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THE INFLUENCE OF EXERCISE ON PERSISTENCE OF FEAR

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THE INFLUENCE OF EXERCISE ON PERSISTENCE OF FEAR

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Dedication

I would like to first dedicate my dissertation to my parents, thank you for always believing in me, even when I didn't believe in myself and reminding me to work hard and play harder.

To Becky Lowndes for being my best friend and constantly motivating me to work harder and be better, you have helped (but mostly convinced) me to see the world and do things I would have never otherwise done.

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The Influence of Exercise on Persistence of Fear

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Pavlovian fear conditioning has been used for almost a century to better understand how fear is acquired and remembered, as well as to find ways to augment the learning and memory process to change memories and create new competitive memories, in an investigation to reduce the persistence of fear. The current work set out to determine if acute and/or chronic exercise could reduce persistence of fear. First, after early life fear exposure (at post-natal day 17 [P17] or 25 [P25]) rats were given a chronic regimen of exercise during late adolescence to determine if exercise could influence memory recall or fear learning (or re-learning) in adulthood. Results indicate a difference in memory based on age of fear exposure, such that rats conditioned at P25 but not P17 show increased levels of freezing when tested in adulthood. While P17 conditioned rats, do not show a fear memory, increases in the average distance run did predict less freezing during the retention test, suggesting an influence on generalized anxiety rather than fear memory directly. Conversely, irrespective of early P25 condition, exercise produced a similar negative correlation on the fear response after adult fear learning; such that increased distances run predicted lower levels of freezing. Following these tests of chronic exercise, acute 30-minutes and 3-hours of exercise prior to extinction/exposure were shown not to enhance reductions in fear on either long-term memory or fear relapse tests, for neither cued nor contextual fear paradigms. Finally, a meta-analytical approach

was employed to disentangle when and under what conditions exercise could enhance fear extinction, but also its influence on other Pavlovian and operant extinction models within the literature. Results indicate that exercise significantly enhanced reductions in responding for operant but not Pavlovian models, with different moderators under both models. Interestingly, it was found that exercising after Pavlovian extinction was critical to larger reductions in responding over extinction alone, which may account for the lack of enhancement seen in my previous findings. Taken together the potential for exercise to reduce the persistence of fear may depend on the timing of application and whether you're targeting learning or memory.

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Chapter 1: Introduction

In the United States alone, 30% of people are affected by anxiety-related disorders in their lifetime (Kessler et al., 2005). Of those, panic and post-traumatic stress disorders (PTSD) make up the vast majority. Both aforementioned disorders have been shown to develop and be maintained through circuitry that overlaps with networks involved in Pavlovian fear conditioning (Delgado, Olsson, & Phelps, 2006; Hofmann, 2008; Milad, Rosenbaum, & Simon, 2014). While learned fear is adaptive, it can persist in instances where a threat is not present leading to a maladaptive state, such as in fear and anxiety related disorders (Rosen & Schulkin, 1998). Rosen and Schulikn (1998) describe anxiety as a state of prolonged anticipatory worry about a potential threat, characterized by an exaggerated fear state, generally without the presence of immediate danger, in which there is a hyper-excitability of the fear circuit that includes hyper-vigilance with increased responsivity.

As numerous people in the United States alone are affected by anxiety-related disorders, it is critical to understand when and under what conditions they develop over time. For example, what is the influence of early life experiences? Additionally, it is important to gain a better understanding of how to both reduce the potential for fear to transition to a maladaptive state and interventions to combat it when it does. Specifically, we know one of the most common approaches to reduce fear responses is through extinction or exposure-based therapies; however, these approaches are subject to relapse and are not 100% effective (for review, see Kaplan, Heinrichs, & Carey, 2011; Vervliet, Craske, & Hermans, 2013). Ultimately, it is critical to find ways to enhance or modify these approaches to produce outcomes that are more favorable.

1.1 FEAR CONDITIONING

Ivan Pavlov first discussed classical conditioning in 1927. He described that through the pairing of a novel stimulus with a stimulus that inherently evokes a response one can train the novel stimulus to elicit a similar response. This paradigm is now commonly discussed in terms of the conditional stimulus (CS) and the unconditioned stimulus (US) eliciting an unconditioned response (UR) which becomes the conditioned response (CR) after the stimuli are paired. In classical Pavlovian fear conditioning in rodents, the CS (typically a tone and/or context) is paired with a US (commonly a footshock) producing a response (freezing). Freezing is a species-specific defensive response, and is thought to represent a normal behavioral response on a continuum based on predatory proximity (Fanselow, 1989). Freezing is conceptualized to represent an ecologically relevant display of associative learning, as rats naturally freeze when a sign or signal (CS) that a predator (US) is close in proximity.

1.2 DEVELOPMENT OF FEAR MEMORIES

The study of the processes that lead to the development of fear and anxiety-related disorders are critical, as aversive or traumatic events early in development can shape later learning (Kosten, Kim, & Lee, 2012). Specifically, exposure to early life aversive or traumatic experiences has been linked to increased rates of anxiety, depression and other psychopathologies (Agid et al., 1999; Carr, Martins, Stingel, Lemgruber, & Juruena, 2013; Fernandes & Osório, 2015; Green et al., 2010; Heim & Nemeroff, 2001; E. A. Young, Abelson, Curtis, & Nesse, 1997).

Previous findings in non-human animal models indicate that fear learning at different periods in early development can lead to different behavioral outputs (B. A. Campbell & Campbell, 1962; J. H. Kim, Li, Hamlin, McNally, & Richardson, 2012),

making it challenging to detect and treat an experience that has the potential to develop into a maladaptive state. Specifically, fear memories can be acquired at post-natal day 12 (P12) indicated by an increased odor avoidance response at P13, but not when trained at P8 (Thompson, Sullivan, & Wilson, 2008). When the period between conditioning and test is increased, Coulter and colleagues (1976) found that animals conditioned at P11-P16 show complete forgetting 42-days after cued fear conditioning. This effect is further highlighted as fear acquired P16-P18 can be exhibited by an increased freezing response 24-hours after training; yet, when these same animals are tested at 48 or more hours post acquisition, the freezing behavior is no longer seen, suggesting the potential of what is called "infantile amnesia" (B. A. Campbell & Campbell, 1962; J. H. Kim et al., 2012; J. H. Kim, McNally, & Richardson, 2006; Li & Richardson, 2013; Travaglia, Bisaz, Sweet, Blitzer, & Alberini, 2016). This pattern of behavior is quite different in animals conditioned a few days later, particularly those conditioned after weaning (P23-25) in which animals show more adult-like behavior of an increased freezing response two or more days after conditioning (B. A. Campbell & Campbell, 1962; J. H. Kim et al., 2012; Travaglia, Bisaz, Sweet, et al., 2016). Spear (1979) suggests that this difference is because events occurring during infancy are easier to "forget".

It is important to note, however, that even in cases of "forgetting" in younger animals, it may not be as if the memory has disappeared or been forgotten, but rather the explicit behavior is no longer seen. Along these lines, previous work has shown persistence in neural activity within the amygdala reflecting that learning has occurred (J. H. Kim et al., 2012), and additional support that after reconditioning in adulthood there is an attenuation in the freezing response, indicating that the original memory may be influencing new memory formation (Sevelinges, Sullivan, Messaoudi, & Mouly, 2008). Additionally, findings from our lab indicate that rats conditioned at P25 (after weaning),

but not P17 (prior to weaning), show an explicit memory for the cue in adulthood, similar to others' previous findings (Akers, Arruda-Carvalho, Josselyn, & Frankland, 2012; Jones & Monfils, 2016; Rudy, 1993; Travaglia, Bisaz, Cruz, & Alberini, 2016; Travaglia, Bisaz, Sweet, et al., 2016). Moreover, the use of a targeted memory reconsolidation-based approach (retrieval+extinction) in adolescence was able to reduce this fear response in rats that showed explicit memory in adulthood. Importantly, rats that were conditioned at P17 did not show explicit memory (freezing to the cue) in adulthood; however, they showed potentiation of fear after experiencing a social reconditioning paradigm. The retrieval-extinction intervention also reduced this potentiated fear responding. These findings suggest that while the rats do not show an initial fear memory in adulthood, the memory is not lost and can be influenced by experiences throughout development. However, when thinking of developing possible translational approaches to target early memories, a cue-specific intervention (such as the one used in Jones & Monfils, 2016), may not be optimal as there may not be an explicit memory for the cue to use during this type of intervention.

1.3 EXTINCTION OF CONDITIONED FEAR

While the acquisition of fear and its memory are widely studied, another behavioral phenomenon, extinction, is a commonly practiced technique to reduce memory of the original CS predicts US relationship. Through presentations of the CS over multiple trials in absence of the aversive US, extinction training leads to decreases in the CR across presentations of the CS alone (Pavlov, 1927; for review see Bouton & Bolles, 1979; Bouton, 2002; Quirk & Mueller, 2008). Over time, the hypothesis as to how this process is reducing responding has varied. Rescorla and Wagner (1972)

suggested there was a decrease in CS associative value, or destruction of the original memory, as a result of prediction error from the US being absent. Others have suggested it may lead to a forgetting effect (Finch & Culler, 1935); yet, some models suggest the possibility of reversal of the acquisition process (Christofi, Nowicky, Bolsover, & Bindman, 1993; Mauk & Ohyama, 2004). Currently, extinction is believed to be a new learning experience that forms a competitive memory: CS does not predict US. Support for this comes from a differentiation of the neural mechanisms involved in the initial acquisition of fear and those recruited during the extinction process (Milad & Quirk, 2002; Quirk, Russo, Barron, & Lebron, 2000; Sotres-Bayon & Quirk, 2010). In addition to initial observations by Pavlov suggesting that while extinction was sufficient to reduce conditioned responding initially, behavior could reemerge or relapse (Pavlov, 1927). This occurred after three distinct memory challenges: renewal, or re-exposure to the original conditioning context (Bouton & Bolles, 1979; Rodriguez, Craske, Mineka, & Hladek, 1999), reinstatement, exposure to the US without the CS (Bouton & Wartzen Truber, 1991; Dirikx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2004; Hermans et al., 2005; Rescorla, 1996), and spontaneous recovery, or the passage of time (Robbins, 1990). Across all three of these phenomena, there is a return of fear expression even after low levels of freezing were achieved during extinction procedures, suggesting that some form of the original memory trace may still exist. Additional work has shown that even after extinction learning and relapse in responding rats re-exposed to the extinction procedure show faster within session extinction behavior, which also suggests there is a persistent memory trace for the extinction memory as well (Quirk, 2002). These findings together may indicate that there is in fact one memory trace for the initial acquisition memory that competes with a new memory for the extinction learning and dependent on other

experiences, such as time and context, may delineate what behavior is expressed in response to the CS.

Interestingly, extinction behavior develops differentially over a life-time, while it has been shown that applying extinction training immediately after conditioning in younger P17 rats can lead to "unlearning", or no memory trace when tested later (J. H. Kim, Hamlin, & Richardson, 2009; J. H. Kim & Richardson, 2007a, 2007b), however the ability to apply extinction immediately in humans in a clinical practice may not be an available option in all circumstances. Additionally, while adolescence is a critical period in which a number of anxiety disorders are first diagnosed (Kim-Cohen et al., 2003), there is also impaired extinction retention during this period (J. H. Kim, Li, & Richardson, 2011; McCallum, Kim, & Richardson, 2010; Pattwell et al., 2012). In particular, P35 (adolescent) rats show similar acquisition of extinction to P24 (preadolescent) and P70 (adult) rats; however, when later tested, they show failure to maintain extinction (J. H. Kim et al., 2009). Taken together, extinction alone may not be sufficient in all instances to reduce the persistence of fear.

1.3.1 Neural basis of fear extinction

Because extinction principles are at the core of exposure models for reducing fear and anxiety in human subjects, the neural mechanisms of extinction have been extensively studied. Particularly, much focus has been on the medial prefrontal cortex (mPFC) in rodents and humans. This region has been of particular interest, as it's been associated with cognitive function and decision making (Euston, Gruber, & McNaughton, 2012; Lapiz & Morilak, 2006; López-Ramos, Guerra-Narbona, & Delgado-García, 2015; Trantham-Davidson et al., 2014). Neural connections between the mPFC, amygdala and

hippocampus have also been shown to regulate fear and emotional memories (Hübner, Bosch, Gall, Lüthi, & Ehrlich, 2014; A. Ishikawa & Nakamura, 2006). The amygdala and hippocampus are thought to be primary neural processing regions for the formation of new associations during fear acquisition in both humans and non-human animals (J. J. Kim & Fanselow, 1992; Knight, Smith, Cheng, Stein, & Helmstetter, 2004; LeDoux, 1992; Phillips & LeDoux, 1992; Romanski, Clugnet, Bordi, & LeDoux, 1993).

Work in humans found that in fact the prefrontal cortex and amygdala are preferentially enhanced during extinction (Gottfried & Dolan, 2004). Additional work indicated that activation in the amygdala was correlated to the CR in early extinction (Phelps, Delgado, Nearing, & LeDoux, 2004), whereas activation in the ventral mPFC (vmPFC) was seen after testing the extinction memory. These results indicate that the vmPFC and amygdala are inversely related and that the vmPFC may regulate fear responses mediated by the amygdala.

In addition to work in humans, a great deal of work in non-human animals has been done to pinpoint the neural mechanisms of extinction at different points of the extinction process. Similarly to work in humans, vmPFC has been shown in rodents to produce an inhibitory response on the lateral amygdala (Rosenkranz, Moore, & Grace, 2003), with lesions of the mPFC producing deficits in extinction retention (Lebrón, Milad, & Quirk, 2004; M. A. Morgan, Romanski, & Ledoux, 1993; Quirk et al., 2000). In particular, after extinction procedures, there is a time dependent change in synaptic plasticity within mPFC neurons (Herry, Vouimba, & Garcia, 1999), with an increase in burst spiking that is also correlated with extinction recall (Santini, Quirk, & Porter, 2008). Further support for the mPFC role in extinction comes from the fact that microstimulation of this region produces a facilitatory response on retention of extinction responding (Milad & Quirk, 2002; Milad, Vidal-Gonzalez, & Quirk, 2004). Interestingly,

spontaneous recovery of fear is associated with a prolonged long-term depression of synaptic transmission within the mPFC in addition to the failure of induction of the immediate-early genes c-Fos and zif268 (Herry & Mons, 2004). This effect on immediate-early gene expression however was not specific to the mPFC but was also shown in the basolateral nucleus of the amygdala (BLA). Additional support for the interconnection of the mPFC and amygdala in fear extinction comes from data indicating that prestimultaion of the mPFC reduces responsiveness from input connections of the insular cortex and BLA to the central nucleus of the amygdala (CeA; Quirk, Likhtik, Pelletier, & Paré, 2003), which is the output to the brainstem and hypothalamic sites mediating behavioral responses (Holstege, Bandler, & Saper, 1996). However the modulation of the mPFC and BLA differ on when they are required for successful extinction, such that inactivation of the infralimbic cortex (IF) of the mPFC prior to extinction training, whereas inactivation of the BLA following limited extinction both facilitate extinction responding (Akirav, Raizel, & Maroun, 2006).

While there are complex interactions between the mPFC and amygdala during the extinction process, there are also critical interactions between the mPFC and the hippocampus. Specifically, there are long-term potentiation (LTP) –like changes in hippocampal inputs into the mPFC immediately following fear extinction, which can be blocked by infusion of a mitogen-activated protein kinase (MAPK) cascade inhibitor into the mPFC or low-frequency stimulation in the hippocampus (Farinelli, Deschaux, Hugues, Thevenet, & Garcia, 2006; Hugues, Chessel, Lena, Marsault, & Garcia, 2006). Additionally, work looking at coupling of theta oscillations during extinction found a decline in the network between the hippocampus-lateral amygdala-mPFC, which during recall coupling only between the amygdala-mPFC and hippocampus-mPFC were present (Lesting et al., 2011). This in addition to work showing that the inactivation of the dorsal

hippocampus does not affect the enhancing effects of norepinephrine in the BLA (Berlau & McGaugh, 2006), which may suggest that extinction responding is not mediated by an interaction between the amygdala and hippocampus directly. The hippocampus alone has also been shown to be critical for extinction behavior, as infusion of the protein synthesis inhibitor anisomycin into the hippocampus immediately after a single retention test was sufficient to block extinction, indicating that the initiation of fear extinction requires protein synthesis (Vianna, Szapiro, Mcgaugh, Medina, & Izquierdo, 2001).

Extinction is also modulated by a variety of neural mechanisms. One critical neuromodulator is brain derived neurotrophic factor (BDNF). Both humans and rodents with a polymorphism that reduces BDNF release (Chen et al., 2006) are impaired in extinction learning (Soliman et al., 2010). Additionally, BDNF within the three brain regions discussed previously is critical throughout the extinction process. Specifically, extinction training increases BDNF mRNA in IL mPFC (Bredy et al., 2007), whereas knocking down BDNF in the hippocampus blocks extinction behavior (Heldt, Stanek, Chhatwal, & Ressler, 2007), similarly blocking BDNF in the amygdala leads to deficits in extinction consolidation (Chhatwal, Stanek-Rattiner, Davis, & Ressler, 2006). Interestingly, recently work has shown that BDNF infused directly into the IL mPFC was sufficient to produce extinction-like levels of responding even in absence of extinction procedures (Peters, Dieppa-Perea, Melendez, & Quirk, 2010; Rosas-Vidal, Do-Monte, Sotres-Bayon, & Quirk, 2014).

Similarly to BDNF, dopamine (DA) has also been found to critically modulate extinction behaviors, such that antagonists not only in the IL mPFC (Hikind & Maroun, 2008; Mueller, Bravo-Rivera, & Quirk, 2010; Pfeiffer & Fendt, 2006), but also the nucleus accumbens (Holtzman-Assif, Laurent, & Westbrook, 2010), a brain region known to increase DA signaling after fear experience (Sorg & Kalivas, 1991; A. M.

Young, 2004), inhibits extinction behavior. A variety of drugs known to upregulate DA have also been shown to enhance extinction, specifically methylphenidate (also known as Ritilan) has been shown to be beneficial when injected systemically (Abraham, Cunningham, & Lattal, 2012), but also when infused directly into the CA1 region of the hippocampus prior to or immediately following extinction procedures (Furini et al., 2017). The drug L-dopa, a dopamine precursor, has also been shown to have various beneficial effects including blocking reinstatement, renewal and spontaneous recovery (Haaker et al., 2013). These effects of L-dopa were also extended to human work in which a group receiving L-dopa showed reduced neural activity in areas related to conditioned fear (such as the amygdala and hippocampus), in addition to enhanced activity in regions critical for extinction and extinction retrieval (including the vmPFC; Haaker, Lonsdorf, & Kalisch, 2015). These enhancing effects of dopamine agonists on extinction can also be modulated through other monoamines action on dopamine, particularly a serotonin 1A receptor (5HT1AR) agonist, blocked deficits in extinction related to early life stress in mice, through an increase in mPFC dopamine release (Saito et al., 2013).

1.3.2 Extinction-based therapies for treatment of anxiety disorders

Currently, one of the most common behavioral approaches to treat a variety of anxiety related disorders in clinical practice is exposure therapy (Morrison & Ressler, 2014). This approach uses extinction-based principles and procedures in attempts to reduce anxiety-related symptoms. During extinction, the CS is presented repeatedly without the US, resulting in diminished CR. During exposure therapy a patient is exposed to the CS or CSs in a controlled setting (e.g. Virtual reality training) in a repeated fashion

similar to extinction, allowing for a new CS-noUS association to form. Using exposure therapy to treat such disorders as PTSD, specific phobias, and social anxiety disorder have been widely successful (Olatunji et al., 2010; Abramowitz, 2013; Cuijpers et al., 2013).

While this approach has shown success, it is not without challenges, as a number of studies have shown that patients with anxiety disorders specifically show deficits in extinction learning compared to healthy controls (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Michael, Blechert, Vriends, Margraf, & Wilhelm, 2007). In addition, there are cases of low treatment acceptance (Choy, Fyer, & Lipsitz, 2007), and 20% or more dropout rates (Barlow et al., 2000; Taylor et al., 2001), as many patients find the therapy itself aversive. Assessments have also found non-response rates as high as 50% (Schottenbauer, Glass, Arnkoff, Tendick, & Gray, 2008). Furthermore, exposure, like extinction, is subject to renewal, reinstatement and spontaneous recovery (for review see Boschen, Neumann, & Waters, 2009). As such, it imperative to better understand the mechanisms through which extinction processes work, and to find ways to improve responding, long-term success, and procedures to reduce the span of therapy such that more patients remain compliant and enrolled in treatment.

1.4 EXERCISE

Exercise has long been studied for its numerous beneficial effects on one's physical health, but in addition to physical health exercise has been shown to produce a variety of positive effects on mental health and the brain, including being neuroprotective and creating stress resilience and resistant states (Cotman, Berchtold, & Christie, 2007; Gates, Killackey, Phillips, & Álvarez-Jiménez, 2015; Knapen, Vancampfort, Moriën, &

Marchal, 2015; J. A. Morgan, Corrigan, & Baune, 2015; Park et al., 2013; van Praag, Fleshner, Schwartz, & Mattson, 2014). The beneficial effects of exercise also extend to enhancing cognitive function (see review Hötting & Röder, 2013) as well as improvements on a variety of learning and memory tasks (Baek, 2016; Cassilhas, Tufik, & de Mello, 2016; Diederich et al., 2017).

1.4.1 Exercise, stress and anxiety

As mentioned above, one of the beneficial effects of exercise is that it promotes stress resistance and resilience (for review see Greenwood & Fleshner, 2011). This is particularly important because exercise could increase resistance to the development of stress-related psychiatric disorders, including depression, anxiety, and PTSD (Asmundson et al., 2013; Greenwood et al., 2013; Sciolino & Holmes, 2012; Sothmann et al., 1996). The effects of exercise on stress are of particular interest because stress has been shown to potentiate fear acquisition (C. D. Conrad, LeDoux, Magariños, & McEwen, 1999; Hoffman et al., 2015; Lukkes, Mokin, Scholl, & Forster, 2009; Zhang & Rosenkranz, 2013), promote fear persistence generally (Yang et al., 2013), enhance anxiety-like behaviors (K. L. Conrad, Louderback, Gessner, & Winder, 2011; Lukkes et al., 2009; Roth et al., 2012), while inhibiting extinction acquisition and memory retrieval (Chauveau et al., 2012; Izquierdo, Wellman, & Holmes, 2006; Knox et al., 2012; for review see Stockhorst, & Antov, 2015), and contributing to the persistence of fear in disorders such as PTSD (Hoffman, Lorson, Sanabria, Foster Olive, & Conrad, 2014). Exercise specifically poses as a candidate option as it promotes better coping strategies by reducing exaggerated negative symptoms under stressed conditions, in humans (Broocks et al., 2001) and other non-human animals (Greenwood, Foley, Burhans, Maier,

& Fleshner, 2005; Greenwood, Strong, Brooks, & Fleshner, 2008; Greenwood, Strong, Dorey, & Fleshner, 2007). In addition, exercise may promote reduction of fear, through lowering levels of anxiety across a variety of conditions (Asmundson et al., 2013; Fuss et al., 2010; Greenwood et al., 2008; Motaghinejad, Fatima, Karimian, & Ganji, 2016; Pietrelli, Lopez-Costa, Goñi, Brusco, & Basso, 2012; Salam et al., 2009). This is important, as those exposed to an early life trauma develop general anxiety symptoms such as increased startle responses (Bazak et al., 2009; Jovanovic et al., 2009), or decreased locomotor behavior (J. Ishikawa, Nishimura, & Ishikawa, 2015), such that exercise may act to reduce these generalized responses.

One mechanism through which exercise may be acting to produce these effects on stress and anxiety is through BDNF (Holmes, 2014). It is known that both chronic and acute exercise increase BDNF within the brain (Huang et al., 2006; Marais, Stein, & Daniels, 2009; Soya et al., 2007; Van Hoomissen, Chambliss, Holmes, & Dishman, 2003). Additionally, selective serotonin reuptake inhibitors (SSRIs), which are commonly used to treat depression and anxiety disorders, produce downstream effects to upregulate BDNF (Nibuya, Morinobu, & Duman, 1995), such that if BDNF's primary receptor trkB is blocked, the beneficial effects are no longer seen (Monteggia et al., 2004; Rantamäki et al., 2007; Saarelainen et al., 2003). SSRIs have also been shown to facilitate extinction responding (Deschaux, Spennato, Moreau, & Garcia, 2011b; Karpova et al., 2011), but this effect is blocked by BDNF depletion and inhibition of the trkB receptor (Heldt et al., 2014). It is important to note that while deficits in hippocampal BDNF caused by stress have been implicated in a variety of anxiety disorders, an effect exercise has been shown to protect against, direct infusion of BDNF into the hippocampus was not sufficient to recover the negative behavioral consequences of stress (Greenwood, Strong, Foley, Thompson, & Fleshner, 2007), suggesting that BDNF may not be the sole mechanism by

which exercise is working to reduce the negative consequences of stress on persistence of fear.

One additional mechanism via which exercise may be operating to reduce persistence of fear is monoamines, as previous reports have shown that under stressed conditions, there is an increase in norepinephrine release in a number of stress-related limbic forebrain regions and that microinjection of an adrenergic antagonist into these regions can block anxiogenic effects of stress (Morilak et al., 2005). Exercise can also mimic this anxiogenic effect as exercise amplifies galanin expression in the locus coeruleus (LC) which in turn suppresses stress-induced activity of the LC, resulting in a reduction of norepinephrine output in LC-target regions (Sciolino & Holmes, 2012). The serotonergic system may also be involved, as agonists of 5-HT2CR reduce social exploration and open arm entries while increasing shock-elicited fear (B. M. Campbell & Merchant, 2003; Christianson et al., 2010; de Mello Cruz et al., 2005), an effect that can be countered by voluntary exercise, which produces changes in 5-HT2CR mRNA in the BLA (Greenwood et al., 2012).

1.4.2 Exercise as a direct augmentation to extinction or exposure therapy

Previously, both behavioral and pharmacological augmentations have shown success in enhancing extinction and exposure outcomes. These include agents such as D-cycloserine (DCS), selective serotonin reuptake inhibitors (SSRIs) and CB1 agonists (Deschaux, Spennato, Moreau, & Garcia, 2011a; Guastella, Lovibond, Dadds, Mitchell, & Richardson, 2007; Karpova et al., 2011; Marsicano et al., 2002; Walker, Ressler, Lu, & Davis, 2002; for review see Fitzgerald, Seemann, & Maren, 2014). Yet, DCS, an agent that is beneficial across fear extinction and exposure-based therapies, has also lead to

persistence of fear responding in some cases (Bolkan & Lattal, 2014; Bouton, Vurbic, & Woods, 2008; Smits et al., 2013; Weber, Hart, & Richardson, 2007).

In addition to these negative responses to pharmacological approaches, people often prefer non-pharmacological interventions as treatment across psychological disorders (Arch, 2014; Mchugh, Whitton, Peckham, Welge, & Otto, 2013; Roy-Byrne, Berliner, Russo, Zatzick, & Pitman, 2003). One such behavioral approach shown to augment extinction pairs a single memory retrieval cue with extinction procedures. In this treatment a single CS is presented in close proximity (at least 10-minutes but less than 6-hours) prior to the extinction session. By using this approach, the single CS presentation initiates a retrieval of the memory making the memory trace liable and subject to modification (Monfils, Cowansage, Klann, & LeDoux, 2009). The modification then occurs through the application of extinction procedures, which produce a reduction of fear, leading to a more persistent reduction in fear responding. Interestingly, the use of this behavioral paradigm has led to augmentation effects across a variety of other extinction models including other Pavlovian models using appetitive stimuli (Flavell, Lambert, Winters, & Bredy, 2013; Olshavsky, Jones, Lee, & Monfils, 2013), operant models (Millan, Milligan-Saville, & McNally, 2013; Xue et al., 2012) and across models using human subjects (Schiller et al., 2010; Xue et al., 2012); for review see Kredlow, Unger and Otto (2016).

This method, while showing success across different models of extinction has also lead to instances where retrieval+extinction did not enhance or produce persistent reductions in responding (Chan, Leung, Westbrook, & McNally, 2010; Flavell et al., 2013; Millan et al., 2013), suggesting that other approaches may be necessary to promote persistent fear reductions. One such approach may be behavioral augmentation of extinction with exercise. As previously mentioned exercise can promote stress resilience

and resistance, as well as improve cognitive flexibility, both of which are critical in extinction and exposure procedures (Cabrera, Chavez, Corley, Kitto, & Butt, 2006; Stockhorst et al., 2015). However, exercise may also work more directly to influence learning and memory processes during extinction. As previously mentioned exercise is known to upregulate BDNF (Huang et al., 2006; Neeper, Gómez-Pinilla, Choi, & Cotman, 1995, 1996; Soya et al., 2007; Uysal et al., 2015), we also know BDNF can enhance a variety of learning and memory tasks (for review see Bekinschtein, Oomen, Saksida, & Bussey, 2011). Specifically, BNDF is critical for extinction of appetitive and aversive memories (Chhatwal et al., 2006; Heldt et al., 2014, 2007; Kirtley & Thomas, 2010), such that direct infusions of BDNF into the IL mPFC can enhance appetitive extinction memories (Otis, Fitzgerald, & Mueller, 2014), whereas BDNF in the IL mPFC during Pavlovian fear extinction is not only necessary but also sufficient on its own to produce fear extinction responses (Peters et al., 2010; Rosas-Vidal et al., 2014). This suggests that if BDNF is not only necessary, but in some cases sufficient to produce extinction behavior, the upregulation of BDNF from exercise paired with extinction procedures may lead to enhanced extinction learning or memory and a more persistent reduction of fear.

A second potential mechanism, DA, could be modulating the effect of exercise on extinction in two ways. First, in extinction or exposure models of substance abuse or those using rewarding stimuli, there is a unique feature not present in aversive or fear based models, in that the drug itself can induce neurological changes. Therefore, periods of abstinence from these drugs can produce negative and stressed states that are related to changes in neural activity. One specific neuroadaptation is a reduction in DA function (Rossetti, Melis, Carboni, & Gessa, 1992), for which exercise can be used to restore signaling (O'dell, Galvez, Ball, & Marshall, 2012; Robertson et al., 2016), thereby

protecting against relapse. Secondly, work in Pavlovian models using both appetitive and aversive stimuli suggest that deficits induced by DA antagonists produce impaired extinction (Hikind & Maroun, 2008; Holtzman-Assif et al., 2010). Conversely, enhancing DA levels strengthens extinction consolidation (Abraham et al., 2012; Abraham, Neve, & Lattal, 2016). Furthermore, L-dopa administered immediately after extinction reduced fear renewal, reinstatement and spontaneous recovery in mice, as well as reduce renewal in humans (Haaker et al., 2013). Taken together with the fact that exercise has been shown to directly upregulate DA, evidence suggests that exercise, too, could facilitate extinction through a DA mechanism (Foley & Fleshner, 2008; Greenwood et al., 2011; Hattori, Naoi, & Nishino, 1994; Heyes, Garnett, & Coates, 1988; Meeusen et al., 1997).

1.5 Specific Aims

In this dissertation, I will explore three specific aims and hypotheses to better understand how exercise can be applied to reduce the persistence of fear. In chapter 2, I will first explore how fear exposure in early life (P17 or P25) affects fear memories in adulthood. Then, through the application of chronic exercise during adolescence, I will determine if exercise can reduce adult fear memories or fear learning after adult conditioning or reconditioning. Next, five experiments in chapter 3 will explore the use of acute exercise prior to extinction, to determine if exercise can augment extinction acquisition or consolidation. These experiments will test exercise's potential effects across humans and rats, as well as across extinction modalities: contextual and cued fear, and multiple tests, including, long-term memory and after potential relapse inducing tests. In my third aim (Chapter 4), I will use a structural equation modeling approach to meta-analysis (meta-SEM) to explore the effects of exercise as an augmentation strategy to

extinction across disciplines. The goal of this work is to determine if exercise applied with extinction procedures (or exposure therapy in human participants) produces a significant positive overall effect. Then, overall data will be tested for potential moderating effects based on extinction model (operant or Pavlovian) and/or stimulus type (appetitive or aversive). Once it is established if either procedure or stimulus type has a significant moderating effect the data will be partitioned based on these effects. Partitioned data will then be tested to determine if these effects are differentially moderated by parameter variables within the primary study design.

Chapter 2: Differential effects of predictable vs. un-predictable aversive experience early in development on fear memory and learning in adulthood: How an adolescent intervention of exercise affects adult memories and learning.

Work within this chapter will be submitted for publication in:

Roquet, R. F., Seo, D., Jones, C. E., & Monfils, M. H. Differential effects of predictable vs. un-predictable aversive experience early in development on fear memory and learning in adulthood.

All authors contributed to experimental design. Data from experiment 1 was collected and analyzed by DS. Whereas experiment 2 was analyzed by CEJ, with behavioral tests run by RFR. The writing of the primary manuscript was shared between RFR and DS. All data from supplemental findings, were collected, analyzed and written up by RFR.

2.1 ABSTRACT

We examined the enduring effects of predictable vs. unpredictable fear acquisition early in life on memory and re-learning in adulthood. At post-natal day 17 or 25 (P17 or P25), rats either remained naïve, or were fear conditioned using paired (predictable) or unpaired (unpredictable) presentations of white-noise and foot-shocks. At 2 months of age (adulthood), each group was fear conditioned (or re-conditioned) with either paired or un-paired training, and then was tested for fear extinction the next day. Initial findings replicate previous work from our lab and demonstrate a difference in memory retention based on age of acquisition. Specifically, rats conditioned at P25, but not P17, using paired conditioning procedures show increased freezing to the cue when tested in adulthood. Following retention tests, our results show that both paired and

unpaired conditioning at P17 potentiated adult paired conditioning using the same cue as in early life. Paired conditioning at P25 potentiated adult conditioning across both adult paired and unpaired groups, whereas unpaired training in early life did not have an effect on memory and reacquisition in adulthood. These findings suggest that early predictable vs. unpredictable aversive learning at P17 or P25 differentially modulate memory retention and future learning, such that follow up tests explored an adolescent intervention of chronic exercise to reduce the persistence of fear. Results show that average distance run during late adolescence of P17 conditioned rat's produces a negative relationship with freezing during the memory retention test in adulthood, such that longer distances run produced less freezing. Secondly, independent of early life conditioning, there is a trend for a negative relationship in freezing after adult conditioning in P25 experimental rats based on the average distance run during late adolescence. Taken together the influence of chronic exercise may be indirectly through its influence on stress and anxiety rather than learning or memory directly.

2.2 INTRODUCTION

Behavioral adjustments through experience are important for organisms to adapt and survive in changing environments. During early life development, learning and memory processes are more plastic than in adulthood, such that experiences during childhood have a profound impact on shaping adult behavior (Arenas, Fernández, & Farina, 2009; Schäble, Poeggel, Braun, & Gruss, 2007). Importantly, evidence suggests differential modulation of fear acquisition and reduction throughout development (Esmorís-Arranz, Méndez, & Spear, 2008; Kim, Hamlin, & Richardson, 2009; Moriceau & Sullivan, 2006; Pattwell, Bath, Casey, Ninan, & Lee, 2011; Travaglia, Bisaz, Sweet,

Blitzer, & Alberini, 2016) with aversive experiences throughout early development producing a variety of outcomes on adult behavior (Hunt et al., 2007; Jones & Monfils, 2016; Sevelinges et al., 2007; Travaglia, Bisaz, Sweet, et al., 2016). Additionally, support from human literature indicates that early life aversive or traumatic experiences can be linked to increased rates of anxiety, depression, and other psychopathologies throughout a life-time (Anda et al., 2006; Carr, Martins, Stingel, Lemgruber, & Juruena, 2013; Fernandes & Osório, 2015; Heim & Nemeroff, 2001).

One phenomena, known as "infantile amnesia", first identified by Campbell and Campbell (1962), states that younger animals readily forget a cue elicited memory (within 7 days of acquisition). More recently, findings from our lab and others have shown that rats conditioned at post-natal day 24 or 25 (P24/P25), but not post-natal-day 17 (P17), show a memory for the conditioned cue in adulthood (Jones & Monfils, 2016; Travaglia, Bisaz, Sweet, et al., 2016). Notably, rat models of fear learning and memory have found the weaning age (or the period when rats are removed from their mothers and become independent), which occurs at approximately postnatal day (P) 21, appears to be a particularly important turning point in shaping the effects of early life experience. For example, cued fear conditioning can readily be acquired before the weaning period, but contextual fear conditioning does not emerge until P23 (Rudy, 1993; Stanton, 2000). In addition, extinction of fear can be acquired in pre-weaning rats, but unlike the post-weaning period, these rats do not require the medial prefrontal cortex (Kim et al., 2009). This recruitment of different neural substrates is thought to be due to the immaturity of relevant connections (e. g. between amygdala and hippocampus) within the circuitry that

is responsible for fear acquisition and modulation (Foster & Burman, 2010; Travaglia, Bisaz, Sweet, et al., 2016; Yap & Richardson, 2005). These immaturities may affect not only long-term retention of early fear memories but potentially lead to differences in later learning (Coulter, Collier, & Campbell, 1976).

The idea that early life fear exposure may affect later learning is supported by additional findings that rats who do not express adult fear retention after P17 conditioning exhibit a potentiation in responding when exposed to a social fear conditioning procedure in adulthood (Jones & Monfils, 2016). This effect, however, was not seen in rats previously conditioned at P25, suggesting a difference in the effect that early life fear exposure may have on learning in adulthood. Work by Sevelinges and colleagues also indicate that contingency of early life fear exposure is critical, as paired but not unpaired conditioning to an odor-shock paradigm at P8-12 produced an attenuation on future fear learning in adulthood (Sevelinges et al., 2007; Sevelinges, Sullivan, Messaoudi, & Mouly, 2008). Taken together, the timing and predictability of conditioning procedures in early life may produce differential effects on later learning, making behavioral disambiguation of the effects of early events on later experience important to enhance our understanding of the factors that influence memory retention and the modulation of future learning. In the current study, the goal was to specifically examine whether there are differential effects of predictable versus unpredictable (paired vs. unpaired) fear acquisition early in life (P17 and P25) on adult fear memory and conditioning (or re-conditioning).

2.3 METHODS

2.3.1 Subjects

Sprague-Dawley rats (250-300 grams, from Harlan) were used for breeding at the University of Texas at Austin. For the first experiment, offspring from 17 breeding pairs were used (n=99). All breeding pairs were left together for approximately two weeks before the male breeder was removed and the female was left housed individually until pups were born. Females were checked daily at 10AM to determine if births had occurred, on the first day pups were present this was marked as the date of birth and considered P0. When pups reached either P17 or P25, all rats except controls that remained naïve, received either paired or unpaired presentations of the CS (white noise) and US (footshock). Only one male per litter was used per group in order to avoid litter effects. All animals were weaned at 21 days of age and housed two or three animals per cage on a 12h/12h light/dark cycle with food and water *ad libitum*. Our basic principle regarding housing for this project was to match the cagemates on their adult experimental treatment condition. As such, at weaning, the rats were housed with cagemates that received a different treatment at P17 or P25 (naïve, paired, and unpaired). For the first experiment, rats from a given litter were used for either the P17 or P25 experiment (not both).

For the second experiment, offspring from six breeding pairs were used to assess long term memory 24-hours after paired conditioning at either P17 or P25 (n=24). All rats were conditioned using paired conditioning procedures detailed below or remained

naïve. All groups included one rat from each breeding pair. Upon weaning, rats were housed with a brother until termination of the experiment. Behavioral training and testing were performed during the rats' dark phase under dim light. The studies were approved by the IACUC of the University of Texas at Austin.

2.3.2 Apparatus and procedures

Four identical conditioning chambers (Coulbourn Instruments, Allentown, PA) were used to fear condition the rats. The chambers were equipped with stainless-steel rod floors (at .5cm thickness, designed for mice such that infant rats could not slip through the bars into the lower pan of the chamber) connected to a shock-generator (Model H13-15; Coulbourn Instruments). Each chamber was enclosed in a sound attenuated box. All experimental procedures were controlled by a computer program (Freezeframe; Coulbourn Instruments) which allowed to precisely administer paired or unpaired white noise and footshock presentations. To provide a different context for the extinction phase, the inside of conditioning chamber was modified with black flat plastic floors (rather than metal grids) and the outside of the plexiglass walls of the conditioning box were covered with paper with alternating black and white lines.

2.3.3 Training

For the first experiment, training was as follows. Pups were conditioned to a CS (white noise 80dB, 10s) and US (0.6mA, 1s) at P17 or P25. After 2-minutes of adaptation, one group received four trials of paired training, where the CS co-terminated with the US. The other group received four trials of unpaired training, where the CS and US did not overlap. The inter-trial interval (ITI) was varied from 85s to 135s with a mean

ITI of 110s. For the unpaired condition, the total duration spent in the conditioning apparatus was kept the same as the paired group. The ITI between stimuli (CSs and USs) varied between 35 and 85, with a mean ITI of 55. During P17 conditioning, vanilla extract (McCormick) was put on the mother's nose to prevent her from discriminating between pups that were removed for training and pups who remained with her. Rats rely on their olfaction to identify their pups and we have found that when applying a small amount of vanilla on the mother's nose, she does not interact differently with the pups that were taken away and those that remained with her. After each session, the conditioning chamber was rinsed with water, followed by 70% ethanol (EtOH). A naïve group of rats were separated from their mother in an opaque plastic box (30 cm×20cm×15 cm) for 10-minutes, the same amount time that animals in other conditions spent for conditioning procedure. Naïve rats did not receive CS or US presentations. At two months of age, each group of animals received either another two trials of CS-US paired or un-paired training, and were tested for fear extinction the next day. For the fear extinction phase, after a 2-minute adaptation period, the 10s CS (80 dB, White Noise) was presented 18 times with a 60s ITI in a different context chamber (Figure 2.1A).

For the second experiment, all rats were tested for long term memory 24-hours after paired conditioning or removal from the homecage (naïve condition). Long-term memory was assessed in the same context half the rats were conditioned in the previous day (Figure 2.1B), using a 5-minute adaptation period followed by four, 10s CS (80 dB, White Noise) presentations with a varied ITI, mean 110s and ranging between 85s to

135s, similar to that used for the conditioning session the previous day, but in a reverse presentation.

