Preexposing a stimulus may enhance its subsequent extinction

by

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Abstract

Extinction, the repeated exposure to a stimulus following conditioning results in decreased conditioned responding (i.e., conditioned fear), has long been used as a therapeutic treatment to attenuate various anxiety disorders, such as phobias and post-traumatic stress disorder (PTSD). But extinction is not the only exposure treatment that can attenuate conditioned fear responses. Exposure to the CS prior to conditioning (CS preexposure) is also known to retard acquisition of conditioned fear. Thus, it seems logical to expect that combining CS preexposure and CS postexposure (extinction) treatments should magnify each treatment's response-attenuating properties. However, previous research suggests otherwise. In three experiments using rat subjects, the present research assessed the combined effects of CS preexposure and extinction, varying the time of introduction of each exposure treatment with respect to conditioning. Extinction performed either immediately or with a delay after conditioning did not differ in the amount of recovery of conditioned fear when the treatments were combined. However, preexposure occurring immediately prior to conditioning (but not long before conditioning) attenuated spontaneous recovery of subsequent extinction, even if few CS preexposures were provided. These results suggest that a combination of CS preexposure and extinction can provide benefits for the long-term attenuation of fear.

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Chapter 1

The ever-growing understanding of the processes that underlie Pavlovian conditioned fear has provided the fundamental grounds to investigate the development of post-traumatic stress disorder (PTSD) and provide tools for treatment of psychological conditions such as phobias and PTSD (Ayres, 1998; Barlow, 1988; Bentz, Michael, de Quervain, & Wilhelm, 2010; Bouton, 1988; Davis & Myers, 2002; Dollard & Miller, 1950; Field, 2006; Levis, 1989; McAllister & McAllister, 1995; Mowrer, 1939; Rauhut, Thomas, & Ayres, 2001). The basic Pavlovian conditioned fear paradigm involves an initially neutral stimulus, which elicits a conditioned fear response (i.e., it becomes a conditioned stimulus, CS) following pairings of the CS and an aversive stimulus that naturally produces a response of fear or anxiety (the unconditioned stimulus, US; i.e., CS-US; Pavlov, 1927).

Extinction

Pavlovian conditioned fear responses can be attenuated through the presentation of the CS in the absence of the US (i.e., CS-US then CS-noUS), which is referred to as *extinction* (Pavlov, 1927). Indeed, in clinical practice, extinction is frequently used as a therapeutic procedure to attenuate pathological fear in what is known as *exposure therapy* (e.g., Craske, 1999; Foa, 2000). Unfortunately, conditioned responding can recover or relapse for a variety of reasons (e.g., Laborda & Miller, 2012). For example, responding may be renewed in a context different from the context in which extinction occurred (e.g., Bouton & Bolles, 1979a), reinstated if the US is experienced following extinction (e.g., Bouton & Bolles, 1979b; Rescorla & Heth, 1975), or spontaneously recovery due to time lapses following extinction (e.g., Pavlov, 1927, Rescorla, 2004a).

Environmental factors that promote relapse from extinction. Extinction is sensitive to context shifts during training, a phenomenon known as *renewal* (Bouton & Bolles, 1979a). Responding at test will emulate the responding acquired in the context in which the test occurs. Thus, when conditioning and extinction occur in different contexts, conditioned responding will be higher if testing occurs in the conditioning context rather than if testing occurs in the extinction context. Returning to the conditioning context is known as ABA renewal (where the first letter refers to the identity of the conditioning context, A, the second letter refers to the identity of the extinction context, B, and the third letter refers to the identity of the test context, A); however, testing in a neutral context can also elicit conditioned responding. Other renewal designs are AAB and ABC renewal where testing occurs in a context that is novel, but conditioned responding still returns (e.g., Bouton & Bolles, 1979b; Bouton & Ricker, 1994).

The effect of renewal doesn't occur solely due to the return to an excitatory context at test (i.e., ABA). Conditioned responding also returns in AAB and ABC renewal preparations. These two types of renewal are important preparations because they demonstrate that the removal from the extinction condition is in part influencing the relapse of responding, rather than the simplified explanation of returning to the excitatory context (Bouton, Winterbauer& Vurbic, 2012). Rescorla (1986) proposed an alternative theory for renewal wherein the context may serve as an occasion setter, or when a relationship between the CS and US is mediated by the presence or absence of another stimulus (Holland, 1983). An occasion setter is more difficult to extinguish than a CS, which is why the behavior returns when placed in a different context even after conditioned responding is reportedly extinguished (Rescorla, 1986).

Similar to Rescorla's (1986) account for renewal that the context serves as an occasion setter, another phenomenon of relapse from extinction known as *reinstatement* may also be explained by incorporating a stimulus as an occasion setter. Behavior is said to be reinstated if the presentation of the US following extinction, but prior to testing, results in an increase of behavior that would not be observed if the US was not presented (Bouton & Bolles, 1979b; Rescorla & Heth, 1975). The US is considered to set the occasion for the previously learned CS-US association, therefore, resulting in more conditioned behavior at test than if the US was not presented. Interestingly, if reinstatement can be explained as an instance of occasion setting, then presenting the US in a different context should attenuate the reinstatement effect. Bouton and Peck (1989) observed that the relapse of conditioned responding was decreased when the US was presented in a context different from the context used at testing than when the US was presented in the same context as testing. Thus, in order for reinstatement to occur the US must be presented in the same context that testing will occur (see also, Bouton & King, 1983; Bouton, 1994).

To further examine the potential context dependency of reinstatement, Westbrook, Irodanova, McNally, Richardson, and Harris (2002) developed several experiments comparing multiple variations in context using a conditioned fear preparation. Experiment 1 replicated the reinstatement effect. The researchers paired a CS with a shock during conditioning, and later extinguished the fear to the CS via CS-alone presentations (Paired condition). The amount of conditioned freezing was increased when the CS-US association was established and the shock was presented following extinction (Paired Reinstatement). If the US was not presented following extinction or the CS and US were never paired together during conditioning, then conditioned freezing was considerably lower than the Paired Reinstatement condition. Westbrook et al. later manipulated the context in which different phases of training occurred (Experiment 2a and Experiment 2b). All subjects received conditioning, extinction, and reinstatement as described above. However, the context in which reinstatement and testing occurred was either Context A or Context B (Experiment 2a). When the reinstatement treatment and testing occurred in the same context as conditioning and extinction, more reinstatement of conditioned fear was observed than if the reinstatement treatment occurred in a different context. Reinstatement of fear was also attenuated if the reinstatement treatment occurred in the same context as both conditioning and extinction, but a novel context at test. Even more intriguing was the fact that fear was reinstated when the US was presented in a novel context (i.e., different from conditioning and extinction), and the subjects were later tested in this same novel context. This suggested that reinstatement of fear is dependent on the context in which reinstatement and testing occur, and the contexts in which conditioning and extinction training occurred do not influence the reinstatement of fear. Therefore, for Experiment 2b, the researchers conditioned subjects in Context A and extinguished in Context B. However, the reinstatement treatment and testing occurred in either Context B or a novel context, Context C. More conditioned fear (i.e., more freezing) was observed when the reinstatement treatment occurred in the same context as testing than if reinstatement occurred in a different context. Thus, based on these results, reinstatement of a conditioned behavior is maximal when testing occurs in the same context as the reinstatement manipulation and decreases as these contexts become more dissimilar.

As is the case with reinstatement, relapse due to spontaneous recovery may also be accounted for in terms of contextual retrieval of the conditioning experience. Spontaneous recovery refers to the reoccurrence of the conditioned behavior due to a time lapse from extinction (e.g., Pavlov, 1927; Rescorla, 2004a). A context may include the physical properties of the room, providing information to the senses via tactile, visual, auditory, and olfactory; however, not all contexts are required to be physical. A context can be discriminable from other contexts by various types of information about the context. The ability to discriminate between contexts can be attributed to physical descriptors of the context, but in some cases, information about a context provided is temporal, thus the same room at different locations in time may establish a separate context (see Bouton, 1993 for further explanation). For example, a high school classroom on a Monday morning has different importance than the same high school classroom on a Sunday morning, thus establishing two temporal contexts. Bouton (1993) argues that, when time elapses between extinction and subsequent assessment of fear elicited by the CS, the context has changed. Thus, the animal must rely on previously learned information at test, which in most cases, is the association that was learned first rather than later.

It is important to note that spontaneous recovery is dependent on the time that elapses between extinction and testing. The time between conditioning and testing does not appear to be a fundamental determinant of whether or not behavior will be recovered at test. For example, Laborda and Miller (2011) conditioned and extinguished a fear response at various intervals from one another. The interval between conditioning and extinction training was short (24 hours) or long (21 days), and the extinction-test interval was also either short (4 days) or long (25 days). For some of the subjects, the US was devalued by presenting the US multiple times prior to testing. Laborda and Miller observed very little spontaneous recovery when the extinction-test interval was short compared to when it was long, independent of whether the conditioning-extinction interval was short or long or if the US was devalued. However, when the extinction-test interval was long, the value of the US was significant in dictating the amount the response was recovered, but the duration of the interval between conditioning and extinction did not factor into the amount of recovery observed. These results seem to suggest that spontaneous recovery is dependent on the strength of the CS-US association when the retention interval is long. However, spontaneous recovery is also dependent on the length of the interval between conditioning and extinction training and not solely dependent on the length of the interval between extinction training and testing (see below for further analysis of the possible interactions of these between-phase intervals with regards to spontaneous recovery).

Behavioral strategies to attenuate relapse from extinction. Extinction can be viewed as a 3-stage procedure, involving acquisition of the fear response (CS-US pairings), extinction of the fear response (CS-noUS presentations), and long-term assessment or retention testing (presentation of the CS under different circumstances to evaluate the maintenance of the extinction-driven attenuation of the fear response). Although extinction is susceptible to relapse, different behavioral strategies have been attempted to thwart recovery of the extinguished fear response. Traditionally, these behavioral strategies have involved manipulation of the conditions of extinction or retention test, and they have had varying degrees of success (see Laborda, McConnell, & Miller, 2011; Miller & Laborda, 2011 for recent reviews[1]). Below is a selective review of recently-developed behavioral strategies which may possibly attenuate relapse by manipulating the conditions under which the fear memory becomes consolidated or reconsolidated for long-term storage.

Although extinction is susceptible to relapse, there are certain procedural manipulations that may increase the effectiveness of extinction and decrease the likelihood of relapse. For instance, delivering a cue at test that was presented during extinction, but not conditioning will attenuate renewal (Brooks & Bouton, 1994; Collins & Brandon, 2002). Due to the association between the cue and extinction training, it is possible that the cue serves to facilitate retrieval of the extinction memory with which it was uniquely associated. Thus, renewal is attenuated because the animal exhibits behavior that is consistent with extinction training rather than conditioning.

Extinction in multiple contexts has also been known to reduce the potential for relapse, with response suppression being observed either when the subject is returned to the context in which the response was acquired (Chelonis, Calton, Hart, and Schachtman, 1999) or when the subject is tested in a novel context (Gunther, Denniston and Miller, 1998). However, Bouton, García-Gutiérrez, Zilski, and Moody (2006) observed that extinction in multiple contexts did not prevent relapse. These incongruent results can be attributed to procedural differences. The ratio of CS alone presentations to CS-US pairings was vastly larger in the studies conducted by Gunther and colleagues (i.e., 4 CS-US, 162 CS-alone) than in the Bouton et al. studies (12 presentations for each training phase). Because of this trial number discrepancy, the number of days over which extinction took place was also significantly different (7 days versus 3 days, respectively), thus providing another potential confound. Denniston, Chang, and Miller (2003) observed attenuation of the renewal effect when extinction was massive (800 extinction trials), but not when extinction was moderate (160 extinction trials). These results were observed in both ABC renewal (Experiment 1) and ABA renewal (Experiment 2). Denniston, et al. concluded that massive extinction makes the CS a signal for US omission (i.e., a conditioned inhibitor), thus attenuating the likelihood of relapse (for more on conditioned inhibition following massive extinction, see Calton, Mitchell, & Schachtman, 1996).

Disruption of consolidation of fear memories: timing of extinction. During the formation of memories, the memory is considered labile until it is consolidated, thus providing the name for the process we refer to as consolidation (McGaugh, 2000). The length of the interval between fear conditioning and extinction can interfere with the consolidation of the fear memory. Thus, it is plausible that a short conditioning-extinction interval will produce less relapse from extinction because the conditioning memory was not successfully consolidated than if conditioning-extinction interval was long.

Within the clinical literature on PTSD, it seems to be unclear whether interventions provided immediately following exposure to a traumatic event are most effective at reducing PTSD symptoms than delaying the onset of treatment. Treatment is considered immediate if it is the counseling or program occurs within two weeks following the traumatic incident, whereas treatment for severe anxiety as a result of a traumatic event or PTSD that occurs months to years following the inciting incident is considered delayed (for reviews see, Ehlers & Clark, 2003; Litz et al, 2002; McNally, Bryant, & Ehlers, 2003; Raphael & Wilson, 2000).

