individuals (Mosig et al., 2014). Conversely, several studies have found that individuals with high trait anxiety are unaware of the contingency between the conditioned stimuli and as a result, do not exhibit physiological differential fear acquisition compared to individuals with low level trait anxiety (Chan & Lovibond, 1996; Grillon, 2002). Grillon, (2002) also found that individuals with high trait anxiety who were unaware of the contingency produced greater physiological responding than participants who were aware of the contingency. Taken together, these findings suggest that trait anxiety may uniquely inhibit reconsolidation given the tendency to experience general physiological arousal and lack of differential fear learning. Thus, it is important for future research to examine reconsolidation across anxiety disorders in order to understand how it can be applied to treatments.

Type and age of memory. The differences in results between the high and low spider fear groups as mentioned above, may also be explained by the type and age of the memory. Scully, Napper, and Hupbach, (2016) found in a meta-analysis that reconsolidation was more salient in older fear memories which are frequently associated with a robust network of linked memories compared to memories that were newly formed. In another meta-analysis reconsolidation was inhibited in older conditioned memories in animal studies, though this effect was not found in human memories which were naturally formed in the context of everyday life (Walsh et al., 2018). Taken together, these findings suggest that conditioned fear memories and naturally developed fear memoires may require different boundary conditions in order to successfully undergo reconsolidation. In Study 2 of this dissertation, it is possible that reconsolidation was found in the high spider fear group because we may have targeted a long-standing network of fear memories associated with spiders, whereas reconsolidation was not found in the low spider fear group because only a young conditioned fear memory created during the study was targeted. It is also possible that reconsolidation was not found in the low spider fear group because the conditioned fear memory was not fully consolidated as it can take up to 24-48 hours for a memory to consolidate (Scully, Napper, and Hupbach, 2016). Given the likely differences with regards to the age of the spider memory, our groups may not be true comparison groups. This may provide further evidence that conditioned and naturally developed memories require different conditions to undergo reconsolidation. Furthermore, this underscores the need to study reconsolidation not only using highly controlled and newly conditioned memories, but also older, naturally developed fear memories.

Post retrieval extinction paradigm. The consistent dissociation between the physiological measures found in this dissertation and other research using both behavioural and

pharmacological interventions (Sevenster, Beckers, & Kindt, 2012; Soeter & Kindt, 2011a) raise the question of whether multiple physiological measures, some of which may interfere with the other, are suitable to study reconsolidation. This dissertation and the above-mentioned studies found that SCR returns following reinstatement, but FPS does not. FPS was measured by a loud startle probe which may have interfered with the measurement of SCR. Neumann and Waters, (2006) found that shocks and a loud tone, similar to the one used in the current study to induce the startle response, are equally effective USs to produce fear acquisition using SCR as a measure of fear. This shows that a loud tone and a shock can both act as USs. This is particularly important because although not formally assessed, participants in this dissertation often stated that they found the loud sound more aversive than the electric shock during the debriefing of these studies. Given that SCR is a measure of expectant arousal (Neumann & Waters, 2006), it is possible that participants were anticipating the tone which may have interfered with using SCR to measure the conditioned fear to the image. Therefore, future researchers should consider having different conditions for physiological measures or use a different probe to elicit FPS.

Additionally, it is possible that the post-retrieval extinction paradigm may not be appropriate to study reconsolidation as some studies have found that reactivation plus extinction/exposure prevents the return of fear (e.g., Steinfurth et al., 2014; Vermes et al., 2020; Telch et al., 2017) and other studies have not found this effect (Chalkia, Oudenhove, & Beckers 2020; Golkar, Bellander, Olsson, & Ohman, 2012; Soeter & Kindt, 2011a). Furthermore, Chalkia, Oudenhove, and Beckers (2020) conducted a verification study of the Schiller et al. (2010) article. Through correspondence, Chalkia, Oudenhove, and Beckers (2020) were informed that Schiller did not apply the exclusion criteria as strictly as originally reported in the

2010 article. Instead, Chalkia, Oudenhove, and Beckers (2020) were informed that a qualitative assessment of physiological measures was used to exclude participants which allowed participants who did not demonstrate differential physiological acquisition to be included in the analysis. Schiller published an amendment in 2018 noting these changes and that the sample used in the original 2010 article was larger than previously reported. Chalkia et al. (2020) found that the ability to replicate Schiller's findings was highly dependent on the exclusion criteria applied. For example, when Chalkia, et al. (2020) analysed the Schiller et al. (2010) dataset using the exclusion criteria originally reported in the 2010 article, they found no evidence to support that reactivation plus extinction prevented the return of fear, whereas they were mostly successful in replicating results using the 2018 exclusion criteria. Taken together, these findings suggest that reactivation plus extinction prevented the return of fear only when subjective experimenter decision were made to exclude participants and thus call in to question the validity of Schiller's 2010 findings.

Given the inconsistent findings using the post-retrieval extinction paradigm, Chalkia et al. (2020) attempted to replicate Schiller's et al. (2010) study using a larger sample size (n = 124) and pre-registered the replication study. In contrast to Schiller et al. (2010), Chalkia (2020) found that fear returned following reinstatement and spontaneous recovery using skin conductance response as a measure of fear when extinction was preceded by reactivation. The researchers argued that the failure to replicate study should be given greater consideration than the original Schiller study because the failure to replicate study was pre-registered and had a larger sample size (n = 124) than Schiller et al. (2010; n = 71). Overall, the study concludes that the effect of reconsolidation using behavioural methods is not robust. This is consistent with the findings from both studies in my dissertation.