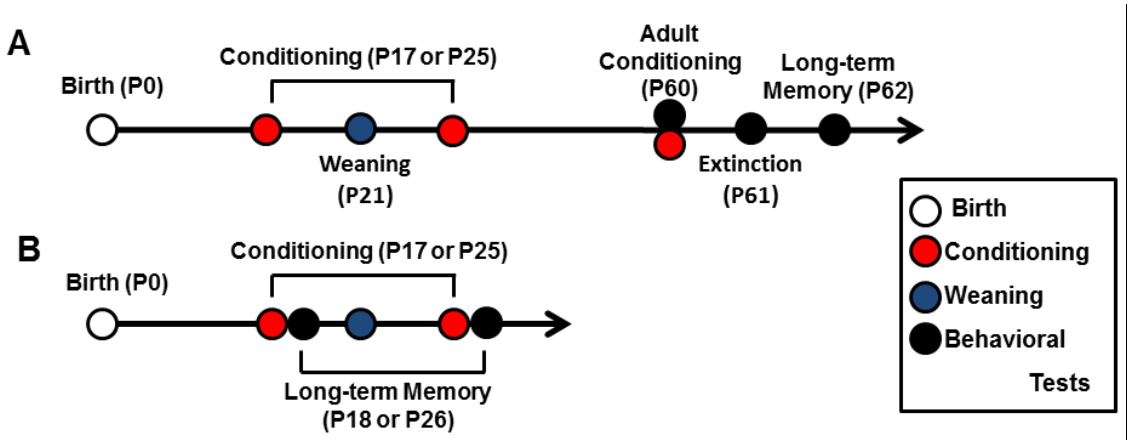

Figure 2.1 Schematic diagrams of the experimental procedures. (A) In experiment 1 pups were conditioned using either paired or unpaired procedures, or remained naïve at P17 or P25. At two months of age, each group was tested for memory to the context and the cue followed by either paired or un-paired CS-US training, 24-hours later rats went through cued fear extinction, and finally tested for LTM the next day. (B) Experiment 2 consisted of pups receiving paired conditioning or naïve procedures at P17 or P25 followed by LTM testing the following day. LTM=Long-term Memory, CS= Conditioned Stimulus and US= Unconditioned Stimulus.

2.3.4 Freezing measurement

Freezing behavior was defined as the absence of all bodily movement, excluding respiration related movement, and was quantified as total percent time during the 10-seconds for each CS presentation. Baseline freezing was collected during the 10-seconds before the 1st CS.

2.3.5 Statistical analysis

All freezing data were analyzed with Prism6 (Graphpad software, La Jolla, CA). All data are presented as mean±SEM. Early fear memory retention was tested with one-way ANOVA, and adult fear extinction freezing was tested with a single-factor repeated-measures ANOVA using 3 different groups as a between subjects factor for each adult training condition (e.g. NP vs. UP vs. PP or NU vs. UU vs. PU; the 1st character represents infant conditioning type, and 2nd represents adult conditioning type: N, Naïve; P, Paired; U, Un-paired). If there were main effects or interactions, *Tukey's test* were used for *post-hoc* analysis. The null hypothesis was rejected at the $p < 0.05$ level.

2.4 RESULTS

Our first experiment tested the effects of predictable vs. un-predictable aversive learning early in development on adult fear conditioning. Rats were first trained at P17 or P25 with a Pavlovian fear conditioning paradigm using either paired or un-paired CS-US training or remained naive, then when rats reached 2 months of age, each group received either paired or un-paired CS-US training. The next day, rats received extinction training followed, 24-hours later, by a long-term memory test. The number of rats used in each group was as follows: NP:9, NU:8, PP:8, PU:8, UP:8 and UU:8 in P17 groups; NP:9, NU:9, PP:8, PU:8, UP:8 and UU:8 in P25 groups. In a second experiment, we examined whether rats that were fear conditioned at P17 or P25 froze to the CS the next day (P18 or P26), both groups included 12 rats each. All rats were included in the statistical analysis.

2.4.1 Experiment 1

2.4.1.1 Early fear memory retention test

During the adult conditioning phase, freezing behavior of the 10-seconds before the 1st CS presentation was used to measure long-term retention of early fear conditioned contextual memory (see Figure 2.2*)*. There were no significant differences in early contextual memory retention both between P17–adult paired groups (NP vs. UP vs. PP; $F_{(2,22)} = 0.4010$; $p = 0.6744$) and P17–adult unpaired groups (NU vs. UU vs. PU; $F_{(2,21)} = 1.829$; $p = 0.1852$). However, both P25-adult paired groups and –unpaired groups showed statistically significant differences in contextual freezing (NP vs. UP vs. PP; $F_{(2,22)} = 6.804$; $p = 0.005$, NU vs. UU vs. PU; $F_{(2,22)} = 3.823$; $p = 0.0376$). *Post-hoc* analysis with *Tukey's test* revealed that, in adult paired groups, early (P25) un-paired group (UP) froze more than naïve group (NP) significantly ($p < 0.01$). There were also trends for early paired groups (PP) to show higher freezing levels than naïve group (NP) and lower freezing levels than un-paired group (UP). Likewise, in adult unpaired groups, early un-paired group (UP) froze significantly more than the naïve group (NP) ($p < 0.05$) to the context, and there was a trend for the freezing level of the early paired group to be greater than the naïve (NU) and lower than the unpaired (UU) groups. Following this the 1st CS presentation in the adult conditioning phase was used for the test of long term retention to the cue. Figure 2.3 shows the mean (±SEM) percentage of freezing time during 1st CS presentation prior to the adult CS-US pairing. As with context retention, there were no significant differences in early CS memory retention between P17 groups (NP vs. UP vs. PP; $F_{(2,22)} = 0.9163$; $p = 0.4147$, NU vs. UU vs. PU; $F_{(2,21)} = 1.000$; $p = 0.3847$). However,

there were dramatic significant differences between P25 groups (NP vs. UP vs. PP; $F_{(2,22)}$ = 35.96; p < 0.0001, NU vs. UU vs. PU; $F_{(2,22)}$ = 11.36; p = 0.0004). *Post-hoc* analysis with *Tukey's test* revealed, that early paired groups froze more than both naïve and early un-paired groups in both adult paired and un-paired groups (PP vs. NP and UP, p < 0.0001; NU vs. PU, p < 0.001; PU vs. UU, p < 0.005). In order to control for potential effects of context on freezing to the cue, we also conducted an Analysis of Covariance, with freezing to context as a covariate. The ANCOVA for the P17 groups was not statistically significant, ($F_{(2,45)}$=2.957, p=0.062). The ANCOVA for the P25 groups was significant, ($F_{(2,46)}$=37.242, p<0.0001). Our results show that early fear experience at P25, but not P17, endured into adulthood in a retention test.

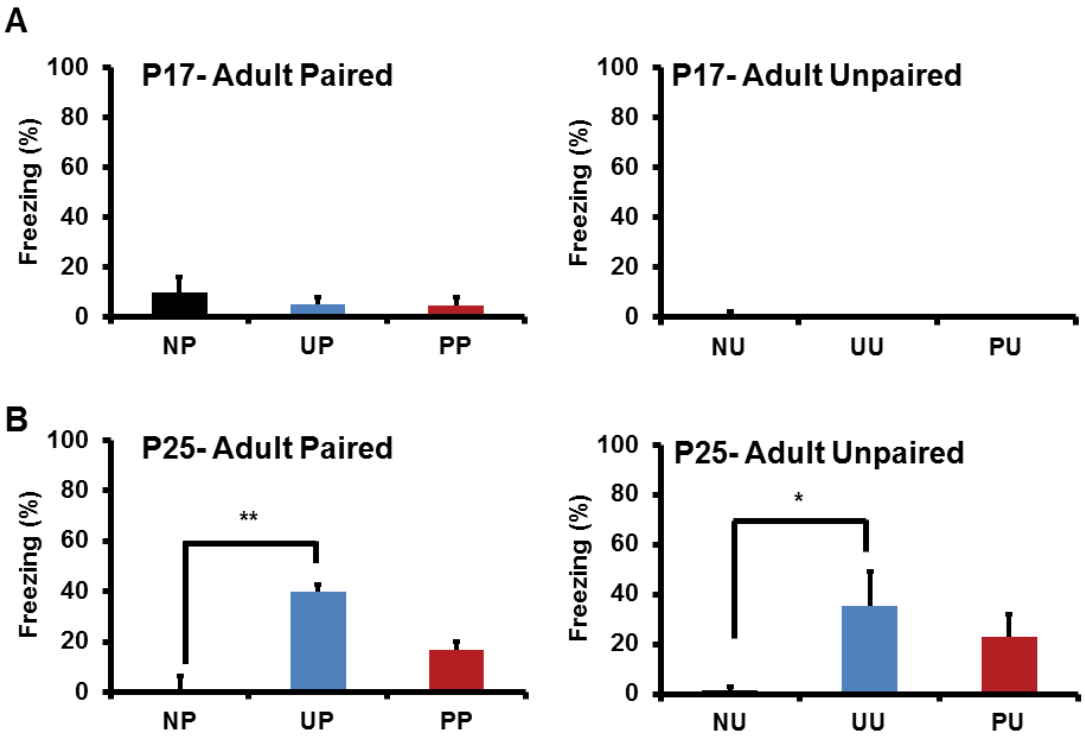

Figure 2.2 **Retention of early contextual memory tested in adulthood.** Each column represents the mean (±SEM) freezing behavior of the 10 seconds before the 1st CS presentation in the adult conditioning phase. (A) All groups trained at P17 froze equivalently with very low level to the context. (B) However, both P25-adult paired groups and –unpaired groups showed early un-paired groups froze more than naïve groups significantly [UP vs. NP , ** $p < 0.01$; UU vs. NU *$p < 0.05$] indicating that early fear experience at P25, but not P17, endured into adulthood in fear contextual retention test. SEM= Standard Error of the Mean, CS= Conditioned Stimulus, NP= Naïve-Paired, NU= Naïve-Unpaired, PP= Paired -Paired, PU= Paired -Unpaired, UP= Unpaired- Paired and UU= Unpaired- Unpaired.

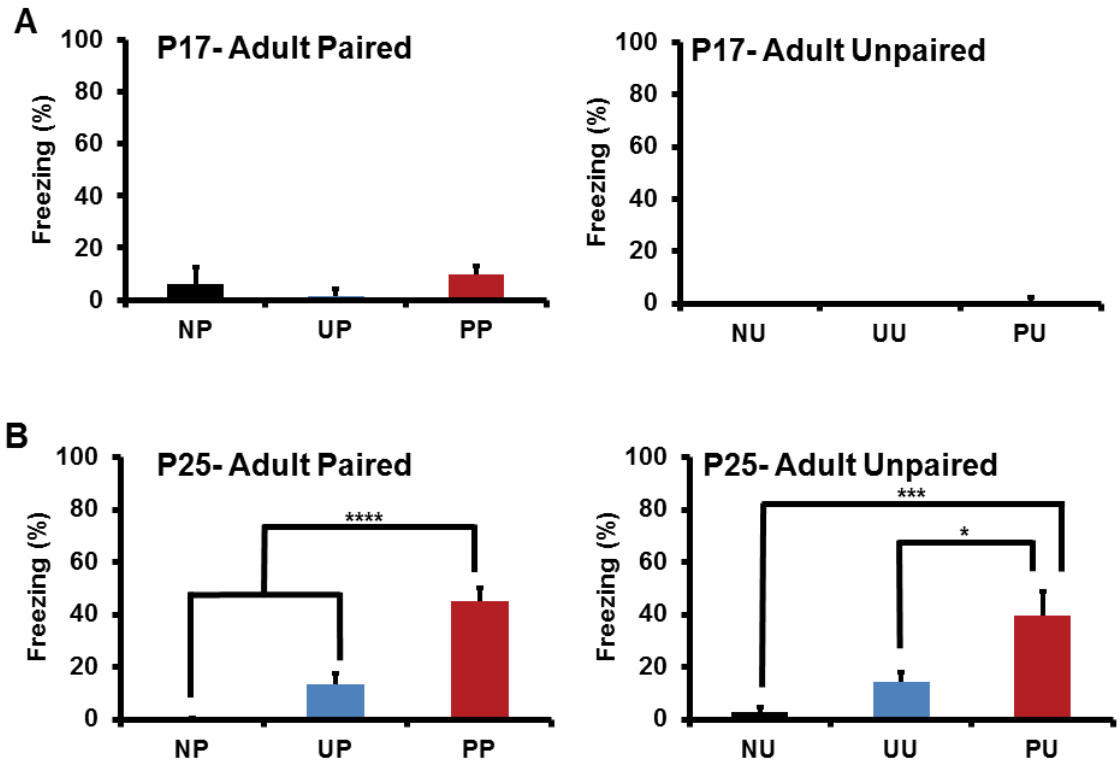

Figure 2.3 **Retention of early cue memory tested in adulthood.** Each column represents the mean (±SEM) freezing behavior of the 1st CS presentation in the adult conditioning phase. (A) All groups trained at P17 froze equivalently with very low level to the cue. (B) However, early paired groups [PP (n=8) and PU (n=8)] froze significantly more than any other groups [NP (n=9), UP (n=8), NU (n=9) and UU (n=8)] indicating that early fear experience at P25, but not P17, endured into adulthood (* indicates $p < 0.05$, *** indicates $p < 0.001$, *** indicates $p < 0.0001$). SEM= Standard Error of the Mean, CS= Conditioned Stimulus, NP= Naïve-Paired, NU= Naïve-Unpaired, PP= Paired -Paired, PU= Paired -Unpaired, UP= Unpaired- Paired and UU= Unpaired- Unpaired.

2.4.1.2 Fear extinction

On the extinction day, there were no differences in baseline context freezing in any group comparisons.

In the P17-adult paired groups, there were significant effects of group ($F_{(2,22)}$ = 10.75; p = 0.0006) and block ($F_{(6,132)}$ = 53.22; p < 0.0001, Figure 2.4A). *Post-hoc* comparisons using *Tukey's test* revealed that the early paired group (PP) froze significantly more than the Naïve (NP) ($p < 0.001$), which suggests that early predictable fear experience potentiated adult fear conditioning. However, the early unpaired (UP) group also froze more than the Naïve (NP) group ($p < 0.01$). The *post-hoc* analysis did not detect significant difference between the UP and the PP group. This result was unexpected. Early unpredictable experience also potentiated adult conditioning in a manner similar to early predictable fear experience at P17. In P17-adult unpaired groups, there were only effects of block ($F_{(6,126)}$ = 8.585; $p < 0.0001$, Figure 4A).

In the P25-adult paired groups, there were significant effects of group ($F_{(2,22)}$ = 10.07; $p < 0.0008$) and block ($F_{(6,132)}$ = 59.70; $p < 0.0001$) and group × block ($F_{(12,132)}$ = 1.959; $p = 0.0329$, see Figure 2.4B). *Post-hoc* comparisons between groups using *Tukey's test* revealed that the early paired group (PP) froze significantly more than early unpaired group (UP) ($p < 0.001$). Further analysis of simple effects within blocks showed significant difference between every combination of groups in block 3 (NP vs. UP and PP, $p < 0.05$; UP vs. PP, $p < 0.0001$). This trend differed from that seen at P17, but the results are consistent with the result that early predictable fear experience potentiates adult fear conditioning. In the P25-adult unpaired groups, there were also significant

effects of group ($F_{(2,22)} = 5.837$; $p = 0.0093$) and block ($F_{(6,132)} = 21.16$; $p < 0.0001$) and group × block ($F_{(12,132)} = 7.033$; $p < 0.0001$). *Post-hoc* comparisons between groups using *Tukey's test* revealed that early paired group (PU) froze significantly more than both naïve (NU) and unpaired group (UU) ($p < 0.05$). This demonstrates that the persistence of early predictable fear memory is strong enough to affect un-predictable conditioning in adulthood.

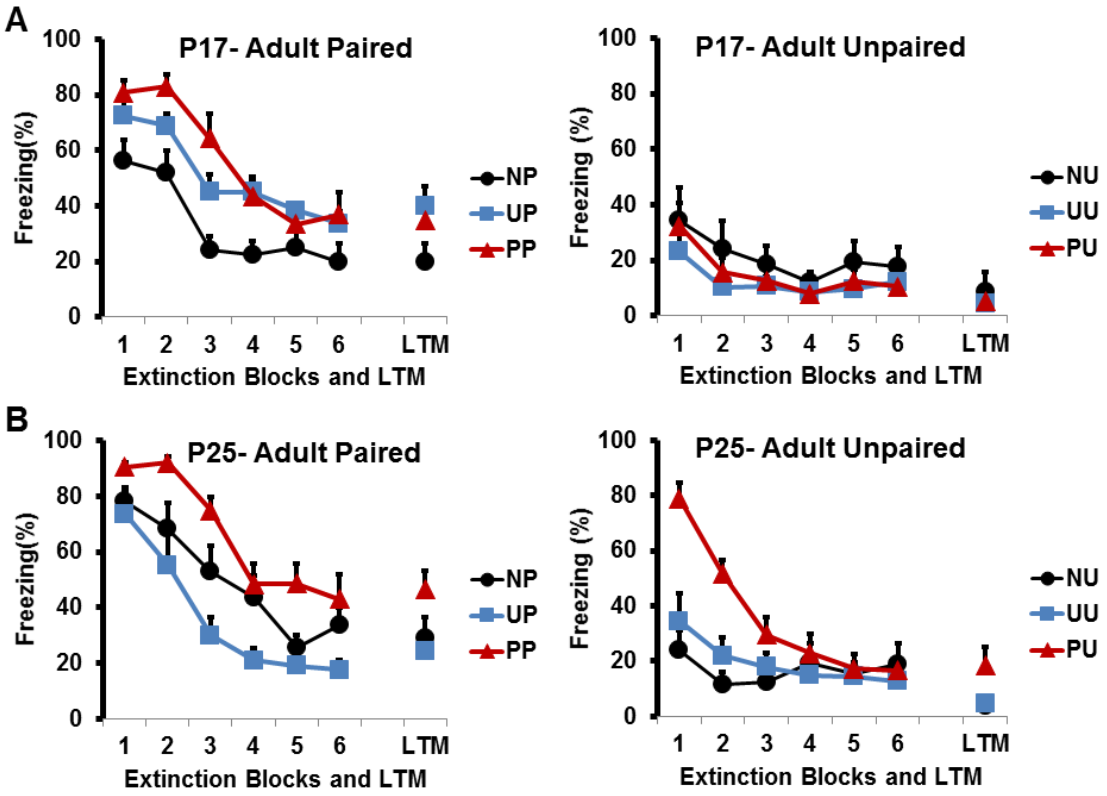

Figure 2.4 **Mean freezing behavior across adult extinction and LTM following adult conditioning.** (A) In the P17-adult paired groups, the early paired group (PP) froze significantly more than the Naïve (NP) ($p < 0.001$), which suggests that early predictable fear experience potentiated adult fear conditioning. Unexpectedly, the early unpaired (UP) group also froze more than the Naïve (NP) group ($p < 0.01$). (B) In the P25-adult paired groups, early paired group froze significantly more than early unpaired group [PP (n=8) vs. UP (n=8), $p < 0.001$) indicating that early predictable fear experience potentiates adult fear conditioning. In the P25-adult unpaired groups, early paired group froze significantly more than any other groups [PU (n=8) vs. NU (n=9) and UU (n=8), $p < 0.05$). This demonstrates that the persistence of early predictable fear memory is strong enough to affect adult un-predictable conditioning. For every group, there was a significant main effect of block, suggesting significant within session decrease in freezing during extinction ($p<.05$). Data are expressed as mean ± SEM. SEM= Standard Error of the Mean and LTM= Long-term Memory.

In summary, conditioning at P17 and P25 yielded different effects on adult conditioning. Both paired and unpaired training at P17 potentiated adult conditioning, but whereas early paired conditioning at P25 potentiates fear acquisition in adulthood, but unpaired conditioning does not.

2.4.1.3 Long-term memory test

At the long-term memory test conducted the day after extinction, only Naïve at P25 - adult unpaired group (P25-NU) showed significant difference in the freezing level between the last block of extinction session and long-term memory test (Figure 2.4B). That group showed a decrease in freezing one day after extinction. Every group showed retention of extinction, and no group showed spontaneous recovery (Figure 2.4).

2.4.2 Experiment 2

2.4.2.1 Long-term memory test

Rats that were conditioned at P17 froze significantly more at P18 than the rats that were not fear conditioned (Figure 2.5A*; t*(10) = 3.204, $p = 0.008$). Additionally, the rats that were fear conditioned at P25 froze significantly more than rats that were not conditioned (Figure 2.5B*; t*(10) = 10.156, $p < 0.0001$).

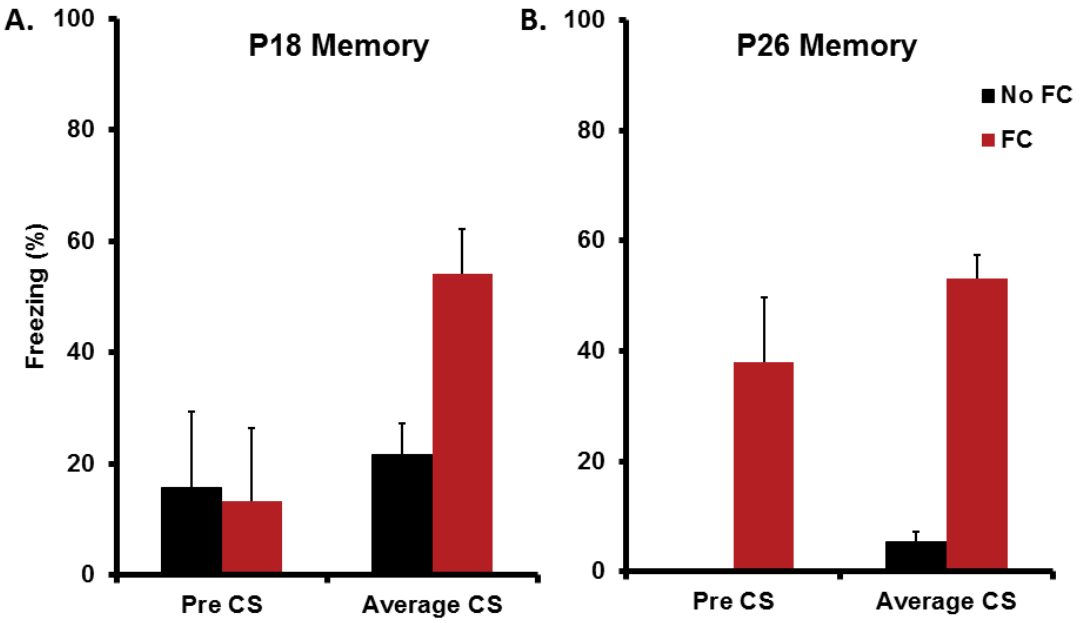

Figure 2.5 **Mean freezing behavior in the LTM memory test performed 24-hours after conditioning at either P17 (A) or P25 (B).** (A) At P18, rats that received paired conditioning at P17 froze significantly more to the CS than rats that were not conditioned ($p<.01$). (B) At P26, rats that received paired conditioning at P25 froze significantly more to the CS than rats that were not conditioned ($p<.0001$). Data are expressed as mean ± SEM. SEM= Standard Error of the Mean, CS= Conditioned Stimulus.

2.5 DISCUSSION

A number of studies have investigated the effects of early life aversive experience on adult behavior. Most, however, have focused on maternal deprivation or other stressors(Callaghan & Richardson, 2011; Guijarro et al., 2007; Kosten, Kim, & Lee,

2012; Madruga, Xavier, Achaval, Sanvitto, & Lucion, 2006; Stevenson, Spicer, Mason, & Marsden, 2009). The current work, similarly to Jones and Monfils (2016), directly examined the retention of early fear conditioned memories, and their enduring effects on fear re-acquisition in adulthood. Our results show that early predictable or unpredictable aversive learning at P17 or P25 differentially modulate fear acquisition and retention in adulthood.

First, paired conditioning at P17 or P25 lead to increased freezing 24-hours later. Yet, in-line with Jones and Monfils (2016), as well as Travaglia et al. (2016), fear memory acquired at P25, but not P17, endured into adulthood, as shown by increased levels of freezing to the context (un-paired early life conditioning) and the cue (paired early life conditioning). Interestingly, experiment one also indicates predictability (paired vs. unpaired) of fear exposure at P17 and P25 yielded differential consequences on fear responding after varying adult conditioning procedures. Both paired and unpaired conditioning at P17 potentiated paired conditioning in adulthood, during extinction and the subsequent long-term memory test. While paired conditioning at P25 potentiated both paired and unpaired conditioning in adulthood, unpaired training at P25 off-set adult paired conditioning.

While results from experiment two replicate those of others in the field, indicating an initial and immediate fear memory when tested within 24-hours after early life conditioning (Kim et al., 2009; Rudy, 1993; Travaglia, Bisaz, Sweet, et al., 2016). The variation in the length from conditioning to test in experiment one may contribute to differential responding seen after a longer delay (months), in which rats conditioned at

P17 do not show evidence of explicit memory in adulthood (i.e., they do not freeze). An effect Spear (1979) describes is due to the fact that memories acquired during infancy are easier to "forget". For example, Akers and colleagues (2012) show that infant contextual fear memory is no longer expressed as soon as 1-week after training, whereas when trained at P30 or P60 freezing was still elicited to the context at least 1-month later. In line with our behavioral timeline, Coulter and colleagues (1976) found that infant P11-P16 animals show complete forgetting 42-days after cued fear conditioning, an effect not seen in the older groups, for which neurological immaturity may underlie the forgetting of earlier events.

Though we did not examine the neural mechanisms of the differential effects of conditioning at P17 vs. P25, there are clues from the literature. One possibility to explain the phenomenon of infantile amnesia or forgetting that we observed in the P17 rats, is the rapid birth of hippocampal cells during early life, which may suggest a lack of maturity in this critical brain region (Frankland, Köhler, & Josselyn, 2013; Josselyn & Frankland, 2012). Importantly, the period between P17 and P24 appears to undergo critical changes in other memory systems as well. Previous work has shown that markers of neuronal activity, plasticity, synaptic maturation, neurite connectivity and myelination, as well as AMPA receptors themselves are differentially expressed after conditioning in P17 versus P24 rats, but that P24 and adult rats share similar responses in AMPA receptors and plasticity markers (Travaglia, Bisaz, Cruz, & Alberini, 2016; Travaglia, Bisaz, Sweet, et al., 2016). These studies emphasize that differing behavioral responses may arise from recruitment of different neural systems during acquisition.

The present study also found contingency of early life conditioning (paired or unpaired) differentially modulated outcomes after reconditioning in adulthood. An effect shown by Sevelinges and colleagues after early life (P8 to P12) fear experience (Sevelinges et al., 2007, 2008). Our results support the importance of contingency as P25 rats exposed to early paired conditioning show a potentiation in freezing after adult paired conditioning, while unpaired experience at the same age blocks this response resulting in no difference initially to previously naive controls but at block three they show significantly less freezing. These data suggest that early memory can modulate adult conditioning, but those effects are dependent on the contingency of early life events. This may be particularly important when we consider the fact that the un-pairing procedure has been shown to produce safety learning to a cue (Ostroff, Cain, Bedont, Monfils, & Ledoux, 2010; Rescorla, 1969; Rogan, Leon, Perez, & Kandel, 2005). In particular, learned irrelevance, a phenomenon in which unpaired training subsequently retards paired conditioning, is not present at P17 or P20, but emerges by P25 (Rush, Robinette, & Stanton, 2001; Stanton, Fox, & Carter, 1998). This reduction in responding after early unpaired then paired training was seen in our P25 rats, however contrary to previous findings, unpaired training at P17 followed by paired training in adulthood potentiated fear responses. The potentiation of fear after P17 conditioning however may be considered a generalized response, as paired conditioning in early life and adulthood also produced an increase in fear responding. This effect is counter to Sevelinges's studies (2007, 2008) in which paired and not unpaired groups at P8 -12 lead to an attenuation rather than potentiation of freezing responses upon reconditioning. This

disparity may arise however, from the earlier developmental time point used during initial conditioning and the paradigm used. Specifically, the odor-shock paradigm used in Sevelinges et al. (2007, 2008) has also been shown to produce an odor preference at earlier developmental time points, P8-9 (Sullivan, Landers, Yeaman, & Wilson, 2000), which may then offset the new aversive learning. In either case these and other previous findings support the hypothesis that long-lasting memory traces acquired early in life can be recruited in adulthood (Stella Li, Callaghan, & Richardson, 2014; Schäble et al., 2007).

One avenue for future research should continue the exploration of ways to reduce the potentiation of fear. The retrieval extinction paradigm shows promise in this regard (Jones & Monfils, 2016). Li and Richardson (2013) found that 14-days after P17 conditioning rats elicit no memory for the conditioned cue (similar to current findings), but that the application of MK-801 (a N-methyl-D-aspartate receptor [NMDAr] inhibitor) blocked the reacquisition of fear. This approach however may not transfer to P25 conditions, as the lack of memory by P31 may be the key, since additional work has shown acquisition (Fanselow & Kim, 1994; Laurent & Westbrook, 2009; Miserendino, Sananes, Melia, & Davis, 1990), but not re-acquisition/reconditioning have been found to be NMDA independent (Roesler et al., 1998; Sanders & Fanselow, 2003; Tayler et al., 2011). The fact that P17 rats do not show a memory for the cue in adulthood makes a cue-targeted approach challenging for translation into clinical populations. Thus, future work should explore alternative and potentially indirect approaches that may work independent of timing of early life aversive or traumatic experience.

In the present work, emotional memory in early life was found to persist and impact adult learning behavior. We show that early predictable or unpredictable aversive experience at P17 or P25 differentially modulates fear memory and reconditioning in adulthood. The most challenging of these findings, with respect to translation to the clinic, is that rats conditioned with either paired or unpaired procedures at P17 exhibiting no initial fear memory but a potentiated response after adult paired procedures, suggesting a more generalized response to aversive experiences in early life. These findings underscore the necessity to develop both direct and indirect approaches to reduce the persistence of fear irrespective of when in early life fear exposure occurred.

2.6 Supplemental Experiments

2.6.1 Introduction

Primary findings from experiment 1 established differences in the influence of early life conditioning on adult memory and reconditioning. In particular, that work shows that a long-term memory trace can exist in pre-weaning rats, despite showing no retention of fear memory behaviorally in adulthood, as both paired and unpaired conditioning at post-natal day 17 (P17) lead to a potentiation after adult paired conditioning. These results suggest that the information encoded in early life still exists even though it is not expressed behaviorally. This type of savings effect was previously found in developmental studies, and supports the idea that memory impairment in early life is due to immaturity of expression related neural connections (Foster & Burman, 2010; Pattwell, Bath, Casey, Ninan, & Lee, 2011; Travaglia, Bisaz, Cruz, & Alberini,

2016; Travaglia, Bisaz, Sweet, Blitzer, & Alberini, 2016). Furthermore, these findings suggest that conditioning at P17 may be less specific than conditioning that occurs at P25. As the same manipulations applied at P25 lead to different outcomes, such that non-predictive or unpaired conditioning at P25 produced no difference in freezing from previously naïve rats, whereas adult paired conditioning similarly potentiated fear after early paired conditioning.

While these findings highlight the different influences early life fear acquisition can have on memory and reconditioning, the question becomes, how can we reduce this persistence and potentiation in fear responses? Adolescence is a critical time for the emergence of anxiety-related disorders and the onset for a variety of treatment regimens (Kim-Cohen et al., 2003; Merikangas et al., 2010). Additionally, work has found that the use of an intervention during adolescence after early life fear experience can reduce the persistence of fear (Jones & Monfils, 2016), in this approach a direct intervention of retrieval + extinction or extinction alone was administered during late adolescence (P45) using the same cue as initial conditioning. While retrieval + extinction was able to reduce initial freezing during the retention test in P25 pair conditioned rats, it also, reduced the potentiation of fear responding in P17 rats after a social reconditioning paradigm in adulthood. This comes in addition to similar results to those in primary analysis and various other works in which rats conditioned prior to weaning exhibit "infantile amnesia" to the cue when tested two or more days after conditioning (Akers, Arruda-Carvalho, Josselyn, & Frankland, 2012; Campbell & Campbell, 1962; Coulter, Collier, & Campbell, 1976; Jones & Monfils, 2016; Li & Richardson, 2013; Travaglia, Bisaz, Cruz,

et al., 2016; Travaglia, Bisaz, Sweet, et al., 2016). With this initial lack of memory, and in thinking of the development of possible translational approaches to target early memories, a cue-specific intervention (such as the one used in Jones & Monfils, 2016), may not be optimal. Additionally, a number of studies have found impaired extinction retention during adolescence (Kim, Li, & Richardson, 2011; McCallum, Kim, & Richardson, 2010; Pattwell et al., 2012). Suggesting a more general approach may be necessary to reduce the potential effects of early aversive or trauma experience on later life.

Chronic exercise may provide an indirect and generalized approach to target these fear responses. While exercise has been shown to have numerous physical health benefits, it also has a variety of positive effects on the brain (Cotman, Berchtold, & Christie, 2007; van Praag, Fleshner, Schwartz, & Mattson, 2014). Specifically, it has been shown to be neuroprotective, create stress resistance, as well as influence a variety of learning and memory tasks, all of which are critical to the development and persistence of fear. In cases where the specific stimulus associated with a trauma cannot be identified exercise may be a suitable intervention to reduce negative outcomes and the potentiation of fear in adulthood. The current experiments set out to determine if chronic exercise during late adolescence could influence the persistence of fear memories and/or act on new memory formation in adulthood.

2.6.2 Methods

First, the current set of experiments choose to explore paired or predictable conditioning procedures in early life and adulthood as findings from primary analysis indicated a similar result in both rats conditioned at P17 and P25 then reconditioned in adulthood using the paired conditioning procedures. Specifically, in both experiments there was a potentiation of fear, additionally previous work from Jones and Monfils (2016) focused on paired conditioning and found an effect using an adolescent intervention. Based on Jones and Monfils (2016) findings we also choose to use a 20s conditioned stimulus (CS) presentation rather than the 10s CS presentation used in primary analysis to be consistent with their previous work exploring possible interventions, as that was the goal of this work.

2.6.2.1 Subjects

Male Sprague-Dawley rats (250-300 grams, from Harlan) and female (215–275g; from either Harlan or retained from previous breeding pairs) were used for breeding. Thirty-eight breeding pairs were used from which 68 male offspring were included across both P17 and P25 experiments. All animals were weaned at 21 days of age and housed two rats per cage on a 12h/12h light/dark cycle with food and water *ad libitum*. Rats from each litter were balanced to different experimental groups for respective P17 or P25 conditions and housed with a non-related rat that had/would be exposed to the same treatment during early life and adolescent intervention. Rats from a given litter were either used for the P17 or P25 experiments (not both) and experiments were run successively not concurrently making direct comparisons between the P17 experiment

and the P25 experiment impossible. All conditioning and testing procedures were performed during the rats' light phase, whereas exercise interventions occurred during the dark phase.

2.6.2.2 Apparatus and procedures

The same apparatus as described in primary analysis of was used for early life conditioning procedures. However because rats were not exposed to unpaired conditioning procedures in early life, it was possible to conduct conditioning/re-conditioning in adulthood in a secondary location; the left portion of a closed shuttle box chamber was used. To maximize differences in the chambers for an ABB design, the second chambers had two metal walls, two black and white striped walls, and stainless-steel rod floors (designed for rats) connected to a shock generator (Coulbourn Instruments, Allentown, PA). In these chambers, experimental procedures were controlled by Graphic State 2 software (Coulbourn Instruments). All behavior was recorded using digital cameras mounted on the top of each chamber. All stimuli used were identical to those used during early life fear experience.

Exercise exposure consisted of individual placement in polycarbonate cages exactly like the ones in which they were housed but with an attached 35.6cm diameter running wheel (Harvard Apparatus) on one-half of the cage. Wheels were fitted with magnetic counters, which recorded every quarter turn of the wheel in either direction. In the sedentary condition, rats were exposed to cages alone without a locked wheel. This was done to reduce the likelihood that they would climb on the wheel and engage in

physical activity nullifying the comparison to freely moving wheels (Koteja, Garland, Sax, Swallow, & Carter, 1999). After each session, cages were rinsed with water, followed by 70% ethanol (EtOH) and fresh bedding was placed in the bottom of the cage.

2.6.2.3 Training

Early life. Pups were first exposed to paired conditioning procedures, in which there were four trials of a CS (White Noise 80dB, 20s) co-terminating with a US (0.6mA, 0.5s) at P17 or P25 or remained naïve. Vanilla extract (McCormick) was put on the mother's nose in P17 conditions to prevent her from discriminating between pups. After each session, the conditioning chamber was rinsed with water, followed by 70% ethanol (EtOH). Naïve rats were separated from their mother or removed from the colony and wheeled into an adjacent room in an opaque plastic box (30 cm×20cm×15 cm) for the same duration that rats in the conditioning group were undergoing conditioning procedures.

Exercise Intervention. All grouping was determined before early life conditioning based on litter, however to gain dexterity and reduce novelty for wheel running, all rats (both those that would be in the sedentary condition and those in the exercise condition) were exposed to three consecutive days of 1-hour access to exercise wheels P40-42. After which all rats were left undisturbed for two days until the start of their intervention condition from P45-65 (21 days). Post-natal day 45 was chosen for the onset of our intervention, as it is generally accepted that adolescence begins between P28-32 (Ojeda & Urbanski, 1994), with its offset after P60, however some changes such

as preputial separation, in male rats, does not occur until P39-45 (Korenbrot, Huhtaniemi, & Weiner, 1977). So to allow for initial transitional changes to occur, we waited until what is considered the mid to late adolescent period, P37-61 (Adriani, Macrì, Pacifici, & Laviola, 2002). Rats were exposed either to an exercise intervention which consisted of single access to a freely rotating exercise wheel for 1-hour within the first 2-hours of the rats dark cycle, or sedentary conditions, rats were exposed to empty cages alone.

Adulthood. After the adolescent intervention period rats were left undisturbed until P90, then one rat per cage was conditioned or reconditioned (depending on early life exposure). Paired conditioning occurred after a 7-minute habituation period in which rats were exposed to three CS-US pairings at the same level as in early life. Twenty-four hours later, all rats were then tested for long-term memory to the CS in the same context as conditioning on the previous day, via three CS presentations in absence of the US after a 10 minute habituation period.

2.6.2.4 Freezing measurement

Freezing behavior was assessed similarly to experiments one and two however it was now quantified as total percent time during the 20-seconds for each CS presentation.

2.6.2.5 Statistical analysis

All freezing data were analyzed with RStudio (Version 0.99.902) using R (Version 3.3.0) and are presented as mean±SEM. Early fear memory retention was tested with a two-way ANOVA of freezing to the first cue presentation before adult conditioning or re-conditioning. To this end, a 2x2 design was used: No-FC_Sedentary, No-FC_Exercise, FC_Sedentary, FC_Exercise (see Figure 2.6A for a timeline of the

experimental procedures). Data from long-term memory was then tested using a three-way ANOVA 2x2x2 design, early life X adolescent intervention X adult condition (see Figure 2.6B.) If there were main effects or interactions, *Tukey's test* were used for *post-hoc* analysis. The null hypothesis was rejected at the $p < 0.05$ level. Additional analyses were done using average distance run across all 21-days of the exercise intervention in a linear model against freezing during the first cue memory, retention test, and long-term memory test in adulthood. In P25 experiments post-hoc general linear hypothesis testing was used to test specific differences in slopes for No Early FC_Exercise_FC compared to Early FC_Exercise_FC and No Early FC_Exercise_Ctl compared to Early FC_Exercise_Ctl, a non-significant difference would indicate that the slopes of the lines are the same and can be collapsed across for further analysis. To be more conservative when looking for null findings a $p > 0.20$ was used to accept the null hypothesis.

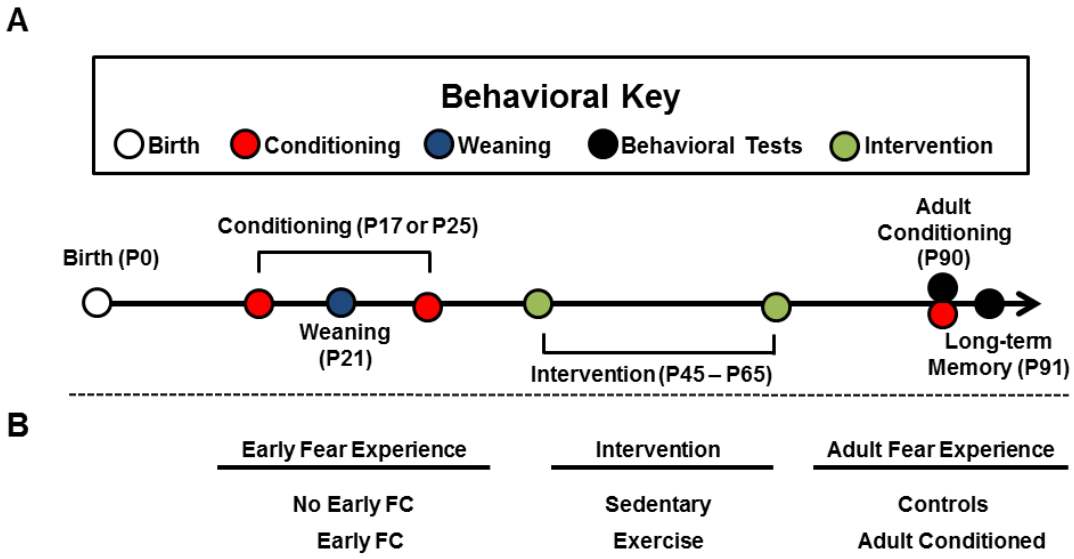

Figure 2.6 **Schematic diagrams of the experimental procedures and conditions.** (A) Timeline of behaviors. (B) Grouping variables. Rats were conditioned using a paired conditioning procedure or remained naïve at P17 or P25. Rats then went through a sedentary or exercise intervention during late adolescence (P45-P65). At 90-days of age, one rat from each cage was tested for memory retention and then received paired CS-US training while the second rat served as a control, the following day both adult conditioned and control rats were tested for LTM.

2.6.3 Results

2.6.3.1 Early fear memory retention test

In adulthood, freezing was assessed during the first CS presentation as a metric of long-term retention of early conditioning, because experiments used an ABB context design pre-CS freezing could not be used as an indicator of contextual memory retention. The number of rats used in each group was eight for the P17 experiment and nine for all P25 groups. Figure 2.7A shows the mean (±SEM) percentage of freezing across rats in P17 conditions first illustrated in the inset showing findings across all groups and then in

the primary figure by collapsing across adolescent intervention, as there was no main effect of intervention ($F_{(1,28)} = 0.0118$; $p = 0.9141$) or interaction between intervention and early life condition ($F_{(1,28)} = 0.6616$; $p = 0.4228$). Similar to previous findings there was no main effect of early life conditioning ($F_{(1,28)} = 0.8601$; $p = 0.3616$) in rats conditioned or not at P17. This effect was then tested under P25 conditions in which there was a main effect of early life experience (Figure 2.7B; $F_{(1,32)} = 7.4876$; $p = 0.0101$). However, there was no main effect of intervention ($F_{(1,32)} = 0.5826$; $p = 0.4509$) or interaction between intervention and early life condition ($F_{(1,32)} = 0.0634$; $p = 0.8029$).