Psychological debriefing (PD), is probably the most widely known form of immediate treatment after trauma. PD is typically conducted within a few days to weeks following a traumatic event with the aim to prevent PTSD symptoms from developing (Raphael & Wilson, 2000). Studies examining the success of PD have shown that PD not only does not reduce the symptoms, but in some cases increases the amount of observed PTSD symptoms (Bisson, Jenkins, Alexander, & Bannister, 1997; Mayou, Ehlers, & Hobbs, 2000; McNally, Bryant, & Ehlers, 2003; Raphael & Wilson, 2000). While examining acute stress victims, Bisson, et al. (1997) observed increased rates of PTSD diagnoses when the victims received PD when compared to burned patients that received any other form of treatment to reduce the rates of PTSD. In a study comparing different types of PD, Sijbrandij, Olff, Reitsma (2006) provided educational debriefing (a verbal narrative that includes factual information, typically conducted in groups) to one group of individuals, emotional ventilation debriefing (a verbal narrative similar to educational debriefing, but includes the individuals reaction to the traumatic event) to another group, and a final group of individuals received no form of PD. Each individual experienced a traumatic event within 2 weeks of the PD, and each person was randomly assigned to one of the different types of PD. At a 6 week follow-up, it was apparent that emotional ventilation debriefing actually increased the rate of PTSD diagnoses more than educational debriefing or when no PD is provided. These results appear to indicate that providing a form of emotional debriefing may enhance the arousal associated with the trauma and lead to the increases in PTSD diagnoses. This explanation may clarify why PD is widely regarded as an ineffective immediate treatment to reduce PTSD symptoms and diagnoses.

Alternative immediate treatments to PD have aided in reducing the anxiety related symptoms due to experiencing a trauma. In order to aid victims of rape during the traumatic events following the attack, Resnick, Acierno, Holmes, Kilpatrick, & Jager, (1999) sought to determine whether a 17 minute video in compound with a typical post-rape treatment was more efficacious at reducing anxiety related symptoms than the typical post-rape treatment alone. The video was designed to alleviate the anxiety pertaining to two primary potentially traumatic events experienced by a rape victim, the trauma of the attack and the adverse reactions during the medical exam that follows. The first portion of the video aimed at reducing anxiety regarding the medical proceedings during the examination following the attack, while the other portion of the video addressed coping with the anxiety, depression, and drug use that may follow the traumatic event itself. The researchers observed a decrease in the initial stress in those participants who watched the video in compound with the typical post-rape treatment provided to the victims, compared to those who only received the treatment. Participants who watched the video also reported less anxiety during the medical procedures, perhaps due to better understanding the these procedures (according to self-report measures). Furthermore, along with reducing the immediate anxiety, there was a reduction in the number of women who met the diagnostic criteria for PTSD after watching the video compared to those that did not. These results indicate that immediate treatment may be effective in reducing anxiety and PTSD diagnoses in some situations.

When alleviating fear, cognitive-behavioral therapy (CBT) will often utilize exposure therapy (a form of extinction) either by presenting the physical object causing the anxiety or by using mental imagery (Foa, Hembree, Rothbaum, 2007). CBT can be provided immediately (i.e., within 2 weeks) following the traumatic event or long after (i.e., several months to even years) the traumatic event (e.g., Foa & Rothbaum, 1998; for a review, see McNally, Bryant, Ehlers, 2003). Initial implementation of CBT immediately following the traumatic event appeared to replicate the same results as PD in that immediate CBT treatment did not reduce anxiety-related symptoms (e.g., Frank, Anderson, Stewart, Dancu, Hughes, & West, 1998; Veronen & Kilpatrick, 1983). More recent reports, however, suggest that CBT sessions provided shortly after an individual experiences the traumatic event may alleviate symptoms of PTSD (for reviews, see Ehlers & Clark, 2003; Litz et al, 2002). In one study examining the benefit of immediate CBT, Bryant, Harvey, Dang, Sackville, and Hasten (1998) gave individuals that suffered a traumatic event five therapeutic sessions that comprised one of the following treatment types: supportive counseling, prolonged exposure, or a combination of prolonged exposure and anxiety management. Upon completion of the treatment, individuals that received some form or exposure therapy (i.e., prolonged exposure or the combined therapy) were less likely to be diagnosed as having PTSD than those that only received counseling.

In another example of how immediate CBT therapies may facilitate recovery, Foa, Hearst-Ikeda, and Perry (1995) examined the effect of providing CBT treatments within two weeks of a traumatic event (in this study, assault on a woman) compared to not receiving any treatments. A few of the sessions used exposure therapy via imagery to alleviate fear related anxiety due to the event. Interestingly, during a 2 month follow-up assessment, women who attended the CBT sessions shortly after their attack were less likely to be diagnosed with PTSD and exhibited overall lower anxiety-related symptoms than the women who did not receive any treatment. However, these differences were no longer present or detectible at the 5 month follow-up assessment. These results suggest that immediate CBT treatments may expedite recovery after experiencing a traumatic event, but the immediate treatment does not provide any benefit with regards to long-term anxiety reduction when compared to natural recovery from the event (i.e., no treatment).

Just as is the case within the clinical literature, basic laboratory research investigating the difference between immediate and delayed extinction in animal models of acquired fear appear to be conflicting. Myers, Ressler, and Davis (2006) observed more resistance to spontaneous recovery when extinction occurred immediately after conditioning (heretofore *immediate extinction*) than when extinction occurred with a delay after conditioning (heretofore *delayed extinction*). Extinction appeared to occur at a slower rate when conducted immediately than when it was delayed, thus one would assume the long-term effectiveness of immediate extinction would also be poor. However, this was not what Myers, et al. observed: less relapse from extinction was observed when extinction was trained 10 minutes following conditioning than when extinction was trained 72 hours following conditioning. Immediate extinction was resilient to relapse of conditioned fear by phenomena spontaneous recovery, renewal, and reinstatement.

Therefore, the researchers conclude that immediate extinction promotes erasure of the conditioning memory (see below for more information on memory erasure). Incongruent with Myers et al., other researchers have failed to observe a benefit for long-term retention of extinction after immediate extinction. That is, under certain circumstances, less spontaneous recovery has been observed when extinction is delayed rather than immediate (Archbold, Bouton, & Nader, 2010; Chang & Maren, 2009; Huff, Hernandez, Blanding, & LaBar, 2009; Maren & Chang, 2006; Norrholm et al., 2008; Rescorla, 2004b; Schiller, et al., 2008; Woods & Bouton, 2008). Johnson, Escobar, and Kimble (2010) examined the discrepancies within the extinction literature. In two experiments, two different between-phase intervals were manipulated. The first study compared the amount of spontaneous recovery of fear observed after conducting extinction immediately (12 minutes from the last conditioning trial to the first extinction trial) or with a delay (24 hours from the last conditioning trial to the first extinction trial) after conditioning when the retention interval between extinction and testing was relatively long (72 h). Under these conditions, they replicated the observations of Myers et al. (i.e., more spontaneous recovery after delayed than immediate extinction). The second study again compared immediate and delayed extinction, however, in this case, the retention interval was also manipulated (it was either 'short' [48 h] or 'long' [7 days]). Johson et al. observed that, when the retention interval was short, delaying extinction after conditioning decreased spontaneous recovery, whereas extinction conducted immediately after conditioning was more resistant to spontaneous recovery following a long retention interval (Johnson, Escobar, & Kimble). The interaction between the conditioning-extinction and extinction-testing interval does not appear to be replicated in the studies examining immediate treatment following a traumatic event previously discussed (e.g., Bryant, et al., 1998; Foa, et al., 1995). However, these studies do not directly compare a short post treatment-assessment interval to a long treatment-assessment interval; but rather, they examine the rates of PTSD at both a short and a long treatment-assessment intervals.

Disruption of reconsolidation of fear memories: memory reactivation prior to extinction. When a memory is acquired it must be *consolidated*. Consolidation was long assumed to result in stability of the memory for long-term storage. However, when that memory is recalled, it may become labile again, potentially allowing for the memory to be updated or altered; these labile memories must be *reconsolidated*. Approximately 6 h after memory reactivation (Davis, & Squire, 1984; Nader, Schafe, & LeDoux, 2000), the memory is no longer plastic. This second lability period is known as the *reconsolidation period* (Debiec, LeDoux, & Nader, 2002; Milekic, & Alberini, 2002; Nader, et al., 2000). Monfils, Cowansage, Klann, and LeDoux (2009) examined the possibility of using the reconsolidation window to erase a fear memory (see also Schiller, Monfils, Raio, Johnson, LeDoux & Phelps, 2010 for a similar procedure in humans). Monfils et al. gave subjects 3 tone-shock pairings. The following day subjects received either a single presentation of the fearful tone in absence of the shock (retrieval cue) or no retrieval cue (Control condition). Extinction occurred at various intervals after the retrieval session. Some of the animals in the retrieval condition received the extinction training within the reconsolidation window (either 10 minutes or 1 hour following the retrieval cue); while others received the extinction training outside the reconsolidation window (either 6 hours or 24 hours following the retrieval cue). Theoretically, if the extinction occurred within the reconsolidation period wherein the fearful memory was recalled, the fear memory should be labile and susceptible to alteration. If extinction alters the fear memory by reducing conditioned fear, providing extinction trials during the reconsolidation period should erase the fear memory. Indeed, Monfils et al. observed extinction that was resistant to relapse when the memory was reactivated and extinguished within the reconsolidation window (prior to 6 hours; also see Clem, & Huganir, 2010; Rao-Ruiz, et al., 2011).

As was the case with extinction during the consolidation period, these findings are not supported by all laboratories. For example, Chan, Leung, Westbrook, and McNally (2010) observed an increase in the relapse of fear when extinction occurred shortly after reactivation (i.e., during the reconsolidation window) when both reactivation and extinction occurred in the same context (see also Chang & Maren, 2011; Constanzi, Cannas, Saraulli, Rossi-Arnaud, & Cestari, 2011; Soeter, & Kindt, 2011; Stafford, Maughan, Ilioi, & Lattal, 2013). Therefore, memory erasure based on conducting extinction during the reconsolidation window is still under debate.

Chapter 2

Extinction refers to attenuation of conditioned responding due to exposure to the CS alone *after* conditioning has taken place (i.e. postexposure). But exposure to the CS alone can also take place *before* conditioning takes place. This procedure is commonly known as the *CS-preexposure effect* or *latent inhibition*, and it is characterized by attenuated conditioned responding due to exposure to the CS prior to conditioning (Lubow & Moore, 1959). Extinction (CS-US then CS-noUS) and latent inhibition (CS-noUS then CS-US) are procedurally and effectively similar: both involve two associations to the target CS which are opposite in value, and both result in reduced responding to the CS. Furthermore, the two procedures are equally sensitive to a variety of manipulations. For example, conditioned responding tends to *spontaneously recover* when time elapses between extinction and test (Pavlov, 1927, Rescorla, 2004a) or latent inhibition training and test (e.g., Aguado, Symmonds, & Hall 1994; Killcross, Kiernan, Dwyer, & Westbrook, 1998; Wheeler et al., 2004). The two procedures are also context dependent, with extinguished responses experiencing *renewal* if extinction occurs in a context different from that of testing (e.g., Bouton & Bolles, 1979a) and CS preexposure having less impact on subsequent conditioning if conditioning occurs in a context different from that of preexposure (e.g., Bouton & Swatzentruber, 1989). Furthermore, exposure to the US alone prior to testing can *reinstate* conditioned responding after both extinction (e.g., Bouton & Bolles, 1979b) and CS preexposure (e.g., Kasprow, Catterson, Schachtman, & Miller, 1984).

Determinants of CS Preexposure. Several variables appear to determine whether CS preexposure effectively retards subsequent acquisition of the conditioned response. Ayres, Philbin, Cassidy and Bellino (1992) observed that varying the number of CS presentations did not have an effect on the magnitude of the CS-preexposure effect, as long as the total time of exposure to the CS was held constant. That is, 12 presentations of a 30 second CS will produce similar decrements in acquisition as 6 presentations of a 60 second CS. The number of stimulus presentations as well as stimulus duration both change, however, the total exposure time remains constant (also see Carlton & Vogel 1967; Crowell & Anderson, 1972; Kremer, 1972; Lubow, Schnur & Rifkin, 1976).