The impact of reconsolidation on behaviour. The second study also examined if the effects of reconsolidation increased approach behaviour during a BAT following reinstatement on Day 3. Inconsistent with our predictions, there were no meaningful differences between participants' predictions on Day 1 of testing compared to their actual behaviours during the BAT on Day 3 following reinstatement. My findings are consistent with Lancaster et al. (2020) who found that reactivation prior to exposure did not produce greater approach behaviour than exposure alone in individuals with high levels of spider or snake fears during BATs immediately following and one-week post treatment. However, another study found that compared to standard extinction, reactivation prior to extinction did not produce greater approach behaviour 24-hours post treatment but did produce greater approach behaviour one-month later (Telch et al., 2017). This suggest that the differences between exposure and reconsolidation may not be immediate but appear over time. Thus, it remains unclear exactly how reconsolidation impacts approach behaviour. However, the results suggest that the distinction between reconsolidation and exposure alone may not be immediately apparent but emerge over time. To understand the clinical utility of reconsolidation, future research should take multiple measures across time post-treatment to establish if there are meaningful differences between reconsolidation and exposure/extinction over time.

Clinical Implications

This dissertation was developed to understand if reconsolidation could be applied to the treatment of specific phobias. Specifically, study two examined a) if memories associated with high levels of spider fear could undergo reconsolidation, b) if reconsolidation increased approach behaviour during a behavioural approach test, and c) if the effects of reconsolidation generalized to other similar feared stimuli. However, both studies had difficulty clearly demonstrating

reconsolidation had occurred. Study 2 was not able to establish and test the effects of stimulus generalization using physiological measures and no effect on behaviour was found. Thus, it remains unclear if it is beneficial to incorporate reconsolidation processes into exposure treatments for specific phobias based on the findings from this dissertation. However, the findings from the current dissertation suggest that it is critical for future research to take measures longitudinally post treatment and to study natural fear memories in order to determine if reconsolidation impacts fear reduction differently than extinction alone.

Limitations and Future Research

There are several limitations that are important to note when considering the results of this dissertation. This section outlines the limitations for both studies and discusses the need for future research.

Study 1. The results from the manipulation check suggest that the groups did not differ in their expectation to receive a shock prior to memory reactivation on Day 2 of testing. This lack of distinction between groups suggests that we did not successfully manipulate the expectation for learning among groups and as a result, limits the interpretations of the findings. Notably, upon reflection, the question used may not have accurately measured the expectation for learning but rather measured participants' expectation to receive a shock. However, the manipulation check was administered on Day 3 during debriefing to ensure that it did not interfere with the memory reactivation process. It is possible that because of the delay, participants' responses might not accurately represent their expectation to receive a shock on Day 2. Future research may consider assessing both the expectation for learning and the expectancy to receive a shock prior to memory reactivation.

Study 2. Study 2 did not control for self-reported snake fear. This is an important limitation to note because a snake image was used as the control image and all participants displayed greater physiological responses to the generalized snake than spider image. Given that there is high comorbidity between snake and spider phobias (Seim & Spates, 2009), it is possible that the high spider fear group was highly fearful of both images.

Another limitation of Study 2 was the inability to demonstrate stimulus generalization using SCR and FPS on Day 1 of testing following fear acquisition. As a result, it was not possible to assess stimulus generalization following post-reinstatement. Study 2 was able to use the BAT as an additional measure of stimulus generalization. However, as mention above, measures were only taken 24 hours following reactivation plus extinction and no effects on behaviour were found. More research is needed to understand if the effects of reconsolidation generalize to other similar stimuli.

Lastly, the sample used in Study 2 consisted of participants with elevated levels of spider fear and thus limit the generalization of the findings which may not translate to clinical samples. Findings from Study 2 suggest that conditioned and natural fear memories may undergo reconsolidation differently. Thus, in order to further understand boundary conditions of reconsolidation, future research should examine reconsolidation of natural memories in clinical samples (i.e., individuals with PTSD, Specific Phobia) with varying levels of symptom severity.

Limitations that apply to both studies.

Notably, several of the interactions in both study one and study two had *p*-values above .05 but had effect sizes in the medium range. Although both studies were sufficiently powered for a medium effect, it is possible that the large variability in participants' responses reduced the ability to detect differences using standard null hypothesis testing. Thus, it is possible that a

Type II error might account for the *p*-values above .05. Given the medium effects and the limited as well as inconsistent research on reconsolidation, it could be beneficial to replicate the studies with a larger sample size to minimize a Type II error and to better understand factors that account for the large variability among participants (e.g., level of acquisition or extinction, conditioned memory versus natural memories), so that these can be better controlled in future research. Larger samples do not influence the calculation of an effect size, but larger samples allow for the effect size to be more precise and more representative of the population being sampled (Fields, 2013). Notably, research using propranolol to study reconsolidation have found medium to large effects (Soeter, & Kindt et al., 2013, 2015a), however, research using behavioural methods have typically not reported on effect size (Schiller et al., 2010; Steinfurth et al., 2014). It is possible that effects using behavioural methods may yield small to medium effects, and thus require larger sample sizes to detect the effect. Smaller effects observed in behaivoural studies may help explain some of the replication issues. Thus, replicating these studies with larger samples and reporting on effect sizes may help understand who is likely to benefit from reconsolidation related interventions.