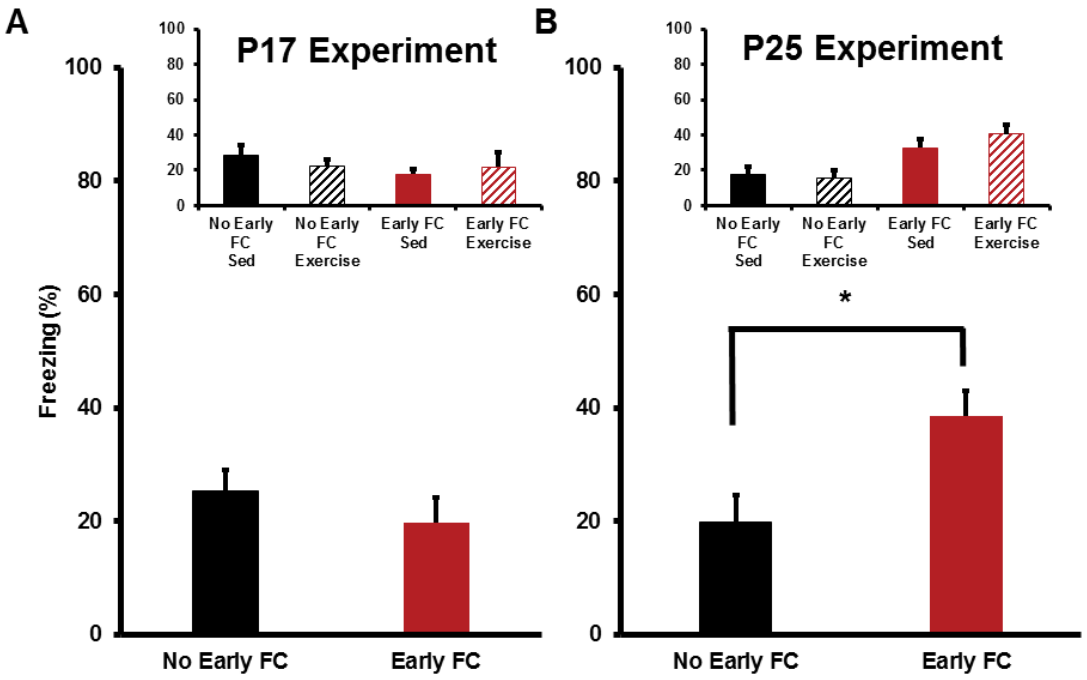

Figure 2.7 **Retention of cue memory tested in adulthood after early life conditioning or not and an adolescent intervention of sedentary or exercise conditions.** Each column represents the mean (±SEM) freezing behavior to the 1st CS presentation (prior to the US onset) in the adult conditioning phase (* indicates $p < 0.05$). (A-Inset) No effects of adolescent exercise intervention were seen so data were collapsed. (A) No differences in freezing were observed in rats conditioned at P17 compared to those that did not receive conditioning. (B-Inset) No effect of adolescent exercise intervention were seen so data were collapsed. (B) Differentially from P17 rats in the P25 experiment rats conditioned in early life froze significantly more than previously naïve rats.

2.6.3.2 Long-term memory test

Following adult conditioning or not, rats were tested via a long-term memory test (LTM). Data across all conditions of the P17 experiment can be seen in the inset of Figure 2.8A, however there were no main effect of intervention ($F_{(1,56)} = 0.0942$; $p = 0.7600$) or early life condition ($F_{(1,56)} = 0.3542$; $p = 0.55383$). Here there was a main effect of conditioning in adulthood ($F_{(1,56)} = 67.874$; $p < 0.001$) and an interaction of early life conditioning and conditioning in adulthood ($F_{(1,56)} = 4.4521$; $p = 0.0393$), so data were collapsed to explore these effects visually. *Post-hoc* comparisons using *Tukey's test* found that there were significant differences between adult conditioned and controls for both rats conditioned at P17 and those that were not, *p*s <.001. However there were no significant differences between adult conditioned rats $p = 0.7086$; indicating there was no potentiation of fear in adult reconditioned rats.

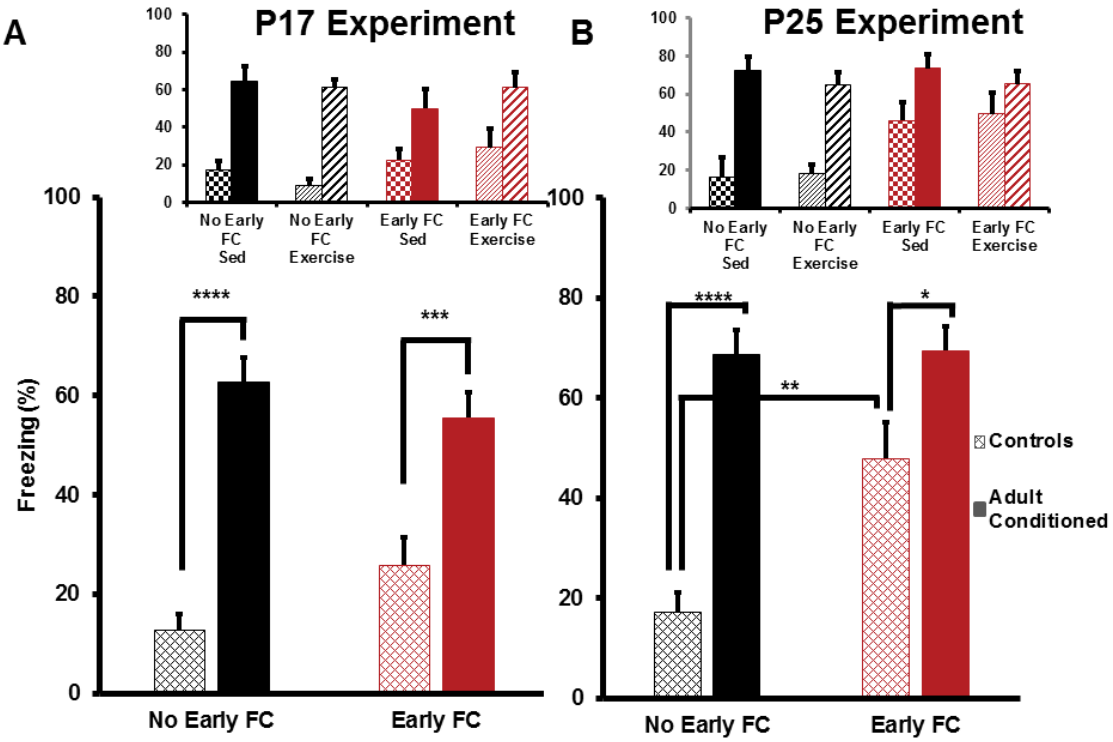

Figure 2.8 **Mean freezing behavior during the LTM memory test performed 24-hrs after adult conditioning.** Adult conditioned rats fall on the right and adult control rats not exposed to the shock are on the left across all comparisons (* indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, **** indicates $p < 0.0001$). (A-Inset) P17 experiment: After adult fear experience or not there were no effects of adolescent exercise intervention so data were collapsed to look at early life and adult conditions. (A) Overall, there were significant differences between adult conditioned rats and controls, as well as an interaction between early life condition and adult conditioning. (B-Inset) P25 experiment: After conditioning in adulthood or no conditioning, there were no effects of adolescent exercise intervention so data were again collapsed. (B) Similar to the P17 experiments, rats in the P25 experiment that were conditioned in adulthood froze significantly more than adult controls. There again was a significant interaction between early life condition and adult conditioning, driven by the significant difference in freezing between rats never previously conditioned (No Early FC, Controls) and those previously conditioned at P25 only (Early FC, Controls)

In addition, rats in the P25 experiment were tested for long-term memory after adult conditioning or not. Again there was no main effect of intervention ($F_{(1,64)} = 0.2328$; $p = 0.63108$). However both early life conditioning ($F_{(1,64)} = 8.2464$; $p = 0.0055$) and conditioning in adulthood ($F_{(1,64)} = 44.5801$; $p < 0.001$) were significant as well as their interaction ($F_{(1,64)} = 7.4958$; $p = 0.008$), data were again collapsed to better visually inspect these findings (Figure 2.8B*)*. *Post-hoc* comparisons found significant differences between adult conditioned and controls for both rats conditioned at P25 and those that were not, $p = 0.035$ and $p <.001$ respectively. Differentially from adult controls in the P17 experiment, rats not conditioned in adulthood did significantly differ in their freezing during the LTM test, $p = 0.001$, such that P25 rats conditioned in early life, but not in adulthood, froze more than rats never previously conditioned. However there were no differences in freezing between adult conditioned and reconditioned rats, $p = 0.9997$. Suggesting no potentiation in fear responding after reconditioning.

2.6.3.3 Exercise intervention

In addition to primary group differences, we wanted to explore the possibility that the voluntary nature of the exercise used may have an influence on either memory retention or adult conditioning, as distances run were variable among rats. First a model with average distance run across all 21 days and early life condition was tested against freezing during cue one prior to adult conditioning/re-conditioning, for which a significant interaction was found (B= -0.0687 p= .0467) indicating a difference in the slopes between rats conditioned at P17 and those that were not, see Figure 2.9. This difference in slopes can be explained by the significant negative correlation seen in P17

conditioned rats (R^2= 0.5517; $F_{(1,12)}$ = 44.5801; p = 0.03477) and the non-significant positive correlation in rats that remained naïve at P17 (R^2= 0.00196; $F_{(1,12)}$ = 0.0118; p = 0.917). This effect was then tested in rats of the P25 experiment for which there was no significant interaction (B= 0.7319 p = .40667; figure not shown).

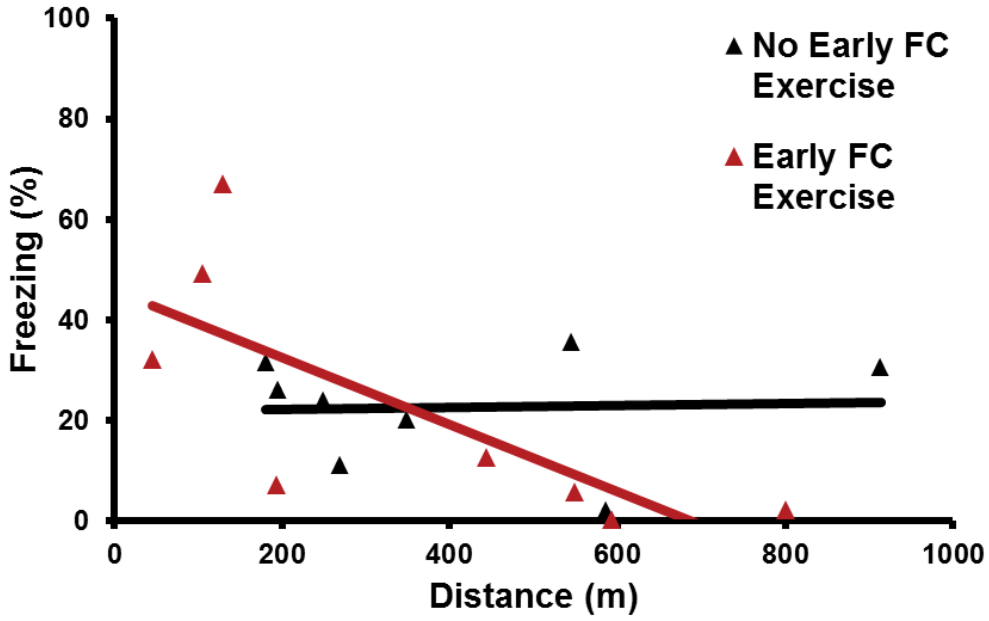

Figure 2.9 **Linear relationships between average distance run across the 21-day exercise intervention and freezing during the memory retention test (cue 1) in adulthood in rats conditioned or not at P17.** Data indicate an interaction between average distance run and early life condition. Data indicate an interaction between average distance run and early life condition (p = 0.047). Rats previously conditioned at P17 show a significant negative correlation (R^2= 0.5517; p = 0.03477), with no correlation in rats that remained naïve at P17 (R^2= 0.00196; p = 0.917).

Exercise could also be influencing adult outcomes through its effects on adult conditioning. This was tested using a model that included early life experience, average distance run during late adolescence and adult conditioning, for which the P17 experiment indicates no significant interactions in the model, *p*'s > 0.05 (figure not shown). Next, data from the P25 experiment were tested, again no significant interactions were found in the model, *p*'s > 0.05. However, after visual inspection a similar pattern was seen in the slopes based on adult conditions (FC vs Ctl) such that general linear hypotheses testing was used to test the differences in slopes. Results found a non-significant difference in adult control rats (*p*= 0.932) and adult conditioned (*p*= 0.932), so data were collapse, Figure 2.10. The model was then re-run including average distance run against freezing during long-term memory based on adult condition, data indicate a trend for a significant interaction (B= 0.0361 *p*= .0773). When broken down a significant negative correlation was seen in adult conditioned rats (R^2= 0.3134; $F_{(1,16)}$ = 7.303; *p* = 0.01569) with a non-significant positive correlation in rats that served as adult controls (R^2= 0.02287; $F_{(1,16)}$ = 0.3745; *p* = 0.5492).

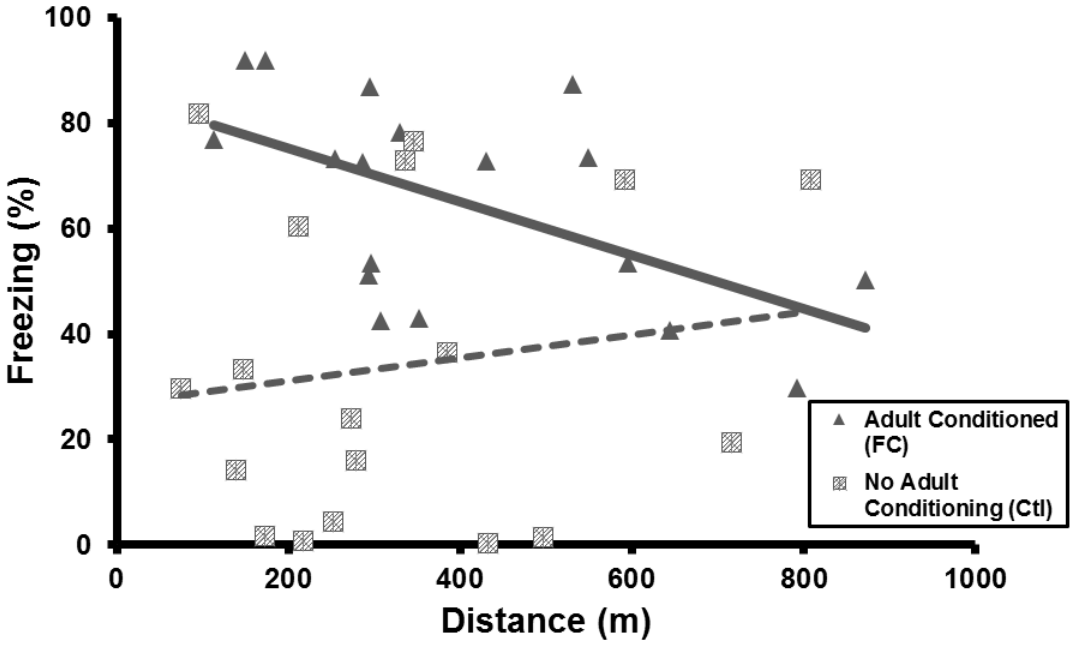

Figure 2.10 **Linear relationships between average distance run across the 21-day intervention period and freezing during LTM in rats conditioned or not in adulthood.** Analysis was initially run across all four groups receiving exercise during late adolescence, for which no effects were seen. However, no differences in slopes were seen for adult conditioned groups (p= 0.932) or no adult conditioning (control) groups (p= 0.932), data were collapsed and are presented in the figure above. A trend for a significant interaction between average distance and adult condition was seen (p= .077). Indicating rats conditioned in adulthood show a significant negative correlation (R^2= 0.3134; p = 0.0157), with no correlation in rats that served as adult controls (R^2= 0.0229; p = 0.5492).

2.6.4 Discussion

Understanding how fear learning and memory develop over time is critical to understand how fear and anxiety-related disorders manifest and persist during a lifetime. The goal of this work was to determine if an intervention of chronic exercise during late adolescence could serve as an indirect approach to reduce persistence of fear. Our results reveal that early predictable aversive learning at P17 or P25 differentially modulate fear retention in adulthood and that these memories and the acquisition of fear in adulthood could be differentially modulated by exercise during late adolescence. Specifically, in the P17 experiment data again suggest no fear memory retention in adulthood, as indicated by no difference in freezing compared to naïve controls. However even without an initial difference in overall fear responding, an increase in average distance run during adolescence predicted lower levels of freezing during this retention test. Conversely, in the P25 experiment data indicate a trend for an interaction between the relationship of distance run and freezing between those rats conditioned in adulthood versus those that were not. However, rats that were conditioned in adulthood exhibit lower levels of freezing at long-term memory, irrespective of early life condition, as levels of previous exercise experience increase. This work replicates the primary findings from chapter 2, as well as a large breath of work which indicates that, when acquired around P17 in rats, fear memories are no longer explicitly expressed two or more days after conditioning (Akers et al., 2012; Campbell & Campbell, 1962; Coulter et al., 1976; Jones & Monfils, 2016; Li & Richardson, 2013; Travaglia, Bisaz, Cruz, et al., 2016; Travaglia, Bisaz, Sweet, et al., 2016). It also contributes to the literature by showing that while rats

conditioned at P17 do not freeze significantly differently from rats not previously conditioned, being exposed to an adolescent intervention of chronic exercise produces a reduction in freezing during the retention test. Of particular interest is the fact that when children who developed PTSD in early life are exposed to an exercise regimen three times a week for 8-weeks (24 days), they show a reduction in PTSD symptoms, as well as a reduction on measures of depression and anxiety (Newman & Motta, 2007). Additional work in non-human animal models has also shown that exercise can serve to produce stress resistance (Greenwood et al., 2003), as well as reduce levels of anxiety (Greenwood, Strong, Brooks, & Fleshner, 2008; Motaghinejad, Fatima, Karimian, & Ganji, 2016; Pietrelli, Lopez-Costa, Goñi, Brusco, & Basso, 2012; Salam et al., 2009). The increased levels of exercise, thus, may be working not on direct fear memories, but to reduce any generalized anxiety response (e.g., increased startle responses; Bazak et al., 2009; Jovanovic et al., 2009, or decreased locomotor behavior Ishikawa, Nishimura, & Ishikawa, 2015), that may have developed as a result of early life trauma. Interestingly, maternal separation, a form of early life stressor, that promotes adult-like potentiation of fear responses rather than persistent reductions of fear,(Callaghan & Richardson, 2011; Kim & Richardson, 2007a, 2007b), also promotes fear memories to persist into adulthood after extinction in P17 rats (Callaghan & Richardson, 2012). Future work should determine if the initial potentiation and persistence of fear through stress exposure can be reduced through the application of chronic exercise.

Interestingly, within these supplemental studies presented here, looking at exercise as a possible intervention to reduce the potentiation of fear after reconditioning

in adulthood, neither P17 nor P25 conditions showed a potentiation of fear as was previously seen in primary analysis, in which both P17 and P25 rats conditioned using a predictable, paired, procedure in early life and in adulthood resulted in a potentiated fear response in adulthood. One reason for these differences may arise from differences in methodology. For example, there was a difference in the cue duration. Previous research has found that not only will a 3-second tone cue produce a significant increase in the freezing response above and beyond that of a 20-second cue, but the freezing response is more resistant to extinction (Kiyokawa et al., 2015). The current findings are also consistent with other work from our lab in which a the use of a 20-second cue during paired conditioning in early life and again during paired reconditioning did not potentiate freezing (Jones & Monfils, 2016). However, rats that exercised in adolescence within the P25 experiment did show an influence on conditioning in adulthood, such that there was a negative correlation between average distance run across the 21-days of exercise access and freezing during the long-term memory test irrespective of early life conditioning or not. This effect, however, was confounded by the fact that there was only a trend for a difference in this effect compared to the relationship seen in adult control rats. Nonetheless, this finding can be supported by the fact that chronic exercise has been shown to produce stress resistance effects, that counter the negative consequences stress has on enhancing fear responses (Greenwood & Fleshner, 2011). While rats within the current set of experiments were not exposed to stress, this effect may indicate exercise acts as a buffer for future fear responding, where the conditioning procedure itself may serve as a stress-inducing event. Yet, this relationship may not significantly differ from

rats not exposed to conditioning in adulthood, as a serious detriment needs to be in place (such as prior uncontrollable stress exposure) for exercise to produce a robust enough effect. It is also important to note that there were no main effects of exercise over sedentary conditions on freezing during this long-term memory test, nor did any level of exercise result in a complete lack of a freezing response, indicating that exercise my not be eliminating the fear response all together but curbing the overall effect. Additionally, previous literature has shown that contextual fear conditioning (Baruch, Swain, & Helmstetter, 2004; Greenwood, Strong, Foley, & Fleshner, 2009; Kohman et al., 2012) cued fear conditioning (Falls, Fox, & MacAulay, 2010) can be enhanced by chronic voluntary exercise. However, less is known about the relationship between distance run and fear responding, but work by Greenwood and colleagues found a correlation similar to ours between fear responding and exercise after stress exposure (Greenwood, Foley, Burhans, Maier, & Fleshner, 2005). Specifically, 6-weeks but not 3-weeks of voluntary wheel access was sufficient to reduce freezing levels to that of non-stressed controls.

The current findings add support to the knowledge that pre-weaning aversive experience produces more generalized neurobehavioral consequences that can be influenced by an indirect approach, such as chronic exercise. This is distinct from findings that aversive experiences acquired after weaning are not influenced in the same way. Future studies should examine the neural mechanisms that underlie the different behavioral effects of early life fear experience and delineate how chronic exercise may be influencing the similar negative correlations presented in this work. The present work highlights the fact that chronic exercise may have the potential to influence fear

responses after adult conditioning, and may serve to buffer, but not eliminate, the fear response.

Chapter 3: Effects of Acute Exercise on Fear Extinction in Rats and Exposure Therapy in Humans: Null Findings from Five Experiments

Significant portions of this chapter were previously published in:

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RFR and MHM determined all experimental designs for experiments 1-4 in rats. JJ, MBP and JAS designed experiment 5 in humans. Experiments 1-4 were conducted by RFR, whereas experiment 5 was done by JJ. Data was analyzed by RFR, JJ and SP with support from DR. Writing the primary manuscript was shared equally by RFR and JJ, with supplemental sections written by RFR.

3.1 Abstract

Background: Exposure therapy is an established learning-based intervention for the treatment of anxiety disorders with an average response rate of nearly 50%, leaving room for improvement. Emerging strategies to enhance exposure therapy in humans and fear extinction retention in animal models are primarily pharmacological. These approaches are limited as many patients report preferring non-pharmacological approaches in therapy. With general cognitive enhancement effects, exercise has emerged as a plausible non-pharmacological augmentation strategy. The present study tested the hypothesis that fear extinction and exposure therapy would be enhanced by a pre-training bout of exercise. Methods: We conducted four experiments with rats that involved a standardized conditioning and extinction paradigm and a manipulation of exercise. In a fifth experiment, we manipulated vigorous-intensity exercise prior to a standardized

virtual reality exposure therapy session among adults with fear of heights. Results: In experiments 1–4, exercise did not facilitate fear extinction, long-term memory, or fear relapse tests. In experiment 5, human participants showed an overall reduction in fear of heights but exercise did not enhance symptom improvement. Conclusions: Although acute exercise prior to fear extinction or exposure therapy, as operationalized in the present 5 studies, did not enhance outcomes, these results must be interpreted within the context of a broader literature that includes positive findings, that used varying timing of exposure to exercise and different intensities. Taken all together, this suggests that more research is necessary to identify optimal parameters and key individual differences so that exercise can be implemented successfully to treat anxiety disorders.

3.2 INTRODUCTION

Exposure-based therapy is an established intervention for treating anxiety disorders (Deacon & Abramowitz, 2004; Hofmann & Smits, 2008; Hofmann, Smits, Asnaani, Gutner, & Otto, 2011); however, there is room for improvement, as non-response rates average nearly 50% (Loerinc et al., 2015). Because exposure therapy is grounded in fear extinction and inhibitory learning principles (Craske et al., 2008; Davis, Ressler, Rothbaum, & Richardson, 2006), it may be prudent to develop and test exposure augmentation strategies that can enhance the acquisition and retention of extinction memories formed during exposure therapy. Support for this approach comes from ongoing research examining the efficacy of a variety of cognitive enhancing drugs shown to augment exposure based therapies (Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015). For example, following early work relating the N-methyl-D-aspartate (NMDA) receptor to fear extinction retention (Davis et al., 2006; Walker, Ressler, Lu, &

Davis, 2002), experiments in rodents and clinical trials in humans have since shown that, when administered prior to a training or therapy session, the NMDA receptor partial agonist d-cycloserine (DCS), can facilitate extinction retention and symptom improvement (Guastella, Dadds, Lovibond, Mitchell, & Richardson, 2007; Mataix-Cols et al., 2017; Otto et al., 2016). However even with these promising research findings, patients seeking care for anxiety disorders generally prefer psychosocial, over pharmacological, approaches (Arch, 2014; McHugh, Whitton, Peckham, Welge, & Otto, 2013). Thus, justifying the development and evaluation of non-pharmacological strategies that can facilitate fear extinction and, by extension, may have the potential to augment exposure therapy outcomes.

Aerobic exercise emerges as one plausible non-pharmacological candidate because it has been shown to broadly affect learning and memory processes, in both acute and chronic forms (Chang & Etnier, 2009; Chang, Labban, Gapin, & Etnier, 2012; Coles & Tomporowski, 2008; Lambourne & Tomporowski, 2010; Perini, Bortoletto, Capogrosso, Fertonani, & Miniussi, 2016; Pesce, Crova, Cereatti, Casella, & Bellucci, 2009; Smith et al., 2010), possibly through a brain-derived neurotrophic factor (BDNF)-dependent mechanism. BDNF has been shown to enhance synaptic plasticity and neuronal excitability (Gomez-Pinilla & Hillman, 2013) as well as mediate extinction memory consolidation (Bramham & Messaoudi, 2005; Chen, Bambah-Mukku, Pollonini, & Alberini, 2012; Peters, Dieppa-Perea, Melendez, & Quirk, 2010; Rattiner, Davis, French, & Ressler, 2004; Schulz-Klaus, Lessmann, & Endres, 2013). Both chronic and acute aerobic exercise have been shown to increase the availability of BDNF in rats and humans (Church et al., 2016; Huang et al., 2006; Marquez, Vanaudenaerde, Troosters, & Wenderoth, 2015; Soya et al., 2007; Szuhany, Bugatti, & Otto, 2015). These increases in BDNF have been associated with exercise training-induced improvements in learning and

cognitive abilities in humans (Kimhy et al., 2015; Vaughan et al., 2014; Winter et al., 2007), as well as increases in human hippocampal adult-neurogenesis (Erickson et al., 2011; Pereira et al., 2007). Accordingly, since exercise has been shown to facilitate learning and memory broadly and has been shown to engage a putative partial mediator of fear extinction retention (i.e., BDNF), acute exercise may have the potential to enhance fear extinction retention and exposure therapy outcomes.

A few studies have tested these hypotheses. For example, Siette et al. (Siette, Reichelt, & Westbrook, 2014) showed that rats with voluntary wheel access for 3-hours immediately before or after fear extinction training showed less freezing at a long-term memory test compared to rats with wheel access 6-hours following extinction training or no wheel access. Moreover, the distance run was correlated with extinction retention. On the other hand, Mika and colleagues (Mika et al., 2015) found that four days of 12-hour wheel access prior to extinction (including the night following conditioning) did not enhance extinction learning or relapse. But, when voluntary wheel access during the extinction session was available, outcomes improved on a relapse test. In humans, a pilot study of nine participants with post-traumatic stress disorder (PTSD) found that 30-minutes of moderate-intensity aerobic exercise immediately before each of the 12 sessions of Prolonged Exposure Therapy (PE) resulted in significantly greater increases in pre- to post-treatment peripheral BDNF levels and greater reductions in PTSD symptoms, compared to PE alone (Powers et al., 2015).

Building upon the aforementioned research, we aimed to provide a comprehensive test of the potential efficacy of acute pre-training administration of aerobic exercise for augmenting exposure therapy. Following a stepped approach to translational research on exposure therapy (see Vervliet, Craske, & Hermans, 2013), we conducted both a test of the augmentation strategy in rats, focusing on the putative

behavioral mechanism (i.e., fear extinction retention), and a test of the augmentation strategy in adult humans with acrophobia, focusing on improved symptom reduction (Rodebaugh, Levinson, & Lenze, 2013).

Specifically, we conducted four separate experiments using rats to determine if exercise timing or exercise duration would augment extinction learning or memory. First, using previous data on the time course of the upregulation of BDNF after 30-minutes of low-intensity forced exercise (Soya et al., 2007), we examined a 30-minute bout of voluntary wheel running both 2-hours and 1-hour before cued extinction training targeting extinction acquisition and consolidation (experiments 1 and 2, respectively). We hypothesized that a 30-minute bout of exercise prior to extinction training would reduce freezing in a subsequent memory test, long-term memory, and decrease relapse of fear responding after reinstatement procedures. Second, because memories are comprised of multiple features including both explicit cue information as well as information about the context in which the learning occurred, we examined an extended, 3-hour bout of wheel access immediately prior to both cued and contextual fear extinction training sessions (experiments 3 and 4). We hypothesized that we would replicate previous findings indicating an effect of voluntary exercise on extinction retention in contextually conditioned rats (Siette et al., 2014) and that these results would extend to a cued fear paradigm. Third, we enrolled humans with height phobia in a single session of virtual reality exposure therapy (VRET) for fear of heights and randomly assigned them to either 30-minutes of aerobic exercise or 30-minutes of rest prior to the VRET session (experiment 5). We hypothesized that participants assigned to aerobic exercise would experience greater symptom reduction compared to those assigned to rest and, following recent findings (Hofmann et al., 2013; Smits et al., 2014; Telch et al., 2014) that fear

level at the end of the VRET session would moderate the relationship between exercise and exposure therapy response.

3.3 METHODS

3.3.1 Experiments 1-3: Effect of Exercise Prior to Cued Extinction Training on Extinction Learning and Memory

The aim of experiments 1-3 was to test if an acute bout of voluntary wheel running prior to extinction training could enhance extinction learning or subsequent memory tests across discrete cue based fear conditioning. The memory tests included a test of long-term memory (LTM; i.e. memory retention from learning that occurred during the extinction training session) and of reinstatement (i.e. how much freezing behavior is reinstated by exposure to the unconditioned stimulus [US] alone without presentation of the conditioned stimulus [CS] after extinction has occurred; (Rescorla & Heth, 1975). We hypothesized that an acute bout of exercise prior to extinction would enhance extinction memories as indexed by reduced levels of freezing at LTM and/or reinstatement tests. We tested three variants of exercise administered prior to extinction, Experiment 1 consisted of 30-minutes of exercise 2-hours prior to extinction, Experiment-2 consisted of 30-minutes of exercise 1-hour prior to extinction and Experiment 3 consisted of 3-hours of exercise immediately prior to extinction. For full details of methodology including equipment, housing and full procedures see Supplemental materials.

3.3.1.1 Subjects

A total of 102 male Sprague-Dawley albino rats (Harlan Laboratories) weighing 275-300g were ordered for the three experiments (34 rats Experiment 1, 32 rats

Experiment 2 and 36 rats Experiment 3). They were housed in pairs throughout the entirety of each experiment. Power analyses conducted in G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) indicated that we would have greater than .80 power to detect an effect size as small as $f = .4$ (a large effect size). All experiments were designed to run 8-12 rats per group (for individual group sizes per experiment see Table 3.1). All procedures in all of our experiments were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Experimental Animals and were approved by the University of Texas at Austin Animal Care and Use Committee (IACUC).

	Experiment 1		Experiment 2		Experiment 3	
Groups (N)						
Exercise	EX (hab) (10)	EX (no hab) (8)	EX (hab) (8)	EX (no hab) (8)	EX (12)	
Control	SED (hab) (8)	SED (no hab) (8)	SED (hab) (8)	SED (no hab) (8)	SED (12)	Novel Object (12)
Voluntary Exercise Condition (EX)						
Duration	30-min		30-min		3-h	
Timing Before Extinction	2-h		1-h		Immediately	
Location	Habituation room		Habituation room		Homecage, both rats present	
Sedentary Control Conditions	No wheel access (SED)				Locked wheel access (SED) Novel object & no wheel access (Novel Object)	
Habituation	Rats were also randomized to receive (hab) or not receive (no hab) habituation to a freely moving exercise wheel (10-min/day)				All rats were habituated to human handling	

Table 3.1: Experimental parameters for cue based extinction experiments 1–3.

3.3.1.2 Procedures

For experiment, specific timelines see Figure 3.1A. Across all cued fear experiments, rats initially went through a conditioning session during which there were three pairings of a 20-second tone cue co-terminating with a foot shock. The following day rats in the exercise condition were exposed to a bout of voluntary wheel running prior to extinction training, whereas control rats remained sedentary (for full details of individual experimental procedures, see Table 3.1). Note that in experiments 1 and 2, prior to conditioning and throughout the entirety of the experiment half of the rats were randomly assigned to 10-minute sessions of access to running wheels in the morning as habituation to exercise wheels. However, there were no difference in distance run prior to extinction in habituated (hab) or no habituation (no hab), therefore rats were grouped into a single exercise group for mega analysis (see Supplemental materials for full details of habituation procedures). This treatment was followed by an extinction training session consisting of 19 presentations of the tone alone. Extinction memory was then tested 24-hours later using nonreinforced tone presentations during a single session (LTM test). After LTM, rats were exposed to unsignaled foot shocks to reinstate freezing behavior and then tested the subsequent day using nonreinforced tone presentations.

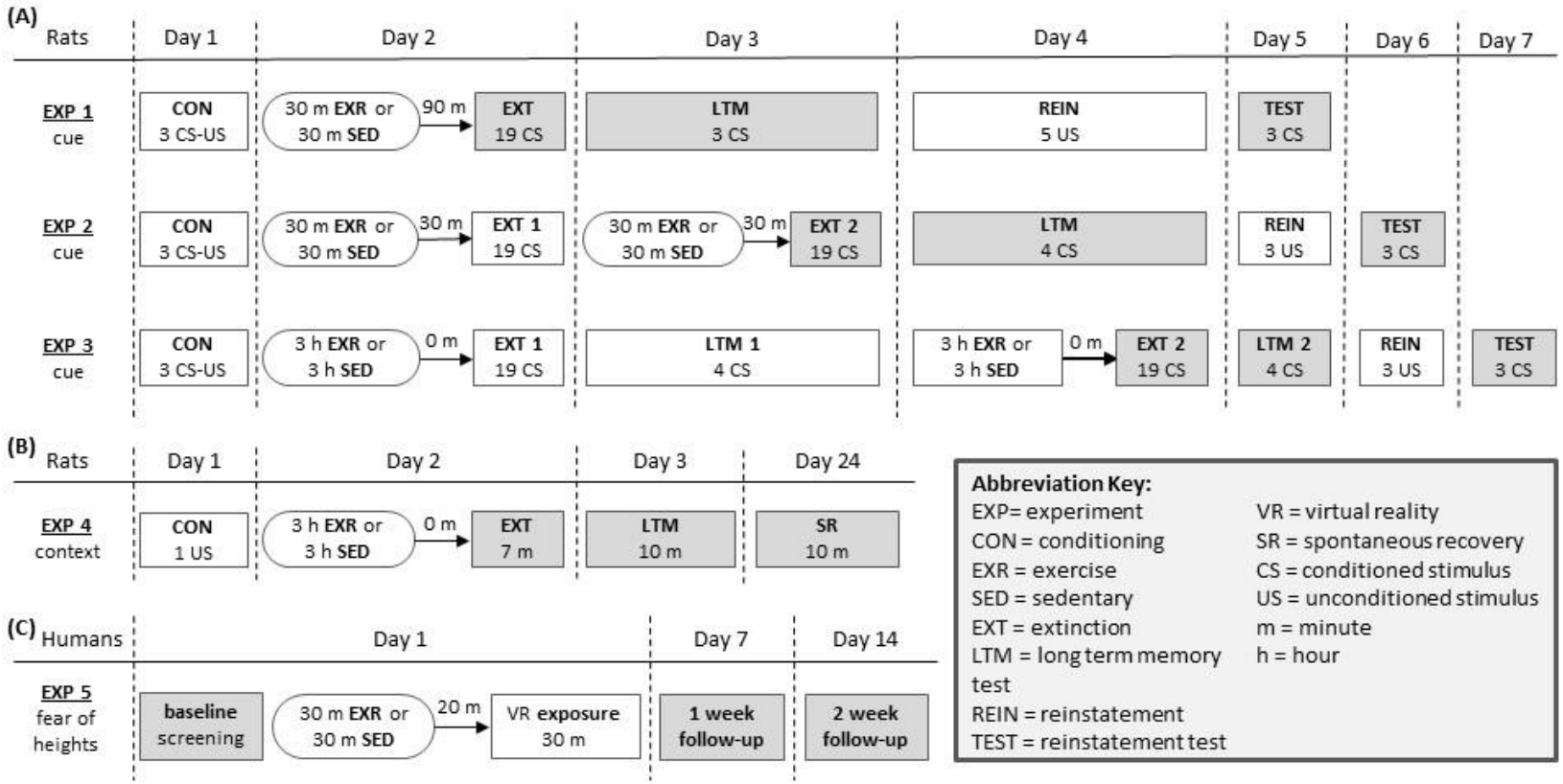

Figure 3.1 **Study timeline indicating the sequence of experimental procedures for all five studies.** Grey boxes indicate data included in analyses.

3.3.1.3 Measures

Freezing during cue presentation was the primary outcome. It was expressed as the proportion of the 20-sec cue presentation during which the rat froze. Freezing was averaged either 1) across all trials (LTM and reinstatement test) or 2) during the first three trials of extinction (early extinction) or 3) during the last three trials of extinction (late extinction).

3.3.1.4 Pooling of Data

Preliminary analyses for experiments 1-3 showed no significant effects of voluntary exercise on fear extinction outcomes. Since all three experiments used similar acquisition, extinction, and testing procedures, we summarized the results of these three independent studies using a mega-analytical approach to pool their data. See supplemental materials for detailed methodology and results.

Pooled data included outcomes from the single extinction session of experiment 1, as well as from the LTM and reinstatement tests. Experiments 2 and 3 had two extinction sessions. Only data from the second extinction session were used in pooled analysis (see grey boxes Figure 3.1A for details of behavioral data used). In addition, data from the only LTM and reinstatement sessions of experiment 2 and the second LTM session and only reinstatement session were used for experiment 3. The data used from each individual experiment in the combined analyses are presented in supplemental material.

3.3.2 EXPERIMENT 4: Effects of Extended 3-Hour Wheel Access on Contextual Fear Extinction Learning and Memory

The aim of experiment 4 was to replicate Siette et al. (2014). They found reductions of fear at LTM after 3-hours of voluntary wheel running immediately before

extinction training on contextual fear extinction. We also attempted to extend their results to an additional memory test—spontaneous recovery. Spontaneous recovery is defined as increased freezing, indicating the initial fear memory is retrieved, after an extended passage of time following successful extinction. One key feature to note is that experiment 4 used a contextual fear conditioning paradigm rather than a cue based paradigm used in experiments 1-3 (see procedures below). For full details of methodology, see Supplemental Materials.

3.3.2.1 Subjects

The preselected sample size of 33 male SD rats was sufficiently powered to detect large overall effects (Faul et al., 2007).

3.3.2.2 Procedures

For a timeline of experimental procedures see Figure 3.1B. Briefly, rats initially went through an acquisition session for which a single foot shock was presented in a distinct conditioning context. The following day all experimental rats were exposed to a 3-hour bout of voluntary wheel running prior to extinction training (n = 10), whereas control rats remained sedentary (locked wheel, n = 12; novel object, n = 11). This treatment was subsequently followed by an extinction training session consisting of 7-minutes in the conditioning context. Memory for extinction was then tested via a 10-minute LTM test in the same context. After LTM rats were left undisturbed for 21 days and then tested during a 10-minute session for spontaneous recovery. These experimental procedures were identical to those of Siette et al. (2014) except that due to housing constraints, our experiments only had two rats per cage with one wheel present, whereas Siette and colleagues had rats housed four rats per cage with two wheels present during the intervention phase.

*3.3**.2.3 Measures***

Different from cued conditioning procedures in Experiments 1-3, contextual extinction was measured as the percentage of time freezing across each minute in the conditioning context. Freezing across the first 3-minutes and last 3-minutes was averaged to quantify early and late extinction, respectively. Finally, across both memory tests, the percentage of freezing was averaged across the entire 10-minute session. It is important to note that this scoring approach differed from that used in Siette et al (2014) as they scored freezing every 2-sec as either freezing vs. not freezing, and then calculated a percentage based on counts of possible observations.

3.3.3 Experiment 5: Testing the Efficacy of Acute Aerobic Exercise for Enhancing Exposure Therapy Outcomes in Humans

Experiment 5 used a randomized controlled experimental research design to test the effect of 30-minutes of aerobic exercise on the effectiveness of an established, single session, virtual reality exposure therapy (VRET) protocol (Ressler et al., 2004; Tart et al., 2013). Outcomes were assessed using several self-report and clinician rated measures of fear of heights administered in person at baseline, approximately 7-days post-treatment (1-week follow-up), and online approximately 14-days post-treatment (2-week follow-up). The level of fear at the conclusion of the exposure therapy session was a hypothesized moderator.

3.3.3.1 Participants

We enrolled 59 eligible participants (69.5% female) through the University of Texas at Austin and surrounding community using flyers and Internet advertisements (an additional participant was excluded at baseline due to motion sickness). Eligible participants were English-speaking adults between the ages of 18-30 with a significant

fear of heights on self-report measures and a significant level of fear in the virtual reality environment. Participants were excluded if they had hearing or visual impairments that would interfere with their ability to participate in the study procedures, if they had any contraindications to performing vigorous aerobic exercise or if they were currently receiving exposure-based treatment for acrophobia. Participants provided written informed consent and all study procedures were approved by the University of Texas at Austin Institutional Review Board and registered on ClinicalTrials.gov [https://clinicaltrials.gov/ct2/show/NCT02361203].

Our sample was predominantly Asian (54.2%) with a mean age of 20.4 ± 2.3 years. Most participants had received some college education (69.5%), nearly half (47.5%) met criterion for a diagnosis of Acrophobia, and 39% engaged in vigorous activity in the 7-days before their baseline session (see Table 3.2 for baseline characteristics of the sample and Table 3.3 for demographic breakdowns by sex). Two participants withdrew from the study before the 1-week follow-up and one participant withdrew from the study before the 2-week follow-up due to unforeseen scheduling conflicts. These three participants' data were included in all the analyses. Power analyses using PinT 3.12 (Power in Two-Level Models; Snijders & Bosker, 1993) indicated that we would have greater than .80 power to detect an effect size of d = .50 for treatment condition differences at either post-treatment assessment.