Timing of conditioning relative to preexposure (preexposure-conditioning interval) and timing of assessment relative to conditioning (conditioning-test interval) are also important determinants of the effectiveness of CS preexposure manipulations. As is the case with extinction, there are some contradictory outcomes when these two intervals are manipulated. Aguado, Symonds and Hall (1994) observed an attenuated CS preexposure effect (i.e., more conditioned responding at test) when a long retention interval was imposed between preexposure and test. Killcross, Kiernan, Dwyer and Westbrook (1998) reported similar results when manipulating the preexposure-conditioning interval. Contradicting these results is a more recent study by De La Casa, Marquez and Lubow (2009), in which a long retention interval between conditioning and testing was observed to enhance the effects of preexposure by retarding the CS-US acquisition more than when a shorter retention interval was used. More research needs to be conducted on the contradictory results to provide a possible explanation; however, reviewing both experiments can provide possible insights into the discrepancy.

Both Aguado et al. (1994) and De la Casa et al. (2009) used rats in a conditioned taste aversion preparation using LiCl as the US. In this preparation, the CS is paired with intraperitoneal LiCl injections that induce malaise and, thus, reject the CS when subsequently offered. The long intervals used in these studies were 12 days (Aguado et al.) and 21 days (De la Casa et al.). De la Casa and colleagues conducted all conditioned taste aversion sessions in experimental chambers different from the animals' home cages, and returned subjects to their home cages for the duration of the retention interval. In contrast, Aguado et al. conducted all sessions in the home cage and, thus, their subjects remained in the experimental context during the retention interval. Thus, changing the location in which the retention interval is spent also appears to determine the effectiveness of the CS-preexposure presentations. Indeed, De la Casa and colleagues directly tested the potential impact of the context in which the animals spent the conditioning-test retention interval, which was either short (1 day) or long (21 days). All experimental manipulations (CS preexposure, conditioning, and testing) were performed in the same context, and the retention interval was spent either in that context (Experiment 2) or a different context (Experiment 3). The effects of CS preexposure were enhanced when a long retention interval occurred in a context different from that of preexposure, but not when the long retention interval occurred in the same context. Furthermore, the effects of CS preexposure were not enhanced when the retention interval was short, regardless of where it was spent.

The context-dependency of the CS preexposure effect is one of its defining characteristics, and serves to differentiate it from similar phenomena such as stimulus habituation (Hall, 1991). When the context for the preexposure phase is different than the context for the conditioning phase, acquisition occurs at a rate similar than if the preexposure had not occurred. That is, preexposure and conditioning must occur in the same context for retardation to be observed (Channell & Hall, 1983; Hall and Honey, 1981; Pearce & Hall, 1979). The contextual dependence of the CS-preexposure effect is further exemplified by the observation that extinction of the CS-context association may abolish subsequent retardation-of-acquisition. In a study conducted by Escobar, Arcediano, and Miller (2002), subjects were placed in the context for extended sessions absent of any stimuli in order to extinguish the CS-context association. Such treatment resulted in rapid subsequent acquisition if the context in which the preexposure occurred underwent extinction, but not if an irrelevant context received extinction treatment (also see Gunther et al., 1994; but see Hall & Minor, 1984; Zalstein-Orda & Lubow, 1995).

Because of the many commonalities between the effects of CS preexposure and extinction, it appears logical to assume that combining these two paradigms would result in an enhancement of their individual response-attenuating properties. Furthermore, if the response attenuating properties of the two paradigms were summative, the combined treatment should also result in a lower likelihood of spontaneous recovery of the conditioned response during the retention test than treatment with either paradigm alone. These assumptions were tested by Leung and Westbrook (2010), who combined CS preexposure and extinction in a conditioned freezing preparation with rat subjects. In their preparation, Stimuli A and B received preexposure, conditioning, and extinction, and were then tested for spontaneous recovery from extinction. Stimulus A was tested after a long retention interval (Remote condition; more spontaneous recovery expected), whereas Stimulus B was tested after a short retention interval (Recent condition; less spontaneous recovery expected). Two further stimuli received either preexposure and conditioning (Experiment 6a) or conditioning and extinction (Experiment 6b) to match the remote (Stimulus C) or recent (Stimulus D) training (see Table 1 for a schematic representation of the experimental designs). Preexposure increased the rate of extinction of A and B, compared to the control stimuli, which had not received preexposure. Thus, it could be anticipated that the combined treatment would also attenuate spontaneous recovery. Spontaneous recovery was assessed by presenting subjects with the AD and BC compounds. Each compound included one element that received the combined treatment and one element that received only one treatment (preexposure or extinction), and one element trained remotely and one element trained recently; thus, they should have elicited similar levels of conditioned responding. However, in all instances, the AD compound exhibited more spontaneous recovery than the BC compound (this was independent of whether C and D had undergone preexposure or extinction). That is, the compound containing Stimulus A, which received remote preexposure and extinction, exhibited more spontaneous recovery than the compound involving Stimulus C, which received either remote preexposure alone or remote extinction alone. Furthermore, providing extensive preexposure to Stimulus A resulted in *more* spontaneous recovery than providing moderate preexposure (Experiment 4), suggesting that CS preexposure has a deleterious effect on subsequent extinction, and that this effect increases with amount of preexposure.

In a related series of experiments, Rosenberg, Holmes, Harris, and Westbrook (2011) used a reinstatement manipulation after combined CS preexposure and extinction in a conditioned freezing preparation. In all experiments, Stimulus A received preexposure. Then, the preexposed stimulus, A, and a novel stimulus, B, each received pairings with the US (i.e., A-US and B-US), and this conditioning was followed by extinction of both stimuli (i.e., A-noUS / B-noUS). In Experiment 1, extinction was followed by a single presentation of the US, which intended to reinstate conditioned fear, and assessment of reinstated fear was conducted on the subsequent day. Fear was again assessed five days later to determine whether reinstatement would interact with spontaneous recovery of the response. Subsequent experiments provided controls for spontaneous recovery without reinstatement (Experiment 2), and for testing at the completion of the spontaneous recovery period when reinstatement occurred shortly after extinction but long before testing (Experiment 3), or long after extinction but shortly before testing (Experiment 4). Consistent with the observations of Leung and Westbrook (2010), extinction progressed faster for Stimulus A, which received the combined treatment, than Stimulus B, which received extinction alone. However, combining CS preexposure and extinction increased the magnitude of reinstatement (Stimulus A exhibited more reinstatement than Stimulus B) *only if* testing was delayed. That is, the combination of the two response-recovery treatments (reinstatement and spontaneous recovery) was required to observe the deleterious effects of preexposure on extinction; preexposure did not enhance recovery after either reinstatement or spontaneous recovery in isolation. Thus, the effects of CS preexposure on extinction may be small but, if detected, they may be enhance, rather than reduce, relapse after extinction.

CS preexposure and permanent fear attenuation. One of the difficulties in observing a long-term benefit of the combined CS preexposure and extinction treatment is that, as is the case with extinction, responding to a preexposed CS tends to spontaneously relapse if retention testing is delayed. That is, if a time lapse is imposed between conditioning and retention test, fear is again observed (Aguado, et al., 1994; Killcross, et al., 1998; Leung & Westbrook, 2010; Leung, et al., 2007; Wheeler, et al., 2004). Thus, responding after both components of the combined preexposure-extinction treatment eventually reverts to fear. Leung and Westbrook proposed that the CS-alone presentations are encoded in a specific local context of nonreinforcement, which is delimited by the temporal proximity between trials during CS preexposure and extinction. As mentioned above, the response-reducing effects of both CS preexposure and extinction are context dependent (e.g., Bouton, 1993; Bouton & Bolles, 1979a; Bouton & Ricker, 1994; Bouton & Swatzentruber, 1989; Channell & Hall, 1983; Escobar et al., 2002; Hall, 1991; Hall & Honey, 1989; Pearce & Hall, 1979); thus, disrupting the local context of CS alone presentations by imposing a delay between extinction and testing (thus making the recovery test temporally distal from either training phase), would favor the recovery of the memories of conditioning (Rescorla, 2004a).

Powell, Escobar and Kimble (2013) reported that, under some conditions, CS preexposure can lead to robust attenuation of fear, even if fear was acquired to maximal levels after preexposure. In their study, rats were repeatedly preexposed to CS X. Then, either immediately (12 min) or long (24 h) after preexposure, rats received conditioning until maximal response suppression was attained. Forty-eight hours later, animals received a retention test. Animals receiving conditioning immediately after preexposure (hereon *immediate preexposure*) showed markedly less fear than animals that received conditioning 24 h after preexposure (hereon *delayed preexposure*). That is, immediate preexposure attenuated spontaneous recovery of fear. In a recent series of studies, Kimble and Escobar (2013) assessed whether immediate preexposure would continue attenuating spontaneous recovery of fear over longer retention intervals. They observed that immediate preexposure resulted in less fear relapse than delayed preexposure, even after a 7-day retention interval. Thus, immediate preexposure may provide a mechanism for permanent attenuation of subsequently-acquired fear responses.

The present studies investigate the effects of combining CS preexposure and conditioning of a fear-producing CS under conditions that may favor maximal fear attenuation after the combined treatment. Specifically, they investigate whether manipulation of the interval between preexposure and conditioning or the interval between conditioning and extinction can determine the effectiveness of the combined treatment. As mentioned above, previous research using the combined CS preexposure and extinction strategy (Leung & Westbrook, 2010; Rosenberg, et al., 2011) failed to observe a benefit of combining these two treatments; however, they used delayed preexposure *and* delayed extinction. Experiment 1 evaluates the effects of providing either immediate or delayed extinction after preexposure and conditioning. Considering that immediate extinction has proven beneficial as a strategy to prevent fear relapse, at least in some instances (Johnson, et al., 2010; Myers, et al., 2006), it is possible that immediate extinction may prove beneficial in the current situation. Experiment 2 evaluates the effects of providing either immediate or delayed preexposure on subsequent fear conditioning and extinction. Previous work from our laboratory (Kimble & Escobar, 2013; Powell, et al., 2013) suggests that immediate preexposure may have long-term, relapse-reducing properties, and thus may also enhance the effectiveness of extinction. Finally, Experiment 3 evaluates the impact of reactivating the memory of the CS long after preexposure but immediately prior to conditioning on subsequent extinction.

Experiment 1

Experiment 1 investigated the effects of CS preexposure on extinction when extinction occurred immediately or with a delay following conditioning (see Table 2). Latent inhibition (CS preexposure) occurred in a single session, and consisted of repeated presentations of the CS alone. On the subsequent day, all animals received CS-US pairings to establish the CS as a robust predictor of the US. Either 12 minutes (Imm condition) or 24 hours (Del condition) following conditioning, the animals underwent extinction in order to attenuate conditioned fear responses to the CS. Recovery of conditioned fear was assessed four days after extinction. Because immediate extinction appeared to result in less spontaneous recovery than delayed extinction over long retention intervals (see Johnson, et al., 2010), it is plausible that combining CS preexposure and immediate extinction will produce similar savings (i.e., less spontaneous recovery).

Methods

Subjects. The subjects were 32 male Sprague Dawley rats (Charles River Labs; weight range: 242-365 g), randomly assigned to four experimental groups (Preex-ImmExt, Control-ImmExt, Preex-DelExt, Control-DelExt, *n*s = 8). Subjects were housed in pairs (cage mates were assigned to different experimental groups) in clear plastic cages with wire lids on a 12 hr light/12 hr dark cycle (lights on at 6:00am). Experimental manipulations occurred around the middle of the light portion of the cycle. All animals had previously served as subjects in a study in which they were trained to leverpress for water reinforcement; the current study used different stimuli and apparatus. The condition to which they were assigned in the previous study was counterbalanced within groups in the present study. All subjects were placed on a water restriction schedule, in which water availability was gradually reduced to 30 min/day, which was provided approximately 2 hours after completion of the daily experimental sessions. Food was available *ad libitum.* Animals were handled three days per week for 30 s prior to initiation of the experiment. All research procedures were conducted in accordance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) and were approved by the Auburn University Institutional Animal Care and Use Committee (IACUC).

Apparatus. The apparatus was eight 30.5 x 24.1 x 21.0 cm Med Associates boxes. The front, top, and back walls of the chamber were made of Polycarbonate with a black and white stripped pattern. The left and right sides of the chamber were made of aluminum sheet metal. The floor was constructed of 4.8 mm stainless steel rods, spaced 1.6 cm center-to-center. The rods were used to deliver a 0.6 mA VDC footshock for 0.5 s, which served as the US. Each individual chamber was housed in a melamime sound attenuation cubicle equipped with an exhaust fan, which provided a constant, 70 dB background noise (this and all other sound pressure level measurements were made using the A scale).

A water niche (5.1 x 5.1 x 5.1 cm) was located 1.5 cm above the grid floors. A response lever could be protracted on a side wall to the left of the niche; depressing this lever led to delivery of a 0.05-ml droplet of distilled water, which served as the reinforcer. The reinforcer was delivered into a cup located inside the niche. Each chamber was dimly illuminated by 100-mA house light (#1820) was located on the side wall opposite the water niche, and each chamber was also equipped with a speaker that could produce a 1000-Hz pure tone, at an intensity of 10 dB above background.