Emerging research on statistics highlights the limitations of relying primarily on *p*-values to understand results (Wasserstein, Schirm, and Lazar, 2019). Specifically, a *p*-value within the traditional threshold (i.e., equal to or below .05) does not confirm that an effect is meaningful or correct. *P*-values below the traditional threshold mean that the variation within the sample can be explained by the model being tested and is less likely to be due to chance (Fields, 2013). However, if the measures being used in reconsolidation research are naturally highly variable, this will make it difficult to replicate findings and attribute the variability within the sample to

the effects of reconsolidation. Future research should consider examining the impact of individual differences on reconsolidation to clarify some of the variability among participants.

Finally, physiological fear acquisition was not consistently demonstrated in either study despite the fact that only participants who could identify the contingency between the shock and the CSs were invited for the remainder of the study. It is important to note that both studies were able to establish that some fear learning took place by Day 2. Analysis in Study 1 showed that participants displayed differential fear learning between the CSs by the beginning on Day 2. Furthermore, visual inspection of the graphs in Study 2 suggested that differential fear learning also took place by the start of Day 2. Some previous studies examining reconsolidation have only included participants who demonstrated differential learning between the CSs in order to demonstrate that any alterations (i.e., updating of non-fearful information) in fear responses throughout testing are attributed to reconsolidation (e.g., Golkar, Bellander, Olsson, & Ohman, 2012; Kindt & Soeter, 2011a; Soeter & Kindt, 2011b; Schiller et al., 2010). However, research has found that the lack of physiological fear acquisition is not predictive of learning during extinction (Gerkicher, Tuscher, & Kalisch, 2019; Lonsdorf et al., 2019). Therefore, using successful discrimination of the CSs during acquisition as an inclusion criterion may unnecessarily discard participants who otherwise should be included. Lonsdorf et al. (2019) highlights that only including participants who demonstrate physiological fear acquisition could limit the generalization of any results because the sample may only include individuals who are exceptional learners and are not representative of the general population. Thus, in the current studies, though the lack of physiological fear acquisition may appear to reduce the internal validity of the studies, it may actually be a more accurate representation of how most people

respond to fear acquisition, and therefore may speak to the external validity, and broader applicability of our findings.

Conclusion

Despite the limitations, the findings from these studies are important and contribute to the understanding of memory reconsolidation. Study 1's main implication is that the verbal manipulation of the expectation for leaning might not be salient enough to reconsolidate conditioned fear memories. Study 2 found some evidence that memories associated with high distress in participants with high levels of spider fear may undergo reconsolidation using FPS as a measure of fear but not using SCR. Furthermore, the inconsistent findings between the SCR and the FPS in both studies call into question if the post-retrieval extinction paradigm is appropriate to investigate reconsolidation using concurrent physiological measures. Overall, both studies underscore the complexity of memory. Specifically, reconsolidation appears to be a nuanced process which still requires more research to fully understand all the factors that influence the ability for a memory to undergo reconsolidation.

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Appendix A: Study 1 consent form

CONSENT FORM FOR RESEARCH PARTICIPATION

Version: August 1, 2016

Title:

Updating fear memories using the reactivation-extinction paradigm

Researcher:
Andrea R. Ashbaugh, Ph.D., C. Psych
University of Ottawa
School of Psychology
136 Jean-Jacques Lussier, VNR 4004
Ottawa, ON K1N 6N5
Andrea.ashbaugh@uottawa.ca
(613)562-5800 x. 4813

Invitation to Participate:

You are being invited to participate in the above mentioned study conducted by the following investigators: Andrea R. Ashbaugh, Ph.D., C.Psych, University of Ottawa (Principal Investigator), Julia Marinos (Ph.D. Student) Stacy MacGregor-Dennique (Research assistant), and Olivia Simioni (Honours Student).

Study Sponsor:

This study is supported by a grant from the Natural Sciences and Engineering Research Council of Canada

Purpose of the Study:

Memory reconsolidation is a process that occurs when information enters a changeable state after a memory has been retrieved, suggesting that old memories can be updated to include new information. The purpose of our research is to investigate the reconsolidation of fearful memories and how the expectation for learning influences this process.

Participation:
Your participation will include three laboratory sessions, spread over a period of three days. Each session will last approximately 30 minutes. At each visit, you will be shown a series of images. Sometimes, the pictures will be accompanied by a mild electrical shock, which you will adjust to a level that is slightly uncomfortable, but not painful, to you. Your skin conductance and Electromyography (EMG) will be measured using electrodes placed on your skin. You will hear loud short bursts of noise throughout the study. In addition you will be asked to fill out some brief questionnaires at the beginning of day 1 and end of day 3. We ask that you refrain

from alcohol or recreational drug consumption 24 hours prior to each testing session, and from ingesting food or caffeine 4 hours prior to each testing session as food, alcohol, and caffeine can affect your psychophysiological response.