	SED (*n* = 30)		EX (*n* = 29)	
	M / %	(SD) / (n)	M / %	(SD) / (n)
Demographics				
Age (years)	20.1	(2.4)	20.7	(2.2)
Gender (female)	66.7%	(20)	72.4%	(21)
Education (some college)	66.7%	(20)	79.3%	(23)
Ethnicity (Hispanic or Latino)	23.3%	(7)	20.7%	(6)
Race				
White	33.3%	(10)	24.1%	(7)
Black or African American	3.3%	(1)	13.8%	(4)
Asian	60.0%	(18)	48.3%	(14)
Not Reported	3.3%	(1)	13.8%	(4)
Engaged in Vigorous Physical Activity in Past 7-Days (Yes)	40.0%	(12)	37.9%	(11)
Fear During Exposure				
Peak SUDS during BAT 1	56.7	(20.4)	58.8	(15.4)
Peak SUDS during VRET	57.3	(14.3)	53.4	(18.4)
Ending SUDS during VRET	21.1	(13.2)	19.9	(14.7)

Note. M = mean, SD = standard deviation, SUDS = subjective unit of distress scale, BAT = behavioral avoidance test, EX = participants received virtual reality exposure therapy after exercising vigorously for 30-minutes, SED = participants received virtual reality exposure therapy after resting for 30-minutes, VRET = virtual reality exposure therapy. When missing data, the adjusted n is provided.

Table 3.2 Descriptive statistics of participants at the baseline session.

	SED (*n* = 30)				**EX (*n* = 29)**			
	Male (*n* = 10)		**Female (*n* = 20)**		**Male (*n* = 8)**		**Female (*n* = 21)**	
	M / %	(SD) / (n)	M / %	(SD) / (n)	M / %	(SD) / (n)	M / %	(SD) / (n)
Demographics								
Age (years)	19.4	(1.5)	20.5	(2.7)	20.6	(1.6)	20.7	(2.4)
Education (some college)	70.0%	(7)	65.0%	(13)	75.0 %	(6)	81.0%	(17)
Ethnicity (Hispanic or Latino)	30.0%	(3)	20.0%	(4)	25.0 %	(2)	19.0%	(4)
Race								
White	40.0%	(4)	30.0%	(6)	12.5 %	(1)	28.6%	(6)
Black or African American	0.0%	(0)	5.0%	(1)	12.5 %	(1)	14.3%	(3)
Asian	60.0%	(6)	60.0%	(12)	62.5 %	(5)	42.9%	(9)
Not Reported	0.0%	(0)	5.0%	(1)	12.5 %	(1)	14.3%	(3)
Engaged in Vigorous Physical Activity in Past 7-Days (Yes)	50.0%	(5)	35.0%	(7)	50.0 %	(4)	33.3%	(7)
Fear During Exposure								
Peak SUDS during BAT 1	47.7	(18.8)	61.3	(20.0)	58.7	(16.2)	58.8	(15.5)
Peak SUDS during VRET	54.5	(16.7)	58.7	(13.2)	59.4	(18.0)	51.2	(18.5)
Ending SUDS during VRET	20.5	(15.7)	21.4	(12.1)	16.3	(9.9)	21.3	(16.2)
AQ								
Baseline	88.7	(22.5)	100.3	(23.2)	88.1	(11.7)	101.2	(21.3)
Week-1 Follow Up	52.8	(30.3)	68.3	(38.5)	50.9	(23.8)	73.3	(32.1)
Week-2 Follow Up	46.4	(35.3)	51.5	(33.7)	36.1	(19.1)	59.5	(33.5)
ATHI								
Baseline	44.8	(3.7)	45.9	(6.2)	45.5	(3.5)	49.0	(5.9)
Week-1 Follow Up	31.9	(9.2)	32.5	(8.8)	30.6	(9.3)	38.6	(8.8)
Week-2 Follow Up	32.1	(8.9)	27.9	(11.5)	20.0	(12.8)	33.6	(11.3)
CGI-S								
Baseline	2.9	(1.3)	3.4	(0.9)	2.8	(0.9)	3.4	(0.9)
Week-1 Follow Up	2.6	(1.3)	2.8	(0.7)	2.3	(0.8)	3.1	(1.1)
Week-2 Follow Up	2.4	(1.1)	2.4	(0.8)	1.9	(0.7)	3.0	(1.1)

Note. M = mean, SD = standard deviation, SUDS = subjective unit of distress scale, BAT = behavioral avoidance test, AQ = Acrophobia Questionnaire, ATHI = Attitude Towards Heights Inventory, CGI-S = Clinical Global Improvement - Severity Index, M = mean, SD = standard deviation, EX = participants received virtual reality exposure therapy after exercising vigorously for 30-min; SED = participants received virtual reality exposure therapy after resting for 30-min.

Table 3.3 Descriptive statistics of participants at the baseline and follow-up sessions by group and gender.

3.3.3.2 Procedures

Randomization. Eligible participants were randomly assigned to receive one of two treatments. Randomization was done using variable-sized permuted block-randomization (block sizes varied from 2 to 4) and was stratified by 1) gender, 2) time of day of exposure therapy (i.e., between 12:00 PM and 6:00 PM [yes, no] to control for potential time-of-day effects of exercise (Chang et al., 2012), and 3) current exercise activity levels (i.e., engaged in vigorous activity in the past week or not). The randomization schedule was created and implemented using the randomization module in REDCap (Research Electronic Data Capture) by study staff members (Harris et al., 2009).

Exercise group. Participants were instructed to refrain from engaging in exercise on the day of the baseline session (e.g. the day of the intervention and exposure therapy) or the preceding day, nor to eat, consume caffeine, or smoke during the 2-hours preceding the session. Aerobic exercise was completed on a treadmill (Cybex 770T) or arc trainer (Cybex 750AT) in a room with ambient temperature at 22° C and normal humidity ranging 40-60% relative humidity. The training program consisted of a 3- to 5-minute warm-up at a progressively increasing speed until the target heart rate (defined below) had been reached. Participants then trained for 30-minutes at the target heart rate and were allowed 3- to 5-minutes to cool down before engaging in subsequent study procedures, similar to methods used in previous studies (Smits, Meuret, Zvolensky, Rosenfield, & Seidel, 2009; Smits et al., 2016). All participants were allowed to watch a comedy television show of their choosing while exercising.

Heart rate reserve (HRR), calculated as 220 minus age of the participants minus their Resting Heart Rate (American College for Sports Medicine, 2013), was used as the normalized measure of exercise intensity, with vigorous defined as 80% (± 5%) of the

subject's calculated HRR (American College for Sports Medicine, 2013). Resting heart rate was measured by automatic calculation (Omron BP760N) after a 20-minute sedentary period. Before exercise initiation, participants were fitted with heart rate monitors (Polar Vantage XL, Polar Electro Inc., Lake Success, NY). Staff supervising the exercise session monitored heart rate once per minute to ensure that participants HR stayed within +/- 5% of their calculated target HR and 94% of participants achieved their target heart rate for the exercise session. While moderate- to vigorous-intensity exercise has been shown to affect BDNF levels (Gustafsson et al., 2009; Laske et al., 2010), we selected vigorous-intensity exercise because it has been shown to be associated with the greatest increase in BDNF (Schmolesky, Webb, & Hansen, 2013).

Sedentary group. The sedentary group did not engage in aerobic exercise during the baseline phase and instead rested for 35-minutes while watching a comedy television show of their choosing. The exercise and sedentary treatments took place approximately 20-minutes before the start of the VRET exposure therapy session.

Virtual Reality Exposure Therapy (VRET). During the approximate 20-minutes between the exercise (or sedentary) treatment and the start of the VRET session, the study therapist reviewed with participants the cognitive-behavioral model of acrophobia, rationale for exposure, as well as the importance of disengaging from avoidance and safety behaviors. During the 30-minutes of VRET, participants went up a virtual glass elevator in a building with 35 floors and roof access using Virtually Better, Inc. software and equipment (www.virtuallybetter.com). In both the virtual elevator and roof, participants were able to look over a virtual edge and virtual railings to gain perspective and virtually move around the space using a remote control. Computerized effects give a real sense of increase in height as the elevator rises. Participants were given the choice of starting their VRET session at either floor 2, 5, or 9 and remained on one floor until there

was a meaningful decrease of SUDS (≥ 50% reduction), at which time the therapist led the participants up to the next floor. The general VRET procedures used in this study are similar to the protocols used in previous studies for the treatment of acrophobia with VRET; however, due to the success of multiple sessions of VRET, a single session of VRET was provided in order to allow for the observation of potential augmentation effects. The use of a suboptimal dose to examine augmentation effects is optimal for initial testing of augmentation strategies (i.e., high-throughput clinical assay; Rodebaugh et al., 2013; Vervliet et al., 2013) and has resulted in the improvement on acrophobia outcome measures (Ressler et al., 2004; Smits et al., 2013; Tart et al., 2013).

1-Week and 2-Week Follow-Up. Participants completed questionnaires approximately 7-days and 14-days following the baseline phase. Participants received up to $100 in Amazon gift cards for completing all three study phases. This level of compensation was selected to ensure efficient recruitment and participation in all sessions including follow-up.

3.3.3.3 Measures

Behavioral avoidance test (BAT). The BAT involved the participant riding a glass elevator (in the virtual reality system) up to the 30th floor and/or roof for 30 seconds. A SUDS rating ≥ 50 on the 30th floor and/or roof, or complete refusal to comply with the BAT due to immense fear, was required for inclusion into the study.

Clinical Outcome Measures. The Acrophobia Questionnaire (AQ) was used as the primary clinical outcome measure as it assesses both avoidance of height related situations and fear of height related situations (Cohen, 1977). This scale is a widely used measure of acrophobia with adequate retest reliability (r = .82–.86) and validity (Baker, Cohen, & Saunders, 1973). A score of ≥ 75 on the AQ was required for eligibility

(Cohen, 1977)[1]. The self-report Attitudes Toward Heights Inventory (ATHI) was used to assesses more general attitudes toward six height-related situations (score range from 0–60 with higher scores indicating greater negative attitudes towards heights (Abelson & Curtis, 1989), and has reasonable internal consistency (α = .81) and acceptable validity (Davis et al., 2006; Rothbaum et al., 1995). Additionally, trained study staff used all available data to obtain the Clinical Global Impressions Severity Scale ratings (CGI; scores range from 1–7 with higher scores indicating greater severity (Busner & Targum, 2007). These clinical outcome measures were completed at the baseline, 1-week, and 2-week follow-up phases.

Fear Attenuation Measures. As is done in many clinical studies, the Subjective Units of Distress Scale (SUDS) was used as the measure of fear levels during exposure to the virtual heights environment. The SUDS is a self-report measure of distress on a 0 to 100 scale (with 100 being the most intense fear). While the SUDS has the drawback of being subjective, it has the advantage of being directly relevant to symptom reduction and has been used extensively as an exposure therapy outcome measure. SUDS ratings were obtained in anticipation of and during the exposures (every 5-minutes as well as at the start of each floor during VRET and the peak SUDS during the BAT).

Exercise Measures. The Physical Activity Readiness Questionnaire-Plus (PAR-Q+) is a self-administered 16-item questionnaire recommended by the American College of Sports Medicine as a minimum test of readiness for physical activity programs and was used to assess study eligibility (Thomas, Reading, & Shephard, 1992). The

[1]The minimum AQ was set to 50 but subsequently raised to 75 because few participants who scored < 75 met the required fear threshold in the VR environment resulting in three participants with an AQ < 75 included in the study.

International Physical Activity Questionnaire-Short (IPAQ) is a well-developed 4-item self-administered instrument used to obtain comparable estimates of current physical activity developed through extensive reliability and validity testing (Ainsworth et al., 2000; Mäder, Martin, Schutz, & Marti, 2006). Whether participants had engaged in vigorous activity the prior week or not was obtained from the IPAQ at baseline and used for randomization stratification purposes.

3.3.4 Statistical Analyses

We conducted three primary analyses: one mega-analysis of pooled data from experiments 1-3, one analysis of experiment 4, and one analysis for experiment 5. Across all three analyses mixed effects models with random intercepts were used to test the effect of Treatment (exercise or sedentary), Phase (Experiments 1-4: early extinction, late extinction, LTM, and reinstatement or spontaneous recovery; Experiment 5: baseline, 1- and 2-week follow-up), and the Treatment x Phase interaction on freezing behavior in experiments 1-4 and AQ total scores in experiment 5. The Treatment x Phase term tested primary hypotheses that treatment would enhance the changes expected between Phases (e.g., from early extinction to late extinction and fear relapse tests, or from baseline to follow-ups). When interactions were nonsignificant, main effects were examined. In the rat experiments, significant effects of phase were followed with planned paired contrasts between consecutive phases to assess change in freezing behavior from early to late extinction, late extinction to LTM, and LTM to reinstatement test (experiments 1-3) or spontaneous recovery test (experiment 4). Similarly, in the human experiment, symptom severity was compared between baseline and 1-week follow-up, and 1- to 2-week follow-

up. Model estimated mean differences with 95% confidence intervals (CI) are reported for these contrasts. No model assumptions were violated across all statistical analyses.

In addition to standard inferential statistics, Bayes Factors (BF) were calculated by comparing Bayesian Information Criteria (BIC) between models of increasing complexity (i.e., no effects, Phase only, Phase + Treatment, Phase + Treatment + Phase x Treatment) using a recommended formula (Wagenmakers, 2007). BFs quantify the level of evidence for one hypothesis over another in general and have been increasingly recommended as a useful metric for interpreting null findings in particular (e.g. Dienes, 2014; Wagenmakers, Morey, & Lee, 2016). While nonsignificant *p*-values on their own are not sufficient evidence that the null hypothesis is "true" (e.g., that two groups are equivalent on a measure as opposed to that data are inconclusive), BFs provide a continuous measure of such evidence (Wagenmakers, 2007). For example, in a Bayesian analysis between the hypothesis that two treatments are different and the null hypothesis that there is no difference, a BF of 10 would indicate that the alternative hypothesis is 10 times more probable than the null hypothesis, a BF of 1 would indicate that the evidence does not favor either hypothesis over the other (i.e., hypotheses are equally supported by the data), and a BF of .10 (i.e., 1/10) would indicate that the null hypothesis is 10 times more probable than the alternative—strong evidence that treatments are equivalent. While BFs are straightforwardly interpretable as continuous indicators, some conventional cutoffs have been provided: 1. strong ($BF > 10$) or moderate ($3 < BF < 10$) evidence in favor of the alternative hypothesis; 2. inconclusive results ($.33 \leq BF \leq 3$); or 3. strong ($0 < BF < 0.10$) or moderate ($0.11 < BF < 0.32$) evidence in favor of the null (Lee & Wagenmakers, 2014). In the current analyses, the strength of the evidence in favor (or against) a treatment effect was evaluated by comparing the full model to the main-effects only model. As such, a $BF > 3$ for the full vs. main-effects only model

comparison would provide evidence of a differential treatment effect in the change between phases, whereas a BF < 0.3 would provide evidence that treatment had no effect on the change between phases, and a BF between 0.33 and 3 would suggest the data are inconclusive. Additionally, to ensure each phases had its intended effect, we evaluated the Phase+Treatment model to the Phase only model. Similarly, a BF > 3 for the Phase+Treatment vs. Phase-only model comparison would suggest an overall treatment effect across Phases, a BF < 0.33 would suggest the absence of an overall treatment effect, and a BF between 0.33 and 3 would suggest the data are inconclusive.

Finally, data were analyzed using traditional ANOVA framework for individual phases of experiments 1–4. Importantly, phases were analyzed independently of each other, and no corrections for multiple comparisons were made. Results from these analyses, which are consistent with the primary analyses described above, are provided in the supplementary material to facilitate comparison with other rodent fear extinction studies that have applied similar analytic approaches.

3.4 RESULTS

3.4.1 Experiments 1-3: Effect of Exercise Prior to Cued Extinction Training on Extinction Learning and Memory

Figure 3.2A shows mean raw freezing percentages during the key phases of Experiments 1-3. In the full model, Phase was significant, $F(3,306) = 120.39$, $p < .001$, Treatment was nonsignificant, $F(1,102) = 2.53$, $p = .12$, and Phase x Treatment was nonsignificant, $F(3, 306) = 0.61$, $p = .61$, (Table 3.4). The main effect of Phase was driven by lower freezing percentages during Late vs. Early Extinction (mean difference = -37.32, 95% CI [-41.46, -33.17], $p < .001$), higher freezing during LTM vs. Late

Extinction (mean difference = 12.57, 95% CI [8.43, 16.72], p < .001), and higher freezing during Reinstatement vs. Late Extinction (mean difference = 14.43, 95% CI [10.28, 18.58], p < .001). Consistent with the nonsignificant interaction, between-Treatment contrasts of freezing during the key phases of the experiment were also nonsignificant (LTM: Exercise = 44.27, 95% CI [37.91, 50.63], Control = 39.15, 95% CI [33.38, 44.91], p = .24; Reinstatement: Exercise = 58.77, 95% CI [52.41,65.13], Control = 53.51, 95% CI [47.74, 59.27], p = .23; note all *p*s uncorrected for multiple comparisons).

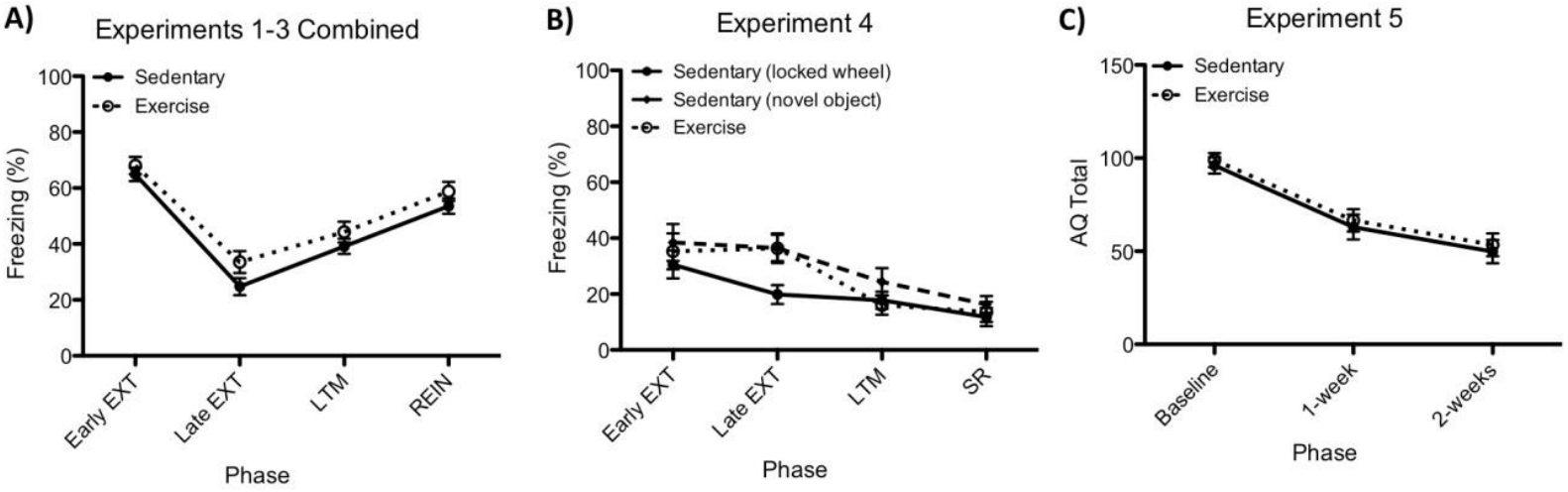

Figure 3.2 **Composite findings across both rat and human experiments.** There were no significant group differences observed in any of the studies. Data are expressed as raw means ± CI. EXT = extinction, LTM = long-term memory, REIN = reinstatement, SR = spontaneous recover. A) On average rats in experiments 1-3 exhibited extinction and a return of fear during long-term memory and reinstatement tests. B) Means for experiment 4 indicate a within session extinction and a reduction in freezing at both long-term memory and spontaneous recovery. C) On average, participants showed a decrease in their fear of heights (measured using the Acrophobia Questionnaire [AQ]) from baseline to 1- and 2-week follow-ups.

	full model statistics		model comparisons	
	F	*p*	*BIC*	*BF*
Experiment 1-3 (combined)				
phase	120.39	< .001	3581.17	> 100
treatment	2.53	.12	3584.68	0.17
phase x treatment	0.61	.61	3600.90	< 0.001
Experiment 4				
phase	28.82	< .001	1086.52	> 100
treatment	1.79	.18	1092.89	0.04
phase x treatment	1.7	.13	1112.49	< 0.001
Experiment 5				
phase	110.74	< .001	1597.34	> 100
treatment	0.15	.70	1602.34	0.08
phase x treatment	0.09	.91	1612.45	< 0.01

Note. Model comparisons reflect Bayesian Information Criteria (BIC) and Bayes Factors (BF) for: phase vs. null model, phase + treatment vs. phase only, phase + treatment + phase x treatment vs. phase + treatment only. Experiment 5 model is for the primary outcome measure (Acrophobia Questionnaire).

Table 3.4 Full model statistics for each main effect and interaction in the final model.

Given the nonsignificant effect of Treatment and of the Phase x Treatment interaction in the full model, Bayesian model comparisons were used to evaluate the strength of the evidence for the null hypothesis that exercise had no effect on freezing levels. A comparison of the full model (Phase + Treatment + Phase x Treatment) to the main-effects-only model yielded a BF < .001, providing strong evidence that exercise did not have an effect on changes in freezing across phases of the experiment. A comparison of the Phase + Treatment model to the Phase-only model yielded a BF = .01, providing strong evidence that exercise did not have an effect on overall freezing levels. Together,

the analyses strongly support the conclusion that exercise did not augment extinction in these experiments.

Further analysis controlling for experiment-level differences in procedures with the addition of experiment as a covariate and all its interactions with the other model covariates showed that treatment remained nonsignificant. Moreover, separate analyses of the individual experiments are consistent with the pooled analysis presented above; these results are summarized in the supplementary material.

3.4.2 Experiment 4: Effects of Extended 3-Hour Wheel Access on Contextual Fear Extinction Learning and Memory

Figure 3.2B shows mean raw freezing percentages during the key phases of Experiment 4. In the full model Phase was significant, $F(3,99) = 28.82$, $p < .001$, Treatment was nonsignificant, $F(2,33) = 1.79$, $p = .18$, and Phase x Treatment was nonsignificant, $F(6, 99) = 1.70$, $p = .13$, (Table 3.4). The main effect of Phase reflected a decrease in freezing during LTM vs. Late Extinction (mean difference = -15.35, 95% CI [-20.44, -10.26], $p < .001$), and a decrease in freezing during Spontaneous Recovery vs. LTM (mean difference = -5.54, 95% CI [-10.63, -0.44], $p = .03$). Consistent with the nonsignificant interaction, between-Treatment contrasts of freezing during the key phases of the experiment were also nonsignificant (LTM: Exercise = 16.07, 95% CI [6.96, 25.18], Locked Wheel Control = 17.80, 95% CI [9.12, 26.48], Novel Object Control = 24.41, 95% CI [16.10, 32.72], all pairwise comparison ps > .18; Spontaneous Recovery: Exercise = 13.59, 95% CI [4.49, 22.70], Locked Wheel Control = 11.79, 95% CI [9.12, 26.48], Novel Object Control = 16.29, 95% CI [7.98, 24.61], all pairwise comparison ps >.46; note all *p*s uncorrected for multiple comparisons).

Given the nonsignificant effect of Treatment and of the Phase x Treatment interaction in the full model, Bayesian model comparisons were used to evaluate the strength of the evidence for the null hypothesis that exercise had no effect on freezing levels. A comparison of the full model (Phase + Treatment + Phase x Treatment) to the main-effects-only model yielded a BF < .001, providing strong evidence that exercise did not have an effect on changes in freezing across phases of the experiment. A comparison of the Phase + Treatment model to the Phase-only model yielded a BF = .04, providing strong evidence that exercise did not have an effect on overall freezing levels. Together, the analyses strongly support the conclusion that exercise did not augment extinction in this experiment. Additional analyses by experimental phase and corresponding figures are presented in the supplemental materials.

3.4.3 Experiment 5: Effect of exercise on VR exposure for acrophobia

3.4.3.1 Primary Analysis

Figure 3.2C shows mean total scores and 95% confidence intervals in the AQ during the key phases of Experiment 5 and Table 3.5 reports the average observed scores for each clinical outcome measure (see Table 3.3 for sex specific averages). In the full model Phase was significant, $F(2, 114) = 110.74$, $p < .001$, Treatment was nonsignificant, $F(1,59) = 0.15$, $p = .70$, and Phase x Treatment was nonsignificant, $F(2, 114) = 0.09$, $p = .91$, (Table 3.4). The main effect of Phase reflected a decrease in AQ total in the 1-week Follow-Up vs. Baseline (mean difference = -31.92, 95% CI [-38.10, -25.73], $p < .001$), which continued through the 2-week vs. 1-week Follow-Up (mean difference = -13.54, 95% CI [-19.79, -7.29], $p < .001$). Consistent with the nonsignificant interaction, between-Treatment contrasts of AQ scores during the follow-up phases of the experiment

were also nonsignificant (1-week Follow-Up: Exercise = 66.96, 95% CI [56.07, 77.84], Control =63.23, 95% CI [52.53, 73.93], p = .63; 2-week Follow-Up: Exercise = 53.08, 95% CI [42.11, 64.06], Control = 50.03, 95% CI [39.33, 60.73], p = .69; note all *p*s uncorrected for multiple comparisons).

	AQ		ATHI		CGI-S	
	M	(SD)	M	(SD)	M	(SD)
SED (n = 30)						
Baseline	96.4	(23.2)	45.5	(5.5)	3.2	(1.0)
1-Week Follow Up	63.0	(36.1)	32.4	(8.8)	2.8	(1.0)
2-Week Follow Up	49.8	(33.7)	29.4	(10.7)	2.4	(0.9)
EX (n = 29)						
Baseline	97.6	(19.8)	48.0	(5.5)	3.2	(0.9)
1-Week Follow Up	67.7	(31.4)	36.6	(9.4)	2.9	(1.1)
2-Week Follow Up	53.4	(31.8)	30.0	(13.0)	2.7	(1.1)

Table 3.5 Statistics of subject outcome measures at baseline, 1-week follow-up, and 2-weeks follow-up.

Given the nonsignificant effect of Treatment and of the Phase x Treatment interaction in the full model, Bayesian model comparisons were used to evaluate the strength of the evidence for the null hypothesis that exercise had no effect on AQ scores. A comparison of the full model (Phase + Treatment + Phase x Treatment) to the main-effects-only model yielded a BF < .01, providing strong evidence that exercise did not have an effect on changes in AQ scores across phases of the experiment. A comparison of the Phase + Treatment model to the Phase-only model yielded a BF = .08, providing strong evidence that exercise did not have an effect on overall AQ levels. Together, the

analyses strongly support the conclusion that exercise did not augment the effect of VR exposure in this experiment. These findings were similar for the secondary outcome measures of ATHI and CGI, see supplemental materials Table 3.6.

	full model statistics		**model comparisons**	
	F	*p*	*BIC*	*BF*
Attitudes Toward Heights Inventory				
phase	107.17	< 0.001	1235.48	> 100
treatment	1.52	0.22	1239.11	0.16
phase x treatment	1.19	0.31	1247.04	0.02
Clinical Global Impressions Severity Scale				
phase	27.56	< 0.001	398.25	> 100
treatment	0.39	0.53	403.03	0.09
phase x treatment	0.73	0.48	411.87	0.01

Note. Model comparisons reflect Bayesian Information Criteria (BIC) and Bayes Factors (BF) for: phase vs. null model, phase + treatment vs. phase only, phase + treatment + phase x treatment vs. phase + treatment only.

Table 3.6 Experiment 5 secondary outcomes full model statistics for each main effect and interaction.

3.4.3.2 Moderation Analysis

Additional secondary analyses tested in the human experiment whether fear at the end of the VRET session moderated the effects of augmentation as per previous findings in the literature reporting on pharmacological enhancers of exposure therapy. These previous studies showed that the effect of the augmentation strategy was significant only among participants with low end SUDS (Hofmann et al., 2013; Smits et al., 2014; Telch

et al., 2014), but see also (de Kleine, Smits, Hendriks, Becker, & van Minnen, 2015). To test for moderation, the outcome models were refit to examine the Treatment x Phase x end SUDS interaction. Across all outcome models this interaction was nonsignificant (all *p*s > .42), and model comparisons provided moderate to strong evidence that end SUDS did not moderate Treatment effect across phases (all *BF*s < .02).

3.5 DISCUSSION

The current studies aimed to provide a robust test of the potential efficacy of acute aerobic exercise, when administered immediately prior to a session, for augmenting exposure therapy. Data from five controlled experiments suggest aerobic exercise administered prior to training (1) does not enhance fear extinction – the putative behavioral mechanism of exposure therapy (Hofmann et al., 2011; Myers & Davis, 2007) and (2) does not facilitate exposure therapy outcomes. While our sample sizes were based on a priori power analyses for large effect sizes in the rat experiments, and medium effect size in the human experiment, we cannot rule out that a smaller effect would have been detected with larger sample sizes. For the human experiment in particular, we chose to power the study to detect medium effect size because detecting a small effect size would have limited practical significance. To further evaluate the null findings we used Bayesian analyses which provided moderate to strong evidence that rather than simply being inconclusive, our experiments showed exercise had no effect on outcomes. While this finding was consistent across our five experiments, prior theoretical and empirical research has provided some evidence of the efficacy of exercise, suggesting that differences in methodological approaches and subject characteristics may play key roles, which are discussed below.

The timing of acute exercise may be paramount to its effects on extinction or exposure therapy. While exercise within 3-hours immediately before or after extinction learning was effective in reducing freezing during long-term memory in rats (Siette et al., 2014), a recent human study showed exercise improved long-term memory on a cued-recall test consolidation when administered 4-hours after (but not before nor immediately after) picture-location association learning (van Dongen, Kersten, Wagner, Morris, & Fernández, 2016). Van Dongen et al (2016) suggest that exercise after learning allows for initial synaptic potentiation to be boosted by exercise later in the consolidation process. While our results are consistent in showing that exercise *before* extinction did not have an effect, future research is needed to explore possible mechanisms more directly such that optimal timing can be determined across procedures.

Another critical factor may be the dose of acute exercise. Our rat experiments used voluntary wheel running instead of forced treadmill exercise because the latter has been shown to induce a stress response in rats, which has the potential to negatively impact fear extinction (Chauveau et al., 2012; Greenwood et al., 2003; Maroun et al., 2013). Rats in our experiments only voluntarily ran 15% of the amount rats ran under forced exercise procedures (Huang et al., 2006; Soya et al., 2007), potentially leading to an insufficient dose to adequately engage the suspected molecular mediator of fear extinction. Although chronic voluntary wheel running has been shown to upregulate BDNF (Adlard, Perreau, Engesser-Cesar, & Cotman, 2004; Venezia, Guth, Sapp, Spangenburg, & Roth, 2016), the effects of acute voluntary exercise on BDNF have been much more limited. The previous work looked at longer bouts of 48, 96 and 168 hours of voluntary wheel access which do show upregulation in various brain regions even after 48-hours of access (Neeper, Gómez-Pinilla, Choi, & Cotman, 1996). Yet, several studies have used acute voluntary exercise (e.g., Mika et al., 2015; Siette et al., 2014) and

observed augmentation effects. None of these studies directly report average distances run during the intervention period; however, we can infer, from the graphical data of the relationship between distance and freezing at long-term memory, that the distances run in those studies seem to be similar on average to those seen in our 3-hour exercise interventions (Siette et al., 2014: approximately 80-210 meters, current experiments 3 & 4: M = 125 meters SD = 113). Interestingly, when the distance scores were regressed on freezing during the final long-term memory test (same as in Siette et al. 2014 study) no effects were seen. This is counter to the Siette et al. 2014 findings, in which there was a significant negative relationship; however, these data were collapsed across all of their exercised groups. It is important to note that 94% of human participants in experiment 5 achieved the prescribed exercise intensity and duration, which has been previously shown to increase BDNF in humans (Schmolesky et al., 2013). Nevertheless, future studies should measure BDNF directly to ensure the alleged target is being engaged.

In addition to timing and duration of acute exercise doses, chronicity may be another key factor impacting the effects of exercise on extinction or exposure therapy. Several experiments have shown beneficial effects of chronic exercise on fear extinction acquisition in rats, but only when exercise is chronic, prior to fear acquisition, and under stressed conditions (Greenwood et al., 2003, 2013; Greenwood, Foley, Burhans, Maier, & Fleshner, 2005; Greenwood, Loughridge, Sadaoui, Christianson, & Fleshner, 2012; Greenwood, Strong, Dorey, & Fleshner, 2007; Greenwood, Strong, Foley, & Fleshner, 2009). However, this observed enhancement effect of exercise in rats might be due to exercise countering the negative consequences stress has on extinction learning rather than augmenting extinction itself. In humans, Hopkins and colleagues showed that a single session of aerobic exercise can enhance object recognition memory, but only when combined with a 4-week exercise program (Hopkins, Davis, Vantieghem, Whalen, &

Bucci, 2012). Unfortunately, we were unable to examine this interaction with the current data due to the low number of individuals reporting having engaged in vigorous exercise in our sample. Future studies with larger samples should test whether chronic exercise in humans interacts with acute exercise prescribed prior to exposure therapy.

An additional subject characteristic that may play a key role in the effect exercise has on augmentation of exposure therapy is sex. Unfortunately, our rat studies only included male rats and our human study was not designed to examine sex differences. Moreover, the small number of males in the human sample (10 in the sedentary group and 8 in the exercise group) precluded us from performing even exploratory analyses. We present the average outcome scores by gender in the supplemental materials (Table 3.3) for hypothesis-generating purposes. It will be important for future studies to enroll an adequate number of males to test any sex-difference hypotheses. Future studies with larger sample sizes can also explore whether other demographic variables reported in Table 3.2, including age, education, race, and ethnicity, are important moderators.

Finally, there may need to be a significant impairment in extinction in order to see any effect of exercise. Powers et al. (2015) found exercise augmentation effects among humans participants diagnosed with Posttraumatic Stress Disorder, a disorder that has been consistently linked with impaired fear extinction (Helpman et al., 2016). However, it is important to note that Powers et al. (2015) was a pilot study consisting of only nine participants. While the presence of any anxiety disorder may suggest resistance to extinction (Duits et al., 2015), less than half our human sample met diagnostic criteria for acrophobia, with mild symptom severity on average, and therefore may represent a population substantially different in their severity and responsiveness to extinction. Indeed, our results mirror those from Guastella et al.'s 2007 study testing the efficacy of DCS to augment exposure therapy for sub-clinical spider fearful patients. Nevertheless,

these findings as well as preclinical rodent experiments (Holmes & Quirk, 2010) suggest that augmentative strategies such as exercise may be particularly effective among participants with diminished response to the behavioral treatment alone.

It is important to discuss the difference in our findings (from experiment 4) and those previously seen in work by Siette and colleagues (Siette et al., 2014). While we attempted to replicate their methodology, two potentially important methodological differences arose. First, the scoring of freezing differed; however, the overall levels of freezing were comparable across experiments. Secondly, in Siette's work, rats were housed in 23cm x 21cm x 23cm cages with four rats per cage; however, in the current study only two rats were house per cage due to 1) protocol regulations by the IACUC dictating that cages 42cm x 20cm x 20 cm can maximally hold three rats and 2) a desire to stay consistent with the proportion of rats per wheel (Siette et al., 2014 had two wheels per four rats). This difference in housing during both the entirety of the experiment and, perhaps more importantly, the fact that the intervention was implemented directly into the homecage may account for differences in our respective findings. Findings from a recent meta-analysis on retrieval + extinction (compared to standard extinction alone) indicate that the number of animals housed together is a significant moderator of overall effects (Kredlow, Unger, & Otto, 2016) and provides support suggesting that the difference in the number of rats per cage may indeed have had a significant impact on the results of experiment 4. An additional concern, specific to our auditory conditioning experiments, might be that exercise could have enhanced contextual fear, and thus, could have overshadowed a potential effect of exercise on auditory fear conditioning. Indeed, previous experiments have shown that exercise can enhance contextual fear acquisition (Baruch, Swain, & Helmstetter, 2004; Greenwood et al., 2009; Kohman et al., 2012; Siette et al., 2014); however, in the present studies, we specifically chose a suboptimal

exposure to the exercise wheels, which would have no effect on fear learning. Our exercise was limited to 10-minutes a day for three days, to simply habituate the rats to wheel access rather than be a full "dose" previously shown to have neurobiological effects on BDNF (Huang et al., 2006; Soya et al., 2007) or behavioral effects on acquisition (Baruch et al., 2004; Greenwood et al., 2009; Kohman et al., 2012; Siette et al., 2014). The effects of our exercise regimen on contextual fear were actually directly tested in experiment 4 (even though this was not the main question of interest), and showed no significant group differences between those that exercised and those that did not.

In conclusion, the current studies provide no evidence that aerobic exercise, when administered acutely and before training, enhances fear extinction or exposure therapy outcomes. Although these findings discourage the use of acute exercise as a blanket strategy for augmenting exposure therapy sessions, the extant literature hints at the possibility that, like is the case for many interventions, the efficacy of exercise for enhancing exposure therapy outcomes may be dependent on intervention and person characteristics and thus provide a possible avenue for future research in this area.

3.6 Supplementary Materials

3.6.1 Experiment 1: Effect of 30-min of exercise 2-h before cued extinction training on extinction acquisition and subsequent memory

The aim of experiment 1 was to test the effect of a single 30-min bout of voluntary wheel running 2-h before extinction training on extinction acquisition and subsequent memory tests. We hypothesized that an acute bout of exercise 2-h prior to extinction would enhance extinction memories as indicated by reduced levels of freezing

at long-term memory (LTM) and/or reinstatement tests. To test these effects, we employed a 2x2 design in which rats underwent one of two habituation conditions in which there was a freely moving wheel available (Habituation) or an empty cage (No Habituation). This was done to reduce neophobia and allow rats to gain dexterity for wheel running, potentially enhancing the amount of voluntary exercise displayed during the exercise treatment prior to extinction. Subsequently rats were then divided into two groups in which prior to extinction training there was wheel access for exercising (Exercise) or a group that remained sedentary with no access to a wheel (Sedentary) (see Figure 3.1 for a timeline of experimental procedures). This resulted in four groups: No Habituation-Sedentary (SED (no hab); n = 8), Habituation-Sedentary (SED (hab); n = 8), No Habituation-Exercise (EX (no hab); n = 8), Habituation-Exercise (EX (hab); n = 10).

3.6.1.1 Methods

Subjects. Thirty-four male Sprague-Dawley albino rats (Harlan Laboratories) were ordered at 275-300 g and housed in pairs throughout the entirety of the experiment. Rats were allowed one week to acclimate to the colony. All rats were housed in 42x20x20 cm polycarbonate cages. Housing rooms were maintained at a constant temperature (21 ± 1°C) and a 12-12 light-dark cycle (lights on at 6:00 and off at 18:00). Food and water were provided ad libitum throughout the entire experiment.

Equipment. All test procedures were conducted in a Habitest Modular System conditioning chamber (Coulbourn Instruments) equipped with metal rod flooring connected to a shock generator and a speaker connected to a tone generator, and enclosed in a sound attenuating chamber. All behavioral testing occurred in the same context across all procedures. Chambers were illuminated with a red light and wiped with disinfectant cleaner between sessions. All videos were recorded from overhead cameras

and output to AVI files, to be scored by a researcher blinded to experimental conditions. Graphic state software controlled video recording and stimulus presentations.

For both the wheel Habituation and/or Exercise treatment sessions, all rats were exposed alone to polycarbonate cages exactly like the ones in which they were housed but with an attached 35.6 cm diameter running wheel (Harvard Apparatus) on one-half of the cage. Wheels were fitted with magnetic counters, which recorded every quarter turn of the wheel in either direction. In No Habituation and/or Sedentary sessions, rats were exposed to cages alone without a locked wheel. This was done to reduce the likelihood that they would climb on the wheel and engage in physical activity nullifying the comparison to freely moving wheels (Koteja, Garland, Sax, Swallow, & Carter, 1999).

Procedures.

Wheel habituation. To reduce novelty to the exercise wheels and to allow rats to gain dexterity for running on the wheels, the rats in the EX (hab) and SED (hab) groups were allowed 10-min of individual access to the exercise wheel three mornings preceding behavioral testing. Rats in the EX (no hab) and SED (no hab) were placed individually in an empty cage for the 10-min. Habituation or No Habituation procedures were continued throughout the experiment to reduce any potential confounds of stress related to removal of wheel access (Greenwood et al., 2012; Nishijima et al., 2013). All habituation procedures occurred in the last hour of the rat's dark cycle (5:00-6:00), with at least 6-h between behavioral testing and habituation procedures. Between sessions, cages were wiped with 70% ethanol and bedding was replaced.

Fear acquisition. All rats were allowed 10-min of habituation to the conditioning chamber before receiving three conditioning trials of a tone (5 kHz for 20-s) co-terminating with a foot shock (0.7 mA for 0.5 s) separated by a variable intertrial interval (ITI) 3-min on average.

Treatment. Previous findings from Soya et al. (Soya et al., 2007) indicate that after a single 30-min bout of low intensity forced exercise there is a maximal 1.5- 2 fold increase of BNDF in the CA1, CA3, and dentate gyrus within the hippocampus 2-h after the start of exercise. This is further supported by Huang et al. (Huang et al., 2006) who also show an up-regulation of BDNF at a 2-h time point after forced exercise. Based on these findings we provided a single bout of voluntary exercise 2-h before the extinction training session expecting that upregulation of BDNF will occur as the extinction session begins to occur (i.e. during extinction acquisition). The use of voluntary exercise rather then forced exercise was chosen based on previous findings suggesting that forced exercise can be stressful, and even an acute stressor such as this has the potential to negatively impact fear extinction (Chauveau et al., 2012; Greenwood et al., 2003; Maroun et al., 2013); thus, by using voluntary wheel running we aimed to reduce exercise type as a potential confound. To measure voluntary running behavior the total distance run by each rat was measured. All wheels were equipped with magnetic counters to track every quarter turn of the exercise wheel, this data was recorded after each exercise session and converted into distance measurements.

Extinction. An extinction training session consisted of 19 presentations of the tone alone separated by a variable intertrial interval (ITI), 3-min on average.

Long-term memory test. 24-h after the extinction session, rats were exposed to three nonreinforced tone presentations separated by a variable intertrial interval (ITI) 3-min on average.

Reinstatement. 24-h after LTM rats were exposed to five unsignaled foot shocks with a fixed intertrial interval (ITI) of 1-min. All foot shocks were at the same (0.7 mA for 0.5-s) level used at the initial fear acquisition.

Reinstatement test. To test the reinstatement of freezing, 24-h after exposer to the unsignaled foot shocks three nonreinforced tone presentations were given, each separated by a variable intertrial interval (ITI) 3-min on average.

Measures.

Freezing behavior. Across all behavioral tests freezing was measured as a total number of seconds and expressed as a percentage of time during each 20-s cue presentation. Freezing was assessed for each individual trial for fear acquisition and extinction, whereas on memory tests the percentage of freezing was averaged across trials.