Procedure.

Restabilization of baseline leverpressing (Day 1). All subjects had been previously trained to depress the response lever to obtain water reinforcement. Thus, they received a one-hour baseline restabilization session in which they were allowed to leverpress for water reinforcement on a variable interval 20 s (VI-20") schedule. This schedule of reinforcement was used throughout all phases of Experiment 1. No nominal stimuli were scheduled to occur during this session.

CS preexposure (Day 2). Subjects assigned to the preexposure (Preex) condition received 36 presentations of the 30-s tone CS, whereas subjects in the control (Control) condition received equivalent context exposure during the 75-min session. CS onsets occurred with an intertrial interval (ITI) of 2(±1) min.

Conditioning and extinction (Days 3 and 4). On Day 3 (Del condition) or 4 (Imm condition; for these subjects, Day 3 was a no-treatment day), all subjects received 6 CS-US pairings with an intertrial interval of 5 (±2) min in a 33-min session. Upon completion of conditioning, all subjects were returned to their home cages, and then returned to the testing chambers for the extinction phase. The interval between the last conditioning trial and the first extinction trial was 12 min (Imm condition) or 24 h (Del condition). During extinction, all subjects received 20 presentations of CS A alone, with a mean ITI of 3 (±1) min in a 60-min session.

Retention interval, restabilization of baseline leverpressing and retention test (Days 5-9). Days 5-7 were no-treatment days, and constituted a retention interval during which spontaneous recovery could be fostered. On Day 8, all subjects were exposed to the experimental context for 60 minutes and allowed to leverpress for water reinforcement in order to reestablish the baseline responding that could have been disrupted by previous experience with shock treatment. On Day 9, all subjects received 3 presentations of the CS to assess the occurrence of spontaneous recovery of the fear response. The ITI was 4 min and the first trial was delivered at 4 min into the session.

For all statistical analyses, significance was established at an alpha level of .05.

Results and Discussion

During conditioning, animals in the Preex condition acquired the CS-shock association more slowly than animals in the Control condition, suggesting that preexposure to the CS delayed acquisition or expression of the CS-US association (see Figure 1). Extinction progressed as expected, with animals that received preexposure exhibiting faster extinction than animals that did not received preexposure. However, responding was equivalent in all groups at the completion of the extinction phase (see Figure 2). Immediate and delayed extinction resulted in similar recovery of conditioned fear. That is, whether extinction occurred immediately or with a delay following conditioning did not provide any benefits in preventing fear relapse (see Figure 3).

Conditioning. The number of conditioning trials selected for this preparation was intended to result in complete response suppression in all groups, regardless of preexposure status. Because this implies that animals should stop leverpressing, the cumulative percentage of animals that continued to leverpress through the session was assessed using a 'response survival analysis.' Behavior was described as not surviving if the animal ceased to leverpress during a trial and did not produce any more responses during subsequent trials. All subjects in the Preex condition and all subjects in the Control condition received equivalent training during preexposure and conditioning. Thus, preexposure condition (Preex *vs.* Control) was used as the only grouping factor for this analysis. During the final conditioning trial, 28.12% (n=9) of all subjects produced a minimum of one response. Response survival was 43.75% (n=7) in the Preex condition and 12.5% (n=2) in the Control condition. A Cox-Mantel test revealed significantly higher response survival in the LI groups than the Control groups, test statistic = 2.56, $p < .05$.

Extinction. Animals in the Imm condition ceased responding towards the end of the extinction session, although they did not exhibit obvious freezing. This was likely due to satiation, as the combined conditioning-extinction session had a total duration of 90 min with a maximum of 270 reinforcers available to the subjects. Therefore, the analysis of extinction was conducted on the first four blocks of four stimuli presentations (i.e., 16 stimuli presentations) rather than all five blocks of stimuli presentations. (Note that, regardless of whether or not they produced the operant response, animals continued to receive the stimulus exposures scheduled for their extinction treatment.) Suppression ratios (Annau & Kamin, 1961) were calculated for the spontaneous recovery presentations of the CS using the formula *suppression ratio = CS responses/(preCS responses + CS responses)*, where CS responses reflects the total number of leverpresses recorded during the 30-second CS presentations, and preCS responses reflects the total number of responses produced during the 90-s period immediately preceding the CS, divided by three. A 2 (condition: Preex *vs.* Control) x 2 (timing of extinction: Immediate Extinction *vs.* Delayed Extinction) x 4 (block of four trials) analysis of variance (ANOVA) revealed main effects of block, $F(3,69) = 20.36$, $MSE = 0.02$, and condition, $F(1,23) = 10.32$, $MSE = 0.06$, as well as a Block x Delay interaction, $F(3,69) = 6.24$, $MSE = 0.02$. Although all subjects showed a decrease in fear responses throughout extinction (main effect of block), the Preex condition exhibited less conditioned fear than the Control condition (main effect of condition), and response attenuation through extinction was faster in the Del than the Imm condition (interaction). Thus, as reported by others (Leung & Westbrook, 2010; Rosenberg, et al. 2011), extinction progressed faster if the CS had received preexposure (Preex condition) than if it had not (Control condition). Surprisingly, delaying extinction increased the rate of response attenuation after CS preexposure, which is contrary to previous reports that immediate extinction progresses at the same rate as (Johnson et al., 2010; Powell et al., 2013) or faster than delayed extinction (Maren & Chang, 2006; Schiller, et al., 2008; Woods & Bouton, 2008).

Spontaneous recovery. A 2 (condition) x 2 (timing of extinction) ANOVA comparing responding during the final block of extinction to responding during the test session (block, within-subjects factor) revealed a main effect of condition, $F(1,25) = 6.43$, $MSE = 0.03$, as well as an effect of block, $F(1,25) = 8.90$, $MSE = 0.04$, indicating spontaneous recovery of conditioned fear, although the Control condition continued to exhibit more overall fear than the Preex condition. However the lack of an interaction suggests that degree of spontaneous recovery was not dependent on the interval between conditioning and extinction. A 2 (condition) x 2 (timing of extinction) ANOVA conducted on the average of the three spontaneous recovery test trials further supported this conclusion, revealing no main effects nor an interaction, all *F*s < 1.0 (see Figure 3). That is, responding was equivalent regardless of whether or not preexposure had occurred and regardless of whether extinction had occurred immediately or with a delay after conditioning. Thus, manipulating the conditioning-extinction interval did not provide any benefits to ameliorate spontaneous recovery, which is consistent with previous reports in the literature (Leung & Westbrook, 2010; Rosenberg, et. al, 2011).

Experiment 2

Experiment 2 investigated the effects of combining CS preexposure and extinction treatments when conditioning occurred immediately or with a delay after preexposure, while maintaining the interval between conditioning and extinction constant. Thus, either 12 minutes (Imm condition) or 24 hours (Del condition) following the final CS preexposure trial, animals received CS-US pairings. Twenty-four hours later, animals received extinction treatment. Recovery of the response was measured four days post extinction. Previous research from our laboratory suggests that immediate preexposure has a long-lasting fear attenuation effect (Kimble & Escobar, 2013). Thus, it seemed likely that immediate preexposure could also enhance the effect of subsequent fear extinction, possibly reducing the magnitude of spontaneous recovery of fear. Table 2 presents the critical aspects of Experiment 2.

Methods

Subjects, apparatus, and procedure. Thirty-two male Sprague Dawley albino rats (Charles River Labs; weight range: 214-285g) served as subjects in Experiment 2, and they were randomly assigned to four experimental groups (*n*s = 8; Preex-Imm, Control-Imm, Preex-Del, Control-Del). Subjects were housed and maintained as described in Experiment 1. All animals had previously participated in a study in which they were trained to leverpress for the water reinforcer. The condition to which they were assigned in the previous study (which used different stimuli and apparatus) was counterbalanced within groups in the present study.

The apparatus, stimuli, and procedures were the same as those used in Experiment 1, with the exception that the timing between preexposure and conditioning, rather than timing between conditioning and extinction, was manipulated (i.e., Immediate Preex *vs.* Delayed Preex rather than Immediate Extinction *vs.* Delayed Extinction; see Table 2).

Results and Discussion

During conditioning, animals in the Preex condition acquired the CS-shock association slower than animals in the Control condition, which suggests that the CS preexposure treatment was effective in retarding subsequent conditioning. Delaying conditioning after preexposure resulted in more retardation (i.e., more robust latent inhibition) than either no preexposure or immediate preexposure (see Figure 4). Extinction progressed as expected, with animals that received preexposure exhibiting faster extinction than animals that did not receive preexposure (see Figure 5). However, immediate preexposure resulted in less recovery of conditioned fear than either delayed preexposure or extinction alone (see Figure 6).

Conditioning. The response survival measure described in Experiment 1 was used to assess response suppression during conditioning. Upon completion of training, 9.38% (n=3) subjects leverpressed during the final stimulus presentation. In Groups Preex-Imm, Control-Imm, and Control-Del, response survival was of 0%. In comparison, response survival in Group Preex-Del was 37.5% (n=3). That is, delayed preexposure appears to result in more retardation than immediate preexposure or no preexposure, χ^2 (3, N=36) = 21.04.

Extinction. A 2 (condition: Preex *vs.* Control) x 2 (timing of conditioning: Immediate *vs.* Delayed) x 4 (block of four trials) ANOVA revealed main effects of condition, $F(1,23) = 6.25$, $MSE = 0.06$, and block, $F(3,72) = 12.93$, $MSE = 0.02$. Neither the main effect of timing of preexposure nor any of the interactions were significant, Fs < 1.04. That is, CS preexposure resulted in less conditioned fear in the Preex condition than the Control condition regardless of the timing of conditioning respect to preexposure. More specifically, faster rates of extinction were observed when the CS had previously received preexposure than if it had not been previously preexposed. These results are similar to what we observed in Experiment 1, in which the timing of extinction did not affect the rate at which fear attenuation occurred.

Spontaneous recovery. A 2 (condition) x 2 (timing of preexposure) x 2 (block, within subjects factor) ANOVA comparing responding during the final extinction block to responding during the testing block revealed a Timing of Preexposure x Block interaction, $F(1,26) = 6.14$, $MSE = 0.04$. There were no main effects of condition or training session, Fs < 1.7, MSEs = 0.03 and 0.04, respectively. The interaction resulted from the Del condition exhibiting more spontaneous recovery at test than the Imm condition in the preexposure condition. Planned comparisons revealed that animals assigned to the Del conditions exhibited more conditioned fear during testing than at the end of extinction, $F(1,26) = 7.68$, $MSE = 0.04$. However, spontaneous recovery was not observed when conditioning occurred immediately following preexposure, $F < 1.0$, $MSE = 0.04$. This conclusion was supported by conducting a 2 (condition) x 2 (timing of preexposure) ANOVA on the average of the three spontaneous recovery test trials. This analysis revealed a Condition x Timing of Preexposure interaction, $F(1,28) = 5.71$, MSE = 0.02. The main effects of condition and timing of conditioning were not statistically significant, Fs < 2.44, MSEs = 0.02. The interaction reflects the observation of less spontaneous recovery when conditioning occurred immediately after preexposure than when conditioning occurred long after preexposure (see Figure 4). Planned comparisons revealed no difference between the two control conditions (Group Control-Imm and Control-Del), $F(1,28) = 0.34$, $MSE = 0.02$. Group Preex-Imm was more resistant to recovery of conditioned fear than a group receiving equivalent treatment but no preexposure (Group Control-Imm), $F(1,28) = 6.89$, $MSE = 0.02$, and a group receiving equivalent preexposure, but long before conditioning (Group Preex-Del), $F(1,28) = 7.8$, $MSE = 0.02$. That is, immediate preexposure appeared to enhance the effectiveness of extinction *and* made such extinction resistant to subsequent spontaneous recovery.

Experiment 3

At least under certain conditions, immediate extinction appears to be more resistant to spontaneous recovery than delayed extinction (Johnson et al., 2010; Myers et al., 2006). A situation potentially similar to immediate extinction can be produced if the memory of the to-be-extinguished stimulus is reactivated by presenting the CS briefly shortly before conditioning. As is the case with immediate extinction, at least under certain conditions, these reactivation manipulations can greatly enhance the effectiveness of extinction and prevent recovery of the fear response (e.g., Monfils, et al., 2009, Rao-Ruiz, et al., 2011, Schiller, et al., 2010, but see Chan, et al., 2010; Chang & Maren, 2011; Constanzi, Cannas, Saraulli, Rossi-Arnaud, & Cestari, 2011; Soeter, & Kindt, 2011; Stafford, Maughan, Ilioi, & Lattal, 2013).

Experiment 2 suggests that recent exposure to the to-be-conditioned stimulus can greatly enhance the effectiveness of subsequent extinction. This is consistent with previous reports that immediate preexposure is less prone to spontaneous recovery of fear than delayed preexposure (Kimble & Escobar, 2013; Powell et al., 2013). Thus, it seems possible that reactivation of the preexposure memory would have an effect on subsequent conditioning similar to the effect that reactivation of the conditioning memory has on subsequent extinction.