Exclusion Criteria:

You may not participate in this study if any of the following apply to you:

- Have had a Heart Transplant
- Have an Artificial cardiac pacemaker
- Been diagnosed with a Cardiac Arrhythmyia (e.g., abnormal heart beat); uncontrolled hyper- or hypo-tension (e.g., low or high blood pressure), myocardial infarction (e.g., heart attack)
- Are currently taking beta-blockers (e.g., acebutolol, atenolol, betaxolol, teaxolol, bioprolol, carteolol, carvedilol, esmolol, labetalol, meoprolol, nadolol, nebivolol, penbutolol, pindolol, propranolol, sotalol, timolol)
- Are currently diagnosed with an anxiety disorder

Benefits

The primary benefit of your participation is that you have helped with the advancement of our knowledge regarding memory reconsolidation and its implications for fearful memories.

Risks

You can expect to experience some slight discomfort due to the mild shock and loud noise that will be administered; however, this should not be any more painful than an electrostatic shock. You will be able to adjust the intensity of the shock so that it is uncomfortable, but not painful. The application process or the electrodes themselves may cause light redness or irritation. Some people find the skin cleaning process for the electrodes' placement on the face somewhat uncomfortable. The skin may be slightly reddened after the removal of the electrodes, but it should disappear within a few hours. In rare cases, the cleaning of the skin may leave a scab that will be apparent for a few days. Please warn the research assistant if the skin cleaning becomes painful, as it may be uncomfortable but not painful.

Voluntary Participation:

Your participation is voluntary. You are free to withdraw from participation at anytime. You can end your participation at any time without any negative consequence to you. If you refuse to participate in this study it will have no impact on your academic program.

Confidentiality and Anonymity:

The information that you will share will remain strictly confidential and will be used solely for the purposes of this research. The only people who will have access to the research data are research personnel of Andrea R. Ashbaugh at the University of Ottawa who are directly involved in the

analysis and publication of results. Results will be published in pooled (aggregate) format and presented at professional conferences, as part of student projects, and in academic journals. The University of Ottawa Institutional Review Board may access the study records and data to ensure the ethical conduct of this study.

Conservation of Data:

Electronic data will be stored on a password protected computer and written data will be stored in a locked cabinet located in the data-analysis space of Andrea R. Ashbaugh, Ph.D. All identifying information (e.g., your signed consent form) will be stored separately from anonymous data.

Compensation:

For participants registered with the ISPR, you will receive one participant pool credit for the each of the first two days of testing for a maximum of two credits. You will receive a $5.00 for your participation on day 3.

For participants not registered with the ISPR, you will receive $5.00 at each visit in exchange for participating (for a total of $15 for participating in all three days).

Contacts:

Please address your questions to Andrea R. Ashbaugh (613)562-5800 x. 4813, andrea.ashbaugh@uottawa.ca.

If you have questions concerning your rights as a study participant, you may contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5.
Tel: (613) 562-5387
Email: ethics@uottawa.ca

Consent:

By signing below you indicate that you have read and understand your participation in this study and your rights as a participant.

_____
Name (First and Last)

_____
Signature

_____
Date

_____

Witness Name (First and Last)

_____

Signature

_____

Date

Appendix B: Study 1 debriefing form

uOttawa

DEBRIEFING FORM FOR RESEARCH PARTICIPATION
Version: August 1, 2016

Thank you for participating in our research.

TITLE:
Updating fear memories using the reactivation-extinction paradigm

INVESTIGATORS AND INSTITUTION:
The study you just participated in is being conducted at University of Ottawa by the following investigators (in alphabetical order): Andrea R. Ashbaugh, Ph.D., C.Psych, University of Ottawa (Principal Investigator), and Julia Marinos (Ph.D. Student).

STUDY PURPOSE AND IMPLICATIONS:
The purpose of this study was to investigate the reconsolidation of fearful memories and how the expectation for learning influenced this process.

Memory consolidation is the process that occurs when information is transferred into long-term memory. It was traditionally thought that these memories became permanent and unchangeable. However, recent research has suggested that these memories enter a period of reconsolidation when they are reactivated. During this period old memories can be altered. Furthermore, research has demonstrated that in order for a memory to be reactivated and return to a state where it can be updated, the expectation for learning something new needs to occur when the memory is retrieved.

In the first session of this study, participants were shown images of spiders. Certain stimuli at times were paired with a mild shock so they would come to elicit a fearful response, which was measured by changes in physiological response (e.g., skin conductance, electromyography (EMG)).

In the second session, some participants had the fearful memory reactivated by the presentation of the fearful stimulus, and told that the relationship between the shock and the image had changed (i.e., to increase the expectation for learning) prior to extinction. Whereas some participants had the fearful memory reactivated by the presentation of the fearful stimulus, and told that they would see the same spider image from yesterday but never receive a shock (i.e., so participants know they will not learn anything new) prior to extinction. Additionally, some participants did not have their memory reactivated and went straight to the 10-minute break followed by extinction. During extinction, participants were presented with the previously conditioned fearful stimulus several times without the mild shock. This was done to eliminate the conditioned fear response by breaking the link between the stimulus and the shock.

During the third session, participants were presented with the fearful stimulus and their physiological response was measured. We predicted that if the fear memory was updated in participants who underwent reactivation with an expectation for learning prior to extinction, those

participants should show lower physiological responses compared to participants who did not undergo reactivation prior to extinction or who underwent reactivation but did not expect to learn anything new. This is because reactivation allows the extinction process to update the old fearful memory, whereas extinction without reactivation creates a new memory about the fearful stimuli, leaving the old memory intact. Creating a new memory results in greater susceptibility to spontaneous recovery of the fear response, compared to an updated memory.