Analysis. A type III sums of squares Analysis of Variance (ANOVA) was used to assess group differences and included 2-factors, habituation condition (Habituation or No Habituation) and treatment (Exercise or Sedentary) across all outcomes. Additional linear models were run to test the relationship between average distance run across treatment session (in groups receiving wheel access) and freezing during long-term memory and reinstatement tests. Data were analyzed using RStudio (Version 0.99.902) using R (Version 3.3.0).

3.6.1.2 Results

Freezing behavior. For individual findings across all behavioral tests see Table 3.7. During fear acquisition a repeated measures ANOVA indicated a significant effect by trial indicating within session acquisition of freezing by CS3 (Figure 3.3A). Following the treatment session rats showed an overall main effect of trial indicating within session extinction occurred across groups (Figure 3.4A) and a significant habituation x treatment x trial interaction, such that there was no difference in extinction behavior until trials 12 to 15 and 17 for which an effect of exercise exists only in rats exposed to the habituation

condition. The next day rats were tested for LTM for which no significant differences in freezing between groups (Figure 3.4B). Finally, after reinstatement procedures a repeated measures ANOVA indicated significant reinstatement of freezing from LTM to reinstatement test across groups but no significant differences between groups in freezing behavior during the reinstatement test (Figure 3.4C).

	Experiment 1			Experiment 2			Experiment 3		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Within Session Acquisition									
habituation	1, 30	0.142	0.708	1, 28	7.416	0.011			
treatment	1, 30	2.184	0.150	1, 28	0.434	0.515	2, 33	0.246	0.783
habituation x treatment	1, 30	1.521	0.227	1, 28	0.805	0.377			
trial	2, 60	297.335	< 0.001	2, 56	181	< 0.001	2, 66	603.53	< 0.001
habituation x trial	2, 60	0.224	0.800	2, 56	2.428	0.097			
treatment x trial	2, 60	0.766	0.469	2, 56	0.061	0.94	4, 66	0.593	0.669
habituation x treatment x trial	2, 60	1.229	0.300	2, 56	0.511	0.603			
Within Session 1 Extinction									
habituation	1, 30	0.702	0.409	1, 28	2.641	0.115			
treatment	1, 30	0.062	0.805	1, 28	0.326	0.573	2, 33	0.219	0.805
habituation x treatment	1, 30	0.076	0.785	1, 28	0.569	0.457			
trial	18, 540	8.524	< 0.001	18, 504	10.482	< 0.001	18, 594	3.625	< 0.001
habituation x trial	18, 540	1.528	0.075	18, 504	1.295	0.185			
treatment x trial	18, 540	1.045	0.407	18, 504	0.636	0.872	36, 594	0.699	0.907
habituation x treatment x trial	18, 540	1.849	0.018	18, 504	0.725	0.786			
Within Session 2 Extinction									
habituation				1, 28	0.810	0.376			
treatment				1, 28	0.962	0.335	2, 33	1.143	0.331
habituation x treatment				1, 28	1.903	0.179			
trial				18, 504	25.052	< 0.001	18, 594	11.5	< 0.001
habituation x trial				18, 504	0.602	0.899			
treatment x trial				18, 504	0.994	0.465	36, 594	0.727	0.88
habituation x treatment x trial				18, 504	1.181	0.271			

Table 3.7 Primary results rat cued extinction experiments.

	Experiment 1			Experiment 2			Experiment 3		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Long-term Memory Session 1									
habituation	1,30	0.985	0.329	1,28	0.172	0.682			
treatment	1,30	0.005	0.947	1,28	0.011	0.916	2,33	0.699	0.504
habituation x treatment	1,30	0.883	0.355	1,28	0.429	0.518			
Long-term Memory Session 2									
treatment							2,33	0.311	0.735
Reinstatement Test									
habituation	1,30	0.039	0.845	1,28	0.134	0.717			
treatment	1,30	0.008	0.928	1,28	0.778	0.385	2,33	1.04	0.365
habituation x treatment	1,30	0.267	0.609	1,28	0.198	0.66			
Long-term Memory Test to Reinstatement Test									
habituation	1,30	0.008	0.930	1,28	0.19	0.666			
treatment	1,30	0.417	0.523	1,28	0.257	0.617	2,33	0.803	0.456
habituation x treatment	1,30	0.042	0.840	1,28	0.387	0.539			
test	1,30	16.000	< .001	1,28	35	< .001	1,33	14.336	0.001
habituation x test	1,30	0.001	0.974	1,28	0.019	0.89			
treatment x test	1,30	0.710	0.406	1,28	0.585	0.451	2,33	0.262	0.771
habituation x treatment x test	1,30	2.630	0.115	1,28	0.127	0.724			

Note. Empty units are data not applicable to that individual experiment

Table 3.7 *cont.* Primary results rat cue based extinction experiment

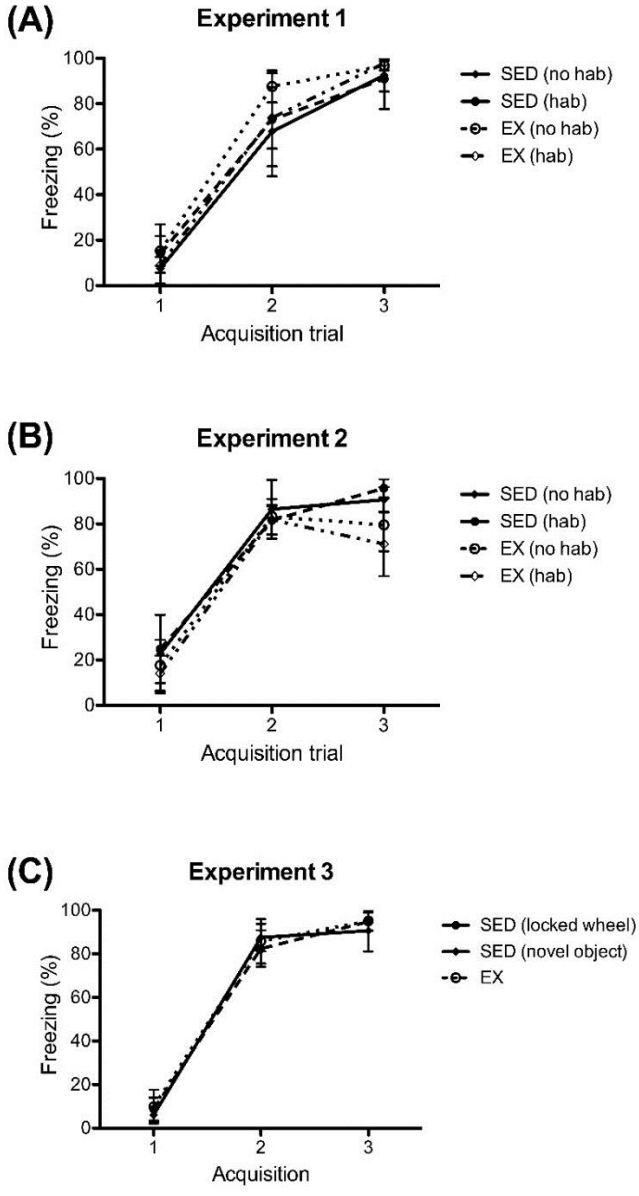

Figure 3.3 **Fear acquisition.** (A-C) Rats in all groups show within session fear acquisition, p's < 0.001. (B) Data indicate a main effect of habituation condition, p = 0.01. Data are expressed as mean ± CI (n = 8–12 per group).

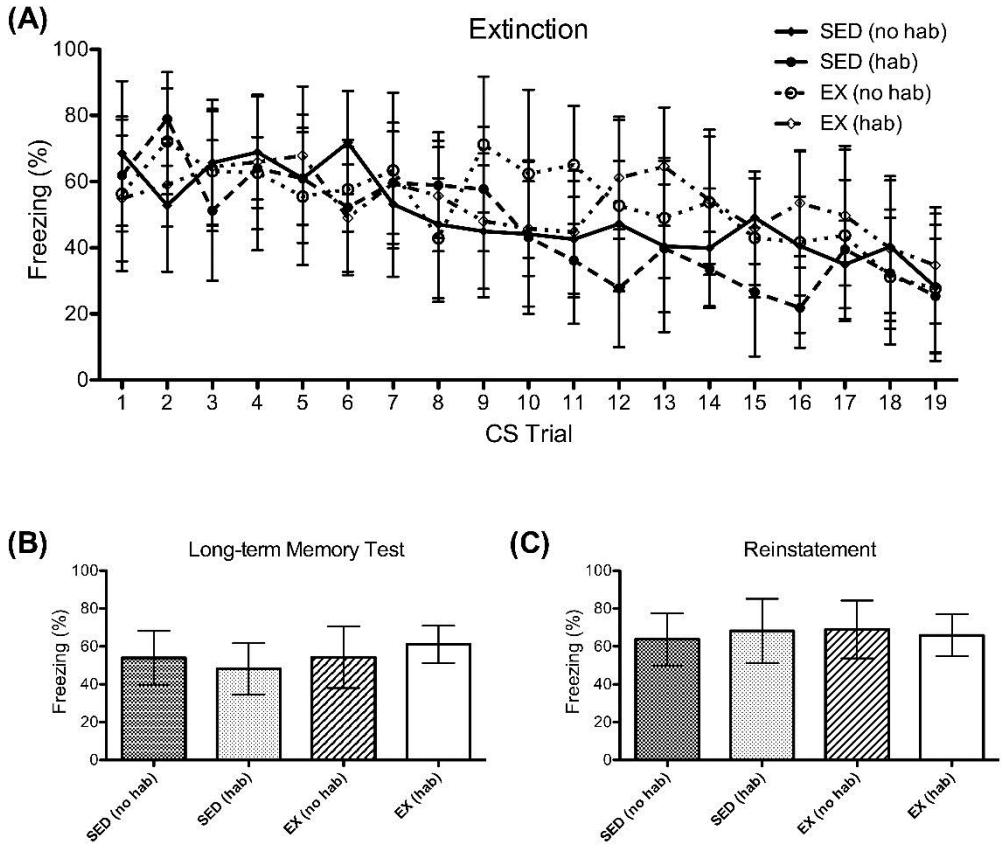

Figure 3.4 **Experiment 1.** (A) within session one extinction, (B) long-term memory and (C) reinstatement data used in cumulative analysis. (A) All rats show within session extinction, $p < 0.001$. (A) Data indicate a significant habituation by treatment by cue interaction, $p = 0.02$. Data are expressed as mean ± CI (n = 8–10 per group).

Voluntary running behavior. On average, both exercise groups ran similar amounts (EX (no hab) M = 55.25, SD = 26.92; EX (hab) M = 68.70, SD = 33.65; $t(16) = 0.94$, $p = 0.36$). For average distances run across both groups during the intervention period, see Table 3.8. Data indicated a non-significant relationship between the average

distance run and later freezing during the LTM test ($r(17) = -0.16$, $p = 0.52$, Figure 3.5A)[2]. However, there was a significant, negative relationship between average distance run and later freezing during the reinstatement test ($r(17) = -0.59$, $p = 0.01$, figure 3.6A). It is important to note that this significant relationship appears to be driven by one outlier who ran a distance 2.67 standard deviations above the mean because when this outlier is removed the negative relationship observed is no longer significant. However, since the purpose of this study was to examine voluntary exercise the outlier was retained for all analyses.

	Average Distance (meters)	**SD**
30-min of access		
Experiment 1	62.72	30.74
Experiment 2	64.31	22.07
3-h of access		
Experiment 3	126.58	99.69
Experiment 4	148.08	128.95

Table 3.8 Average distance in meters run across each experiment.

[2]Initial analysis found no differences between the EX (no hab) and EX (hab) groups in the relationship between average distances run prior to the extinction sessions and later freezing behavior during the LTM test or the reinstatement test. Therefore, data from the EX (no hab) and EX (hab) were combined.

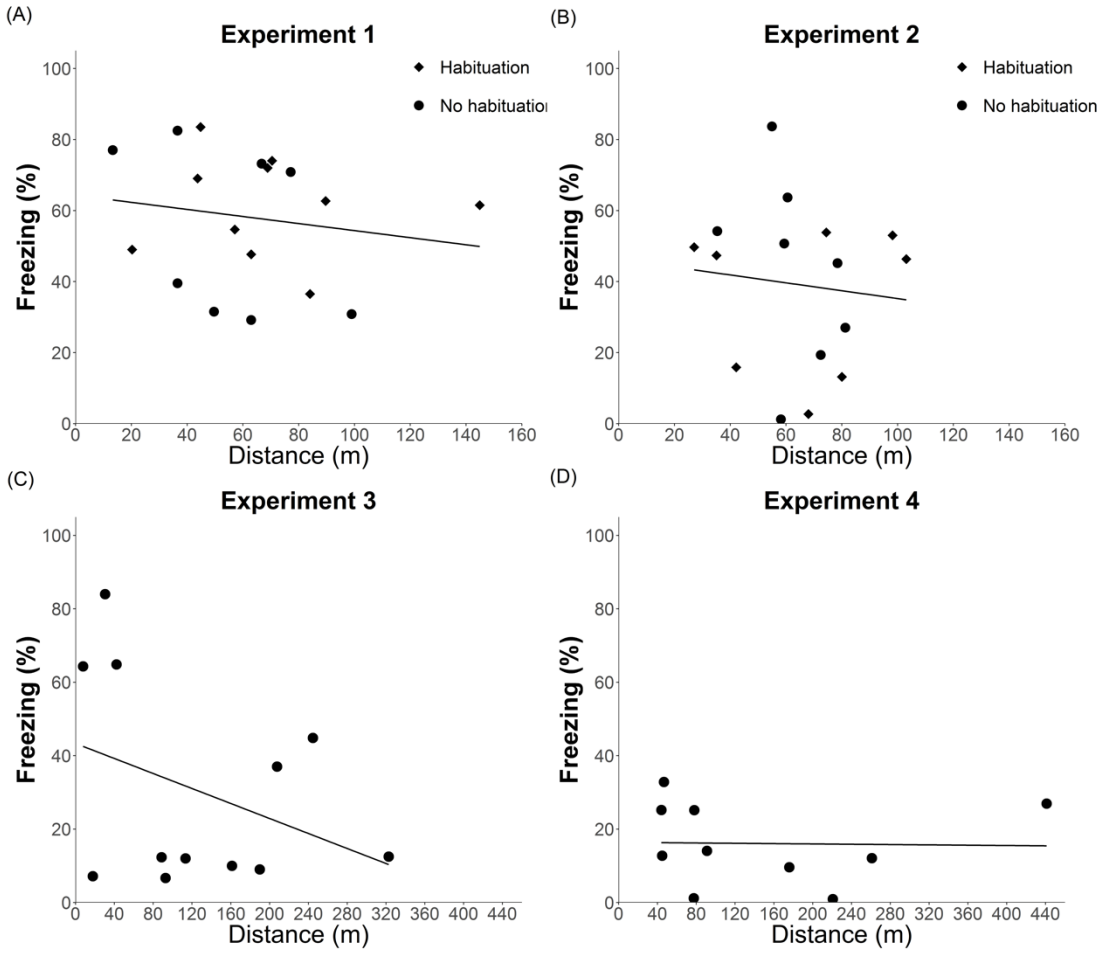

Figure 3.5 **Linear relationship between average distance run before extinction session(s) and freezing during the long-term memory test.** (A, B, D) and the second (final) long-term memory test (C) after extinction. No significant relationships were observed across all experiments. (A-B) Geometric shapes indicate habituation condition; there were statistically no differences between groups so data was combined. *Note:* experiments 1 and 2 rats were allowed 30-min of wheel access whereas experiments 3 and 4 there was 3-h of access.

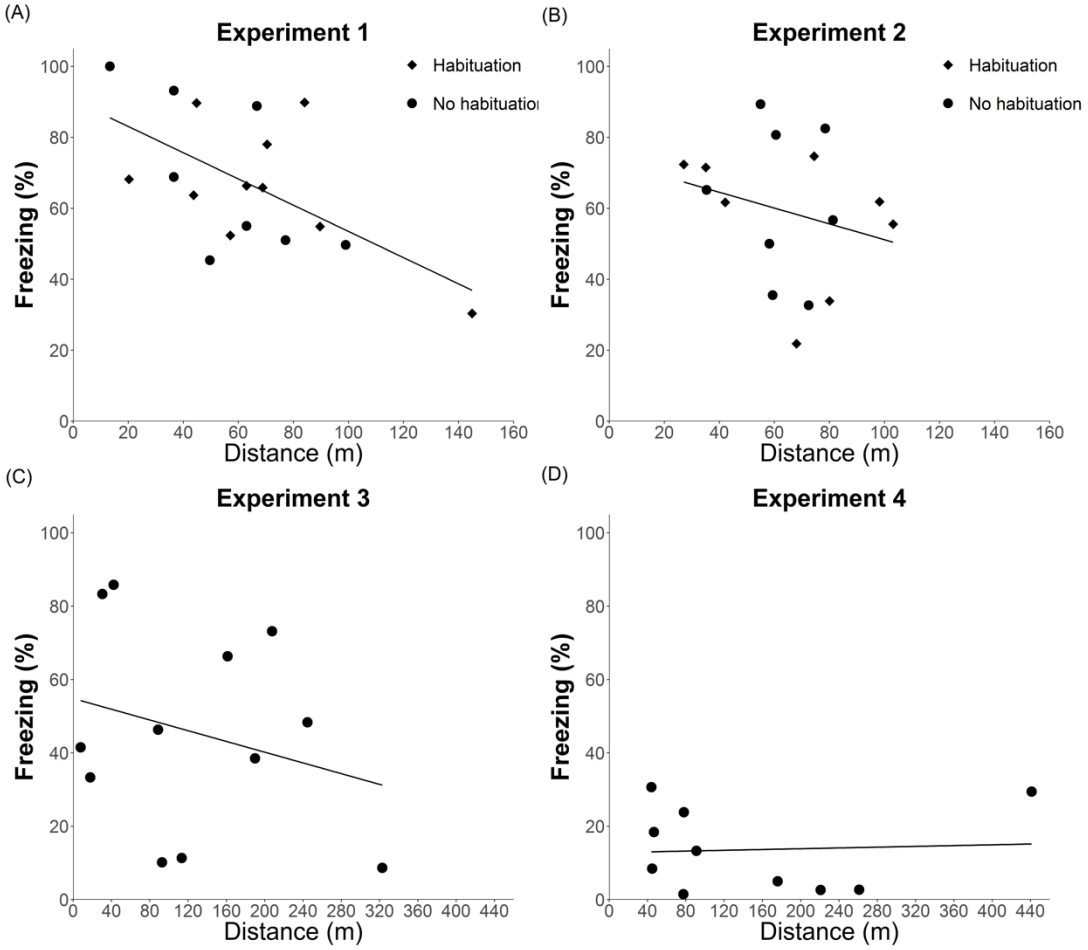

Figure 3.6 **Linear relationship between average distance run before extinction session(s) and freezing during relapse tests**. Reinstatement (A-C) or spontaneous recovery (D). (A) Data indicate a significant negative relationship between distance run and freezing, R^2 = .3486 p = .01, however data are driven by one rat who ran a significantly greater distance, see result section for greater details. (B, C, D) No significant relationships were observed. (A-B) Geometric shapes indicate habituation condition; there were statistically no differences between groups so data was combined. *Note:* experiments 1 and 2 rats were allowed 30-min of wheel access whereas experiments 3 and 4 there was 3-h of access.

3.6.2 Experiment 2: The effect of 30-min of exercise 1-h before cued extinction training on extinction consolidation and subsequent memory

The aim of experiment 2 was nearly identical to experiment 1; however, the single 30-min bout of voluntary wheel running occurred 1-h before extinction training. It was hypothesized that an acute bout of exercise 1-h prior to extinction may enhance the consolidation of extinction memories resulting in reduced levels of freezing at LTM and/or during the reinstatement test. To this end, we used an identical 2x2 design: No Habituation-Sedentary (SED (no hab); n = 8), Habituation-Sedentary (SED (hab); n = 8), No Habituation-Exercise (EX (no hab); n = 8), Habituation-Exercise (EX (hab); n = 8), see Figure 3.1B for a timeline of the experimental procedures.

3.6.2.1 Methods

Subjects. A total of 32 male Sprague-Dawley rats were used in this experiment, with all the same specifications, characteristics, and housing conditions as in experiment 1.

Equipment. All equipment used was identical to what was used in experiment 1.

Procedures.

Wheel habituation. All habituation procedures were identical to those used in experiment 1.

Fear acquisition. The fear acquisition procedures were identical to those used in experiment 1.

Treatment. A time delay of 1-h between onset of exercise and initiation of extinction sessions was implemented to potentially produce maximal up-regulation of BNDF during extinction consolidation, based on the 2-h time point found by Soya et al. (Soya et al., 2007). This timing was determined based on 1-h from exercise onset to extinction session onset plus 1-h and 10-min for the extinction session. The same

treatment preceded each extinction session for each rat. Voluntary running behavior was assessed similarly to experiment 1.

Extinction. Fear extinction procedures were identical to those used in experiment 1, except that there were two extinction sessions. An additional extinction session was added to try and reach larger decreases in freezing by the end of extinction possibly reducing the potential of a ceiling effect seen at the reinstatement test in experiment 1.

Long-term memory tests. The LTM test procedures were identical to those used in experiment 1, except that there were four CS alone presentations.

Reinstatement. The reinstatement procedures were identical to those used experiment 1 except to reduce the likelihood of ceiling effects during the reinstatement test there were three unsignaled foot shocks.

Reinstatement test. The reinstatement test procedures were identical to those used in experiment 1.

Measures.

Freezing behavior. Freezing behavior was scored in a similar fashion as in experiment 1.

Analysis. Similar statistical approaches and software were used as in experiment 1.

3.6.2.2 Results

Freezing behavior. For individual findings across all behavioral tests see Table 3.7. During fear acquisition a repeated measures ANOVA indicated that all groups showed within session fear acquisition with a main effect of habituation condition such that rats previously exposed to wheel habituation froze less (Figure 3.3B). While there were significant differences in acquisition between the habituation and no habituation

conditions, there were no significant group differences in freezing behavior during the first three CS presentations of the first extinction session suggesting no difference in the overall level of retention ($F(3, 28) = 0.78$, $p = 0.52$). A repeated measures ANOVA indicated a significant main effect of trial during the first extinction session indicating a reduction in freezing during the session across groups (Figure 3.7A). A similar significant within-session reduction in freezing was observed during the second extinction session (Figure 3.8A). Following two days of extinction there were no significant group differences in freezing behavior during the LTM test (Figure 3.8B). Then using a reinstatement procedure it was found that across groups there were no differences at the reinstatement test (Figure 3.8C). Nevertheless, repeated measures ANOVA did indicate significant reinstatement of freezing from LTM to the reinstatement test.

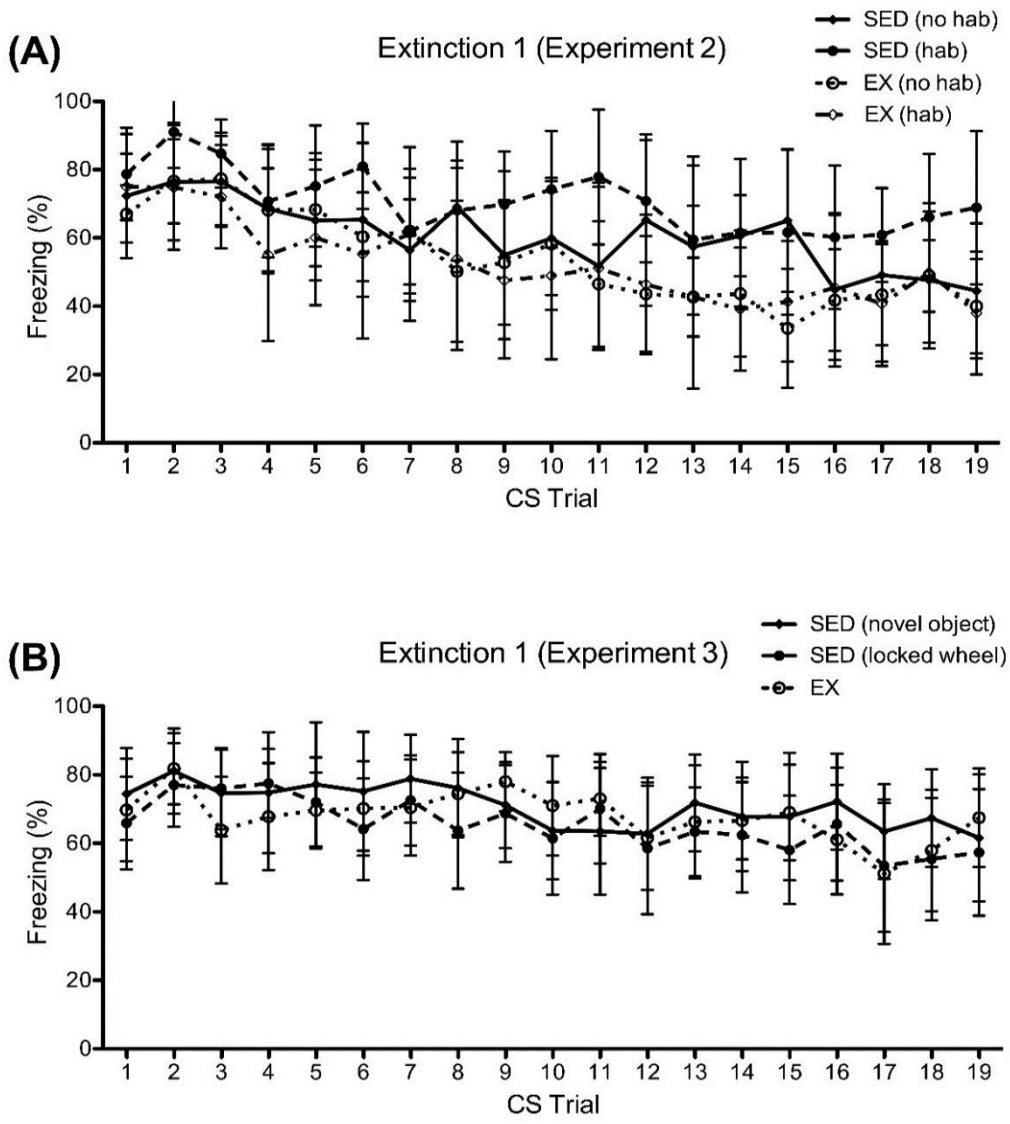

Figure 3.7 **First extinction session**. After completion of experiment 1 high levels of freezing across groups observed at the end of a single extinction session (>35%), this was also observed in the figures below, warranting a second day of intervention plus extinction exposure, to reduce the potential of ceiling effects at the reinstatement test. (A-B) All rats show within session extinction, p's < .001. Data are expressed as mean ± CI (n = 8–12 per group).

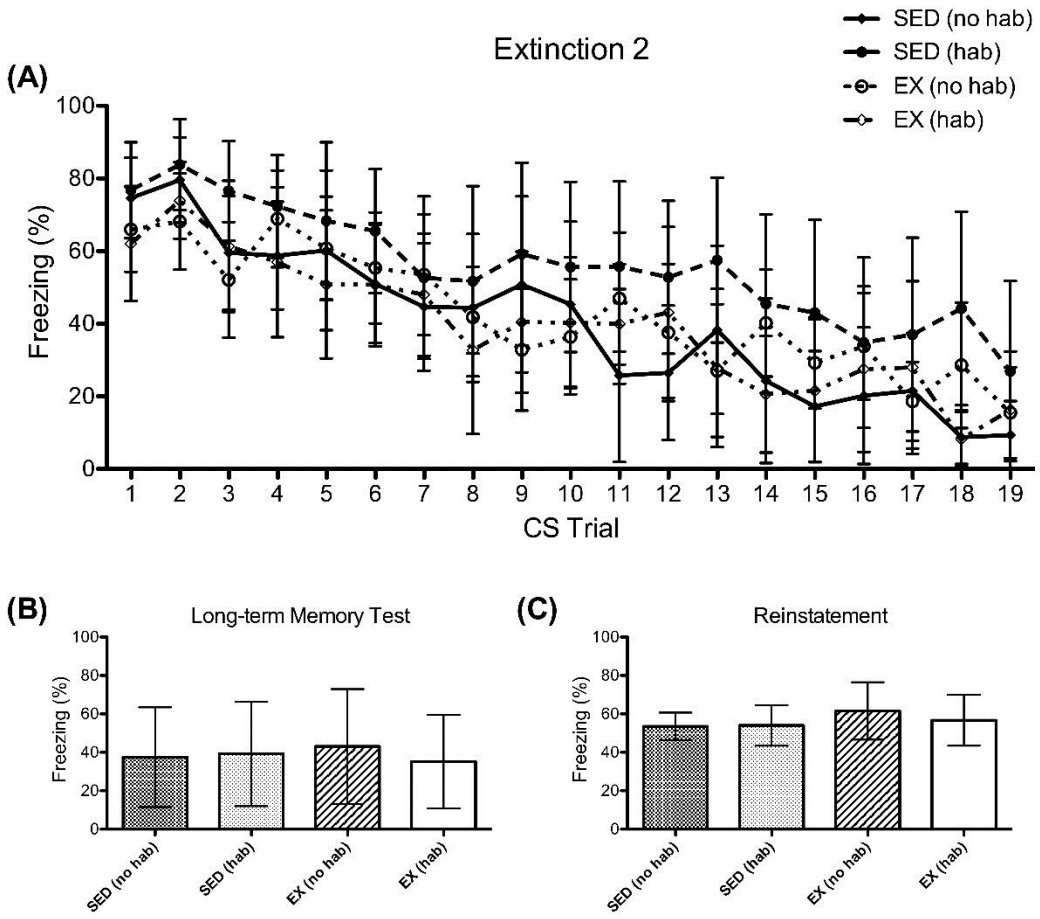

Figure 3.8 **Experiment 2.** (A) within session two extinction, (B) long-term memory and (C) reinstatement data used in cumulative analysis. (A) All rats show within session extinction, $p < 0.001$. Data are expressed as mean ± CI (n = 8 per group).

Voluntary running behavior. On average both exercise groups ran similar amounts (EX (no hab) M = 62.59, SD = 14.79; EX (hab) M = 66.04, SD = 28.61; $t(14) = 0.30$, $p = 0.77$). For which the average distances run across both groups during the intervention period are shown in Table 3.8. Data indicated a non-significant relationship between the average distance run and later freezing during the LTM test ($r(15) = -0.10$, p

= 0.70, Figure 3.5B) as well as a non-significant relationship between the average distance run and later freezing during the reinstatement test (r(15) = -0.25, p = 0.36, Figure 3.6B)[3].

3.6.3 Experiment 3: Effects of extended 3-h wheel access on cued fear extinction and subsequent memory

The aim of experiment 3 was to test the effect of 3-h of voluntary wheel running immediately before extinction training on cued fear extinction and subsequent memory. We hypothesized that we would extend previous findings from Siette and colleagues (2014) to cued fear extinction and show a reduced level of responding at LTM and further test if these effects carried over after reinstatement procedures. To test our predictions, we employed a 3-cell design in which rats either 1) had access to a wheel for exercise, 2) had access only to a locked wheel (sedentary), or 3) had access only to a novel object, to control for the novelty effect of a wheel in the cage (see Figure 3.1C for a timeline of the experimental procedures).

3.6.3.1 Methods

Subjects. Final experimental procedures included 36 male Sprague-Dawley rats (Novel Object, n = 12; Sedentary, n = 12; Exercise, n = 12) with all the same specifications, characteristics, and housing conditions as in experiment 1. All behavioral tests were started 2-h into the rat's light cycle (8:00) and were concluded by (16:00).

[3]Initial analysis again found no differences between the EX (no hab) and EX (hab) groups in the relationship between average distance run prior to the extinction sessions and later freezing behavior during the LTM test or the reinstatement test. Therefore, data from the EX (no hab) and EX (hab) were combined.

Experiment 3 did not include wheel habituation procedures, so rats habituated to handling for 2-min for four days prior to the first behavioral test.

Equipment. All exercise and conditioning equipment was similar to experiment 1 with the following changes. To replicate the methods of Siette, et al. (2014), sedentary rats were exposed to a locked wheel. Wheels were locked by inserting a metal tab on both sides of the wheel rendering them unable to rotate. The novel object group, unique to experiment 3, used a piece of PVC tube 18 cm long with a diameter of 11 cm as the novel object. This tubing was large enough for rats to lie in the tube and observe from the outside but rats were unable to climb on top or move the tube within the cage.

Procedures.

Fear acquisition. The fear acquisition procedures were identical to those used in experiment 1.

Treatment. On the days in which rats went through the fear extinction procedure (i.e. days 2 & 4), the session was immediately preceded by a 3-h treatment session. During this session rats remained in their homecages and either a free moving wheel, a locked wheel, or a novel object was placed in the cage. Unique to experiment 3, all treatments were implemented in the homecage with both rats present. To determine which rat used the exercise wheel, rats were recorded using a Sony Handycam HD and videos were later scored for counts run by each individual animal. The voluntary running behavior was otherwise assessed using the same procedures as in experiment 1.

Extinction. The fear extinction procedures were identical to those used in experiment 1. However, they occurred on day 2 and 4 with a LTM session on day 3.

Long-term memory test. LTM test procedures were identical to those used in experiment 1, but occurred on both days 3 and 5.

Reinstatement. The reinstatement procedures were identical to those used experiment 1 and occurred 24-h after the second LTM session.

Reinstatement test. The test for reinstatement procedures were identical to those used in experiment 1 and occurred 24-h after reinstatement procedures.

Measures. Freezing behavior was scored in a similar fashion as in experiment 1.

Analysis. Similar statistical approaches and software were used as in experiment 1, with the exception that this experiment only had one factor (treatment) with 3-levels (Exercise, Locked and Novel Object).

3.6.3.2 Results

Freezing behavior. For individual findings across all behavioral tests see Table 3.7. First all groups show within-session fear acquisition by CS3 (Figure 3.3C). After the first treatment rats were run through extinction procedures for which a repeated measures ANOVA indicated a significant main effect of trial, indicating within-session reduction in freezing during the first extinction session, (Figure 3.7B) with no significant group differences. The subsequent day rats were then tested for LTM where no significant group differences in freezing behavior were seen (Figure 3.9). After a second exposure to wheel or sedentary conditions, all rats again show within-session extinction (Figure 3.10A). For which again there were no significant group differences in freezing behavior when tested during a second LTM test (Figure 3.10B). Finally across all groups there was a significant increase in freezing from the second LTM test to the reinstatement test but no significant between group differences in freezing during the reinstatement test (Figure 3.10C).

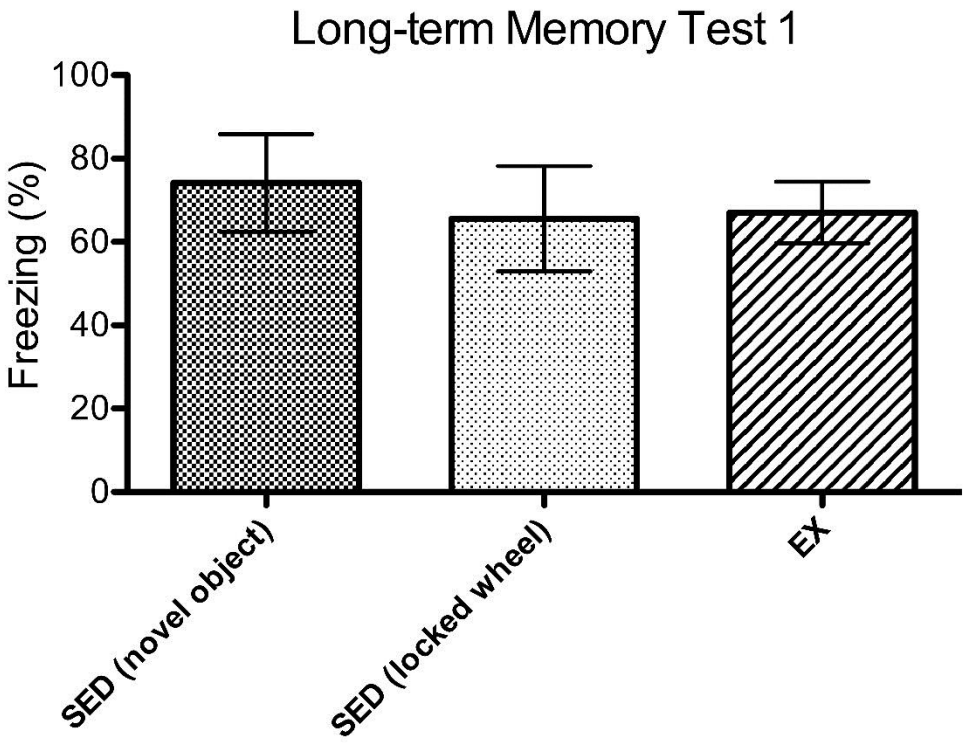

Figure 3.9 **Freezing during the first session of long-term memory of experiment 3.** Data are expressed as mean ± CI (n = 8–12 per group).

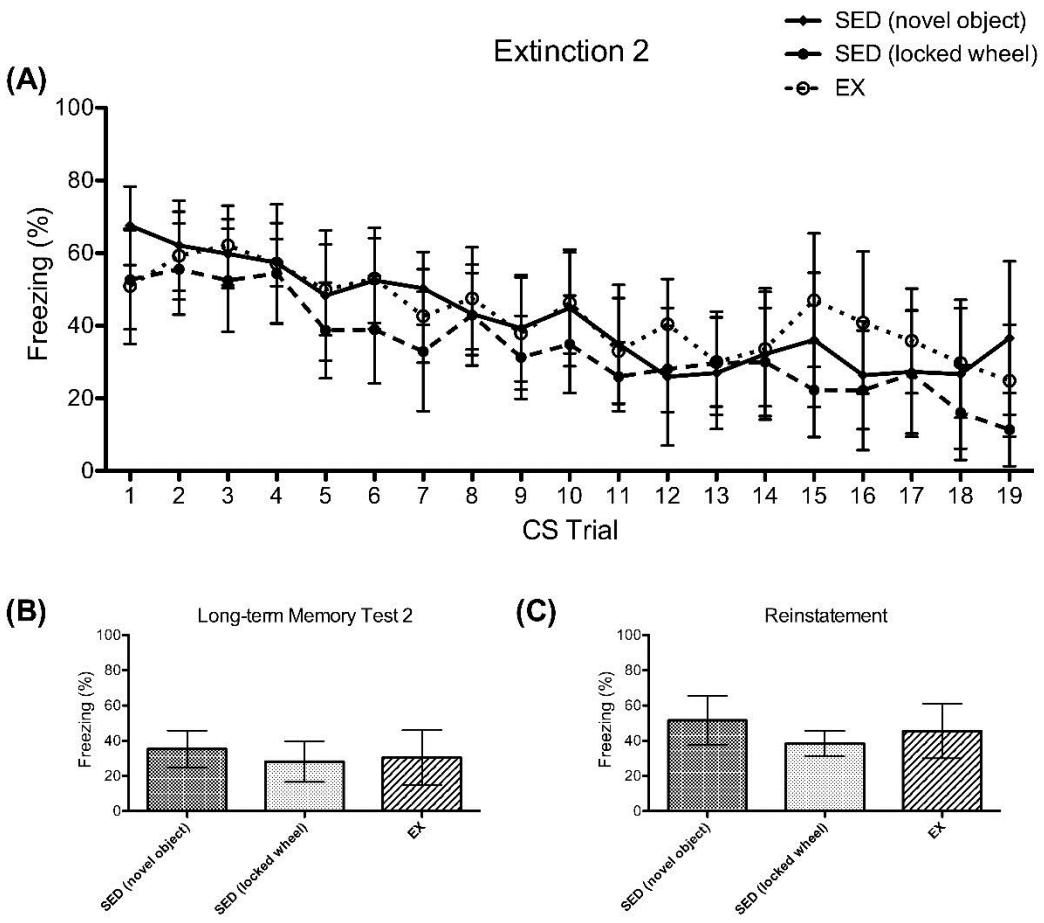

Figure 3.10 **Experiment 3.** (A) within session two extinction, (B) long-term memory session two and (C) reinstatement data used in cumulative analysis. (A) All rats show within session extinction, $p < 0.001$. Data are expressed as mean ± CI (n = 8–12 per group).

3.6.4 Experiment 4: Effects of extended 3-h wheel access on contextual fear extinction and subsequent memory

Experiment 4 aimed to replicate the reduction of fear seen at LTM after 3-h of voluntary wheel running immediately before extinction training on contextual fear extinction as previously shown by Siette and colleagues (Siette et al., 2014), as well as extend their results to an additional memory test—spontaneous recovery. To test this, identical groups were used as in experiment 3 but for a unique timeline of procedures see Figure 3.1D.

3.6.4.1 Methods

Subjects. A total of 33 male Sprague-Dawley rats were ultimately used in this experiment (Novel Object, n = 12; Sedentary, n = 11; Exercise, n = 10)[4] with all the same specifications, characteristics, and housing conditions as in experiment 1 and the same handling procedures as in experiment 3.

Equipment. All equipment was identical to that used in experiment 3.

Procedures.

Habituation. Animals were first habituated to the conditioning chamber across two sessions lasting 5-min; each session was separated by 3-h and occurred the day prior to conditioning.

[4]Two cagemates from the exercise group were excluded from the data analysis because both ran less than 5% of the average distance run by all other rats due to one burying the wheel at the beginning of the treatment session. In addition, one rat from the sedentary condition due to equipment error was not exposed to the US during acquisition and was not included in any of the analyses.

Fear acquisition. Different from the previous experiments, during conditioning all rats received a single foot shock (0.7 mA for 0.5-s) 5-min after being placed in the conditioning chamber. All rats remained in the chamber for two additional min after the foot shock.

Treatment. All implementations of behavioral treatments were identical to experiment 3. Voluntary running behavior was acquired similarly to experiment 3.

Extinction. The fear extinction procedures were different from the previous experiments. The extinction session consisted of rats being place in the conditioning chamber for 7-min.

Long-term memory test. To test LTM rats were placed in the conditioning chamber for 10-min.

Spontaneous recovery test. Rats were placed in the conditioning chamber for 10-min.

Measures.

Freezing behavior. Freezing during acquisition was assessed for 1-min following the single foot shock. On the subsequent day, the percentage of time freezing was assessed for each of the 7-min of the extinction session. Finally, across both memory tests, the percentage of freezing was averaged across the entire 10-min session.

Analysis. Similar statistical approaches and software were used as in experiment 3.

3.6.4.2 Results

Freezing behavior. For individual findings across all behavioral tests, see Table 3.9. First during acquisition there were no significant differences in freezing across groups, *(*Figure 3.11A). During extinction there was a significant effect of time across the

extinction session (p = 0.002, Figure 3.11B) but no between group differences. When tested for LTM there were no differences in freezing between groups (Figure 3.11C). Finally when tested for spontaneous recovery 21 days after LTM there was a significant reduction in freezing behavior with no significant between group differences during the spontaneous recovery test (Figure 3.11D).