Experiment 3 investigated the possibility that reactivation of the CS preexposure memory immediately prior to conditioning would have similar effects on extinction as immediate latent inhibition, even if conditioning occurred long after CS preexposure. CS preexposure occurred 24 hours prior to conditioning for all subjects. Prior to conditioning, some of the animals received 3 reminder presentations of the CS alone in order to reactivate the CS-preexposure memory, while the remaining animals did not receive the reactivation treatment. Twelve minutes following reactivation of the preexposure memory, conditioning began. Extinction occurred the following day, and spontaneous recovery of conditioned fear was assessed four days following extinction.

Methods

Subjects, apparatus, and procedure. The subjects were thirty-two male Sprague Dawley albino rats (Charles River Labs; weight range: 209-332g), housed and maintained as described in Experiment 1. All animals had previously participated in a study in which they were trained to leverpress for water reinforcement, but which used different stimuli and environmental cues. The condition to which they were assigned in the previous study was counterbalanced within groups in the present study. With these restrictions, animals were assigned to one of four groups (Preex-Reactivate, Control-Reactivate, Preex-NoReactivate, and Control-NoReactivate, *n*s = 8). The apparatus and stimuli were the same as those described in Experiment 1.

The procedure was identical to that described for the Del condition of Experiment 1, with the following exceptions. Twenty-four hours after completion of CS preexposure (Day 3), subjects in the Reactivate condition received 3 presentations of the CS alone with an intertrial interval of 2 (±1) min in a 8-min session. Subjects in the NoReactivate condition received equivalent exposure to the context. After completion of this session, all subjects were removed from the experimental chambers, returned to their home cages, and then returned to the testing chambers for the conditioning session. The interval between the last reactivation stimulus presentation and the first conditioning stimulus presentation was 12 min. Spontaneous recovery testing occurred four days later, as described for Experiments 1 and 2.

Results and Discussion

Consistent with the observations of Experiments 1 and 2, subjects in the Preex condition exhibited slower acquisition of fear and faster extinction rates than subjects in the Control condition. More importantly, animals receiving the reactivation manipulation exhibited less fear than animals not receiving the reactivation manipulation (see Figure 7). The reactivation procedure increased the rate of extinction, and this rate increase was semi-independent of whether subjects had previously received preexposure to the CS (see Figure 8). Furthermore, the Reactivate condition exhibited less recovery of conditioned fear than the NoReactivate condition. As was the case with increased extinction rate, this attenuation of spontaneous recovery was observed independent of whether the CS received many preexposure presentations and then few reactivation presentations or only a few reactivation presentations (see Figure 9).

Conditioning. Response survival was assessed by comparing the proportion of animals that leverpressed during each trial for Groups Preex-Reactivate, Control-Reactivate, Preex-NoReactivate, and Control-NoReactivate. Upon completion of the conditioning phase, response survival was 0% for all subjects in all groups. However, the Preex condition exhibited retarded conditioning as compared to the Control condition, χ^2 (3, N = 36) = 15.47.

Extinction. A 2 (condition: Preex *vs.* Control) x 2 (reactivation manipulation: Reactivate *vs.* NoReactivate) x 4 (block of 4 trials) ANOVA revealed a main effect of block and a

Reactivation x Block interaction, *F*s(3,69) = 36.13 and 3.55, respectively, *MSE* = 0.01, as well as main effects of condition and reactivation manipulation, *F*s (1,23) = 9.47 and 9.03, respectively, *MSE* = 0.05. All remaining interactions were not significant, *F*s < 1.86, *MSE*s = 0.05, and 0.01, respectively. These observations are consistent with those of Experiments 1 and 2, in which animals receiving the combined CS preexposure and extinction treatment showed faster extinction of fear than animals receiving extinction alone. However, the fact that the reactivation manipulation did not interact with condition suggests that a few CS-alone presentations shortly before conditioning were sufficient to increase the rate of extinction. Indeed, suppression during the last block of extinction was marginally lower in Group Control-Reactivate than Group Control-NoReactivate, *t*(13) = 1.96, *p* = .07.

Spontaneous recovery. A 2 (condition) x 2 (reactivation manipulation) x 2 (training session) ANOVA conducted on the final extinction block and the average of three stimulus presentations at test revealed main effects of Condition, and Reactivation, *Fs*(1,26) = 6.13 and 8.3, respectively, *MSE* = 0.04. There was also a main effect of Training Session, *F*(1,26) = 13.93, *MSE* = 0.01, as well as a Condition x Training Session interaction, *F*(1,26) = 7.95, *MSE* = 0.01. All other main effects and interactions were not significant, *F*s < 3.42. These results suggest that preexposing the CS did result in spontaneous recovery of conditioned fear, but reactivation of the preexposure memory decreased this spontaneous recovery. Although conditioned fear was apparently not extinguished in the control conditions as it was in the Preex conditions, brief preexposure immediately prior to conditioning maintained all extinction-driven gains in Group Control-Reactivate and not Group Control-noReactivate. The Condition x Training Sesssion interaction indicates greater spontaneous recovery when the CS was preexposed than the controls. Planned comparisons revealed no difference in the Control condition between the final extinction block and the testing block, $F > 0.39$, $MSE = 0.01$, however, more conditioned fear was observed at test than during the final extinction block in the Preex condition, $F(1,26) = 23.25$, $MSE = 0.01$, indicating there was recovery of conditioned fear when the CS was preexposed, but not when the CS was not preexposed. This observation could simply reflect an apparent inflation of the extinction effect in the Preex condition.

A 2 (condition) x 2 (reactivation manipulation) ANOVA conducted on the average of the three spontaneous recovery trials revealed a main effect of reactivation manipulation, $F(1,27) = 4.83$, $MSE = 0.02$, and no other main effects or interactions, *F*s < 1.37. That is, the reactivation manipulation successfully decreased spontaneous recovery, even if the conditioning session occurred long after the preexposure session (equivalent to Group Preex-Del of Experiment 2). However, the reactivation manipulation did not completely prevent spontaneous recovery. There was significantly more fear at test than during the last extinction block in both the Preex-Reactivate and Preex-NoReactivate groups, $t(7) = 5.83, 2.44$, *ps* <.05, respectively, indicating that the two groups were equivalent, $t(14) = 1.28$, $p = .22$. Interestingly, the reactivation manipulation had a similar effect of decreasing relapse of conditioned fear *independently* of whether or not there had been a CS preexposure session the previous day. Furthermore, although extinction was not complete in Group Control-Reactivate, the fear reduction achieved during the extinction session was maintained through the retention interval in this group, $t(7) = 1.01$, $p = .35$ by comparing the final extinction block to test. The maintenance of extinction obtained in Group Control-Reactivate made the amount of fear at test virtually equivalent to the Preex-Reactivate group, $t(14) = 0.71$, $p = .48$. Thus, it seems that even a small number of presentations of the CS by itself prior to conditioning are enough to enhance the effects of extinction and attenuate fear relapse, as long as these presentations are given very shortly before conditioning. The implications of these results are discussed in the General Discussion.

General Discussion

The present studies provide evidence that combining CS preexposure and extinction can ‘protect’ extinction from subsequent spontaneous recovery more effectively than providing extinction alone. Consistent with previous studies, combining CS preexposure and extinction appeared to enhance the rate of extinction (Leung & Westbrook, 2010; Rosenberg, et. al, 2011). The main manipulation introduced in these studies was the timing of extinction (Experiment 1) and preexposure (Experiment 2) with respect to conditioning. Contrary to our expectations, providing extinction immediately after conditioning did not provide any protection against spontaneous recovery, regardless of whether preexposure had or had not taken place prior to conditioning. However, consistent with what we anticipated, providing preexposure immediately prior to conditioning and extinction did provide protection against relapse of conditioned fear. Furthermore, the ‘relapse protection’ effect of preexposure could be achieved in a situation in which conditioning was delayed after preexposure if the CS preexposure memory was reactivated immediately prior to conditioning (Experiment 3). Surprisingly, protection from relapse was relatively independent of the number of preexposure trials: CS preexposure attenuated spontaneous recovery as long as such preexposure occurred shortly before conditioning, independently of whether there were many or few CS preexposure presentations.

Both extinction and the CS-preexposure effect are highly context dependent, the usual observation being that, unless the context of testing serves to retrieve the memories of CS-alone exposure, relapse of fear is observed (see Bouton, 1994). ‘Context’ can be broadly defined to include physical and temporal characteristics of a learning experience. Thus, it is possible that if preexposure and conditioning occur in close temporal proximity, they are likely encoded as occurring within a single temporal context and both experiences (CS-noUS and CS-US) come to have common retrieval cues (see Kimble & Escobar, 2013). Thus, the CS will provide ambiguous information about the subsequent delivery of the shock US (US delivery or omission). A different situation would take place if conditioning occurs with a delay after preexposure because the time interval imposed between preexposure and conditioning would create separate local contexts that are clearly delineated. Each of these contexts would have its own retrieval cues for the information they signal (US delivery or omission). Because the test context is temporally different from the preexposure context, spontaneous recovery should be more likely to occur after delayed than immediate preexposure. Possibly, the CS-alone presentations of extinction strengthen the CS-noUS memory and make it more likely to be retrieved. However, this 'strengthening' should benefit the delayed condition more than the immediate condition because nonreinforcement during extinction should differentially retrieve the memory of nonreinforcement during preexposure. This suggests that the impact of immediate preexposure may be at the level of encoding of the information, which would be consistent with the observation that spontaneous recovery after immediate preexposure does not occur even if testing is delayed for seven days (Kimble & Escobar, 2013).

There are many future directions that stem from this research. If indeed, immediate preexposure results in CS preexposure and conditioning being encoded in the same temporal context, then increasing the difference between the preexposure and conditioning experiences (e.g., by shifting physical contexts) should attenuate the benefit of immediate preexposure. The context change should delineate the contexts for each training phase, where a shift in physical contexts will create a separate preexposure context, a conditioning context, and an extinction context even with immediate preexposure. Thus, changing the physical context of the preexposure training from conditioning and extinction should emulate similar patterns of responding as when conditioning is delayed from preexposure. Therefore, the separate contexts (both physical and temporal) will have distinct associations between the context and the presence or absence of the US, and each context will provide independent cues for which association is appropriate. Even though the temporal context will be similar between preexposure and conditioning, the physical shift in context between preexposure and conditioning should result in greater spontaneous recovery than if immediate preexposure is conducted in the same physical context as conditioning.

Although the present experiments suggest that immediate preexposure (but not immediate extinction) provides long-term protection from relapse of conditioned fear, it is possible that the interval imposed between conditioning and extinction may still be of importance. Johnson et al. (2010) reported that immediate extinction results in less fear relapse than delayed extinction when the retention interval between extinction and testing was long (7 days) rather than short (48 hours). In stark contrast with the effects of immediate preexposure, we failed to observe a benefit of immediate extinction after CS preexposure. However, this may be due to the length of our retention interval, which was shorter (4 days) than that used by Johnson et al. (7 days). Possibly, if the retention interval is lengthened to 7 or more days in duration, we would observe less spontaneous recovery than if extinction treatment is provided alone.

Following memory consolidation, recalling that memory makes it susceptible to updating until it is reconsolidated. The time during which a memory is considered labile after reactivation is approximately 6 hours (Davis, & Squire, 1984; Nader, Schafe, & LeDoux 2000). It is during this time window that a memory may be altered or ‘updated’ by further experience. If the altering of a memory during the reconsolidation period results in an updated memory, then providing preexposure to the CS immediately prior to conditioning should result in increased conditioned fear when compared to the control condition because the preexposure memory will be updated with the memory of fear conditioning. For example, Monfils, et al. (2009) extinguished fear to the CS following conditioning training within the reconsolidation window (i.e., less than 6 hours; Davis, & Squire, 1984; Nader, et al., 2000), thus *updating* the fear memory with the information acquired during the extinction phase (see also Schiller, Monfils, Raio, Johnson, LeDoux & Phelps, 2010). Their conclusion was that extinction provided during the reconsolidation period ‘erased’ the previously acquired fear memory. The current experiments provide evidence contrary to the notion of memory updating. Experiment 3 demonstrates that reactivating the preexposure memory immediately prior to conditioning did not result in that memory being updated with the new information acquired during conditioning. Therefore, the present data suggest that providing contradictory training (i.e., CS-noUS then CS-US or CS-US then CS-noUS) within six hours of memory reactivation does not automatically result in the new memory coming to substitute for the reactivated memory.