This study has a number of important implications. Firstly, this study will build on previous research and provide further understanding regarding the conditions that need to be met (i.e., the expectation for learning something new) in order for a memory to return to a malleable state and be updated. Secondly, the study may have important implications for the treatment of specific clinical populations. If fearful memories can be updated by combining memory reconsolidation with existing extinction paradigms, this can provide clinicians with a simple and non-invasive method to help those struggling with anxiety, posttraumatic stress disorder, and phobias.

As testing is ongoing we ask that you not discuss the hypotheses or purpose of this study with others.

To learn more about fear conditioning, extinction, and memory reconsolidation and its implications you can also consult the following articles:

Auber A., Tedesco V., Jones C.E., Monfils M.H., & Chiamulera C. (2013). Post-retrieval extinction as reconsolidation interference: methodological issues or boundary conditions? *Psychopharmacolgy, 226*, 631-647.

Boschen, M.J., Neumann, D.L., & Waters, A.M. (2009). Relapse of successfully treated anxiety and fear: Theoretical issues and recommendations for clinical practice. *Australian and New Zealand Journal of Psychiatry, 4,* 89-100.

Schiller D., Monfils M.H., Raio C.M., Johnson D.C., LeDoux J.E., & Phelps E.A. (2010). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature, 463*, 49-53.

Sevenster, D., Beckers, T., & Kindt, M. (2012). Retrieval per se is not sufficient to trigger reconsolidation of human fear memory. *Neurobiology of Learning and Memory, 97*, 338-450.

If you have any questions about your participation in the study or have experienced distress as a result of participating in the study you can contact the principal investigator, Dr. Andrea Ashbaugh at (613) 562-5800 x. 4813 to arrange an appointment to discuss your feelings pertaining to your participation and to receive additional resources for help in the Ottawa area.

If you have any questions concerning your rights as a research participant please contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5.
Tel: (613) 562-5387
Email: ethics@uottawa.ca

Appendix C: Instructions for all stages of the study

Day 1-Acquisition:

- **Condition:** All three conditions get the same instructions.
- **Instructions:** We are going to start. There will be two images presented. The shock will only be paired with one image. Monitor the relationship between the image you are seeing and when a shock is received.

Day 2-Reactivation

- **Condition:** Reactivation with expectation for learning group
- **Instructions:** We are going to start. Shortly you will see the images you saw yesterday. The relationship between the shock and the images has changed. Please observe how it has changed. Please keep your eyes on the screen at all times.

Day 2-Reactivation:

- **Condition:** Reactivation with no expectation for learning group
- **Instructions:** We are going to start. You will see the same images you saw yesterday. However, today you WILL NEVER receive a shock at any point during the experiment. Please keep your eyes on the screen at all times.

Day 2-Extinction:

- **Condition:** All three conditions get the same instructions.
- **Instructions:** We are going to start. Please monitor the relationship between the image and when a shock is received. Please keep your eyes on the screen at all times. Are you ready?

Day 3-Reinstatement:

- **Condition:** All three conditions get the same instructions.
- **Instructions**: We are going to start. Please monitor the relationship between the image and when a shock is received. Please keep your eyes on the screen at all times. Are you ready?

Day 3- Re-extinction:

- **Condition:** All three conditions get the same instructions.
- **Instructions**: We are going to start. Please monitor the relationship between the image and when a shock is received. Please keep your eyes on the screen at all times. Are you ready?

Appendix D: Study 1 consent to use data form

All of your data was recorded with a six digit de-identified number. That means there is no way to link your personal information with the data recorded during your participation in the current study. Please indicate below if we are able to use your de-identified data in our analysis.

☐ I consent to the use of my data.

☐ I do not consent to the use of my data.

Appendix E: Study 2 consent form

CONSENT FORM FOR RESEARCH PARTICIPATION

Version: January 2018

Title: Updating fear memories using the reactivation-extinction paradigm

Researcher:
Andrea R. Ashbaugh, Ph.D., C. Psych
University of Ottawa
School of Psychology
136 Jean-Jacques Lussier, VNR 4004
Ottawa, ON K1N 6N5
Andrea.ashbaugh@uottawa.ca
(613)562-5800 x. 4813

Invitation to Participate: You are being invited to participate in the above mentioned study conducted by the following investigators: Andrea R. Ashbaugh, Ph.D., C.Psych, University of Ottawa (Principal Investigator), Julia Marinos (Ph.D. Student), Stacy MacGregor-Dennique (Research assistant), and Olivia Simioni (Honours Student).

Study Sponsor: This study is supported by a grant from the Natural Sciences and Engineering Research Council of Canada

Purpose of the Study: Memory reconsolidation is a process that occurs when information enters a changeable state after a memory has been retrieved. The purpose of our research is to investigate the reconsolidation of fearful memories, how the strength of the fear influences this process, and how this process generalizes to other feared stimuli.

Participation: Your participation will include three laboratory sessions, spread over a period of three days. Each session will last approximately 45 minutes. At each visit, you will be shown a series of images. Sometimes, the pictures will be accompanied by a mild electrical shock, which you will adjust to a level that is slightly uncomfortable, but not painful, to you. Your skin conductance and Electromyography (EMG) will be measured using electrodes placed on your skin. You will hear loud short bursts of noise throughout the study. In addition you will be asked to fill out some brief questionnaires at the beginning of day 1 and at the end of day 3. You will be asked to complete behavioural tasks to assess your level of spider fear on day 1 and day 3.