	Experiment 4		
	df	*F*	*p*
Acquisition			
treatment	2, 30	0.101	0.904
Within Session 1 Extinction			
treatment	2, 30	2.132	0.136
min	6, 180	3.618	0.002
treatment x min	12, 180	0.871	0.578
Long-term Memory Session 1			
treatment	2, 30	1.246	0.302
Spontaneous Recovery			
treatment	2, 30	0.502	0.61
Long-term Memory Test to Spontaneous Recovery			
treatment	2, 30	0.996	0.381
test	1, 30	8.616	0.006
treatment x test	2, 30	0.75	0.481

Table 3.9: Primary results from experiment 4 context based extinction

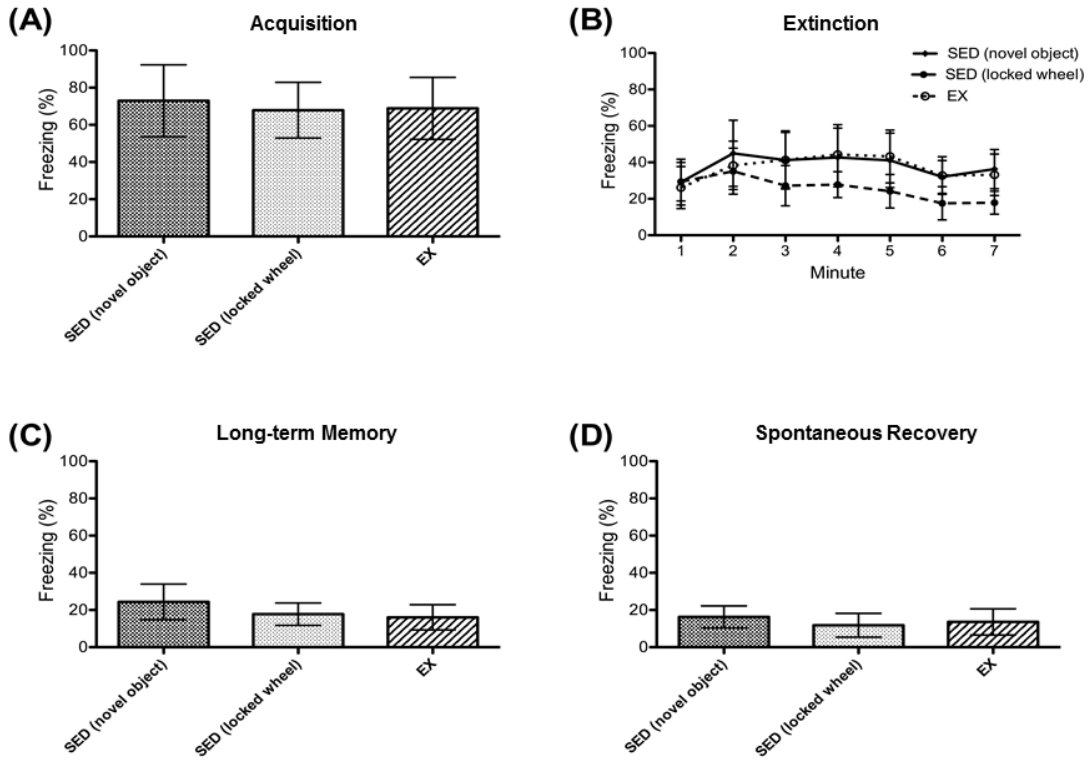

Figure 3.11 **Experiment 4.** (A) Rats in all treatment groups show fear acquisition with no differences across groups. (B) There was an effect of within session extinction, $p = 0.002$, but no effect of treatment. (C-D) Rats show no differences in freezing at the long-term memory test or during the spontaneous recovery test. Data are expressed as mean ± CI (n = 10–12 per group).

Voluntary running behavior. Voluntary distance run during the 3-h session prior to extinction was not significantly related to freezing during the LTM test ($r(9) = -0.02$, $p = 0.95$, Figure 3.5D) or during the spontaneous recovery test ($r(9) = 0.16$, $p = 0.65$, Figure 3.6D).[5] For average distances run across the intervention period, see Table 3.8.

[5]One rat voluntarily ran 2.79 standard deviations greater than the mean during the exercise session, however with the removal of this rat there was still a non-significant relationship and because voluntary exercise was the desired behavior all data were included in analysis.

Chapter 4: Does Exercise Augment Operant and Pavlovian Extinction: A Meta-Analysis

4.1 ABSTRACT

Background Exposure therapy, a behavioral approach to reduce symptomology in fear, anxiety, and drug related psychiatric disorders, is based on learning and memory principles of extinction, and is subject to relapse. As such, it is important to find ways to enhance outcomes. One such way is through exercise. **Objectives** Identify if exercise augments extinction behavior, and whether this depends on the experimental paradigm used (i.e. operant or Pavlovian) and/or stimulus (i.e. appetitive or aversive). Additionally, determine which moderating variables influence the effects of exercise on extinction learning. **Methods** A literature search was conducted and a Hedges' g calculation was employed to conduct a meta-analysis (metaSEM) using a structural equation modeling approach. This approach was chosen because of its ability to account for dependencies in effect sizes. **Results** We found a significant effect of exercise as an augmentation over extinction alone (g= 0.37, p< .001), with extinction paradigm producing a moderating effect (B= 0.43, p= .030), whereas stimulus did not. Data were then split by extinction paradigm, with operant extinction models having a significant effect (g= 0.55, p< .001), and number of extinction sessions moderating aggregate effects. Pavlovian models did not have significant overall effects (g= 0.11, p= .3976), but were moderated by the number of animals housed together and exercise after extinction. **Conclusions** The effects of exercise on extinction learning are differentially modulated by the type of paradigm used, the number of extinction sessions, when the exercise treatment was applied (after extinction) and the housing conditions.

4.2 INTRODUCTION

Clinicians working with psychiatric patients face the challenge of varying response rates, and the potential for relapse after treatment. Disorders such as anxiety affect approximately 30% of the U.S. population in a lifetime, and substance use disorders affected approximately 270,000 Americans in 2015 alone (Kessler et al., 2005; SAMHSA, 2015), underscoring the necessity to provide treatments that both work in the short-term, but are also resistant to relapse. Both anxiety-related and substance use disorders are thought to develop and be maintained through learning and memory mechanisms, and rely on behavioral principles of operant and Pavlovian conditioning (for review see Britton, Lissek, Grillon, Norcross, & Pine, 2011; Milton & Everitt, 2012). As such, they both allow for the potential use of standard extinction-based behavioral approaches to reduce levels of responding (G. B. Kaplan, Heinrichs, & Carey, 2011). Over the last few decades, exposure-based therapies (which possess elements that overlap with extinction) have been used in clinical populations (Foa & Mclean, 2016; Krijn, Emmelkamp, Olafsson, & Biemond, 2004). While these therapies have shown efficacy over control conditions, as well as above and beyond pharmacotherapies, there are still patients who do not show improvements (Barlow et al., 2000; Bystritsky, 2006; Foa et al., 2005; Schottenbauer, Glass, Arnkoff, Tendick, & Gray, 2008; Simpson et al., 2013). In addition, patients treated with exposure based therapies are still subject to extinction relapse (as evidenced by reinstatement, renewal, and spontaneous recovery; Bouton, 2002). Such phenomena have lead us to the understanding that while extinction can reduce levels of responding, it does not erase the initial memory, but rather leads to the formation of new inhibitory learning, which may compete with the initial memory.

With the knowledge that a new memory is formed during extinction/exposure training, additional research has focused on developing avenues to augment extinction

efficacy using pharmacological and behavioral approaches, to improve behavioral/treatment outcomes and reduce the potential for relapse (for review see Fitzgerald, Seemann, & Maren, 2014). Specifically, one pharmacological agent, D-cycloserine (DCS), has shown success across both extinction and exposure therapy (Ressler et al., 2004; Santa Ana et al., 2009; Walker, Ressler, Lu, & Davis, 2002); however, it has also been associated with persistence of responding under certain conditions (Bolkan & Lattal, 2014; Myers & Carlezon, 2012; Smits, Rosenfield, Otto, Marques, et al., 2013; Weber, Hart, & Richardson, 2007). Additionally, work looking at methylene blue a neurometabolic enhancing drug found similar results to DCS in humans and animals. Such that when a session of extinction/exposure was successful, as measured by low levels of fear at the end of session, methylene blue was beneficial however when high levels of fear were displayed subjects were worse off at follow-up tests (Auchter, Shumake, Gonzalez-Lima, & Monfils, 2017; Telch et al., 2014). With these mixed results from augmentation through pharmacotherapy, there has been a push to find alternative approaches to facilitate exposure outcomes. Non-pharmacological treatment avenues have been considered, with patients indicating that they prefer behavioral, cognitive and psychological approaches over pharmacotherapies (Arch, 2014; Mchugh, Whitton, Peckham, Welge, & Otto, 2013; Roy-Byrne, Berliner, Russo, Zatzick, & Pitman, 2003). In addition, both anxiety disorders and substance use disorders often develop in adolescent populations for which pharmacological interventions are controversial due to ongoing neurodevelopment, resulting in a limited range of approved pharmacotherapies (Ipser, Stein, Hawkridge, & Hoppe, 2009; G. Kaplan & Ivanov, 2011). One such behavioral approach is through exercise. Introduced independently, exercise has been shown to reduce anxiety, stress and depression, as well as increase measures of well-being (Asmundson et al., 2013; Dunn, Trivedi, Kampert, Clark, &

Chambliss, 2005; Manger & Motta, 2005; Muller, Dennis, & Gorrow, 2006; Stonerock, Hoffman, Smith, & Blumenthal, 2015), all of which are predictive of treatment success. When applied broadly to supplement varying behavioral therapy types, exercise has also been shown to be beneficial (Brown et al., 2010; Merom et al., 2008); however, as the articles in the present work will highlight, exercise appears to have mixed effects on extinction: positive (Lynch, Piehl, Acosta, Peterson, & Hemby, 2010), negative (Peterson, Hivick, & Lynch, 2014), null (Sanchez, Moore, Brunzell, & Lynch, 2014) and even mixed findings within a single article (Zlebnik, Saykao, & Carroll, 2014).

As the field of exercise to augment extinction/exposure has developed, researchers have used a wide variety of study designs to maximize overall effects. Specifically, some employ weeks (or continuous bouts) of exercise whereas others only use a single (or acute) bout. Others use forced exercise on treadmills whereas some experimenters allow voluntary access for an allotted amount of time. There is also a large range of timing of exercise, such as before, during, or after extinction, and even during the test session. This variation in exercise regimens alone warrants a better understanding of which parameters predict significant overall effects— in particular, those that produce a persistent reduction of responding over standard extinction. In the present analysis, we explore both the global effects of exercise on extinction, as well as determine whether exercise can have differential effects based on type of stimuli used (i.e. appetitive or aversive) or based on operant and Pavlovian extinction models. The overarching goal, through the use of a structural equation modeling approach to meta-analysis, is to determine not only if exercise can have a significant enhancement on extinction by producing a greater and persistent reduction in responding, but delineate what parameters are the strongest predictors of its success.

4.3 METHODS

4.3.1 Search Strategy

Currently, there are no systematic reviews of the effects of exercise on extinction or exposure therapy so primary searches were conducted to find articles. A search of PubMed and EBSCOhost was conducted on articles published through August 8, 2016. The terms searched (Exercise AND Extinction), (Exercise AND Exposure Therapy), (Wheel AND Extinction), (Wheel AND Exposure Therapy), (Running AND Extinction), and (Running AND Exposure Therapy).

4.3.2 Selection

Both human and non-human animal studies looking at the effect of exercise on extinction or exposure based therapies alone were included in analysis. Experiments using any amount of exercise as a manipulation were included. Experiments were included in the analysis if their design met the following criteria: 1) examined aversive or appetitive memories after an operant or Pavlovian extinction procedure; 2) compared an exercise plus extinction/exposure group to a control group who was also exposed to extinction procedures; and 3) assessed responses after extinction or exposure therapy through a test of long-term memory, reinstatement, relapse, or spontaneous recovery. Studies that met any of the following exclusion criteria were eliminated: 1) extinction of exercise behavior; 2) experiments comparing across special populations (e.g. rats bred for high anxiety compared to low anxiety); 3) abstinence only (i.e. no active extinction or exposure procedures); 4) tests precipitated by non-conditioned stimuli (e.g. pharmacological methods); 5) exercise employed only before initial acquisition or at test; 6) forced swim exercise (used to induce stress); 9) articles using combinations of therapies not just extinction/exposure alone and 8) cases where data was used in multiple

papers (Peterson, Abel, & Lynch, 2014; Sanchez, Moore, Brunzell, & Lynch, 2013), in these cases data from the most current papers were used (Peterson, Hivick, et al., 2014; Sanchez et al., 2014).

4.3.3 Data Abstraction

Articles meeting the selection criteria were collected and data were abstracted for analysis by the first author and independently checked for accuracy by the senior author. Errors were reconciled through discussion amongst the authors. In instances were not enough data was available in the text to calculate true effect sizes authors were first contacted on June 27, 2016, these initial emails were followed by up to two follow up emails between July 11, 2016 and August 8, 2016. All authors responded with the requested data within this period so analysis commenced.

4.3.3.1 Study design

All studies included for analysis used standard experimental methods for operant and Pavlovian extinction procedures (for review see Todd, Vurbic, & Bouton, 2014). The primary experimental methods seen across both tasks include an extinction based procedure and a post-test of responding. Briefly, in tasks using a Pavlovian model of extinction after conditioning, the conditioned stimulus (CS) was repeatedly displayed in absence of the unconditioned stimulus (US), thereby leading to a progressive reduction in the conditioned response (CR). This CR was then tested directly via a long-term memory test or tests for return of responding such as reinstatement, relapse, or spontaneous recovery. In the selected operant tasks, a stimulus (S) is followed by a positive consequence (reinforcer) after a response is produced (R), leading to the S becoming a CS associated with the reinforcer. During extinction, the response no longer leads to the consequence and thus leads to a reduction in the response. This reduction in responding

can then be tested similarly to Pavlovian models. It is important to note that for the present analysis, all operant experiments looked at reinstatement of responding. This was done either by re-exposure to the reinforcer and/or the S depending on the stimuli that were extinguished.

Both between- and within-subject designs were used in the present meta-analysis. For between-subject designs, subjects were randomly distributed into groups that received an exercise intervention or remained sedentary. One experiment used a within-subject design, in which subjects were tested both under control conditions (no wheel present) and with access to an exercise wheel during the experimental test (Zlebnik, Anker, Gliddon, & Carroll, 2010).

With a wide range of inclusion criteria, it was often possible for multiple comparisons from a single experiment to be made, for which three cases arose: 1) two or more exercise groups were present within one experiment; 2) multiple outcome measures were conducted on the same subjects; and 3) a combination of multiple exercise groups and multiple outcome measures. In all cases, groups were included in the analyses using a clustering approach to account for potential dependence within the data. Additionally, some studies included multiple control groups, in which case those that had received extinction procedures and the least or no exercise access were used for comparison.

All studies employing exercise as a modifier of extinction were included, as such; exercise was implemented in a variety of different ways. In some cases, our search terms yielded studies in which exercise was only applied prior to acquisition (which could confound interpretations of the effect of exercise specifically on extinction) or studies in which exercise only had a direct influence on the outcome test (e.g. when it was applied during the outcome test only). Owing to possible confounds, we opted to exclude such cases from our analyses. If exercise begun prior to acquisition but continued until

extinction, we included the experiments in our analysis. In addition, if exercise was applied prior to testing (e.g. post extinction) and then again during the outcome test, we included the data in our analysis.

4.3.3.2 Study outcomes

In the current meta-analysis, the following tests were examined to evaluate the rate of responding after extinction (for review, see Bouton, 2002): exposure to the CS alone (long-term memory), the unexpected presentation of the CS and/or US (reinstatement), presentation of CS after the passage of time (spontaneous recovery), and presentation of the CS in a novel context after the passage of time (relapse; Mika et al., 2015).

4.3.3.3 Effect size data

Methods of study design and data analysis varied considerably across studies; however, for all between- subject studies, exercise and control groups were compared directly on their outcome test behavior. For the within-subject studies, effect sizes were calculated by comparing scores on two control tests to a test where exercise was available. Note that in an experiment done by Zlebnik et al. (2014), rats either had access to running wheels or not during extinction (between subjects) then, at test, all rats were assessed with and without wheel access (within subjects). While tests using a within-subjects design are more powerful, the purpose of the present analysis is to explore the effects of exercise on extinction, so we choose to calculate effect size measures based on whether rats had access to running wheels or not during extinction procedures.

For the calculation of effect sizes, means and standard deviations were preferred, but F statistics, p-values, t-values, as well as reported effect size calculations were used, depending on availability. Whenever possible, data reported directly in the original text

were used; however, authors were contacted directly when there was insufficient information to accurately determine overall effect sizes. If exact p-values were not reported (i.e. $p<.05$) a conservative approach was taken using the value provided (i.e. $p=.05$). Additionally, if there was no mention of the tails used for the statistical test, it was assumed to be two-tailed.

4.3.3.4 Moderator variables

Data regarding study design, participant characteristics, conditioning procedures and exercise parameters were abstracted to assess moderating effects.

Between/within-subject designs. Studies included in this analysis used both between- and within-subject designs, however, since only one experiment used a within-subject design there was not a sufficient sample to test design as a moderator.

Participant characteristics.

Gender. Previous research has shown that both operant and Pavlovian extinction can be influenced by gender, more specifically sex hormones (Chang et al., 2009; Graham & Milad, 2013; Kerstetter, Aguilar, Parrish, & Kippin, 2008; Yuan & Chambers, 1999), so moderator analysis was done to explore differences between male, female and a combination of both genders (mixed).

Subjects. Included in analysis were experiments that used human, mouse or rat subjects. Because previous research has found that rats enjoy running and find it rewarding (Greenwood et al., 2011; Heyse, Brenes, & Schwarting, 2015; Rasmussen & Hillman, 2011) difference in voluntary running behavior could be influenced by subject.

Housing (animal only). Previous research has shown that social buffering can affect fear conditioning outcomes (Kiyokawa, Honda, Takeuchi, & Mori, 2014; Kiyokawa, Takeuchi, & Mori, 2007). Furthermore, a recent meta-analysis looking at

retrieval+extinction (a procedure aimed at improving treatment outcomes and reduce return of fear) found a significant moderating effect of number of animals housed together in fear, but not appetitive, conditioning experiments (Kredlow, Unger, & Otto, 2016). The present analysis also explored the potential moderating effects of number of animals housed together. This was done in two ways. We first used a continuous scale; however, because number of animals is actually an integer and previous work in the field has focused on differences between animals housed alone versus multiple animals we also tested this variable as a dichotomous factor. Two comparisons from one experiment were not included in this analysis because animals were housed alone for 12 hours a day and 12 hours with a cagemate making classification impossible (Mika et al., 2015).

Age (rat only). Previous research has shown that a rat's age can influence extinction behavior (McCallum, Kim, & Richardson, 2010). The vast majority of studies included in the present paper used rat subjects; as such, we chose to include only age as a moderator for the rats, and opted not to include data from other species as age in days is not comparable across species.

Conditioning procedures.

Stimuli. Of the experiments included in the analysis, most used discrete conditioned stimuli such as a tone or light; however, context was also used as a conditioned and/or test stimulus. While it is known that context plays a critical role in operant conditioning and extinction retention (Bouton & Todd, 2014), only one experiment explored responses to context cues. In the Pavlovian experiments, both context and discrete cues were tested. This is of particular interest, because contextual fear and appetitive memories are known to be hippocampal-dependent (Ferbinteanu & Mcdonald, 2001; Phillips & LeDoux, 1992) and exercise has been shown to both upregulate brain-derived neurotrophic factor (BDNF) and increase neurogenesis in the

hippocampus (Huang et al., 2006; Soya et al., 2007; Van Praag, Christie, Sejnowski, Gage, & Stevens, 1999), both of which could be influencing extinction behavior. Because of the known influence of exercise on the hippocampus and its importance in contextual memories, the stimuli used to test behavior (i.e. discrete vs. context) were assessed for their effects in experiments using a Pavlovian extinction model.

In addition, due to differences in the types of conditioning used (i.e. Pavlovian and operant) experiments using operant models may or may not have the CS present during the extinction session which could influence later tests of responding. To this effect, all operant models used a test of reinstatement behavior, for which the reinstatement of responding could be precipitated by the CS, reinforcer or both. Both parameters were tested for their effects on the aggregate operant analysis.

One additional parameter that could influence behavioral responding is the intensity of the stimulus. Across all operant tasks, a dose of drug was given on a range of milligrams per kilogram. Pavlovian experiments used both appetitive and aversive stimuli, and as such both stimuli are on different scales (i.e. drug dose vs. shock intensity) this parameter was not tested as a moderator on Pavlovian models.

Number of sessions and timing of procedures. Additional parameters that could moderate overall effects are the number of extinction sessions used, as well as the time from extinction to test. Effectively, previous research has shown that spontaneous recovery may occur following extinction training (Rescorla, 2004). Exercise could have an influence on this effect.

Test parameters.

Test type. Because the purpose of this analysis was to explore a test following extinction, the type of test may be important to the overall outcome. Unfortunately, isolating this parameter was not possible, for two reasons: First, across Pavlovian

experiments, a wide variety of tests were used and due to the inconsistency in methods used for testing outcomes, the analysis would not have a sufficient sample per level (< 3). Second, across operant experiments, there was insufficient variability in the outcome test, because all experiments used a test of reinstatement. While insufficient variability was remedied when data were combined across conditioning types, there remained inconsistency of methods, making it unadvisable to test this parameter.

Exercise parameters.

Timing. Exercise across experiments was classified to have occurred during one of the following five time points: prior to acquisition, prior to extinction, during extinction, after extinction and during the outcome test. All experiments were coded as having access or not at each time point based on when exercise occurred, and each time point was tested individually in the analysis. One point to note, is that because exercise prior to acquisition and after exposure therapy could not be accurately accounted for in human experiments (as participants could have exercised following their exposure session(s), whereas they were asked specifically not to exercise prior to coming into their exposure sessions(s), these data points were left blank and not included in the analysis. In addition, exercise prior to extinction is defined as exercise that occurred after acquisition but before initiation of extinction that targeted extinction learning. Conversely, exercise after extinction was defined as any exercise that occurred after the cessation of extinction procedures, but before the memory test(s) applied to influence extinction memory.

Length and type. All experiments included the time subjects exercised; this was then converted into a value in minutes and analyzed as a continuous potential moderator variable. In addition, because Pavlovian models used two different types of exercise, forced or voluntary, this variable was analyzed as a potential dichotomous moderator variable only under the Pavlovian data subset.

Location. Exercise location also varied depending on the study: a number of experiments allowed for exercise in the home environment, while others were done in a different, distinct location. Because of known negative effect of handling on conditioning (Hoffman, Armstrong, Hanna, & Conrad, 2010), exercise in the homecage versus a secondary location was tested.

Operant specific parameters. Operant experiments using appetitive stimuli often incorporate parameters into the experimental design that are not commonly used in aversive conditions. We chose to explore two of these design characteristics.

Extended access/maintenance. After meeting acquisition requirements, some experiments then allowed the animals to have continued access to the reinforcer before extinction procedures were carried out. Unfortunately, only one experiment did not use an extended access period in their design so data are reported but no analysis was done to test this difference as a moderator.

Abstinence. One experimental parameter, implemented in some operant paradigms, was an abstinence period. This is an extended period after initial acquisition for which the appetitive stimuli are no longer accessible, and which could possibly lead to a withdrawal-like state. Because abstinence and withdrawal have been shown to produce a negative affective state and influence relapse of responding (Koob, 2009), as well as the fact that physical exercise during drug withdrawal has been shown to reduce self-rated measures of anxiety, depression, and craving (Bock, Marcus, King, Borrelli, & Roberts, 1999), we wanted to explore its potential to moderate overall operant effects.

4.3.4 Details of Analyses

4.3.4.1 Effect size analyses

Individual effect sizes were calculated using the *metafor* package in R (Viechtbauer, 2010). Hedges's g (Hedges, 1981) was used as the effect size measure. It was chosen because it corrects for small sample sizes. Interpretation of this effect size measure can be done using Cohen's standards (Cohen, 1977): small (0.2), moderate (0.5), and large (0.8). Both overall, as well moderator analysis, were conducted using the *metaSEM* program in the OpenMx package of the R statistical environment (Cheung, 2015; Cheung, Finch, & Buzick, 2015). This program was used because of its ability to handle the potential for statistically-dependent effect sizes, for which it uses a three-level Structural Equation Modeling (SEM) approach (Cheung, 2014; Van Den Noortgate, López-López, Marín-Martínez, & Sánchez-Meca, 2012) to handle potential dependence from the three situations previously discussed: 1) two or more exercise groups present within one experiment; 2) multiple outcome measures conducted on the same subjects; and 3) a combination of multiple exercise groups and multiple outcome measures. This approach was used in place of, for example, averaging effect sizes into one mean effect size per study or selecting one effect size per study, both of which would result in loss of information. In using this approach for meta-analysis, the first level of the model represents participants in the primary studies, the second level represents effect sizes within a single experiment, and the third level represents the multiple effect sizes across experiments. Using this multi-level approach, heterogeneity is partitioned at both level two (indicating within study variability) and level three (indicating between study variability). Aggregate effect sizes were calculated across all experimental data as well as subdivided by those testing operant vs. Pavlovian models of extinction.

4.3.4.2 Moderator analyses

To further explore what experimental parameters could be influencing our aggregate effects, moderator analysis was conducted using *metaSEM* in R on all the parameters discussed above (Cheung, 2015; Cheung et al., 2015). Both categorical and continuous moderators were included in analysis. For categorical variables with two levels, separate effect sizes were calculated for both groups, and the beta calculation of the difference in effect sizes was used to test for moderator effects. Effect sizes for categorical variables that included 3 or more levels were calculated, and a chi-square difference test comparing the base or overall model to a model that included the potentially moderating variable with all levels was performed, in which a significant difference would suggest a moderating effect. For continuous moderators, unstandardized regression coefficients were computed and evaluated for a significant relationship. To reduce the likelihood of Type I errors, moderator analyses were conducted on categorical variables only when a total of 10 or more effect sizes were included in the moderator analysis, and at least three effect sizes within a level came from at least two experiments. For example, if a single level of the moderating factor included data from only one experiment even if that one experiment had six effect sizes measures (i.e. six effect sizes contributing to that level) this factor was not explored as a potential moderator, as this may relate to a study effect rather than a moderator effect. This would result in "inconsistency" in methods. In addition, if a there was only one level of a moderating factor (e.g. all operant experiments tested reinstatement) this moderating factor was not tested, due to insufficient variability. For the same reason as those listed for categorical variables, continuous moderators were not tested if insufficient data of fewer than 10 effect size measures were available.

4.3.4.3 Publication Bias

To determine if publication bias could be influencing overall findings, funnel plots were created and the Trim and Fill method using the *metafor* package in R was used to determine how many studies might be missing from the analysis (Duval & Tweedie, 2000; Viechtbauer, 2010). To reduce skew from clustering effects using the metaSEM approach, all effect sizes were treated as independent. Funnel plots for each of the analyses were visually inspected for asymmetries in the number of effect sizes that fell above and below the mean effect size. Then, the Trim and Fill method was applied to identify and characterize asymmetry in the included studies. Trim and Fill analysis augments the observed data and imputes effect sizes to balance out asymmetric funnel plots. Funnel plots can also be used to visually assess heterogeneity in the data. A lack of heterogeneity is indicated by a large number of effects sizes falling outside the confidence intervals of the funnel. Heterogeneity is defined as the variability of true score estimates in a population (Higgins, 2008). When violations to heterogeneity exist, it is suggested that the exploration of moderators may be useful to account for variability within the sample.

4.4 RESULTS

4.4.1 Trial flow

From the search strategy discussed above, a total of 1789 articles were initially found using the stipulated criteria. Next, all duplicate articles were removed, and 1694 articles remained. Then, through initial screening by the authors looking at the articles abstracts, 40 potential articles were screened further. Approximately 400 articles were specifically excluded because procedures of exposure therapy are often called exercises, which include things like cognitive tests or other tasks that are not actual physical

exercise. In addition, approximately 1000 articles were excluded because the term "exercise exposure" is used to describe an exercise intervention for numerous diseases and disorders. Of the 40 remaining articles that used methodologies appropriate for this analysis, 14 were found to meet all criteria previously discussed. In addition to these 14 articles, data in one unpublished article, which has since been accepted for publication, from the current authors was included in analysis (Jacquart et al., 2017). Extracted from the 15 articles were 24 total experiments with 73 comparisons meeting all inclusion and exclusion criteria (see Figure 4.1). Data from all potential articles and experiments were obtained by the authors and used in analysis.

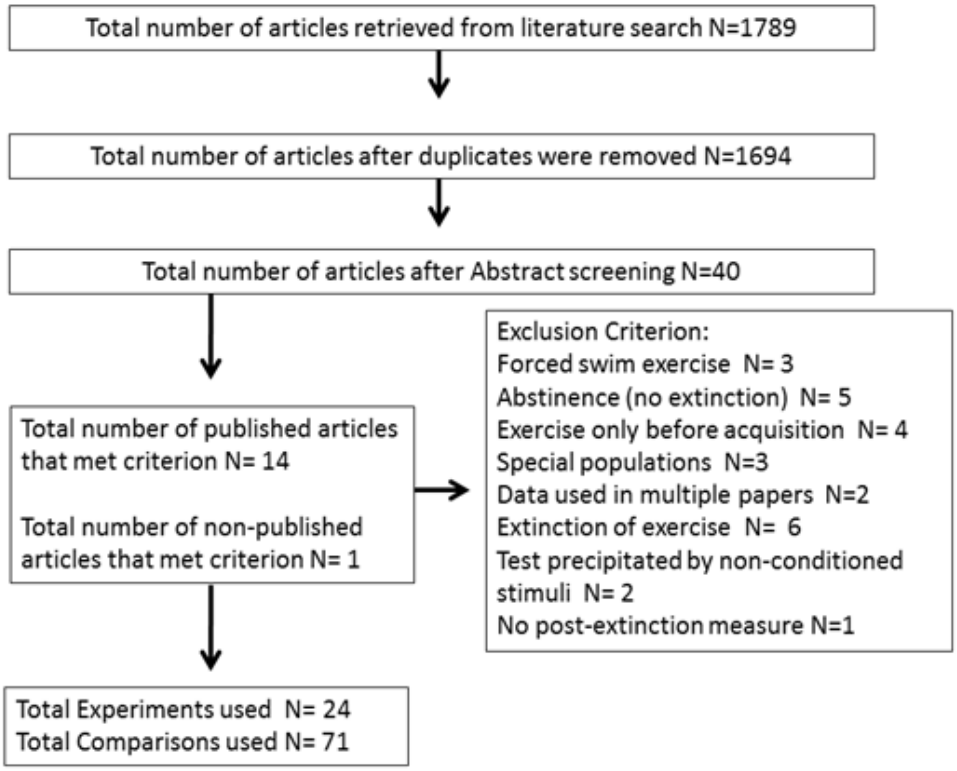

Figure 4.1. **Trial flow diagram of the article search and selection process.**

4.4.2 Full analysis

4.4.2.1 Main effects.

The first test of this analysis looked at the overall effects of exercise on post extinction outcomes across all effect sizes, and we found a significant effect of lower levels of responding above standard extinction alone (g= 0.40, 95% CI [0.17, 0.62], p< .001, n= 71). Such that exercise plus extinction produced lower levels of responding at memory test(s). For individual and aggregate effects, see Figure 4.2.

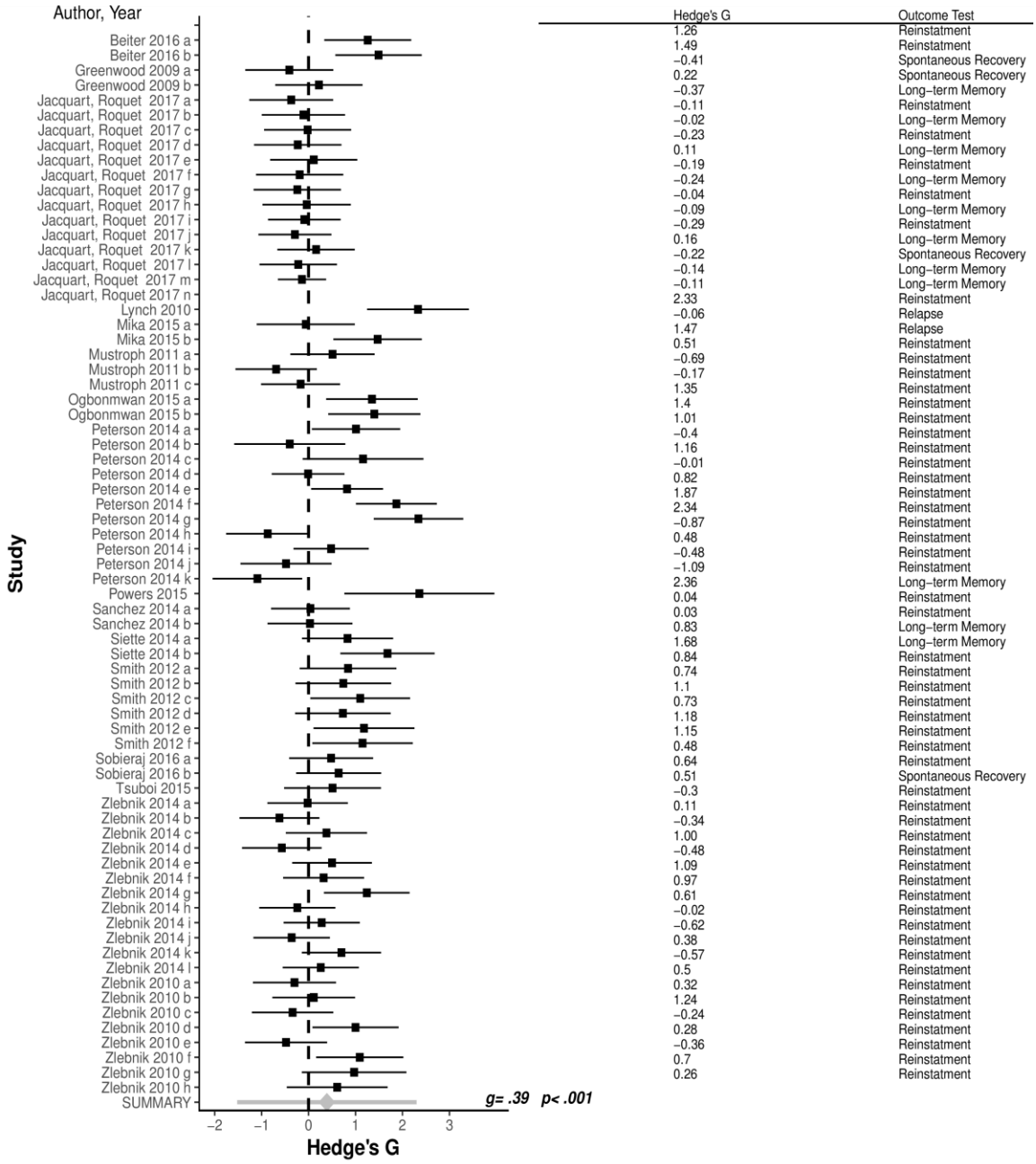

Figure 4.2 **Forest plot of individual study effects for exercise plus extinction over extinction alone on memory outcome tests across all extinction types.**

Publication Bias & Heterogeneity. To test for publication bias in the experiments used for analysis, funnel plots were constructed and examined for asymmetry. No asymmetries were visually seen in the full analysis (see Figure 4.3). To confirm the visual inspection, the model was then run using the Trim and Fill method (Duval & Tweedie, 2000) in which there were no cases where data points were added. It should be noted, however, that as numerous data points did fall outside both the 90% and 95% confidence intervals, further analysis indicates significant heterogeneity (Q[*df*=70] = 180.11, $p < .001$) warranting exploration of potential moderators.

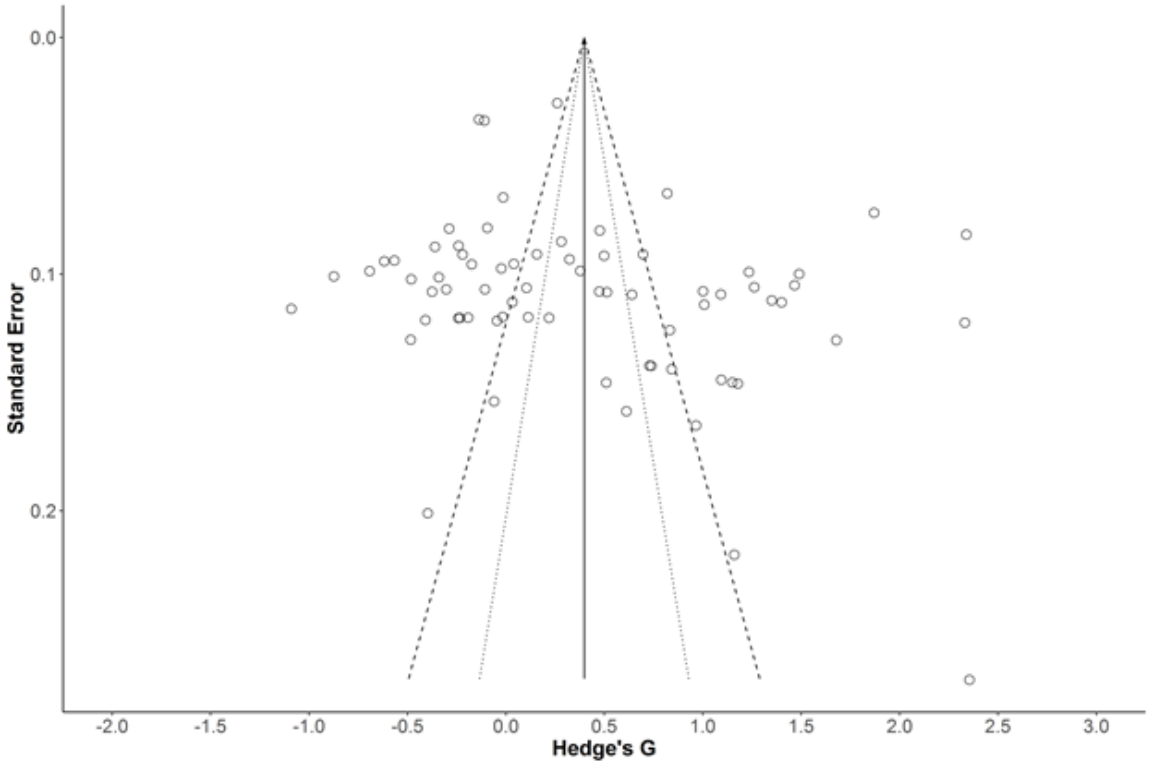

Figure 4.3 **Funnel plot of the individual study effects of exercise applied with extinction over extinction alone across all models.** Dotted lines represent 95% confidence intervals (CI) and dashed lines represent 99% CI.

Moderators. Overall analysis was followed by a test for a moderating effect to address our primary question of whether type of extinction procedure (operant or Pavlovian) moderated aggregate effects. Our analysis revealed a significant moderating effect (B= 0.46, SE= 0.21, p= .031), leading to our future exploration in the analysis below. In addition to the type of extinction, we wanted to determine if the type of stimuli used, which was closely related to conditioning type, would also have an effect; however, data indicate no difference between appetitive and aversive experiments (B= -0.33, SE= 0.21, p= .111). Specifically, experiments using appetitive stimuli had a significant effect (g= 0.52, 95% CI [0.24, 0.79], p< .001, n= 49), whereas experiments testing aversive stimuli did not (g= 0.18, 95% CI [-0.16, 0.51], p= .298, n= 21).

While the primary question of the global analysis was to determine if type of extinction or stimuli type were significant moderators, all other potential moderators were tested and data can be found in Supplemental Materials. Of the 15 potential moderators tested, one parameter was found to moderate overall analyses: whether exercise was implemented after extinction. For a summary across all findings, see Table 4.1.

	ALL		**OPERANT**		**PAVLOVIAN**	
		Moderator effect		Moderator effect		Moderator effect
OVERALL	**g=0.40**		**g= 0.58**		g= 0.14	
TYPE (OPERANT/PAVLOVIAN)		**B= 0.46**	NA		NA	
STIMULUS TYPE		B= -0.33		**		B= 0.32
APPETITIVE	**g= 0.52**				g= -0.13	
AVERSIVE	g= 0.18				g= 0.20	
GENDER		*X2*=0.43		B= -0.42		B= 0.25
FEMALE	g= 0.28		g= 0.33			
MALE	**g=0.44**		**g= 0.76**		g= 0.11	
MIXED	g= 0.39				g= 0.36	
SUBJECT		*X2*= 1.56		**		*X2*= 0.90
HUMAN	g= 0.38				g= 0.34	
MICE	g= -0.12				g= -0.13	
RAT	**g= 0.44**				g= 0.17	
CONDITIOING PARAMETERS						
# EXT SESSIONS		B= 0.05		**B= -0.25**		B= 0.06
EXT SESSION RANGE		B=- 0.13		**B=- 0.48**		B=0.26
Limited	**g= 0.38**		**g= 0.68**		g= 0.11	
Extended	g= 0.25		g= 0.21		g= 0.33	
EXT to TEST		B= 0.00		B= 0.03		B= -0.06
EXERCISE PARAMETERS						
EX PRIOR ACQ		B= -0.09		B= -0.32		B= -0.10
No exercise	**g= 0.43**		**g= 0.72**		g= 0.20	
Exercise	g= 0.34		**g= 0.40**		g= -0.22	
EX BEFORE EXT		B= -0.42		*B*= -0.0		*
No exercise	**g= 0.77**		g= 0.60			
Exercise	**g= 0.33**		**g= 0.58**			
EX DURING EXT		B= 0.13		*B*= -0.03		B= 0.21
No exercise	**g= 0.36**		g= 0.60		g= 0.10	
Exercise	**g= 0.48**		**g= 0.57**		g= 0.30	
EX AFTER EXT		**B= 0.79**		*		**B= 1.04**
No exercise	**g= 0.30**				g= -0.08	
Exercise	**g= 1.07**				**g= 0.94**	
EX DURING TEST		B= 0.14		*B*= 0.06		B= -0.32
No exercise	**g= 0.35**		**g= 0.56**		g= 0.20	
Exercise	**g= 0.49**		**g= 0.62**		g= -0.13	
EXERCISE LENGTH		***		B= 0.20		B= -0.08
EXERCISE TYPE		B= 0.02		**		B= 0.12
Forced	g= 0.42				g= 0.40	
Voluntary	**g= 0.40**				g= -0.09	

Table 4.1 Summary of effects across all analyses

	ALL		**OPERANT**		**PAVLOVIAN**	
		Moderator effect		Moderator effect		Moderator effect
ANIMAL SPECIFIC						
AGE-DAYS		B= 0.17		B= 0.15		NED
HOUSING #		B= -0.01		**		**B= 0.33**
HOUSING: SINGLE/MULTIPLE		B= -0.41		**		B= 0.09
Single	**g= 0.47**				g= -0.03	
Multiple	g= 0.07				g= 0.09	
EXRECISE IN HOMECAGE		B= 0.36		**		B= -0.10
In cage	**g= 0.48**				g= 0.08	
Alternative location	g= 0.10				g= 0.16	
OPERANT PARAMETERS						
STIMULUS INTENSITY (DOSE)		NA		B= 0.18		NA
CS @ EXT		NA		B= -0.42		NA
No-CS			**g= 0.63**			
CS			g= 0.31			
TEST STIMULUS		NA		*X2*= 0.20		NA
BOTH			g= 0.56			
CS			**g= 0.60**			
Reinforcer			g= 0.44			
EXTENDED ACCESS		NA		B= -0.57		NA
Access			**g= 0.95**			
No access			g= 0.38			
ABSTINENCE		NA		B= 0.31		NA
Drug abstinence			**g= 0.76**			
No abstinence			**g= 0.46**			
PAVLOVIAN PARAMETERS						
EXTINCTION STIMULUS		NA		NA		B= -0.04
CTX					g= 0.12	
CUE					g= 0.16	

Table 4.1 *cont.* Summary of effects across all analyses

4.4.3 Partitioned data

Of the 71 comparisons included in the analysis, 46 used operant extinction models (46 non-human animal comparisons) and 25 used Pavlovian extinction models (22 non-human animal comparisons; 3 human comparisons). A total of 706 subjects were used across all experiments included in analysis, of that, operant experiments were comprised of 411 non-human animal subjects (100% rats; 58% males, 42% females) and Pavlovian studies included 239 non-human animals (76% rats, 24% mice; 100% males) and 66 human participants of both genders. Individual characteristics for each comparison are described in Table 4.2 A-B (Operant) and Table 4.3 A-B (Pavlovian).