In some cases, CBT initiated immediately (i.e., within 2 weeks) after a traumatic event have been proven successful at decreasing the overall rates of PTSD diagnoses (for reviews see Ehlers & Clark, 2003; Litz, et al., 2002) or reducing the initial rates of PTSD (e.g., Foa, Hearst-Ikeda, Perry, 1995). The current studies suggest a form of training that may promote resiliency to conditioned fear and/or facilitate subsequent treatment should conditioned fear develop. Under certain conditions, the use of combined pre- and post-fear conditioning can result in more effective long-term attenuation of fear than using either treatment alone. Therefore, these results do provide an alternative manner in which relapse of fear can be attenuated by combining two types of CS exposure. It is possible that the combination of CS preexposure and extinction can be used as a behavioral strategy to prevent relapses of fear in fields that have a proclivity for traumatic events. For example, exposing a soldier to olfactory or auditory cues of a battlefield without any aversive emotional outcomes prior to deployment could be analogous to the preexposure training. Upon experiencing a traumatic event during the deployment, therapy can be provided, and include exposure therapy which would equate to the extinction training used in the present experiments. The present research would suggest that, in order to maximize treatment effectiveness, preexposure must occur shortly before prior to the traumatic event. This preexposure does not have to be lengthy; a short “booster” session involving brief preexposure to the to-be-encountered cues can provide long-term protection benefits against conditioned fear (Experiment 3). The fact that brief preexposure proved beneficial suggests that the combined treatment presented here can be realistically applied to the real world in situations in which lengthy preexposure may not be practical or possible. In the previous example, a brief 5 minute preexposure to one of the cues used during training immediately prior to a mission will potentially reduce relapse when combined with subsequent therapy. Therefore, using latent inhibition as training and extinction as a therapeutic technique, the incidences of PTSD as well as other anxiety disorders can be greatly reduced.

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Footnotes

[1] Pharmacological strategies have been attempted to facilitate therapeutic treatments, but they will not be reviewed here; the interested reader may wish to consult the recent reviews by Anthony, Ledley, & Heimberg, 2005; Bentz, et al., 2010; de Bitencourt, Pamplona, & Takahashi, 2012; Davis, Myers, Chhatwal, & Ressler, 2006)

Table 1

Leung & Westbrook, 2010 Experimental Designs

Experiment 6a							
Preexpose	Cond	Ext	Retention	Preexpose	Cond	Ext	Test
A-	A+	A-		B-	B+	B-	AD
C-	C+			D-	D+		BC

Experiment 6b							
Preexpose	Cond	Ext	Retention	Preexpose	Cond	Ext	Test
A-	A+	A-		B-	B+	B-	AD
	C+	C-			D+	D-	BC

Note: Each experiment represents a within subjects design. Preexpose represents the preexposure phase of training, Cond represents the conditioning phase of training, and Ext repersents the extinction phase of training. A, B, C and D represent different audiovisual stimuli, and '+' and '-' represent the presence and absence of the US, a mild footshock, respectively.

Table 2

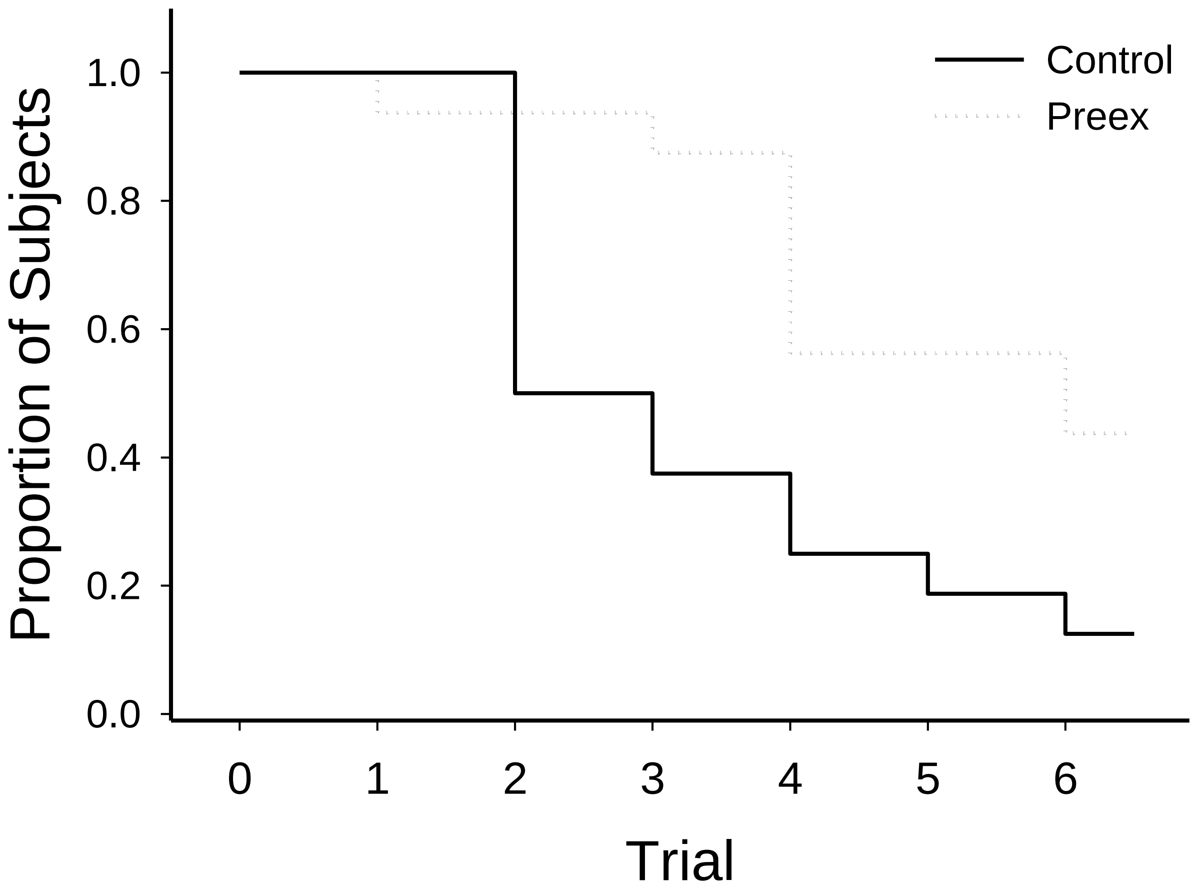
Experimental Designs

	Experiment 1				
	Preex	Conditioning	Interval	Extinction	Test
Preex-ImmExt	36 CS-	6 CS+	10 min	20 CS-	3 CS -
Control-ImmExt	Context -	6 CS+	10 min	20 CS-	3 CS -
Preex-DelExt	36 CS-	6 CS+	24 h	20 CS-	3 CS -
Control-DelExt	Context -	6 CS+	24 h	20 CS-	3 CS -
	Experiment 2				
	Preex	Interval	Conditioning	Extinction	Test
Preex-Imm	36 CS-	10 min	6 CS+	20 CS-	3 CS -
Control-Imm	Context -	10 min	6 CS+	20 CS-	3 CS -
Preex-Del	36 CS-	24 h	6 CS+	20 CS-	3 CS -
Control-Del	Context -	24 h	6 CS+	20 CS-	3 CS -
	Experiment 3: LI Reactivation				
	Preex	Reactivation	Conditioning	Extinction	Test
Preex-Reactivate	36 CS-	3 CS-	6 CS+	20 CS-	3 CS -
Control-Reactivate	Context -	3 CS-	6 CS+	20 CS-	3 CS -
Preex-NoReactivate	36 CS-	Context -	6 CS+	20 CS-	3 CS -
Control-NoReactivate	Context -	Context -	6 CS+	20 CS-	3 CS -

Note: The + designates the presence of the US, a mild footshock. The CS was a low frequency tone. Context- indicates the animals will remain in the chamber for duration of the session with no nominal stimuli being presented.

Figure 1

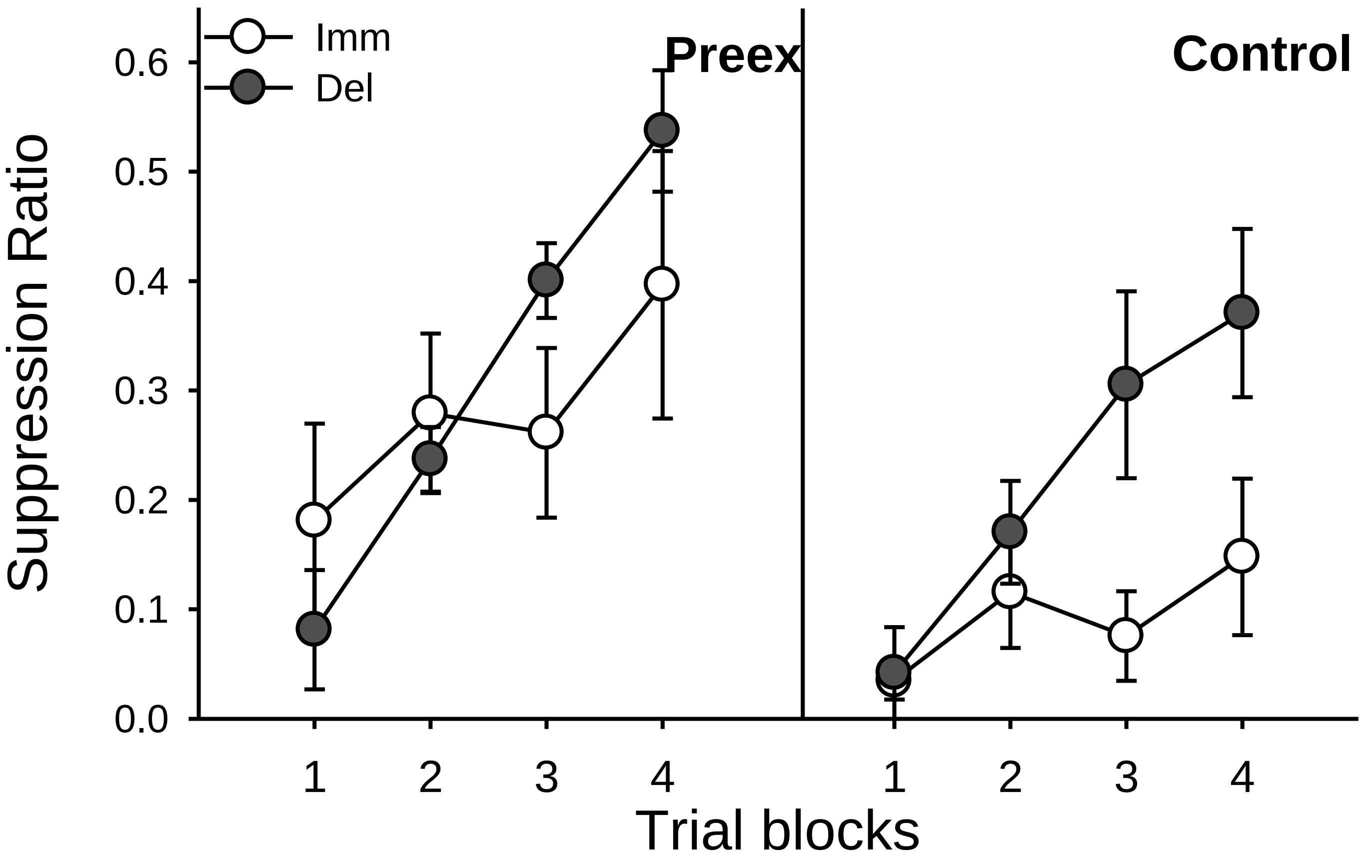
Experiment 1: Conditioning



Note. Response survival for conditioning in Experiment 1. ’Trial’ refers to each period of presentation of the CS. Each descent in the graph represents the proportion of animals that ceased to leverpress during that trial and all subsequent trials, indicating complete suppression to the CS. See text for details.