Please refrain from alcohol or recreational drug consumption 24 hours prior to each testing session, and from ingesting food or caffeine 4 hours prior to each testing session as these can affect your psychophysiological response.

Exclusion Criteria: You may not participate in this study if any of the following apply to you:

- Have had a Heart Transplant
- Have an Artificial cardiac pacemaker
- Been diagnosed with a Cardiac Arrhythmyia (e.g., abnormal heart beat); uncontrolled hyper- or hypo-tension (e.g., low or high blood pressure), myocardial infarction (e.g., heart attack)
- Are currently taking beta-blockers (e.g., acebutolol, atenolol, betaxolol, teaxolol, bioprolol, carteolol, carvedilol, esmolol, labetalol, meoprolol, nadolol, nebivolol, penbutolol, pindolol, propranolol, sotalol, timolol)
- Are currently diagnosed with an anxiety disorder

Benefits: The primary benefit of your participation is that you have helped with the advancement of our knowledge regarding memory reconsolidation and its implications for fearful memories.

Risks: You can expect to experience some slight discomfort due to the mild shock and loud noise that will be administered; however, this should not be any more painful than an electrostatic shock or hearing a very loud noise. You will be able to adjust the intensity of the shock so that it is uncomfortable, but not painful. It is possible that you may also experience some discomfort during the behavioural task. You will be provided with a list of self-help and professional resources, should you be interested in receiving more information about emotional difficulties. These resources include, but are not limited to:

- Ottawa Distress Centre, 613-238-3311
- Centre d'Aide 24-7, 819-595-9999
- Ottawa Academy of Psychology, 613-235-2529
- University of Ottawa Student Academic Success Service, http://www.sass.uottawa.ca/about/mental-health-wellness.php

The application process or the electrodes themselves may cause slight redness or irritation. Some people find the skin cleaning process for the electrodes' placement on the face somewhat uncomfortable. The skin may be slightly reddened after the removal of the electrodes, but it should disappear within a few hours. In rare cases, the cleaning of the skin may leave a scab that will be apparent for a few days. Please warn the research assistant if the skin cleaning becomes painful, as it may be uncomfortable but should not be painful.

Voluntary Participation: Your participation is voluntary. You are free to withdraw from participation at anytime. You can end your participation at any time without any negative consequence to you. If you refuse to participate in this study it will have no impact on your academic program.

Confidentiality and Anonymity: The information that you will share will remain strictly confidential and will be used solely for the purposes of this research. The only people who will have access to the research data are research personnel of Andrea R. Ashbaugh at the University of Ottawa who are directly involved in the analysis and publication of results. Results will be published in pooled (aggregate) format and presented at professional conferences, as part of

student projects, and in academic journals. The University of Ottawa Institutional Review Board may access the study records and data to ensure the ethical conduct of this study.

Conservation of Data: Electronic data will be stored on a password protected computer and written data will be stored in a locked cabinet located in the data-analysis space of Andrea R. Ashbaugh, Ph.D. All identifying information (e.g., your signed consent form) will be stored separately from anonymous data.

Compensation: For participants registered with the ISPR, you will receive one participant pool credit for the each of the first two days of testing for a maximum of two credits. You will receive $15.00 for your participation on day 3.

For participants not registered with the ISPR, you will receive $45.00 in exchange for participating ($15 per visit).

Any participant that chooses to withdraw from the study will be compensated for the session they have started.

Contacts: Please address your questions to Andrea R. Ashbaugh (613) 562-5800 x. 4813, andrea.ashbaugh@uottawa.ca.

If you have questions concerning your rights as a study participant, you may contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5.
Tel: (613) 562-5387
Email: ethics@uottawa.ca

Consent: By signing below you indicate that you have read and understand your participation in this study and your rights as a participant.

_____
Name (First and Last)

_____
Signature

_____
Date

_____
Witness Name (First and Last)

_____
Signature

_____
Date

Appendix F: Study 2 debriefing form

uOttawa

DEBRIEFING FORM FOR RESEARCH PARTICIPATION
Version: January 2018

Thank you for participating in our research.

TITLE: Updating fear memories using the reactivation-extinction paradigm

INVESTIGATORS AND INSTITUTION: The study you just participated in is being conducted at University of Ottawa by the following investigators (in alphabetical order): Andrea R. Ashbaugh, Ph.D., C.Psych, University of Ottawa (Principal Investigator), and Julia Marinos (Ph.D. Student).

STUDY PURPOSE AND IMPLICATIONS: The purpose of our research is to investigate the reconsolidation of fearful memories, how the strength of the fear influences this process, and how this process generalizes to other feared images.

Memory consolidation is the process that occurs when information is transferred into long-term memory. It was traditionally thought that these memories became permanent and unchangeable. However, recent research has suggested that these memories enter a period of reconsolidation when they are reactivated (e.g., recalled). During this period old memories can be altered. Furthermore, research has inconsistently found that the strength of the fear inhibits the ability for a memory to be updated. Also, research has inconsistently found that once a memory is updated with non-fearful information, the information generalizes to other images of the same category (i.e., all spiders instead of just the spider that was used to reactivate the memory).