A

Author	Year	Experiment	Comparison	Effect Size/ Direction	Gender	Subject	Number of animals housed together	Age (days)
Beiter	2016	1	a	1.26	Male	Rat	1	
Beiter	2016	1	b	0.15	Male	Rat	1	
Lynch	2010	1		2.33	Male	Rat	1	90
Ogbonmwan	2015	1	a	1.35	Male	Rat	1	53
Ogbonmwan	2015	1	b	1.40	Male	Rat	1	53
Peterson	2014	1	a	1.01	Male	Rat	1	
Peterson	2014	1	b	-0.40	Female	Rat	1	
Peterson	2014	1	c	1.16	Female	Rat	1	
Peterson	2014	2	d	-0.01	Male	Rat	1	
Peterson	2014	2	e	0.82	Male	Rat	1	
Peterson	2014	2	f	1.87	Male	Rat	1	
Peterson	2014	2	g	2.34	Male	Rat	1	
Peterson	2014	2	h	-0.87	Female	Rat	1	
Peterson	2014	2	i	0.48	Female	Rat	1	
Peterson	2014	2	j	-0.48	Female	Rat	1	
Peterson	2014	2	k	-1.09	Female	Rat	1	
Sanchez	2014	1	a	0.04	Male	Rat	1	30
Sanchez	2014	1	b	0.03	Female	Rat	1	30
Smith	2012	1	a	0.84	Female	Rat	1	91
Smith	2012	1	b	0.74	Female	Rat	1	91
Smith	2012	1	c	1.10	Male	Rat	1	91
Smith	2012	1	d	0.73	Male	Rat	1	91
Smith	2012	1	e	1.18	Female	Rat	1	105
Smith	2012	1	f	1.15	Male	Rat	1	105
Sobieraj	2016	1	a	0.48	Male	Rat	1	56
Sobieraj	2016	1	b	0.64	Male	Rat	1	56

Table 4.2 Operant Extinction Characteristics

Table 4.2 *cont.* Operant Extinction Characteristic

Author	Year	Comparison	US	Dose (mg/kg)	CS During Extinction	Extinction Sessions	Time from Extinction to Test	Memory Test	Test Stimulus
Beiter	2016	a	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Beiter	2016	b	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Lynch	2010		Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Ogbonmwan	2015	a	Cocaine	0.5	D- Light		21	Reinstatement	CS
Ogbonmwan	2015	b	Cocaine	0.5	D- Light		21	Reinstatement	CS
Peterson	2014	a	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	b	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	c	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	d	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	e	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	f	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	g	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	h	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	i	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	j	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Peterson	2014	k	Cocaine	1.5	D- Light	6	0	Reinstatement	CS
Sanchez	2014	a	Nicotine	0.26	D- Light	5	0	Reinstatement	CS
Sanchez	2014	b	Nicotine	0.47	D- Light	5	0	Reinstatement	CS
Smith	2012	a	Cocaine	0.5	D- Tone	7	3	Reinstatement	Both
Smith	2012	b	Cocaine	0.5	D- Tone	7	5	Reinstatement	Both
Smith	2012	c	Cocaine	0.5	D- Tone	7	3	Reinstatement	Both
Smith	2012	d	Cocaine	0.5	D- Tone	7	5	Reinstatement	Both
Smith	2012	e	Cocaine	0.5	D- Tone	5	1	Reinstatement	CS
Smith	2012	f	Cocaine	0.5	D- Tone	5	1	Reinstatement	CS
Sobieraj	2016	a	Methamphetamine	0.5	D- Light	6	1	Reinstatement	CS
Sobieraj	2016	b	Methamphetamine	0.5	D- Light	6	2	Reinstatement	CS

A

Author	Year	Comparison	Exercise Prior to Acquisition	Exercise Prior to Extinction	Exercise During Extinction	Exercise After Extinction	Exercise During Test	Exercise Length (min)	Exercise Type
Beiter	2016	a	No	Yes	No	No	No	1680	Voluntary
Beiter	2016	b	No	Yes	No	No	No	840	Voluntary
Lynch	2010		No	Yes	No	No	No	1680	Voluntary
Ogbonmwan	2015	a	No	No	No	Yes	No	31680	Voluntary
Ogbonmwan	2015	b	No	No	No	Yes	Yes	30240	Voluntary
Peterson	2014	a	No	Yes	No	No	No	840	Voluntary
Peterson	2014	b	No	Yes	No	No	No	1680	Voluntary
Peterson	2014	c	No	Yes	No	No	No	5040	Voluntary
Peterson	2014	d	No	Yes	No	No	No	20160	Voluntary
Peterson	2014	e	No	Yes	No	No	No	1260	Voluntary
Peterson	2014	f	No	Yes	No	No	No	1260	Voluntary
Peterson	2014	g	No	Yes	No	No	No	12600	Voluntary
Peterson	2014	h	No	Yes	No	No	No	12600	Voluntary
Peterson	2014	i	No	Yes	No	No	No	1680	Voluntary
Peterson	2014	j	No	Yes	No	No	No	1680	Voluntary
Peterson	2014	k	No	Yes	No	No	No	1680	Voluntary
Sanchez	2014	a	No	Yes	No	No	No	1200	Voluntary
Sanchez	2014	b	No	Yes	No	No	No	1200	Voluntary
Smith	2012	a	Yes	Yes	Yes	No	Yes	117000	Voluntary
Smith	2012	b	Yes	Yes	Yes	No	Yes	117000	Voluntary
Smith	2012	c	Yes	Yes	Yes	No	Yes	117000	Voluntary
Smith	2012	d	Yes	Yes	Yes	No	Yes	117000	Voluntary
Smith	2012	e	Yes	Yes	Yes	No	Yes	117000	Voluntary
Smith	2012	f	Yes	Yes	Yes	No	Yes	117000	Voluntary
Sobieraj	2016	a	No	Yes	Yes	No	Yes	29820	Voluntary
Sobieraj	2016	b	No	Yes	Yes	No	Yes	29820	Voluntary

Table 4.2A *cont.* Operant Extinction Characteristics

A

Author	Year	Comparison	Exercise in the Homecage	Extended Drug Access	Abstinence
Beiter	2016	a	Yes	Yes	Yes
Beiter	2016	b	Yes	Yes	Yes
Lynch	2010		Yes	Yes	Yes
Ogbonmwan	2015	a	Yes		No
Ogbonmwan	2015	b	Yes		No
Peterson	2014	a	Yes	Yes	No
Peterson	2014	b	Yes	Yes	No
Peterson	2014	c	Yes	Yes	No
Peterson	2014	d	Yes	Yes	No
Peterson	2014	e	Yes	Yes	No
Peterson	2014	f	Yes	Yes	No
Peterson	2014	g	Yes	Yes	No
Peterson	2014	h	Yes	Yes	No
Peterson	2014	i	Yes	Yes	No
Peterson	2014	j	Yes	Yes	No
Peterson	2014	k	Yes	Yes	No
Sanchez	2014	a	Yes	Yes	Yes
Sanchez	2014	b	Yes	Yes	Yes
Smith	2012	c	Yes	No	No
Smith	2012	d	Yes	No	No
Smith	2012	e	Yes	No	No
Smith	2012	f	Yes	No	No
Smith	2012	c	Yes	No	No
Smith	2012	d	Yes	No	No
Sobieraj	2016	a	Yes	Yes	Yes
Sobieraj	2016	b	Yes	Yes	Yes

Table 4.2 *cont.* Operant Extinction Characteristic

B

Author	Year	Experiment	Comparison	Effect Size/ Direction	Gender	Subject	Number of animals housed together	Age (days)
Zlebnik	2010	1	a	-0.30	Female	Rat	1	90
Zlebnik	2010	1	b	0.11	Female	Rat	1	90
Zlebnik	2010	1	a	-0.34	Female	Rat	1	90
Zlebnik	2010	1	b	1.00	Female	Rat	1	90
Zlebnik	2010	1	c	-0.48	Female	Rat	1	90
Zlebnik	2010	1	d	1.09	Female	Rat	1	90
Zlebnik	2010	1	e	0.97	Female	Rat	1	90
Zlebnik	2010	1	f	0.61	Female	Rat	1	90
Zlebnik	2014	1	a	-0.02	Female	Rat	1	
Zlebnik	2014	1	b	-0.62	Male	Rat	1	
Zlebnik	2014	1	a	0.38	Female	Rat	1	
Zlebnik	2014	1	b	-0.57	Male	Rat	1	
Zlebnik	2014	1	c	0.50	Female	Rat	1	
Zlebnik	2014	1	d	0.32	Male	Rat	1	
Zlebnik	2014	1	e	1.24	Female	Rat	1	
Zlebnik	2014	1	f	-0.24	Male	Rat	1	
Zlebnik	2014	1	g	0.28	Female	Rat	1	
Zlebnik	2014	1	h	-0.36	Male	Rat	1	
Zlebnik	2014	1	i	0.70	Female	Rat	1	
Zlebnik	2014	1	j	0.26	Male	Rat	1	

Table 4.2 *cont.* Operant Extinction Characteristic

B

Author	Year	Comparison	US	Dose (mg/kg)	CS During Extinction	Extinction Session(s)	Time from Extinction to Test	Memory Test	Test Stimulus
Zlebnik	2010	a	Cocaine	0.4	D- Light	14	3	Reinstatement	US
Zlebnik	2010	b	Cocaine	0.4	D- Light	14	3	Reinstatement	US
Zlebnik	2010	a	Cocaine	0.4	D- Light	14	5	Reinstatement	US
Zlebnik	2010	b	Cocaine	0.4	D- Light	14	5	Reinstatement	US
Zlebnik	2010	c	Cocaine	0.4	D- Light	14	7	Reinstatement	US
Zlebnik	2010	d	Cocaine	0.4	D- Light	14	7	Reinstatement	US
Zlebnik	2010	e	Cocaine	0.4	D- Light	14	9	Reinstatement	US
Zlebnik	2010	f	Cocaine	0.4	D- Light	14	11	Reinstatement	US
Zlebnik	2014	a	Cocaine	0.4	D- Light	14	10	Reinstatement	CS
Zlebnik	2014	b	Cocaine	0.4	D- Light	14	10	Reinstatement	CS
Zlebnik	2014	a	Cocaine	0.4	D- Light	14	10	Reinstatement	CS
Zlebnik	2014	b	Cocaine	0.4	D- Light	14	10	Reinstatement	CS
Zlebnik	2014	c	Cocaine	0.4	D- Light	14	10	Reinstatement	US
Zlebnik	2014	d	Cocaine	0.4	D- Light	14	10	Reinstatement	US
Zlebnik	2014	e	Cocaine	0.4	D- Light	14	10	Reinstatement	US
Zlebnik	2014	f	Cocaine	0.4	D- Light	14	10	Reinstatement	US
Zlebnik	2014	g	Cocaine	0.4	D- Light	14	10	Reinstatement	Both
Zlebnik	2014	h	Cocaine	0.4	D- Light	14	10	Reinstatement	Both
Zlebnik	2014	i	Cocaine	0.4	D- Light	14	10	Reinstatement	Both
Zlebnik	2014	j	Cocaine	0.4	D- Light	14	10	Reinstatement	Both

Table 4.2 *cont.* Operant Extinction Characteristic

B

Author	Year	Comparison	Exercise Prior to Acquisition	Exercise Prior to Extinction	Exercise During Extinction	Exercise After Extinction	Exercise During Test	Exercise Length (min)	Exercise Type
Zlebnik	2010	a	Yes	No	Yes	No	No	5040	Voluntary
Zlebnik	2010	b	Yes	No	Yes	No	Yes	7560	Voluntary
Zlebnik	2010	a	Yes	No	Yes	No	No	5040	Voluntary
Zlebnik	2010	b	Yes	No	Yes	No	Yes	7560	Voluntary
Zlebnik	2010	c	Yes	No	Yes	No	No	5040	Voluntary
Zlebnik	2010	d	Yes	No	Yes	No	Yes	7560	Voluntary
Zlebnik	2010	e	Yes	No	Yes	No	No	5040	Voluntary
Zlebnik	2010	f	Yes	No	Yes	No	No	5040	Voluntary
Zlebnik	2014	a	Yes	Yes	No	No	Yes	7920	Voluntary
Zlebnik	2014	b	Yes	Yes	No	No	Yes	7920	Voluntary
Zlebnik	2014	a	Yes	Yes	Yes	No	Yes	9360	Voluntary
Zlebnik	2014	b	Yes	Yes	Yes	No	Yes	9360	Voluntary
Zlebnik	2014	c	Yes	Yes	No	No	Yes	7920	Voluntary
Zlebnik	2014	d	Yes	Yes	No	No	Yes	7920	Voluntary
Zlebnik	2014	e	Yes	Yes	Yes	No	Yes	9360	Voluntary
Zlebnik	2014	f	Yes	Yes	Yes	No	Yes	9360	Voluntary
Zlebnik	2014	g	Yes	Yes	No	No	Yes	7920	Voluntary
Zlebnik	2014	h	Yes	Yes	No	No	Yes	7920	Voluntary
Zlebnik	2014	i	Yes	Yes	Yes	No	Yes	9360	Voluntary
Zlebnik	2014	j	Yes	Yes	Yes	No	Yes	9360	Voluntary

Table 4.2 *cont.* Operant Extinction Characteristic

B

Author	Year	Comparison	Exercise in the Homecage	Extended Drug Access	Abstinence
Zlebnik	2010	a	Yes	Yes	No
Zlebnik	2010	b	Yes	Yes	No
Zlebnik	2010	a	Yes	Yes	No
Zlebnik	2010	b	Yes	Yes	No
Zlebnik	2010	c	Yes	Yes	No
Zlebnik	2010	d	Yes	Yes	No
Zlebnik	2010	e	Yes	Yes	No
Zlebnik	2010	f	Yes	Yes	No
Zlebnik	2014	a	Yes	Yes	No
Zlebnik	2014	b	Yes	Yes	No
Zlebnik	2014	a	Yes	Yes	No
Zlebnik	2014	b	Yes	Yes	No
Zlebnik	2014	c	Yes	Yes	No
Zlebnik	2014	d	Yes	Yes	No
Zlebnik	2014	e	Yes	Yes	No
Zlebnik	2014	f	Yes	Yes	No
Zlebnik	2014	g	Yes	Yes	No
Zlebnik	2014	h	Yes	Yes	No
Zlebnik	2014	i	Yes	Yes	No
Zlebnik	2014	j	Yes	Yes	No

Table 4.2 *cont.* Operant Extinction Characteristic

A

Author	Year	Experiment	Comparison	Effect Size/ Direction	Gender	Subject	Number of animals housed together	Age (days)
Greenwood	2009	1	a	-1.35	Male	Rat	1	
Greenwood	2009	2	b	0.22	Male	Rat	1	
Jacquart, Roquet	2017	1	a	-0.37	Male	Rat	2	
Jacquart, Roquet	2017	1	b	-0.11	Male	Rat	2	
Jacquart, Roquet	2017	1	c	-0.02	Male	Rat	2	
Jacquart, Roquet	2017	1	d	-0.23	Male	Rat	2	
Jacquart, Roquet	2017	2	e	0.11	Male	Rat	2	
Jacquart, Roquet	2017	2	f	-0.19	Male	Rat	2	
Jacquart, Roquet	2017	2	g	-0.24	Male	Rat	2	
Jacquart, Roquet	2017	2	h	-0.04	Male	Rat	2	
Jacquart, Roquet	2017	3	i	-0.09	Male	Rat	2	
Jacquart, Roquet	2017	3	j	-0.29	Male	Rat	2	
Jacquart, Roquet	2017	4	k	0.16	Male	Rat	2	
Jacquart, Roquet	2017	4	l	-0.22	Male	Rat	2	

Table 4.3 Pavlovian Extinction Characteristic

A

Author	Year	Comparison	CS During Extinction	US	Extinction Session(s)	Time from Extinction to Test	Memory Test
Greenwood	2009	a	D- Tone	Shock	9	14	Spontaneous Recovery
Greenwood	2009	b	D- Tone	Shock	13	14	Spontaneous Recovery
Jacquart, Roquet	2017	a	D- Tone	Shock	1	1	Long-term Memory
Jacquart, Roquet	2017	b	D- Tone	Shock	1	3	Reinstatement
Jacquart, Roquet	2017	c	D- Tone	Shock	1	1	Long-term Memory
Jacquart, Roquet	2017	d	D- Tone	Shock	1	3	Reinstatement
Jacquart, Roquet	2017	g	D- Tone	Shock	2	1	Long-term Memory
Jacquart, Roquet	2017	h	D- Tone	Shock	2	3	Reinstatement
Jacquart, Roquet	2017	i	D- Tone	Shock	2	1	Long-term Memory
Jacquart, Roquet	2017	j	D- Tone	Shock	2	3	Reinstatement
Jacquart, Roquet	2017	k	D- Tone	Shock	2	1	Long-term Memory
Jacquart, Roquet	2017	l	D- Tone	Shock	2	3	Reinstatement
Jacquart, Roquet	2017	m	Context	Shock	1	1	Long-term Memory
Jacquart, Roquet	2017	n	Context	Shock	1	21	Spontaneous Recovery

Table 4.3 *cont.* Pavlovian Extinction Characteristic

A

Author	Year	Comparison	Exercise Prior to Acquisition	Exercise Prior to Extinction	Exercise During Extinction	Exercise After Extinction	Exercise During Test	Exercise Length (min)	Exercise Type	Exercise in the Homecage
Greenwood	2009	a	No	Yes	No	No	No	60480	Voluntary	Yes
Greenwood	2009	b	No	Yes	No	No	No	10080	Voluntary	Yes
Jacquart, Roquet	2017	a	No	Yes	No	No	No	30	Voluntary	No
Jacquart, Roquet	2017	b	No	Yes	No	No	No	30	Voluntary	No
Jacquart, Roquet	2017	c	No	Yes	No	No	No	30	Voluntary	No
Jacquart, Roquet	2017	d	No	Yes	No	No	No	30	Voluntary	No
Jacquart, Roquet	2017	g	No	Yes	No	No	No	30	Voluntary	Yes
Jacquart, Roquet	2017	h	No	Yes	No	No	No	30	Voluntary	Yes
Jacquart, Roquet	2017	i	No	Yes	No	No	No	30	Voluntary	Yes
Jacquart, Roquet	2017	j	No	Yes	No	No	No	30	Voluntary	Yes
Jacquart, Roquet	2017	k	No	Yes	No	No	No	180	Voluntary	No
Jacquart, Roquet	2017	l	No	Yes	No	No	No	180	Voluntary	No
Jacquart, Roquet	2017	m	No	Yes	No	No	No	180	Voluntary	No
Jacquart, Roquet	2017	n	No	Yes	No	No	No	180	Voluntary	No

Table 4.3 *cont.* Pavlovian Extinction Characteristic

B

Author	Year	Experiment	Comparison	Effect Size/ Direction	Gender	Subject	Number of animals housed together	Age (days)
Jacquart, Roquet	2017	5	m	-0.14	Both	Human		
Jacquart, Roquet	2017	5	n	-0.11	Both	Human		
Mika	2015	1	a	-0.06	Male	Rat		63
Mika	2015	1	b	1.47	Male	Rat		63
Mustroph	2011	1	a	0.51	Male	Mouse	1	
Mustroph	2011	2	b	-0.69	Male	Mouse	1	
Mustroph	2011	3	c	-0.17	Male	Mouse	1	
Powers	2015	1		2.36	Both	Human		
Siette	2014	2	a	0.83	Male	Rat	4	42
Siette	2014	2	b	1.68	Male	Rat	4	42
Tsuboi	2015	1		0.51	Male	Rat	1	

Table 4.3 *cont.* Pavlovian Extinction Characteristic

B

Author	Year	Comparison	CS During Extinction	US	Extinction Session(s)	Time from Extinction to Test	Memory Test
Jacquart, Roquet	2017	e	Context	Heights	1	0	Long-term Memory
Jacquart, Roquet	2017	f	Context	Heights	1	0	Long-term Memory
Mika	2015	a	D- Tone	Shock	1	7	Relapse
Mika	2015	b	D- Tone	Shock	1	7	Relapse
Mustroph	2011	a	Context	Cocaine	4	1	Reinstatement
Mustroph	2011	b	Context	Cocaine	4	1	Reinstatement
Mustroph	2011	c	Context	Cocaine	4	1	Reinstatement
Powers	2015		D- Cues	Trauma	12	0	Long-term Memory
Siette	2014	a	Context	Shock	1	1	Long-term Memory
Siette	2014	b	Context	Shock	1	1	Long-term Memory
Tsuboi	2015		D- Object	Lithium Chloride	7	14	Spontaneous Recovery

Table 4.3 *cont.* Pavlovian Extinction Characteristic

B

Author	Year	Comparison	Exercise Prior to Acquisition	Exercise Prior to Extinction	Exercise During Extinction	Exercise After Extinction	Exercise During Test	Exercise Length (min)	Exercise Type	Exercise in the Homecage
Jacquart, Roquet	2017	e		Yes	No		No	34	Forced	
Jacquart, Roquet	2017	f		Yes	No		No	34	Forced	
Mika	2015	a	Yes	Yes	No	Yes	No	7200	Voluntary	No
Mika	2015	b	Yes	Yes	Yes	Yes	No	7228	Voluntary	No
Mustroph	2011	a	No	Yes	Yes	No	Yes	43200	Voluntary	Yes
Mustroph	2011	b	Yes	Yes	Yes	No	Yes	43200	Voluntary	Yes
Mustroph	2011	c	Yes	Yes	Yes	No	Yes	43200	Voluntary	Yes
Powers	2015			Yes	No		No	360	Forced	
Siette	2014	a	No	Yes	No	No	No	180	Voluntary	Yes
Siette	2014	b	No	No	No	Yes	No	180	Voluntary	Yes
Tsuboi	2015		No	No	No	Yes	No	420	Forced	No

Table 4.3 *cont.* Pavlovian Extinction Characteristic

4.4.4 Operant studies: Main effects.

When examining the overall effects of exercise on post extinction outcomes, there was significantly less responding in those subjects that exercised across experiments that used operant extinction procedures (g= 0.58, 95% CI [0.25, 0.92], p< .001, n= 46). For individual and aggregate effects, see Figure 4.4.

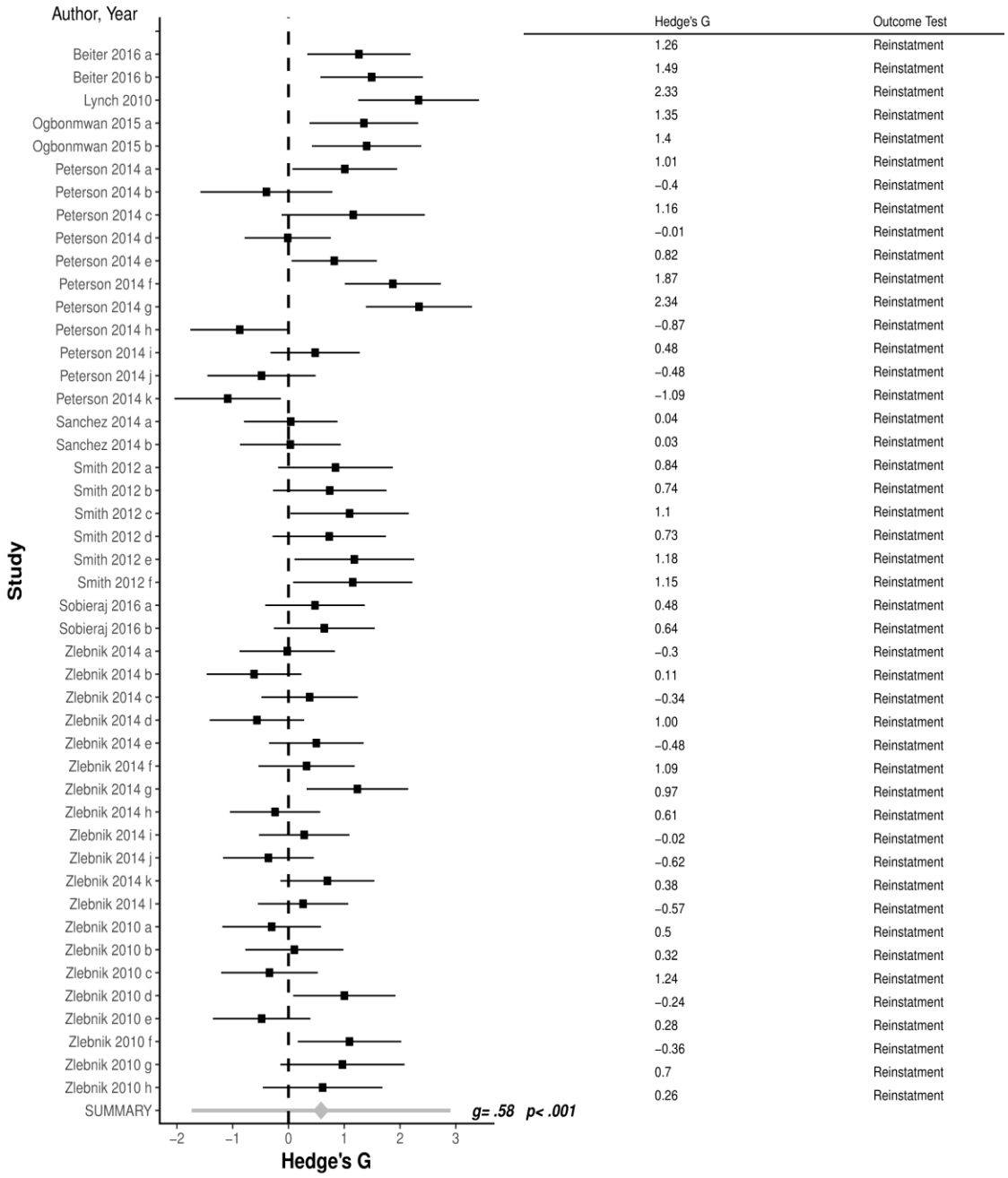

Figure 4.4 **Forest plot of individual study effects for exercise plus extinction over extinction alone on memory outcome tests across operant extinction paradigms**.

Publication Bias & Heterogeneity. To test for potential publication bias across operant experiments, funnel plots were constructed, for which no asymmetries were seen (see Figure 5). To confirm the visual inspection, the models was then run using the Trim and Fill method (Duval & Tweedie, 2000) and there were no cases where data points were added to any the plot. However, again as numerous data points did fall outside the confidence intervals, further analysis indicates significant heterogeneity (Q[*df*= 45] = 126.78 , *p*< .001), warranting exploration of potential moderators.

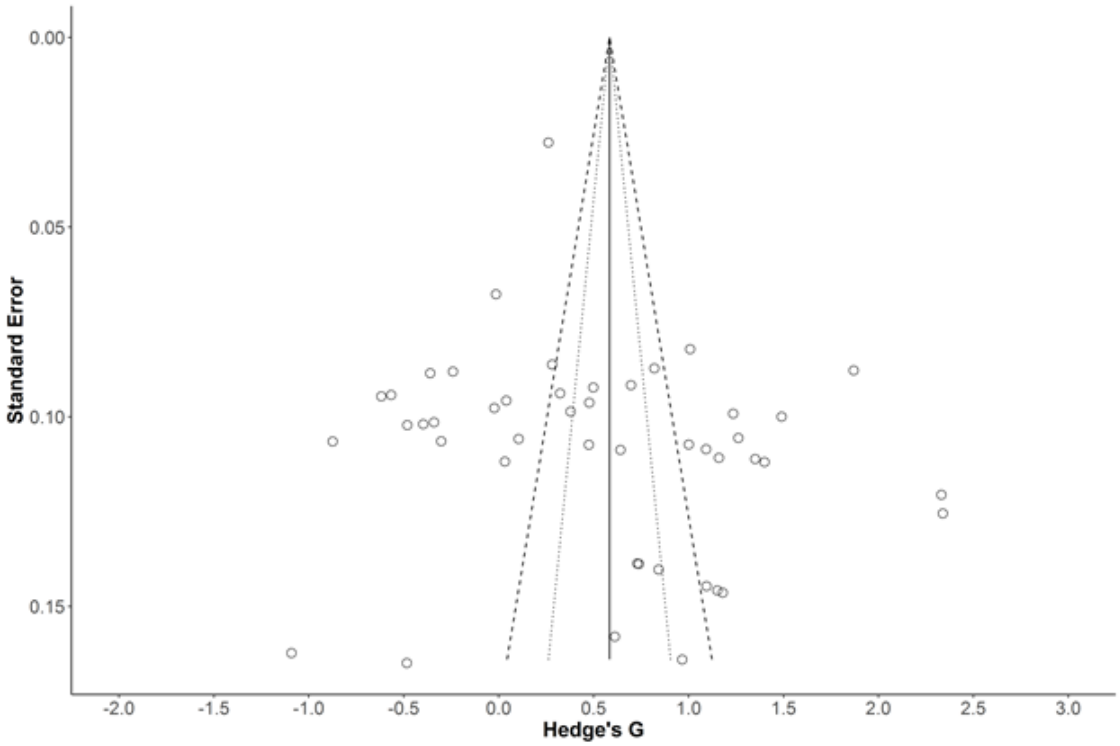

Figure 4.5 **Funnel plot of the individual study effects of exercise applied with extinction over extinction alone across operant extinction models.** Dotted lines represent 95% confidence intervals (CI) and dashed lines represent 99% CI.

Moderators.

Participant characteristics.

Gender. The gender of subjects indicated a trend for a significant moderating effect (B= -0.42, SE= 0.23, p= .061). This is shown through a large overall effect in male subjects (g= 0.76, 95% CI [0.40, 1.11], p< .001, n= 32), with a smaller trend for an effect in female subjects (g= 0.33, 95% CI [-0.04, 0.71], p= .082, n= 24). When controlling for the significant effect seen for the number of extinction sessions, this effect is no longer a trend (p= 0.364).

Subject. Across all experiments only rats were used as subjects, so no moderator analysis was done.

Non-human animal experiment specific.

Animal housing. Number of animals housed together was not tested because all experiments included had animals housed individually.

Rat experiment specific.

Age in days. Age in days looking at rat subjects only did not have a moderator effect on overall main effects (*B*= 0.15, *SE*= 0.15, *p*= .319, *n*= 21).

Conditioning procedures.

Stimuli. The dose or stimulus intensity was tested as a continuous moderator for which there was a non-significant effect (*B*= 0.18, *SE*= 0.14, *p*= .223, *n*= 46). One unique feature of operant extinction is that because a response predicts both the CS and reinforcer, one or both stimuli can be extinguished. A trend was found, depending on whether the CS was present or not during extinction (*B*= -0.42, *SE*= 0.23, *p*= .071, *n*= 46). When controlling for the significant effect seen for the number of extinction sessions, this effect is no longer a trend however (*p*= 0.528). For cases where the CS was not present, there was a moderate to large significant overall effect (*g*= 0.63, 95% CI

[0.36, 1.10], p< .001, n= 22). There was only a trend for a small to moderate effect in those experiments where the CS was present (g= 0.31, 95% CI [-0.03, 0.65], p= .077, n= 24). Because different stimuli can be extinguished, this allows various stimuli to be used to test reinstatement of responding, for which we found no overall moderating effect (X^2= 0.20, df= 2, p= .904). Under conditions where the CS was used, there was a significant effect (g= 0.60, 95% CI [0.25, 0.95], p< .001, n= 26), whereas when the reinforcer was employed to reinstate behavior, there was no effect (g= 0.44, 95% CI [-0.21, 1.09], p= .181, n= 12). Finally, when a combination of both stimuli were used there was a trend for an effect (g= 0.56, 95% CI [-0.05, 1.16], p= .070, n= 8).

Timing of procedures. The number of extinction sessions significantly moderated overall analysis, such that fewer extinction sessions produced greater overall effects (B= -0.25, SE= 0.10, p= .014, n= 44; see Figure 6) which accounted for 8% of the variance. However, this effect was further supported by moderate overall effects seen in cases of limited extinction (1-7 sessions; g= 0.68, 95% CI [0.44, 0.92], p< .001, n= 24). With extended extinction (8-14 sessions), producing a small overall effect (g= 0.21, 95% CI [-0.10, 0.52], p= .192, n= 20), with the difference producing a significant moderating effect (B= -0.48, SE= 0.21, p= .026). The time from the end of extinction to test was also tested and found not to moderate overall analysis (B= 0.03, SE= 0.15, p= .838, n= 46).

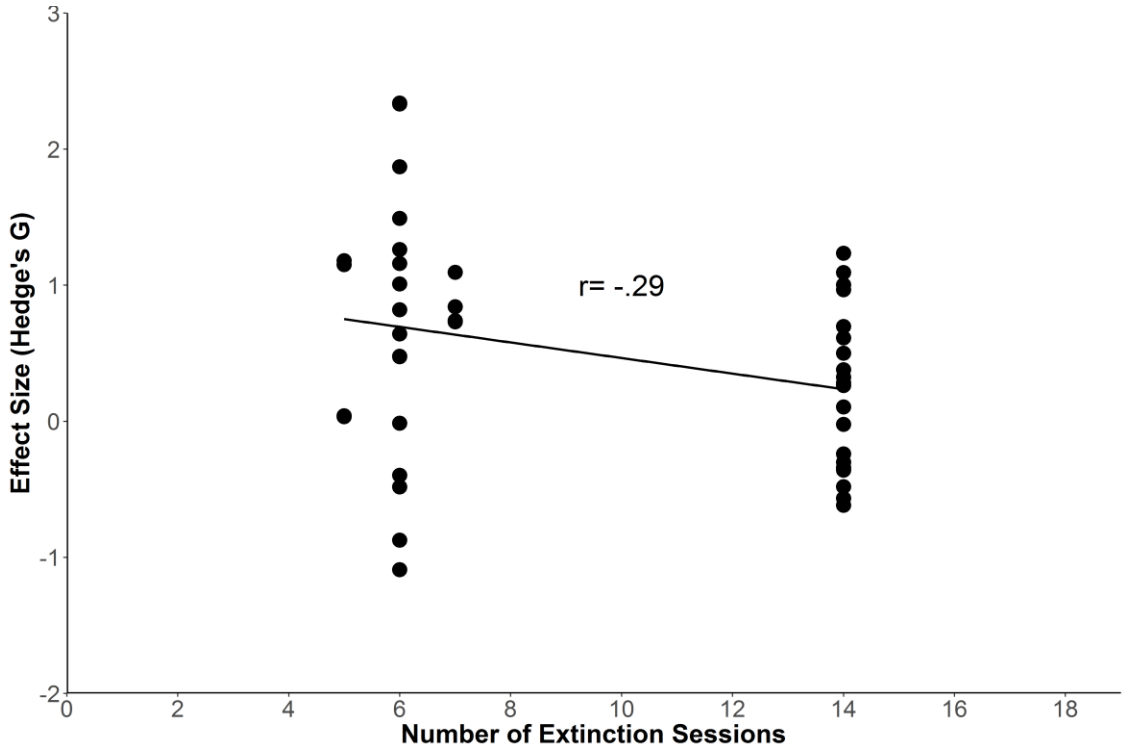

Figure 4.6 **Regression plot of the effect sizes from post extinction tests relation to the number of extinction sessions across operant extinction training paradigms.** Moderator analysis found a significant overall effect, such that fewer extinction sessions produced greater overall effects.

Exercise parameters. Of the various tests of when exercise occurs in relation to extinction procedures, exercise after extinction did not have sufficient consistency within methods to be tested as only two comparisons from the same experiment were included in analysis. However, all other measures were possible. First explored was whether exercise prior to acquisition had a moderator effect on tests after extinction, for which no significant effect was seen (*B*= -0.32, *SE*= 0.26, *p*= .220). With further assessment, we found that both those that used exercise prior to acquisition (*g*= 0.40, 95% CI [0.02,

0.77], p= .040, n= 26), and those that did not (g= 0.72, 95% CI [0.31, 1.13], p< .001, n= 20), show significant effects. Exercise prior to extinction, again, did not have a moderator effect on overall findings (B= -0.02, SE= 0.35, p= .958). This was due to the fact that effects were moderate in both experimental conditions, where exercising prior to extinction produced a significant overall effect (g= 0.58, 95% CI [0.21, 0.95], p= .002, n= 36), with a trend in those that did not (g= 0.60, 95% CI [-0.05, 1.25], p= .071, n= 10). Both conditions in which exercise was present during extinction or not (g= 0.57, 95% CI [0.12, 1.01], p= .012, n= 22; g= 0.60, 95% CI [0.20, 0.99], p= .003, n= 24) show a significant overall effect. Again, because these effects were similar and moderate under Cohen's scale, no moderator effect was seen (B= -0.03, SE= 0.26, p= .913). Experiments using exercise during post extinction test(s) or not were found to have significant effects (g= 0.62, 95% CI [0.12, 1.113], p= .015, n= 24; g= 0.56, 95% CI [0.16, 0.96], p= .006, n= 22) for which there was not a moderator effect (B= 0.06, SE= 0.29, p= .827).

Exploration of exercise time was tested only in voluntary exercise as no operant experiments used forced exercise. Overall a non-significant effect was seen (B= 0.20, SE= 0.13, p= .112, n= 46).

Non-human animal specific. All operant experiments used exercise in the homecage to test exercise as an augmentation strategy.

Operant specific parameters. Across operant experiments, some studies employed an abstinence period (g= 0.87, 95% CI [0.28, 1.45], p= .004, n= 7), however this did not moderate the overall effects (B= 0.40 SE= 0.34, p= .238, n= 46) as there was no difference from those that were not exposed to abstinence (g= 0.47, 95% CI [0.14, 0.80], p= .005, n= 39).

4.4.5 Operant studies: Summary.

Of the 14 potential parameters tested, the number of extinction sessions was found to moderate aggregate effects, such that more sessions produced less of an overall effect. In addition, there were trends for an effect of gender and whether the CS was present at extinction; however, when controlling for the number of extinction sessions, there were no longer a trend in these effects. For a summary of results, see Table 1.

4.4.6 Pavlovian studies: Main effects.

When examining the overall effects of exercise on post extinction outcomes, experiments employing Pavlovian extinction models show a small and non-significant overall effect (g= 0.14 95% CI [-0.15, 0.42], p= .336, n= 25). For individual and aggregate effects, see Figure 7. Exploring further the effect of stimuli, neither experiments using appetitive stimuli (g= -0.13, 95% CI [-0.78, 0.53], p= .708, n= 3) or aversive stimuli (g= 0.20, 95% CI [-0.12, 0.51], p= .224, n= 22) show a significant effect, nor do they moderate overall analysis (B= 0.32, SE= 0.37, p= .387).

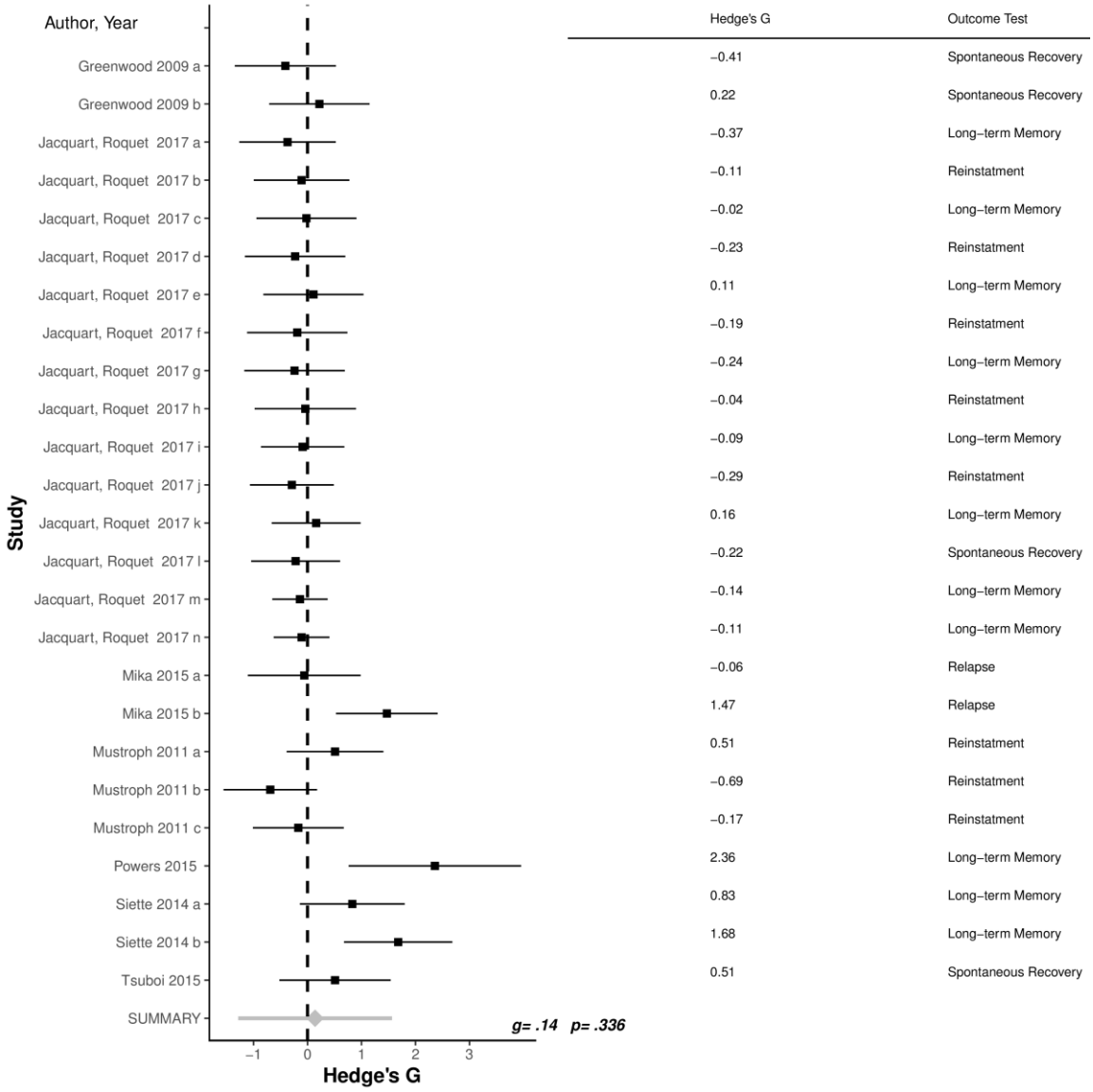

Figure 4.7 **Forest plot of individual study effects for exercise plus extinction over extinction alone on memory outcome tests cross Pavlovian extinction paradigms.**

Publication Bias & Heterogeneity. Publication bias across Pavlovian experiments were tested through the construction of funnel plots and examined for asymmetries, for which none were seen (see Figure 4.8). After visual inspection, the Trim and Fill method (Duval & Tweedie, 2000) was then applied and no additional points were added to the figure. However, numerous data points did fall outside the confidence intervals, and further analysis indicates significant heterogeneity (Q[*df*= 24] = 39.59 , *p*= .024) warranting exploration of potential moderators.