Figure 2

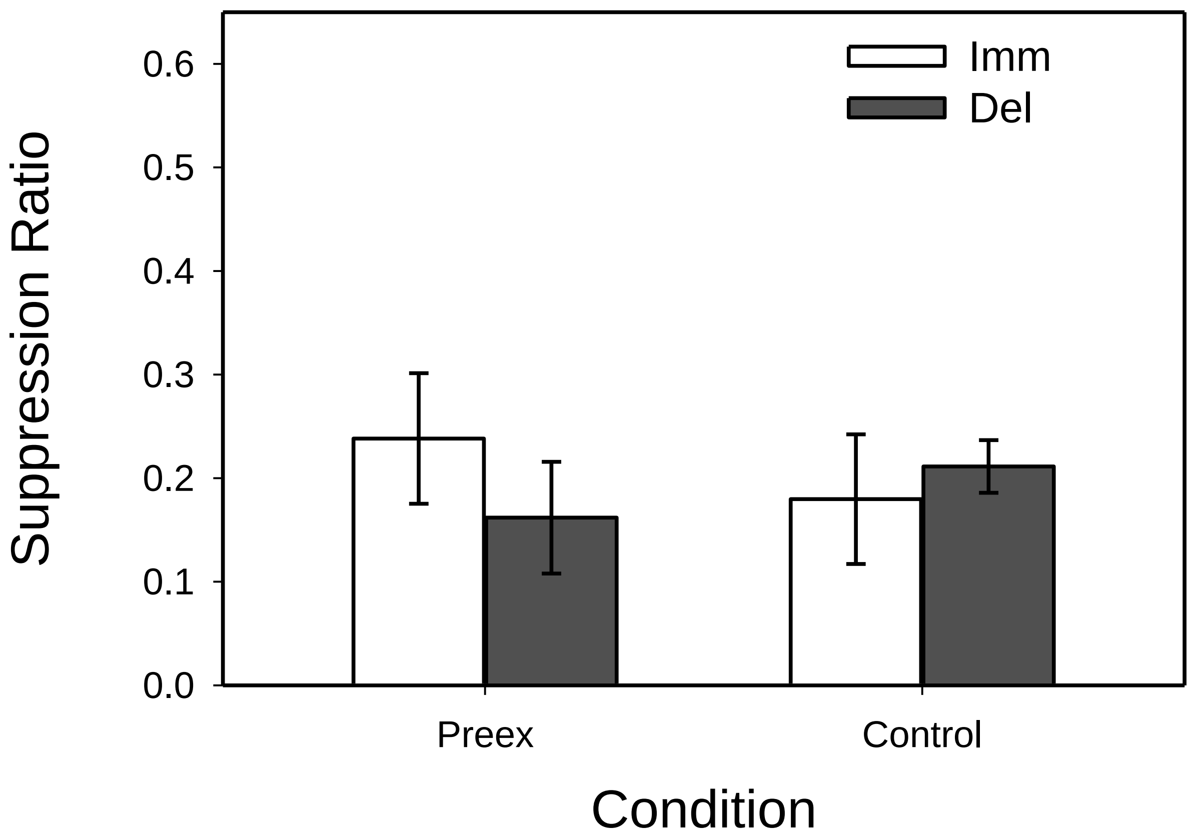
Experiment 1: Extinction



Note. Extinction in Experiment 1. The left panel presents the Preex condition, which received preexposure prior to conditioning, whereas the right panel presents the Control condition, which did not receive preexposure. The Imm condition received extinction 12 min after conditioning, whereas the Del condition received extinction 24 h after conditioning. All subjects received preexposure 24 h prior to conditioning. See text for details.

Figure 3

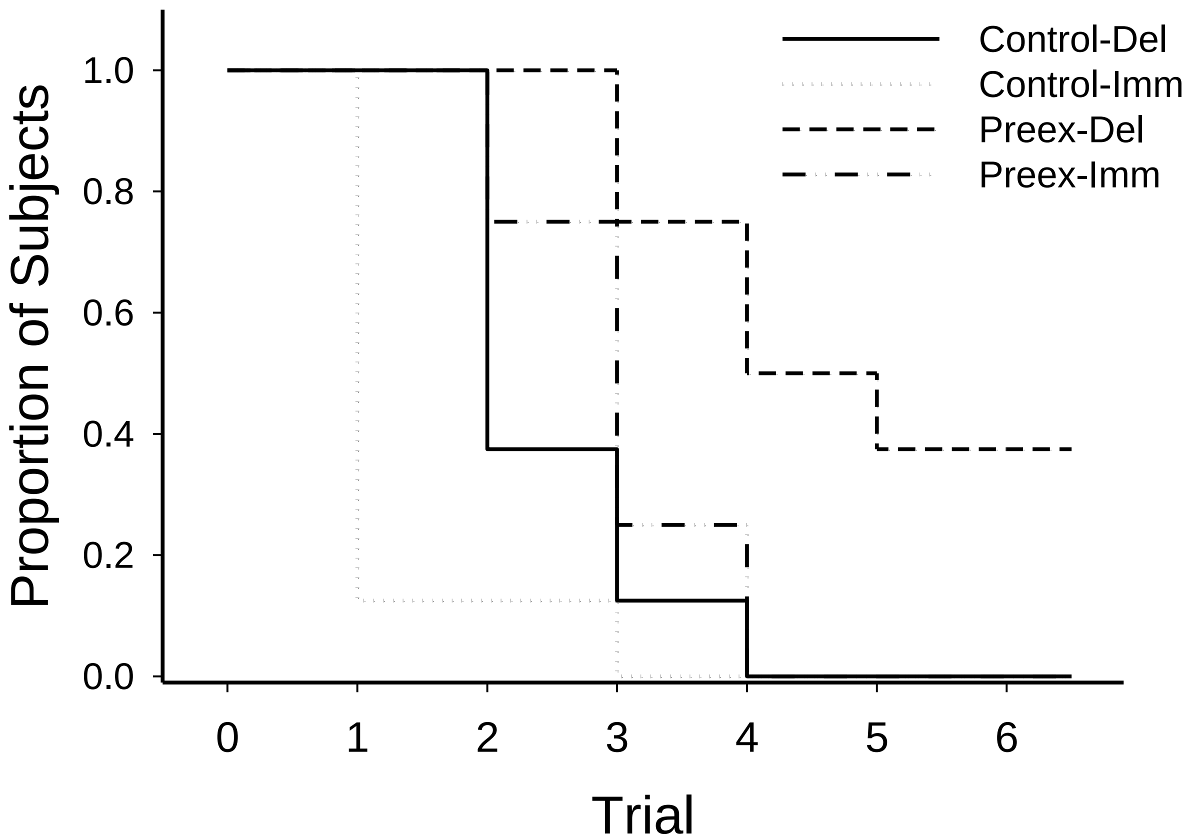
Experiment 1: Spontaneous recovery test



Note. Spontaneous recovery test in Experiment 1. The Preex condition received both CS preexposure and extinction, whereas the Control condition received only extinction training. The Imm condition received extinction 12 min after conditioning, whereas the Del condition received extinction 24 h after conditioning. All subjects received preexposure 24 h prior to conditioning. See text for details.

Figure 4

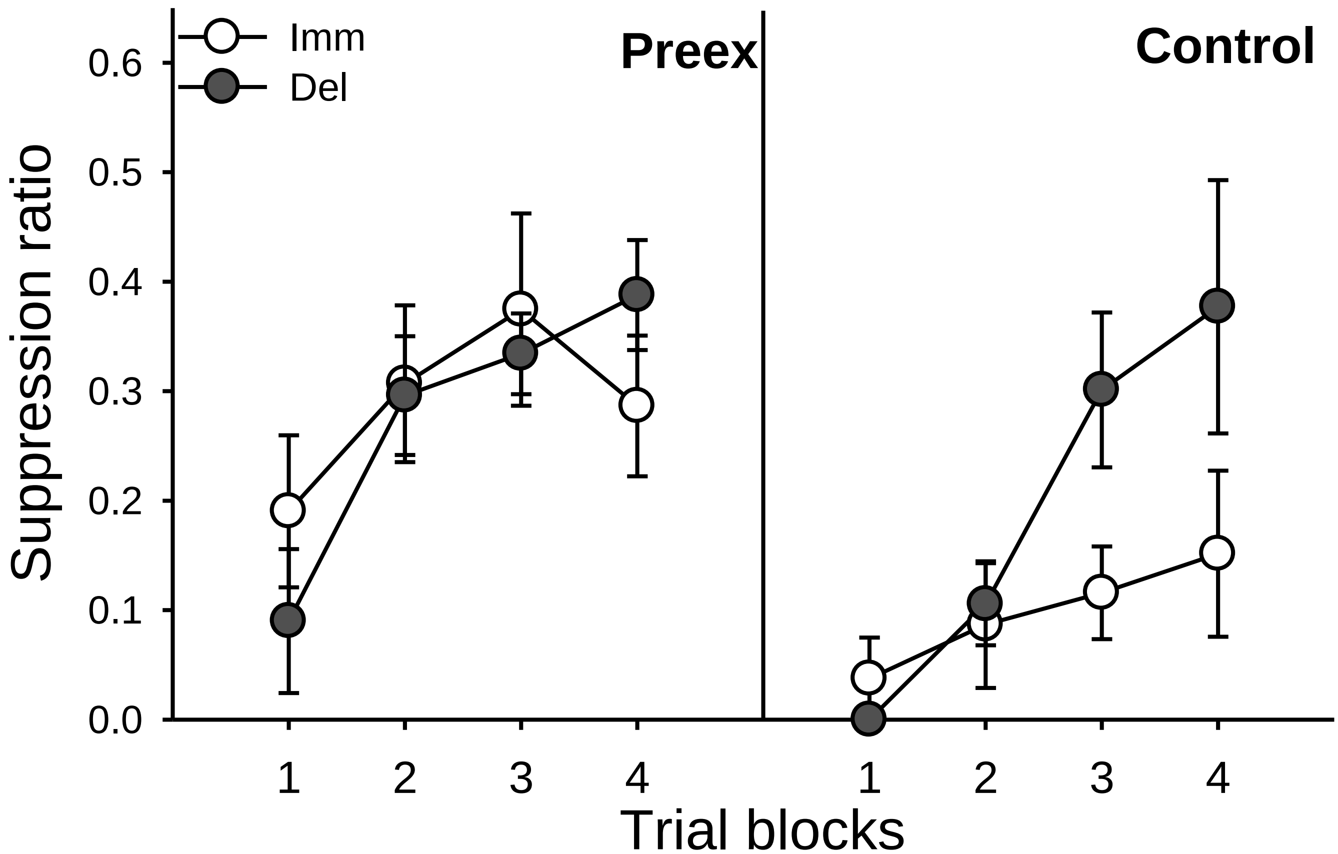
Experiment 2: Conditioning



Note. Response survival for conditioning in Experiment 2. ‘Trial’ refers to each period of presentation of the CS. Each descent in the graph represents the proportion of animals that ceased to leverpress during that trial and all subsequent trials, indicating complete suppression to the CS. See text for details.

Figure 5

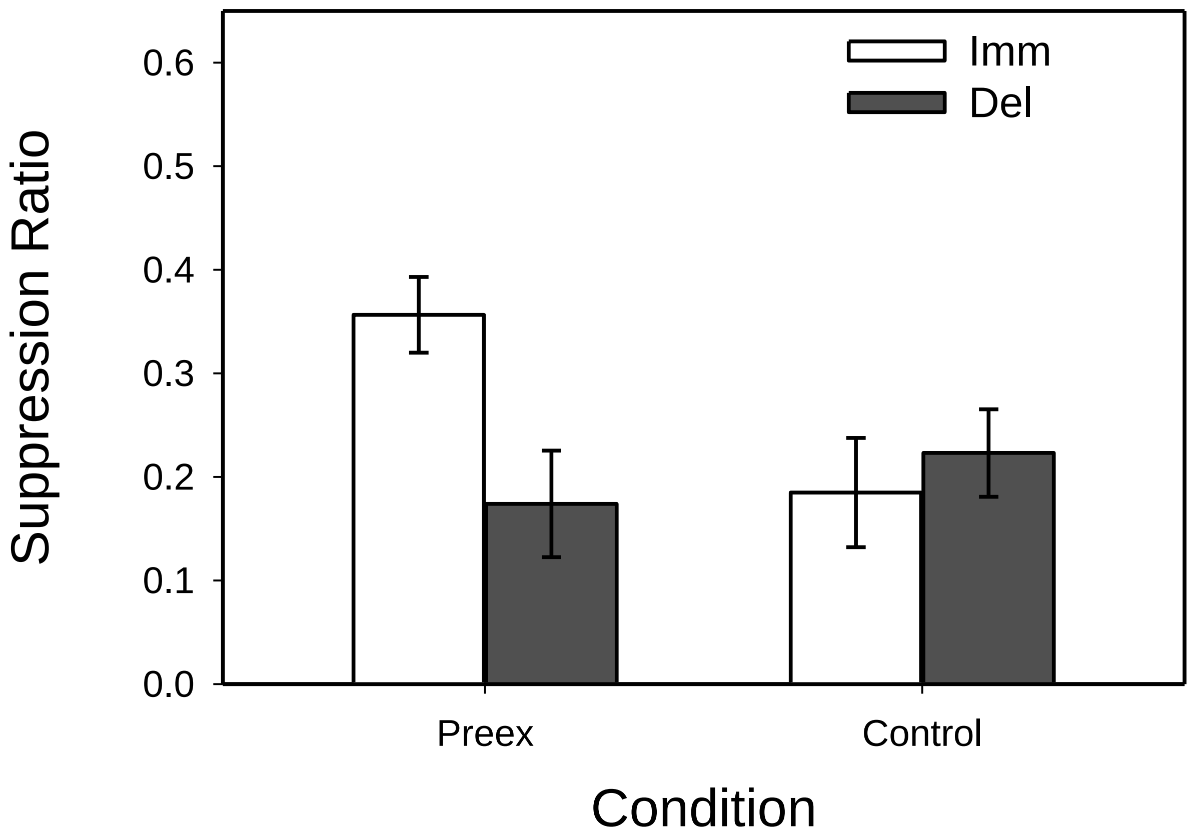
Experiment 2: Extinction



Note. Extinction in Experiment 2. The left panel presents the Preex condition, which received preexposure prior to conditioning, whereas the right panel presents the Control condition, which did not receive preexposure. The Imm condition received extinction 12 min after conditioning, whereas the Del condition received extinction 24 h after conditioning. All subjects received preexposure 24 h prior to conditioning. See text for details.