One purpose of the study was to examine if high versus low spider fear impact the updating of fearful memories. We expect that fear memories would be harder to update in individuals with high spider fear compared to those with low spider fear. The second purpose of the study was to examine if the updating effect of reconsolidation influences similar fear memories. If it does generalize, we expect that individuals will have a reduced fear response (i.e., reduced physiological responses) when viewing all spider images on day three of the study compared to their responses day one.

We also predict that if the fear memory is reconsolidated (i.e., updated with non-fearful information about the spider image) then individuals in the high spider phobia group will be more willing to place their hand in the jar during the behavioural task on day three compared to their baseline predictions on day one. Conversely, the individuals with low spider fear will display no change in their behaviours during this task because they did not have an increased fear of spiders at the start of the study.

Participants were told that there was a probability that a spider was in each of the eight jars during the behavioural task. However, no spiders were present in any of these jars. This was done so participants would experience anxiety during this task without having to use animals in the study. This deception was a necessary and an important component of our experiment.

This study has a number of important implications. First, this study will build on previous research and provide further understanding regarding the conditions that need to be met (i.e., whether the strength of fear influences reconsolidation) in order for a memory to return to a malleable state and be updated. Secondly, the study may have important implications for the treatment of specific clinical populations. If fearful memories can be updated by combining memory reconsolidation with existing extinction paradigms, this can provide clinicians with a simple and non-invasive method to help those struggling with anxiety, posttraumatic stress disorder, and phobias.

As testing is ongoing we ask that you not discuss the hypotheses or purpose of this study with others.

To learn more about fear conditioning, extinction, and memory reconsolidation and its implications you can also consult the following articles:

Auber A., Tedesco V., Jones C.E., Monfils M.H., & Chiamulera C. (2013). Post-retrieval extinction as reconsolidation interference: methodological issues or boundary conditions? *Psychopharmacolgy, 226*, 631-647.

Bjˆrkstrand, J., Agren, T., Ahs, F., Frick, A., Larsson, E.M., Hjorth, O., . . . Fredrikson, M. (2016). Disrupting reconsolidation attenuates long term fear memory in the human amygdala and facilitates Approach Behavior. *Current Biology*, *26(19),* 2690-2695. doi:10.1016/j.cub.2016.08.022

Boschen, M.J., Neumann, D.L., & Waters, A.M. (2009). Relapse of successfully treated anxiety and fear: Theoretical issues and recommendations for clinical practice. *Australian and New Zealand Journal of Psychiatry, 4,* 89-100.

If you have any questions about your participation in the study or have experienced distress as a result of participating in the study you can contact the principal investigator, Dr. Andrea Ashbaugh at (613) 562-5800 x. 4813 to arrange an appointment to discuss your feelings pertaining to your participation and to receive additional resources for help in the Ottawa area.

You can also find out more information about anxiety disorders and other mental health problems at the following websites:

- http://www.cpa.ca/psychologyfactsheets/
- http://www.anxietycanada.ca/english/brochures.php

If you would like to receive support or help for psychological problems in the Ottawa area, the following resources may be of use:

- Ottawa Distress Centre, 613-238-3311
- University of Ottawa Student Academic Success Service, http://www.sass.uottawa.ca/about/mental-health-wellness.php

If you are interested in seeking self-help resources, the Association of Behavioral and Cognitive Therapies maintains a searchable database of recommended books for a series of concerns, which can be found online: http://www.abct.org/SHBooks/

If you have any questions concerning your rights as a research participant please contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, Room 154, Ottawa, ON K1N 6N5.
Tel: (613) 562-5387
Email: ethics@uottawa.ca

Appendix G: Instructions for behavioural approach task

For this next task, we'll be over here (***direct participant to the jars on the table and have them sit in front of the table). Remove cover and place it neatly on the floor out of the way.*** For this task, we'll be progressively going through each of these jars. If at any point you would like to stop, please let me know.

Day 1 protocol:

JAR 1: The first container is empty—"How willing would you be to place your hand in jar 1 (0= willing to 100= very unwilling) on day 3?"
(RECORD RESPONSE ON SHEET)

JAR 2: The second container is empty but previously contained a house spider "How willing would you be to place your hand in jar 1 (0= willing to 100= very unwilling) on day 3?"
(RECORD RESPONSE ON SHEET)

JAR 3:There is a 20% chance that there is a house spider in the third container — "How willing would you be to place your hand in jar 1 (0= willing to 100= very unwilling) on day 3?"
(RECORD RESPONSE ON SHEET)

Repeat all instructions for Jars 4-7, with the following percentages:

Jar 4: 40%

Jar 5: 60%

Jar 6: 80%

Jar 7: 100%

Jar 8: There is a nonpoisonous tarantula in the eighth container. Are you willing to put your open hand into the container up to your wrist for 30 seconds on day 3?"

WHEN THE PARTICIPANT SAYS THEY ARE NOT WILLING TO PUT THEIR HAND IN THE JAR AND WISHES TO NO LONGER CONTINUE ONTO THE NEXT JAR: Thank you, we can stop there.

Day 3 protocol:

JAR 1:The first container is empty—are you willing to put your open hand into the container up to your wrist for 30 seconds?"

If the participant says "Yes","Please put your hand into the jar now. The 30 seconds will start when I say go and you will be told when to take your hand out. Go." ***(START STOPWATCH)***

When the 30 seconds are up: You can take your hand out now. On a scale from 0 to 100, with 0 being not anxious at all and 100 being very anxious, how did you find putting your hand in the jar? ***(RECORD RESPONSE ON SHEET)***

JAR 2:The second container is empty but previously contained a house spider —are you willing to put your open hand into the container up to your wrist for 30 seconds?"