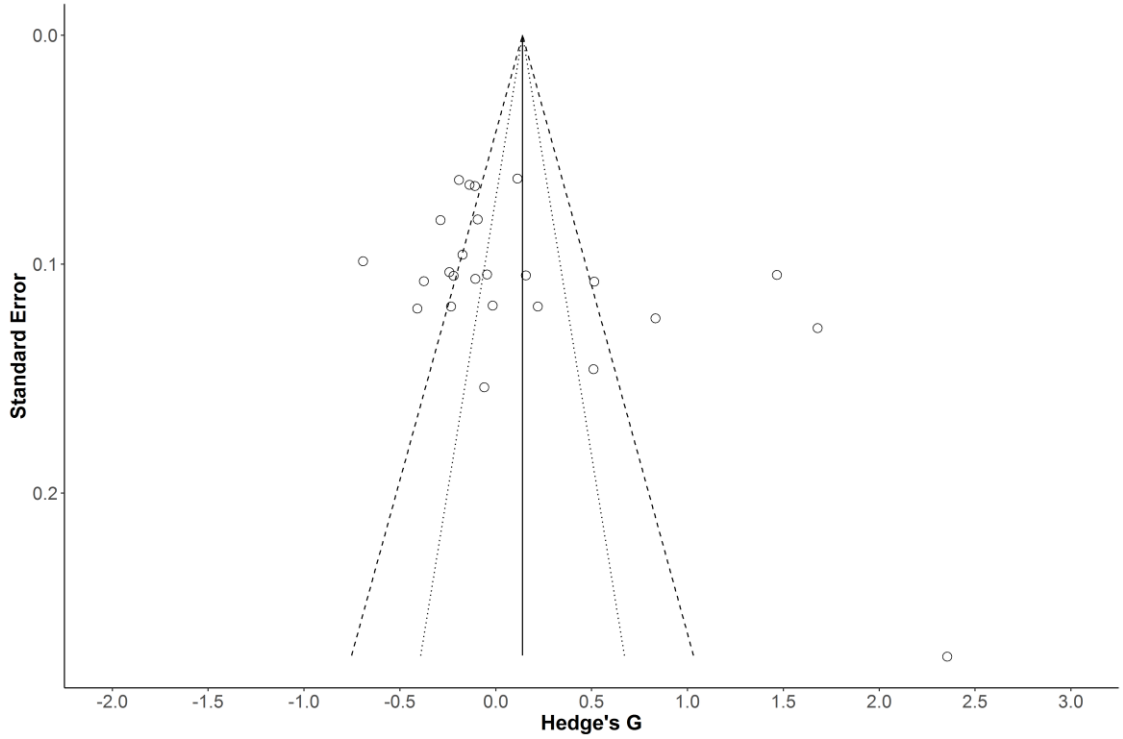

Figure 4.8 **Funnel plot of the individual study effects of exercise applied with extinction over extinction alone across Pavlovian extinction models.** Dotted lines represent 95% confidence intervals (CI) and dashed lines represent 99% CI.

Moderators.

Participant characteristics.

Gender. The gender of participants did not significantly moderate the overall findings (B= 0.25, SE= 0.47, p= .599). In both experiments with male subjects (g= 0.11, 95% CI [-0.20, 0.42], p= .482, n= 22) and those that included a mix of genders (g= 0.36, 95% CI [-0.51, 1.23], p= .420, n= 3) no effect was seen.

Subject. Across all subject groups, there were small to moderate non-significant effects; rats (g= 0.17, 95% CI [-0.17, 0.52], p= .324, n= 19), mice (g= -0.13, 95% CI [-0.79, 0.54], p= .714, n= 3) and humans (g= 0.34, 95% CI [-0.51, 1.19], p= .434, n= 3). Additionally, there was no overall moderating effect (X^2= 0.90, df= 2, p= .639).

Non-human animal experiment specific.

Animal housing. Number of animals housed together did moderate analysis when tested as a continuous variable (B= 0.33, SE= 0.12, p= .006, n= 20). Specifically, this relationship indicates that the more animals housed together, the larger the overall effect, accounting for approximately 37% of the variance in effect sizes (see Figure 4.9). However, when testing between housed individually versus housed in groups, no effect was seen (B= 0.09, SE= 0.35, p= .798). Neither animals housed alone (g= -0.03, 95% CI [-0.47, 0.42], p= .906, n= 6), nor individuals housed with at least one other animal (g= 0.09, 95% CI [-0.28, 0.45], p= .645, n= 14) showed a significant effect on their own.

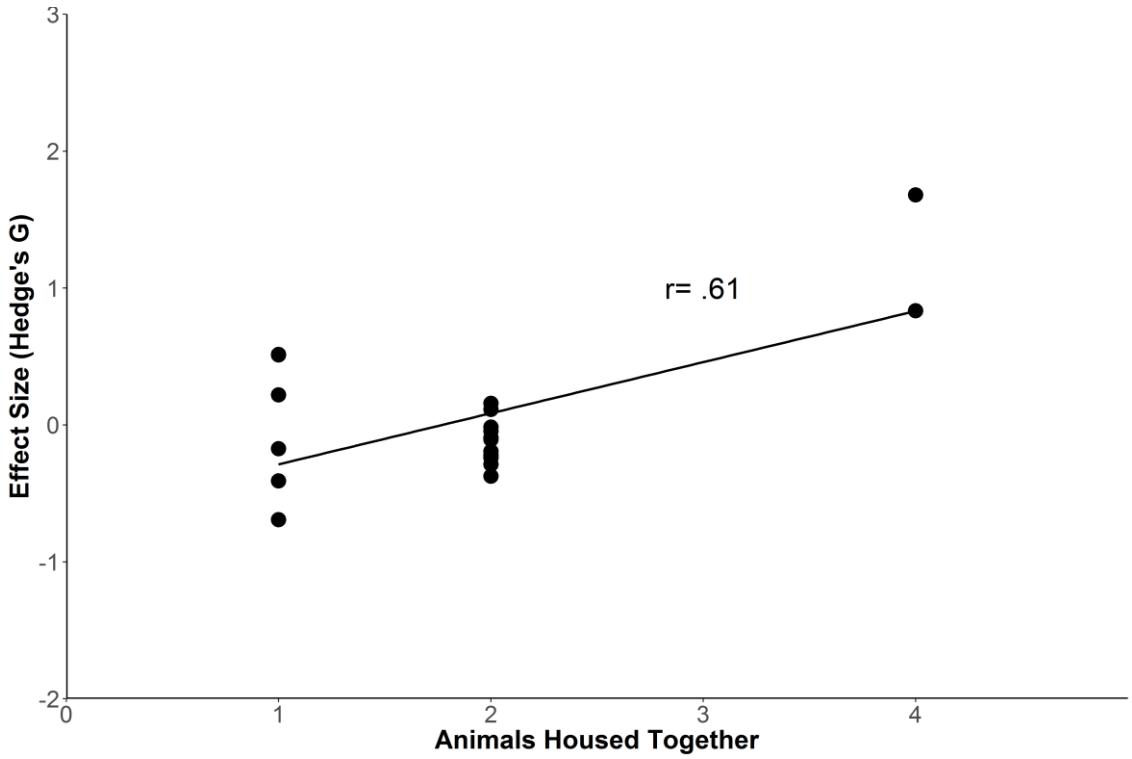

Figure 4.9 **Regression plot of the effect sizes from post extinction tests relation to the number of animals housed together across Pavlovian extinction training paradigms.** Number of animals housed together did moderate overall analysis, the relationship indicates that the more animals housed together, the larger the overall effect.

Rat experiment specific.

Age in days. Age in days was not explored as only two experiments reported age which only produced five total comparisons.

Conditioning procedures.

Stimuli: We found that neither a discrete cue (g= 0.16, 95% CI [-0.23, 0.54], p= .423, n= 16) nor context (g= 0.12, 95% CI [-0.30, 0.54], p= .574, n= 9) produced significant effects. Furthermore, stimuli did not have a moderating effect on the aggregate Pavlovian model (B= -0.04, SE= 0.29, p= .892).

Timing of procedures. Neither the number of extinction sessions (*B*= 0.06, *SE*= 0.14, *p*= .689, *n*= 25), nor time from the end of extinction to test (*B*= -0.06, *SE*= 0.12, *p*= .595, *n*= 25) provide moderator effects. Under further exploration, neither cases of limited extinction (1-7 sessions; *g*= 0.11, 95% CI [-0.20, 0.41], *p*= .493, *n*= 22) or extended extinction (8-14 sessions; *g*= 0.36, 95% CI [-0.41, 1.14], *p*= .355, *n*= 3) produced significant overall effects, ultimately leading to a non-significant moderation effect (*B*= 0.26, *SE*= 0.42, *p*= .536).

Exercise parameters. A variety of different exercise parameters were measured, the first of which was whether exercise prior to acquisition had a moderator effect on tests after extinction, with no significant effect observed (*B*= -0.10, *SE*= 0.38, *p*= .785). Under both conditions of no exercise prior (*g*= 0.20, 95% CI [-0.09, 0.50], *p*= .182, *n*= 18), or available exercise (*g*= -0.22, 95% CI [-0.54, 1.07], *p*= .189, *n*= 4), significant effects were seen. Exercise prior to extinction was not analyzed as only two comparisons did not use exercise prior to extinction. Next, whether experiments used exercise during the extinction session(s) or not was found to have non-significant effects (*g*= 0.30, 95% CI [-0.28, 0.89], *p*= .308, *n*= 5; *g*= 0.10, 95% CI [-0.22, 0.41], *p*= .551, *n*= 20). This parameter did not moderate overall effects (*B*= 0.21, *SE*= 0.33, *p*= .525). Exercise implemented after extinction procedures produced a large significant effect (*g*= 0.94, 95% CI [0.45, 1.44], *p*< .001, *n*= 3) leading to a moderating effect on overall analysis (*B*= 1.04, *SE*= 0.27, *p*< .001) when compared to experiments that did not have subjects exercise after extinction (*g*= -0.08, 95% CI [-0.29, 0.12], *p*= .434, *n*= 19). Finally, neither experiments that used exercise during post extinction test(s) (*g*= -0.13, 95% CI [-0.78, 0.53], *p*= .706, *n*= 3), or those that did not (*g*= 0.20, 95% CI [-0.12, 0.51], *p*= .224, *n*= 22) indicate significant effects. While effects were in opposite directions, both were small, indicating no overall moderating effect of this parameter (*B*= -0.32, *SE*= 0.37, *p*= .387).

Further exploration of exercise time in both forced and voluntary exercise experiments indicate no effect of time (*B*= -0.08, *SE*= 0.13, *p*= .551, *n*= 25). When exploring the potential of exercise type: forced (*g*= 0.40, 95% CI [-0.33, 1.13], *p*= .334, *n*= 4) or voluntary (*g*= -0.09, 95% CI [-0.23, 0.41], *p*= .592, *n*= 22), neither was found to have a significant effect or moderate overall analysis (*B*= 0.12, *SE*= 0.15, *p*= .442).

Non-human animal specific. Overall there was no effect in animals that exercised in their homecage (*g*= 0.08, 95% CI [-0.30, 0.45], *p*= .697, *n*= 11), versus those that exercised in an alternative environment (*g*= 0.16, 95% CI [-0.38, 0.70], *p*= .557, *n*= 11). These differences did not moderate analysis (*B*= -0.10, *SE*= 0.32, *p*= .768).

4.4.7 Pavlovian studies: Summary.

Of the 17 potential moderators tested, two parameters were found to moderate overall findings. First the effect of number of animals housed together remained significant after controlling for the effect of exercise after extinction (*p*= .038). Additionally, because the effect of housing could be influenced by the fact that some animals exercised in the homecage, an effect that was not found to be a significant moderator, we controlled for this parameter in the model for number of animals housed together. Again the moderating effect was maintained (*p*= .005). It is important to note, however, that when exploring whether animals were housed alone as opposed to two or more, there was no moderating effect. Second, when controlling for number of animals housed together on the effect of exercise after extinction (*p*= .006) remained significant. These findings suggest that number of animals housed together and exercise after extinction may be the most critical moderators of exercises effect on Pavlovian extinction. For a summary of all results, see Table 4.1.

4.5 DISCUSSION

4.5.1 Overall Main Effects

The current analysis set out to provide a comprehensive review and meta-analysis of exercise's influence on extinction across models of learning and memory. Overall, findings from 71 comparisons suggest that exercise has a small (g= 0.40) significant effect. One primary *a priori* moderator of interest was to test differences between operant and Pavlovian extinction models. This effect was found to significantly moderate overall analysis (*p*= .031). The data were then subset based on extinction type, and revealed a significant moderate effect (g= 0.58) in operant experiments, but a small, non-significant effect for Pavlovian experiments (g= 0.14). The overall moderator effect and differences in aggregate analysis based on extinction model led to further exploration of potential model specific moderators. Across all analyses, there were no parameters that significantly moderated all three tests. There was, however, overlap in the full set of analysis and Pavlovian models (exercise vs no exercise after extinction). This effect will be discussed further under Pavlovian models.

4.5.2 Moderators of Exercises Effects Across Operant Models

A number of previous experiments have explored the effects of exercise on abstinence alone and withdrawal symptoms finding positive outcomes (Taylor, Ussher, & Faulkner, 2007; Thanos et al., 2013; Werme, Lindholm, Thorén, Franck, & Brené, 2002). One of the key components of the present analysis, however, was to look at the potential effect of exercise on learning and memory (specifically when extinction procedures are also applied). Following a literature review, 46 comparisons were tested to determine the overall effect of exercise applied with extinction over extinction alone, followed by

additional tests of specific parameters that could moderate aggregate effects. Overall analysis found a significant moderate effect (g= .58).

4.5.2.1 Number of extinction sessions.

Of the 14 possible moderating variables, number of extinction sessions was found to be significant, both when tested as a continuous variable and when classified into a dichotomous comparison. Specifically, the number of extinction sessions produced a small negative (*r*= -.29) relationship with effect size outcomes accounting for about 8% of the variance. Next, we also tested limited (1-7 sessions) versus extended (8-14 sessions) of extinction for which there was also a significant moderator effect. This effect can be explained by a floor effect, whereby a behavior or response can only go so low (e.g. there cannot be negative responding). In the case of extinction, the goal is to reduce the level of responding through repeated sessions in which the response is no longer reinforced. When the procedure is repeated a number of times, possible responding reaches a minimum, such that there may not be the possibility for exercise to further decrease post extinction responding above and beyond what was learned across an increasing number of extinction sessions. Although previous findings have shown that an increase in treatment duration leads to greater overall outcomes (Zhang, Friedmann, & Gerstein, 2003), in cases where there is a lack of long-term accessibility or limitations due to insurance restrictions (Horgan & Merrick, 2002; Samet et al., 2001), the effect of adding exercise to those treatment regimens could produce larger reductions in responding in the future.

One important factor to point out for operant models is that exercise across all time points had a moderate effect, but so did not having exercise access at each time point. This may suggest that just having any exercise is beneficial, with all time points

producing a moderate range (.40-.72) of effect sizes independent of exercise access, and that other factors, such as number extinction sessions, are more critical to the success of exercise as an augmentation strategy. These findings may be consistent with a dopamine hypothesis, that during periods of abstinence from drugs of abuse stress and other aversive symptoms relate to changes in neural activity. Specifically neuroadaptations that reduce dopamine function (Rossetti, Melis, Carboni, & Gessa, 1992). For which exercise could possibly reduce dysregulation in dopamine action and signaling after removal of the appetitive stimuli (O'dell, Galvez, Ball, & Marshall, 2012; Robertson et al., 2016). If true, exercise at any point could be beneficial to extinction outcomes by regulating neural activity and negative states. An additional factor that could have influenced the fact that timing of exercise did not have a moderating effect may be how the data were coded. For example, exercise could have occurred before, during and after extinction; however, each time point was tested separately rather than having a factor that included all three-time points. As such, there could be instances where exercise was only available at one-time point for one comparison but could have occurred at all three time points but was tested individually at each time point of interest for other comparisons. Ultimately, while individual time points may be important, there could also be a cumulative effect; however, due to the large variability in when and in what combination exercise was employed across comparisons, we chose to test individual time points. But as more experiments explore different combinations of when exercise is applied, increasing the number of comparisons that would fall in each grouping, it would be critical to further test these combinations. Ultimately however, the current approach was sensitive enough to see significant moderator effects in overall analysis, as well as in Pavlovian extinction models.

It is also important to note that after all data was extracted and received from primary authors an additional article was published that would have met criterion for inclusion under operant models, (Somkuwar et al., 2016) . While there is not enough data in the text to determine an overall effect size, data indicate a significant effect of exercise to reduce reinstatement to alcohol responding, such that inclusion may have contributed to the positive overall effects, as well as to the effect seen in operant models. This experiment also used six extinction sessions, which may have influenced moderator analysis, however this effect would have been included in the limited extinction group which was where large significant effects were seen, and while I cannot determine the magnitude of the effect from the data available the direction of the effect is the same as the current findings.

4.5.3 Moderators of Exercises Effects Across Pavlovian Models

Pavlovian models included 25 comparisons, for which 13 possible moderating variables were teste. Of those, 'number of animals housed together' and whether there was 'exercise access or not after extinction' were found to moderate Pavlovian effects.

4.5.3.1 Number of animals housed together.

The parameter 'number of animals housed together' was first tested as a continuous variable and was found to significantly moderate overall Pavlovian effects. Specifically, the more animals housed together, the larger the overall effect (r= .61). Two caveats are important to note. First, when housing was also tested as a dichotomous variable looking at animals housed alone vs. animals housed with one or more cagemates, there was no longer a moderating effect. Second, in all cases where animals were housed four animals per cage (the greatest number of possible animals housed together), these animals also exercised in their homecage, and while initial analysis did not find

exercising in the homecage to be a moderating factor on its own, the combination of parameters could possibly have had an effect. Additional analyses, however, revealed that when including whether exercise was available in the homecage in the model together with number of animals housed together, the effect of housing remained significant. Still, while the continuous moderating effect remained after including exercising in the home cage, all effect size calculations for four animals housed per cage came from one experiment, such that under the exclusion criterion for discrete variables this moderator would not have been tested, and should be interpreted with caution.

4.5.3.2 Exercising after extinction.

One exercise parameter significantly moderated Pavlovian effects; specifically, 'whether exercise was administered after extinction or not'. This effect of augmentation after extinction has also shown success across a number of pharmacological approaches including amino acid receptor modulators (Bicuculline; Berlau & McGaugh, 2006), monoamine modulators (Norepinephrine; Berlau & McGaugh, 2006), and cannabinoid modulators (Cannabidiol; Das et al., 2013). Additionally, work in d-cycloserine (DCS; a NMDA receptor partial agonist) has indicated an enhancing effect on extinction, but only when sufficient reductions of fear were achieved during the extinction session (Bolkan & Lattal, 2014; Smits, Rosenfield, Otto, Powers, et al., 2013). Unfortunately, with the information available in the current analysis we could not assess whether this was also the case for exercise, because in fact only one experiment used level of fear at the end of extinction as a moderator of potential outcome effects. Further exploration may be needed to systematically determine if exercise has differing effects based on within-session extinction behavior or if it can produce a persistent reduction of free regardless of within-session responses.

Additional support for these findings also come from two potential neurotransmitter systems that are influenced by exercise: BDNF and/or DA (Foley & Fleshner, 2008; Greenwood et al., 2011; Greenwood, Strong, Foley, & Fleshner, 2009; Griffin et al., 2011; Hattori, Naoi, & Nishino, 1994; Heyes, Garnett, & Coates, 1988; Huang et al., 2006; Meeusen et al., 1997; Soya et al., 2007), which are also critical for Pavlovian extinction (Gourley, Howell, Rios, Dileone, & Taylor, 2009; Heldt, Stanek, Chhatwal, & Ressler, 2007; Hikind & Maroun, 2008; Holtzman-Assif, Laurent, & Westbrook, 2010; Kirtley & Thomas, 2010). Interestingly, previous work has shown that BDNF is required during extinction consolidation (a process that would occur after extinction procedures), but not during encoding (Chhatwal et al., 2006). If exercise is working to upregulate BDNF, the application of excise after extinction could promote a known mechanism required for extinction consolidation, leading to a larger effect over extinction alone. Consistent with this explanation, the literature looking at DA and its effects on enhancing extinction also suggests that both the application of methylphenidate and L-Dopa *after* extinction were successful at enhancing extinction outcomes (Abraham, Cunningham, & Lattal, 2012; Haaker et al., 2013). While the present analysis cannot address the mechanism engaged with exercise, there is support that its application after extinction may provide the best opportunity to enhance extinction outcomes, and that such an effect would be consistent with a possible engagement of BDNF and/or DA.

4.6 CONCLUSIONS

A number of past meta-analyses explored the effectiveness of exposure-based therapies for various anxiety related disorders, but less information is available on potential augmentations for exposure, in particular, behavioral interventions. In addition, there is a lack of systematic statistical reviews of the effect of augmentation of extinction

procedures in non-human animal models using either pharmacological or behavioral interventions, with the exception of Kredlow and colleagues (2016). While a variety of review papers have evaluated and summarized both the mechanisms and possible augmentation strategies for extinction/exposure in operant and Pavlovian models, the use of a statistical approach such as a meta-analysis allows a reader the ability to see individual effects from primary analysis, as well as the global effects and specific parameters that contribute the largest overall influence. We believe the present work will provide researchers with a better foundation for the use of exercise as an augmentation strategy for extinction/exposure based procedures.

4.7 Supplemental Results

4.7.1 All studies

Moderators.

Participant characteristics.

Gender. The gender of participants did not significantly moderate the overall findings (X2= 0.43, df= 2, p= .807). However, there was significantly less responding for male participants who exercised (g= 0.44, 95% CI [0.19, 0.69], p< .001, n= 43). With no significant effects were seen in female participants (g= 0.28, 95% CI [-0.09, 0.66], p= .143, n= 24) or experiments including both genders (g= 0.39, 95% CI [-0.54, 1.32], p= .409, n= 3).

Subject. Overall there was no significant moderating effect across subjects; humans, mice and rats (X2= 1.56, df= 2, p= .458). Although experiments using rats (g= 0.44, 95% CI [0.20, 0.68], p< .001, n= 65) did indicate a significant effect. Whereas in

both mice (g= -0.12, 95% CI [-0.95, 0.70], p= .771, n= 3) and humans (g= 0.38, 95% CI [-0.53, 1.28], p= .416, n= 3) there was no significant effect.

Non-human animal experiment specific.

Animal housing. Number of animals housed together did not have a moderator effect on overall main effects when tested as a continuous variable (B= -0.01, SE= 0.11, p= .915), but produced a trend when tested between housed alone versus multiple animals per cage (B= -0.41, SE= 0.24, p= .091). When exploring housing as a dichotomous variable we were able to show that animals housed alone show a significant overall effect (g= 0.47, 95% CI [0.23, 0.72], p< .001, n= 52), however animals housed with at least one other animal did not (g= 0.07, 95% CI [-0.34, 0.48], p= .737, n= 14).

Rat experiment specific.

Age in days. Age in days looking at rat subjects only did not have a moderator effect on the overall main effects (B= 0.17, SE= 0.13, p= .286, n= 25).

Conditioning procedures.

Stimuli. Due to inconsistencies in methods used across experiments adequate assessments for whether CS, US or US intensity were moderators of overall effects were not possible.

Timing of procedures. The number of extinction sessions was found not to have a significant moderator effect when tested as a continuous variable (B= 0.05, SE= 0.13, p= .661, n= 69), nor when tested as a dichotomous variable (B= -0.13, SE= 0.27, p= .640). For which cases of limited extinction produced – overall effects (1-7 sessions; g= 0.38, 95% CI [0.15, 0.61], p= .001, n= 47). Whereas with extended extinction (8-14 sessions)

there was a small overall effect (g= 0.25, 95% CI [-0.14, 0.64], p= .203, n= 23). In addition the time from the end of extinction to test did not moderate overall effects (B= 0.00, SE= 0.10, p= .970, n= 71).

Exercise parameters. A variety of different exercise parameters were explored the first of which was whether exercise prior to acquisition had a moderator effect on tests after extinction, for which no significant effect was seen (B= -0.09, SE= 0.24, p= .707), due to both conditions having similar small overall effects; exercise prior to acquisition (g= 0.34, 95% CI [-0.04, 0.72], p= .083, n= 30), no exercise prior to acquisition (g= 0.43, 95% CI [0.15, 0.71], p= .003, n= 38). Exercise prior to extinction again did not have a significant moderator effect (B= -0.42, SE= 0.32, p= .181). Here we see that both experiments that used exercise prior to extinction and those that did not show a significant overall effects (g= 0.33, 95% CI [0.09, 0.57], p= .007, n= 60; g= 0.77, 95% CI [0.17, 1.34], p= .011, n= 11). Similarly experiments that utilized exercise during the extinction session or not were found both to have significant effects (g= 0.48, 95% CI [0.13, 0.84], p= .008, n= 46; g= 0.36, 95% CI [0.09, 0.62], p= .007, n= 25). This parameter however did not moderate overall effects (B= 0.13, SE= 0.21, p= .539). Differentially from findings at other time points when exercise was implemented after extinction procedures, there is a significant moderator effect (B= 0.79, SE= 0.32, p= .015). This was driven by the large effect of exercising after extinction (g= 1.07, 95% CI [0.47, 1.67], p< .001, n= 5) as compared to a small effect in those that did not (g= 0.30, 95% CI [0.11, 0.50], p= .002, n= 63). Experiments utilizing exercise or not during post extinction test(s) were found to have significant effects (g= 0.49, 95% CI [0.11, 0.87], p=

.012, n= 27; g= 0.35, 95% CI [0.09, 0.32], p= .009, n= 44). There again however was no moderating effect based on this parameter (B= 0.14, SE= 0.23, p= .541).

Further exploration of exercise time across both forced and voluntary exercise experiments found that the model was unable to converge on a result, likely because exercise time ranged from 30-minutes to 117,000 minutes across all studies. But whether exercise type forced (g= 0.42, 95% CI [-0.38, 1.21], p= .305, n= 4) or voluntary not (g= 0.40, 95% CI [0.16, 0.63], p= .001, n= 67) significantly moderated data, however it did not (B= 0.02, SE= 0.42, p= .961).

Non-human animal specific. While across animals exercising in the homecage there was a significant effect (g= 0.48, 95% CI [0.22, 0.68], p< .001, n= 57), this effect was small and not different from those animals that did not (g= 0.10, 95% CI [-0.39, 0.58], p= .698, n= 11; B= 0.36, SE= 0.28, p= .195).

Chapter 5: General Discussion and Conclusions

5.1 Overview of research

Fear learning and memory can be adaptive. Learned fear promotes survival in threatening and dangerous situations. In some cases, however, learned fear can become maladaptive. In anxiety disorders, cues associated with threat or danger can elicit fear even when an immediate threat is no longer present. This can result in a persistent state of worry or vigilance. One intervention that may be able to attenuate this persistent reactivity is physical exercise. Exercise has been shown to influence stress and anxiety-related behaviors (Asmundson et al., 2013; Fuss et al., 2010; Greenwood & Fleshner, 2011; Newman & Motta, 2007; Stonerock, Hoffman, Smith, & Blumenthal, 2015), possibly via the same molecular mechanisms that mediate aspects of learning and memory (Baek, 2016). The present work set out to determine when and under what conditions exercise could be applied to reduce the persistence of fear.

Initial work first established differences in how the predictability of early life fear exposure influenced memory and reconditioning. Our results suggest that rats conditioned with either paired (predictable) or unpaired (unpredictable) procedures at post-natal day 17 (P17) show no initial fear memory; yet, after paired conditioning there was a potentiation of fear responses in adulthood, whereas those rats exposed to paired conditioning at P25 and again in adulthood show a potentiation, suggesting a less specific effect in P17 conditioned rats. As an attempt to reduce this persistence of fear, chronic exercise was introduced in adolescence as an intervention after early life aversive experience to determine if a non-specific approach could reduce persistence of fear.

Across all tests, exercise was found to have various effects on fear memory and learning. Specifically, rats conditioned at post-natal day 17 (P17) did not show memory for the experience (i.e., the tone); however, increased amounts of exercise in adolescence predicted less freezing during this retention test. In rats conditioned at P25, more exercise in adolescence also predicted less freezing, but only after adult conditioning (and irrespective of early life experience). We next explored whether exercise could be used as a potential augmentation strategy to reduce the persistence of fear when applied prior to extinction, a known learning procedure to reduce fear responding. We found that varying the time from exercise onset to extinction procedures, allowing for different durations of exercise, as well as using multiple conditioning and extinction procedures, did not produce better outcomes than extinction alone. Previous work in the field had shown exercise to be a successful augmentation strategy under certain conditions. In light of our own results, we decided to revisit the literature and used meta-analytical approaches to determine when and under what experimental parameters exercise had enhanced extinction outcomes. Our findings indicated that extinction in operant but not Pavlovian memory models were significantly enhanced by exercise. Operant extinction was moderated by the number of extinction sessions. Pavlovian extinction was moderated by the number of animals housed together and timing of exercise relative to extinction.

5.2 Can exercise reduce the persistence of fear?

Previous work has explored the beneficial effects of exercise on mental health (Cotman, Berchtold, & Christie, 2007; Gates, Killackey, Phillips, & Álvarez-Jiménez,

2015; Knapen, Vancampfort, Moriën, & Marchal, 2015; Park et al., 2013) and its enhancing effects on learning and memory (Baek, 2016; Cassilhas, Tufik, & de Mello, 2016; Diederich et al., 2017). The current work set to determine if exercise could also reduce the persistence of fear.

One way exercise may reduce the persistence of fear is through indirect effects on stress and anxiety. Specifically, exercise in it of itself can produce a mild stress response, as indicated by increased levels of the stress hormone corticosterone (CORT; Chen et al., 2016). Despite higher post-exercise baseline CORT, Chen et al. (2016) found the ability of a "large" or uncontrollable stressor (e.g. a forced swim test) to stimulate CORT is diminished after extended exercise. Exercise prior to this uncontrollable stressor may be providing controllable stress training, which was previously shown to buffer an uncontrollable stress response and reduce its negative effects on depression and anxiety-related behaviors (Amat, Aleksejev, Paul, Watkins, & Maier, 2010; Amat, Paul, Zarza, Watkins, & Maier, 2006). Interestingly, while 3-weeks of exercise is sufficient to produce neurological changes after an uncontrollable stressor, behaviorally, the ability for exercise to reduce the detrimental effects of stress were only found after at least 6-weeks of exercise exposure (Greenwood, Foley, Burhans, Maier, & Fleshner, 2005). These findings suggest that while initial neurological changes may emerge earlier, continued training is necessary for behavioral responses to be altered. While the experiments in the present thesis used 3-weeks of exercise in both P17 and P25 conditioning experiments, exercise could conceivably be working to provide a controllable stress training experience. As such, when exposed to the tone after early life conditioning (P17), or upon

exposure to a fear conditioning procedure, exercise treatment dose dependently dampens the freezing response.

Early life trauma can increase the likelihood of developing fear and anxiety-related disorders in the future (Carr, Martins, Stingel, Lemgruber, & Juruena, 2013; Fernandes & Osório, 2015; Heim & Nemeroff, 2001); however, an intervention of exercise was found to reduce PTSD symptoms, as well as depressive and anxiety-related responses (Newman & Motta, 2007). Importantly, in our P25 experiment, exercise did not completely eliminate learned fear; however, exercise dose dependently dampened fear behavior, suggesting that it may have affected subjects' stress and anxiety states. This is not to say that chronic voluntary exercise cannot influence learning and memory directly. It has been shown to have direct effects on contextual fear conditioning (Baruch, Swain, & Helmstetter, 2004; Greenwood, Strong, Foley, & Fleshner, 2009a; Kohman et al., 2012), with additional but limited support for direct effects in cued fear conditioning (Falls, Fox, & MacAulay, 2010). However, across all these studies, exercise treatment was applied immediately prior to or after conditioning procedures, whereas in the current experiments exercise treatment ceased 25-days before the memory retention and conditioning procedure, limiting its immediate and direct effect.

While both experiments utilizing chronic exercise were able to show an effect of exercise on fear responding (chapter 2), all experiments in chapter 3 indicate that acute exercise prior to extinction or exposure procedures did not enhance extinction acquisition, memory retention or prevent return of fear in relapse tests. One key factor that may affect the effect of exercise on extinction or exposure therapy is chronicity.

Several experiments have shown beneficial effects of chronic exercise on fear extinction acquisition in rats, but only when exercise is chronic, prior to fear acquisition, and after stress exposure (Greenwood et al., 2003, 2013, 2005; Greenwood, Strong, Foley, & Fleshner, 2009b; Greenwood, Loughridge, Sadaoui, Christianson, & Fleshner, 2012; Greenwood, Strong, Dorey, & Fleshner, 2007). Again, these benefits may derive from the ability of exercise to act as a mild controllable stressor that buffers later negative effects of exposure to an uncontrollable stressor. Additionally, in humans, Hopkins and colleagues show that a single session of aerobic exercise could enhance object recognition memory, but only when combined with a 4-week exercise program (Hopkins, Davis, Vantieghem, Whalen, & Bucci, 2012), suggesting that chronicity enables the acute beneficial effect of exercise.

A "prepping" effect could explain how chronic exercise enables beneficial acute effects of exercise on learning and memory. Exercise is a stressor, but it may be a "perfect stressor". Chronic mild stress (here I am suggesting that it may occur as a result of chronic exercise) promotes cell death in the adult hippocampus (Gu et al., 2014; Jayatissa, Bisgaard, West, & Wiborg, 2008). However, chronic exercise has also been shown to promote the birth and maturation of new cells in the adult hippocampus (Van Praag, Christie, Sejnowski, Gage, & Stevens, 1999). Thus, exercise treatment could conceivably prune unnecessary or "weak" neurons through its stress effects, while simultaneously promoting integration of new information via new neurons and new connections in the hippocampus. Considering the hippocampus may already be primed for integration from prior chronic exercise exposure, when acute exercise is applied prior

to a hippocampus-dependent cognitive task (such as object recognition memory in the example above), the memory then has room to be stored via the availability of new neurons, enhancing the overall learning and memory process. This hypothesis remains to be examined.

If chronicity of exercise is critical for exercise to be beneficial, such that this neural system may be "prepped" for maximal augmentation of extinction, the current studies may not have allowed for sufficient access, as there was only minimal (10-minutes for 3-days) or no access to exercise wheels prior to the full treatment. Follow up studies should test a similar implementation of a chronic exercise regimen prior to an acute bout of exercise paired with extinction procedures as previously shown to enhance object recognition memory in Hopkins et al. (2012), to determine if prior chronic exercise exposure is critical to observe an extinction-enhancing effect and a reduction in the persistence of fear. In addition, follow-up studies should explore whether blocking the mechanisms required for exercise to enhance neurogenesis, such as brain derived neurotrophic factor (BDNF) or N-Methyl-D-aspartic acid (NMDA; van Praag, 2008), would lead to only the detrimental effects of stress on fear extinction.

5.3 When and under what conditions can exercise enhance extinction?

After the findings from chapter 3 suggested that acute exercise applied prior to extinction or exposure therapy were not sufficient to enhance extinction further exploration was done to take a broader look at when and under what circumstances exercise could be used to augment extinction procedures throughout the literature. This

work was particularly critical as there was a large variability in how and when exercise was applied across experiments making it hard to disentangle what factors are critical for beneficial outcomes, so as to establish a foundation for future research. Data indicate across all 71 comparisons there was a moderate positive overall effect in procedures that used exercise applied with extinction over extinction alone, this effect was significantly moderated by model type (operant versus Pavlovian). Data were then divided by model type and reanalyzed for each model individually, for which exercise had a moderate significant effect on operant models and a small non-significant effect across Pavlovian models.

These differences in the benefits of exercise applied with extinction in operant versus Pavlovian models may result from the stimuli used in both of these models. As previously mentioned one primary difference in operant and Pavlovian models is that operant models almost always (and 100% of the studies included in this analysis) use appetitive stimuli that are also drugs of abuse. While our analysis did explore the moderating effect of appetitive versus aversive stimuli, which did not result in a moderator effect, this difference in an effect could stem from differences in operant and Pavlovian models, in which the amount of the appetitive stimulus received is controlled by the subjects responding, leading to higher overall exposure to the drug in operant vs. Pavlovian models. These drugs in particular, while being rewarding and promoting the conditioning process, have additional pharmacological properties that with repeated access can lead to drug dependence, which in turn can lead to psychological and physiological withdrawal symptoms when drug access ceases. This is particularly

important when thinking about a common theme discussed throughout this dissertation: the negative influence of stress on persistence of fear, leading to the inhibition of fear extinction processes, which is also true for extinction across other learned behaviors, such as those in operant models. In one theory of addiction, coined the "dark side" of addiction, the perpetuation of drug taking behaviors (responding) is due to an exaggerated stress response after continued drug use, that leads to a negative affective state that continually perpetuates attempts to restore oneself back to a homeostatic level, typically through continued drug taking (Koob & Le Moal, 2005). The idea is that the stress responses brought on by the negative consequences of drug withdrawal subject the user to seek a relief, which is remedied by increased drug taking (responding); however, this response simultaneously maintains the negative and stress-inducing cycle. In this case, the application of exercise is working to reduce the responsivity to stress and allows extinction to be unperturbed by the inherent detrimental effects drugs of abuse have on the extinction process. This action of exercise to function as a positive influence to combat the negative impact drugs of abuse have on stress responses and their influence on extinction may explain why having exercise access or not at any given time point was found to have a moderate positive effect across operant models. This non-specific effect may indicate that exercise is not influencing learning and memory directly, but acting as a buffer to the consequences of stress. In addition to overall effects, operant models were found to be moderated by number of extinction sessions, such that more sessions produced less of an enhancing effect of exercise with extinction over extinction alone. This is important as the results indicate a potential floor effect. With an increasing

number of extinction sessions, the level of responding reaches a minimum and the application of exercise cannot further reduce the level of responding. While none of the experiments included in this analysis directly tested varying numbers of extinction sessions, this could be a question addressed in the future, as it could be beneficial to include exercise into a treatment regimen in humans patients who are seeking treatment for drugs of abuse, especially in instances were limited exposure sessions are available, to try and maximize recovery outcomes.

Pavlovian extinction models, on the other hand, were moderated by two variables. First, the number of animals housed together had a positive moderating effect, such that more animals housed together resulted in larger reductions in responding. However, as previous literature suggests a difference would be expected based on whether animals were housed alone versus housed with any other number of animals, as rodents are known to be social animals and are negatively impacted by social isolation (Okada, Matsumoto, Tsushima, Fujiwara, & Tsuneyama, 2014; Skelly, Chappell, Carter, & Weiner, 2015; Voikar, Polus, Vasar, & Rauvala, 2004; Weiss, Pryce, Jongen-Relo, Nanz-Bahr, & Feldon, 2004). When data were partitioned into this dichotomous variable (alone vs. multiple), the moderating effect was no longer seen. Additionally as both data points in the highest number of animals housed together (four) came from the same experiment, there may be other factors within this experiment that may be influencing findings. The second variable to produce a moderating effect was whether subjects exercised after extinction or not. While it may be counter intuitive that exercise after extinction may be beneficial based on previous discussion that exercise is influencing fear responding

through stress and chronicity, we know, that chronic exercise upregulates BDNF (Adlard, Perreau, Engesser-Cesar, & Cotman, 2004; Venezia, Guth, Sapp, Spangenburg, & Roth, 2016), with chronic exercise also enhancing the BDNF response to an acute bout of exercise (Szuhany, Bugatti, & Otto, 2015). Additionally, BDNF is critical for the consolidation of extinction memories (Chhatwal, Stanek-Rattiner, Davis, & Ressler, 2006), so an upregulation of BNDF during the consolidation process via an acute bout of exercise, given after prior chronic exercise exposure/experience, may yield the most benefit by having a system that is already primed to maximize an effect. Interestingly, one of the experiments included in the meta-analysis allowed exercise prior, during and after Pavlovian extinction which produced a large overall effect within that primary study. Together, these findings suggest that future research should replicate and extend these results to determine if exercise during extinction was critical or if prior to and after is sufficient.

5.4 Conclusion

Overall, the current compilation of work showed that exercise may reduce the persistence of both fear and appetitive responding, but that its effectiveness relies on when and how exercise is applied across tasks. Particularly, chronic exercise applied in adolescence reduced freezing responses in rats previously conditioned at P17 as a function of distance run in adolescence, whereas prior conditioning at P25 was not influenced, but rather after conditioning in adulthood, similarly, lead to reductions in freezing as distance run increased. One way chronic exercise may be producing these reductions in the persistence of fear is through its influence on stress and anxiety states.

This idea is also supported by findings within the meta-analysis in which operant, but not Pavlovian, extinction was significantly enhanced by exercise. An effect that could relate to the known stress response induced during withdrawal from drugs of abuse, suggesting that exercise at any time point around the extinction session may be countering the negative effects stress has on extinction processes.

When acute exercise was applied prior to extinction or exposure therapy, we did not observe any augmentative effects above and beyond extinction alone. We hypothesize that exercise itself produces a stress response and that to see beneficial effects of exercise, there needs to be some level of regular or chronic exercise first, leading to a "priming" effect on the memory system involving the hippocampus. The results from our meta-analysis however indicate that exercise after, but not before, extinction was sufficient to moderate overall Pavlovian analysis, such that future research could focus on applying exercise after extinction, as well as explore pairing acute exercise after extinction with prior chronic exercise exposure as a way to maximize BDNF upregulation, during extinction consolidation. Overall, our data indicate that exercise can be used to reduce persistence of fear, but only under very specific circumstances.

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