Figure 6

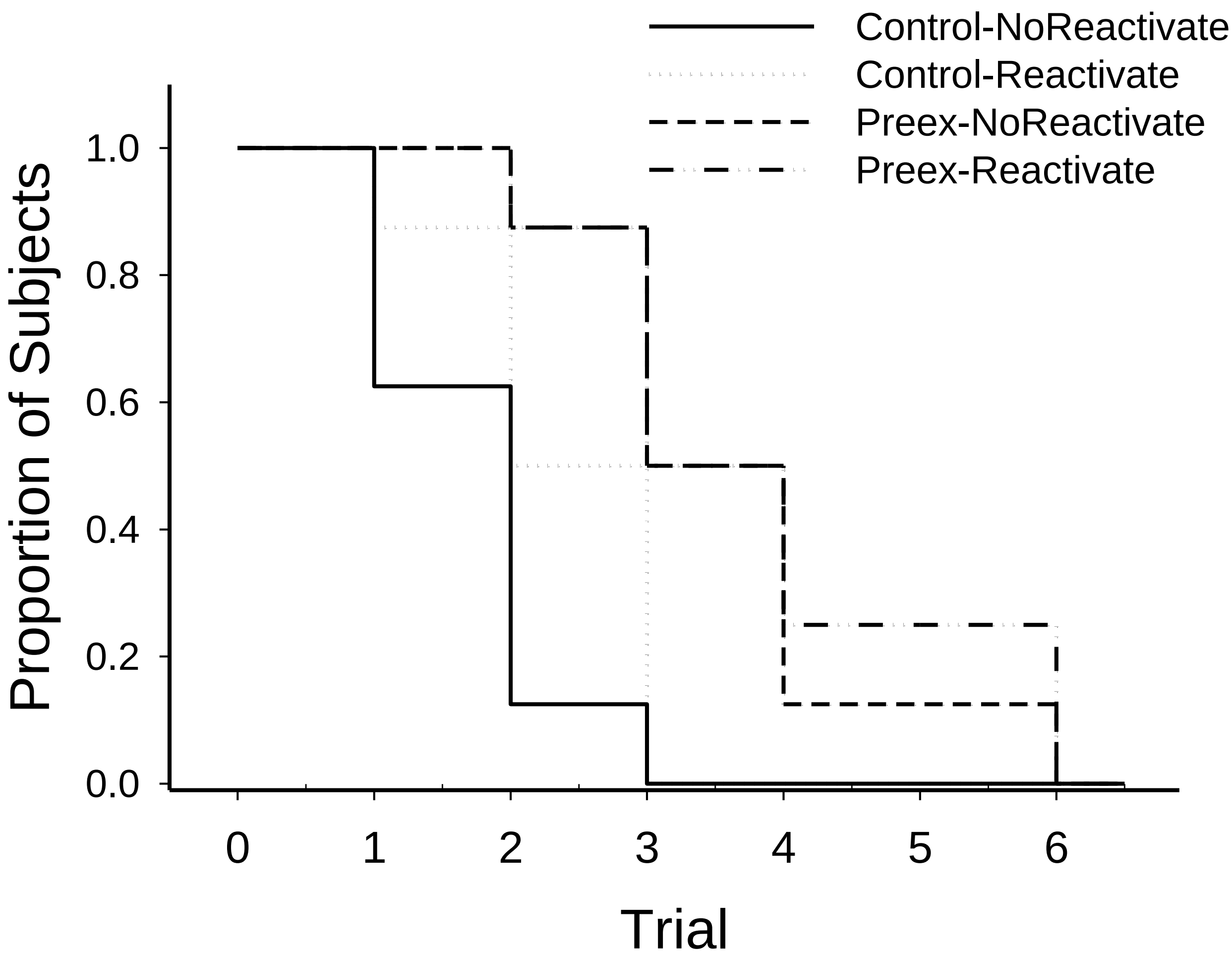
Experiment 2: Spontaneous recovery test



Note. Spontaneous recovery test in Experiment 2. The Preex condition received both CS preexposure and extinction, whereas the Control condition received only extinction training. The Imm condition received extinction 12 min after conditioning, whereas the Del condition received extinction 24 h after conditioning. All subjects received preexposure 24 h prior to conditioning. See text for details.

Figure 7

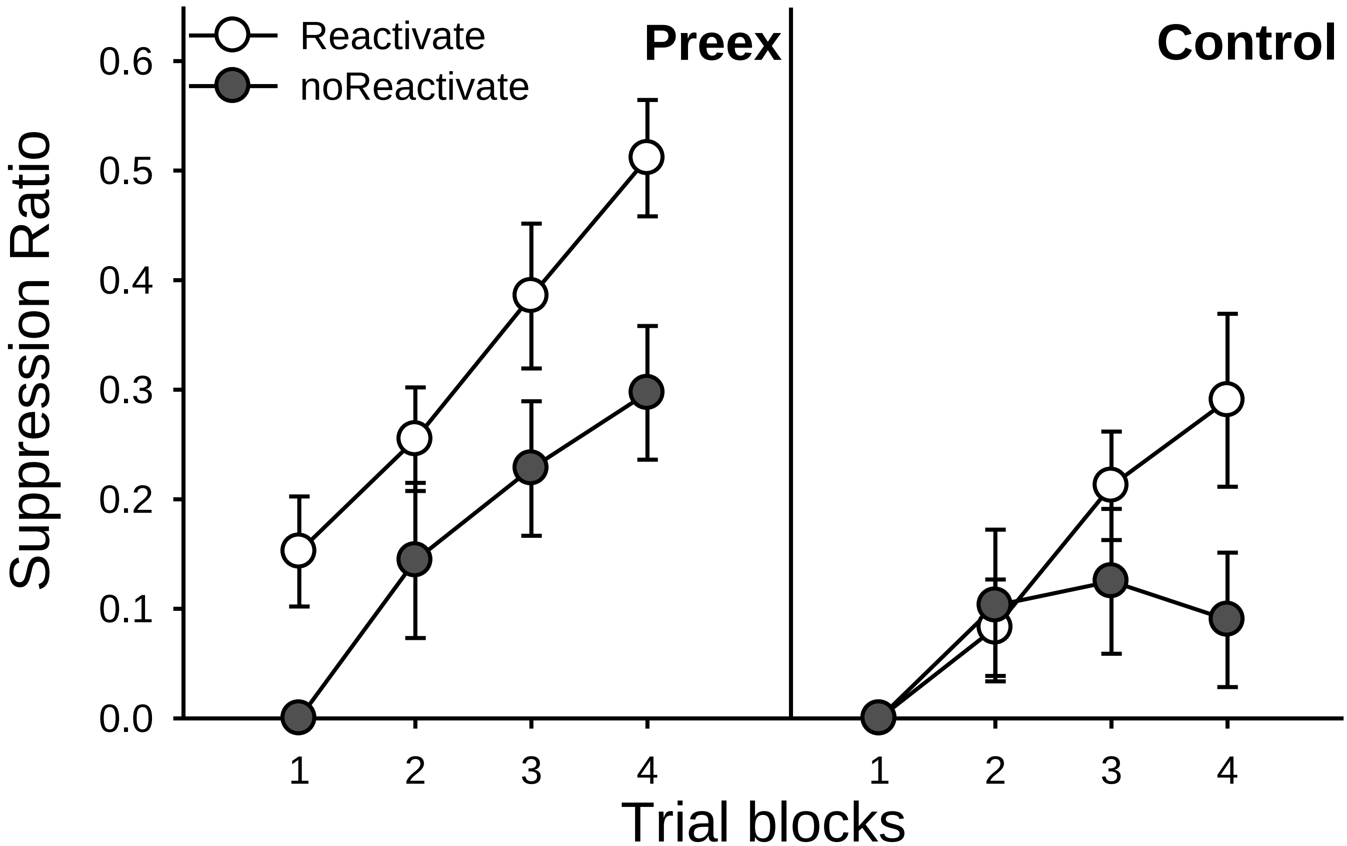
Experiment 3: Conditioning



Note. Response survival for conditioning in Experiment 3. ‘Trial’ refers to each period of presentation of the CS. Each descent in the graph represents the proportion of animals that ceased to leverpress during that trial and all subsequent trials, indicating complete suppression to the CS. See text for details.

Figure 8

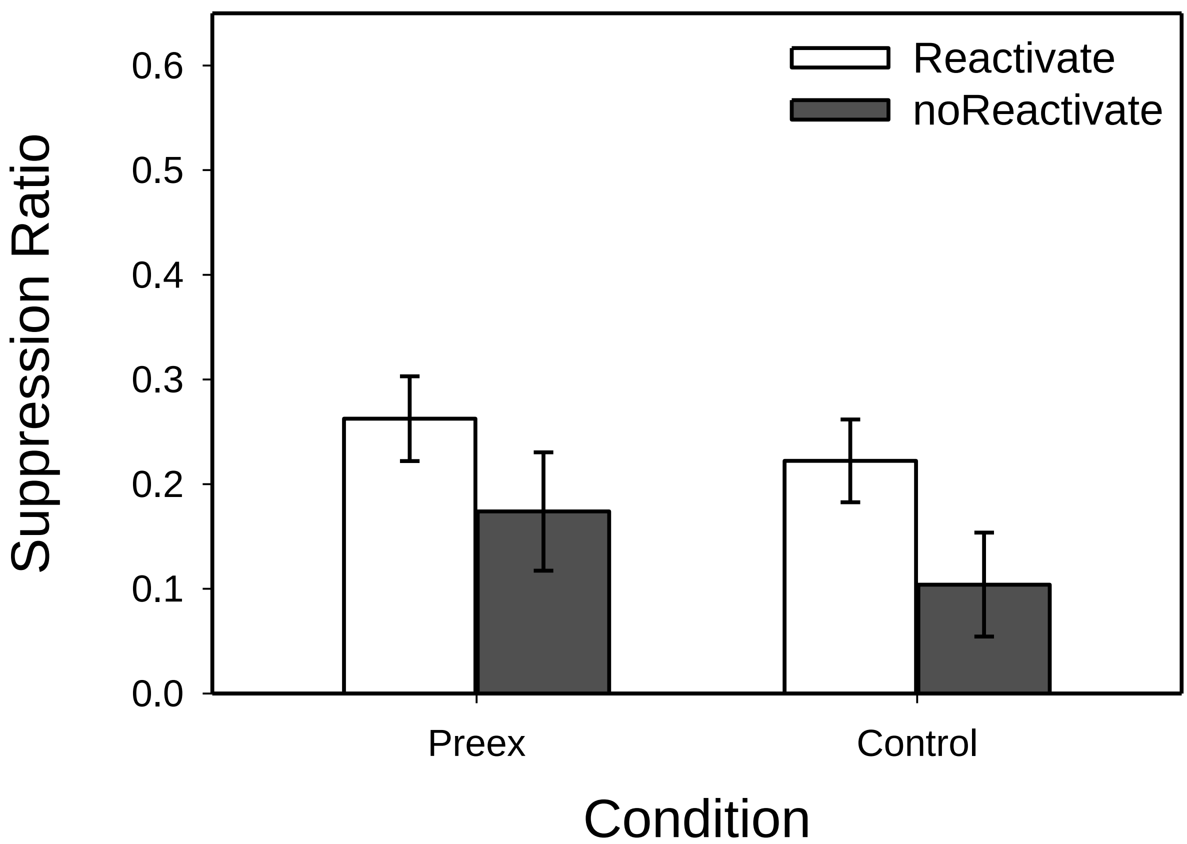
Experiment 3: Extinction



Note. Extinction in Experiment 3. The left panel presents the Preex condition, which received preexposure prior to conditioning, whereas the right panel presents the Control condition, which did not receive preexposure. The Imm condition received extinction 12 min after conditioning, whereas the Del condition received extinction 24 h after conditioning. All subjects received preexposure 24 h prior to conditioning. See text for details.

Figure 9

Experiment 3: Spontaneous recovery test



Note. Spontaneous recovery test in Experiment 3. The Preex condition received both CS preexposure and extinction, whereas the Control condition received only extinction training. The Imm condition received extinction 12 min after conditioning, whereas the Del condition received extinction 24 h after conditioning. All subjects received preexposure 24 h prior to conditioning. See text for details.