If the participant says "Yes","Please put your hand into the jar now. The 30 seconds will start now. Go." ***(START STOPWATCH)***

When the 30 seconds are up: You can take your hand out now. On a scale from 0 to 100, with 1 being not anxious at all and 100 being very anxious, how did you find putting your hand in the jar? ***(RECORD RESPONSE ON SHEET)***

JAR 3: There is a 20% chance that there is a house spider in the third container —are you willing to put your open hand into the container up to your wrist for 30 seconds?"

If the participant says "Yes","Please put your hand into the jar now. The 30 seconds will start now. Go." ***(START STOPWATCH)***

When the 30 seconds are up: You can take your hand out now. On a scale from 0 to 100, with 1 being not anxious at all and 100 being very anxious, how did you find putting your hand in the jar? ***(RECORD RESPONSE ON SHEET)***

Repeat all instructions for Jars 4-7, with the following percentages:

Jar 4: 40%

Jar 5: 60%

Jar 6: 80%

Jar 7: 100%

Jar 8: There is a nonpoisonous tarantula in the eighth container. Are you willing to put your open hand into the container up to your wrist for 30 seconds?"

WHEN THE PARTICIPANT SAYS "NO" AND WISHES TO NO LONGER CONTINUE ONTO THE NEXT JAR: Thank you, we can stop there.

Appendix H: Study 2 consent to use data form

All of your data was recorded with a six digit de-identified number. That means there is no way to link your personal information with the data recorded during your participation in the current study. You have now been fully informed of the purpose of the study and the deception involved. Please indicate below if we are able to use your de-identified data in our analysis.

_____

Name (First and Last)

_____

Signature

_____

Date

Appendix I: Spider Phobia Questionnaire

Answer each of the following statements either True or False as you feel they generally apply to you. If the statement is true most of the time or mostly true for you, you should answer true. If it is mostly false or false most of the time, mark it false. Indicate your answer by selecting the appropriate option.

1. I avoid going to parks or on camping trips because there may be spiders about (T or F)	17. Some spiders are very attractive to look at (T or F)
2. I would feel some anxiety holding a toy spider in my hand (T or F)	18. Some spiders are very attractive to look at (T or F)
3. If a picture of a spider crawling on a person appears on the screen during a motion picture, I turn my head away (T or F)	19. I don't believe anyone could hold a spider without some fear (T or F)
4. I dislike looking at pictures of spiders in a magazine (T or F)	20. The way spiders move is repulsive (T or F)
5. If there is a spider on the ceiling over my bed, I cannot go to sleep unless someone keels it for me (T or F)	21. If I came upon a spider while cleaning the attic I would probably run (T or F)
6. I enjoy watching spiders build webs (T or F)	22. I am more afraid of spiders than any other animal (T or F)
7. I am terrified by the thoughts of touching a harmless spider (T or F)	23. I would not want to travel to Mexico or Central America because of the greater prevalence of tarantulas (T or F)
8. If someone says that there are spiders anywhere about, I become alert and on edge (T or F)	24. I am cautious when buying fruit because bananas may attract spiders (T or F)
9. I would not go down to the basement to get something if I thought there might be spiders down there (T or F)	25. I have no fear of non-poisonous spiders (T or F)
10. I would feel uncomfortable if a spider crawled out of my shoe as I took it out of the closet to put it on (T or F)	26. I wouldn't take a course in biology if I thought I might have to handle live spiders (T or F)
11. When I see a spider, I feel tense and restless (T or F)	27. Spider webs are very artistic (T or F)
12. I enjoy reading articles about spiders (T or F)	28. I think that I'm no more afraid of spiders than the average person (T or F)
13. I feel sick when I see a spider (T or F)	29. I would prefer not to finish a story if something about spiders was introduced into the plot (T or F)
14. Spiders are sometimes useful (T or F)	30. Even if I was late for a very important appointment, the thought of spiders would stop me from taking a shortcut through an underpass (T or F)
15. I shudder when I think of spiders (T or F)	31. Not only am I afraid of spiders, but millipedes and caterpillars make me feel anxious (T or F)
16. I don't mind being near a harmless spider if there is someone there in whom I have confidence (T or F)	

Appendix J: Spielberger Trait Anxiety (Trait)

A number of statements which people have used to describe themselves are given below. Read each statement and then select the appropriate option to the right of the statement to indicate you generally feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	Almost Never (1)	Sometimes (2)	Often (3)	Almost Always (4)
1. I feel pleasant				
2. I feel nervous and restless				
3. I feel satisfied with myself				
4. I wish I could be as happy as others seem to be				
5. I feel like a failure				
6. I feel rested				
7. I am “calm, cool, and collected”				
8. I feel that difficulties are piling up so that I cannot overcome them				
9. I worry too much over something that really doesn’t matter				
10. I am happy				
11. I have disturbing thoughts				
12. I lack self-confidence				
13. I feel secure				
14. I make decisions easily				
15. I feel inadequate				
16. I am content				
17. some unimportant thoughts runs through my mind and bothers me				
18. I take disappointments so keenly that I can’t put them out of my mind				
19. I am steady person				
20. I get in a state of tension or turmoil as I think over my recent concerns and interests				