AVOIDANCE-BASED PAVLOVIAN-INSTRUMENTAL INTERACTIONS

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ABSTRACT OF THE DISSERTATION

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How are aversive associations formed in the brain, and how do they subsequently influence behavior? Imagine an individual who experienced a harrowing flight, and subsequently acquired an intense fear of flying. The sight of airports or planes in flight – previously innocuous stimuli – may now trigger an aversive response within the individual. If the individual continues to fly without facing additional negative experiences, the aversive response may be updated, or *extinguished*. However, this individual may choose to avoid air travel in order to relieve anxiety, even though future flights would likely not be coupled with negative events. Here, the temporary relief of anxiety renders the avoidance behavior adaptive. However, avoidance can also be maladaptive. For instance, continually avoiding air travel limits the ability to visit family and friends that live afar. While avoidance behaviors are often performed without consequence in everyday life, they also play a role in the persistence of many clinical disorders. The avoidance of an anxiety-provoking stimulus is a defining behavior in anxiety disorders. Similarly, negative reinforcement-based models of addiction posit that avoidance of withdrawal symptoms is a major factor in sustained drug-seeking and

relapse. In both of these cases, aversive Pavlovian conditioned stimuli (CS) modulate instrumental avoidance behaviors and vice versa. This dissertation sought to better understand the flexibility of aversive CS-US relationships and how these relationships can motivate avoidance behaviors. A combination of behavioral, neuroimaging and physiological measures were used.

The first goal of this dissertation was to understand how aversive Pavlovian CS-US associations are formed and updated in the brain. The second goal was to examine the behavioral and neural correlates of aversive Pavlovian control over instrumental avoidance behavior using the Pavlovian-to-instrumental Transfer (PIT) task, which tests the ability of Pavlovian CS to motivate instrumental behavior. The third goal of this dissertation was to understand how stress, a real-world variable that is often comorbid with anxiety and addiction, affects the ability of aversive CS to motivate instrumental avoidance behavior. Overall, these studies shed light on clinical disorders involving extinction failure and excessive avoidance responses, such as drug addiction, anxiety and post-traumatic stress disorder (PTSD).

Dedication

To Mom, for teaching me the importance of perseverance

To Jamie, for your unconditional patience and love

To Phoebe, for re-opening my eyes to the simple wonders of the world

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Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

1.1 General Introduction

Our behaviors are often motivated by our perception of stimuli in the environment. For instance, negative past experiences with a stimulus may lead to avoidance of this stimulus upon future encounters. In this situation, an interaction is occurring between a previous stimulus-outcome association (i.e. a stimulus leading to a negative outcome) and a response-outcome contingency (i.e. an avoidance response leading to elimination of the outcome). The former contingency corresponds to a learned Pavlovian response, while the latter is instrumental in nature. The behavioral and neural basis of Pavlovian and instrumental learning has been the subject of many studies in psychology and neuroscience. However, research has only recently begun to explore the neural basis of interactions between these two systems.

The research presented in this dissertation aims to explore unanswered questions related to interactions between the Pavlovian and instrumental learning systems during avoidance learning. This dissertation begins by outlining the behavioral, neural and physiological components of various affective learning processes, as well as factors that influence these learning processes and implications for clinical models. Three studies, utilizing a variety of methods, are then discussed. The aim of these three studies is to better characterize Pavlovian-instrumental interactions by examining: the neural correlates of appetitive and aversive extinction learning, and how regions involved in extinction learning function in tandem with large-scale brain networks (Chapter 2), the neural correlates of avoidance-based Pavlovian-to-instrumental transfer (Chapter 3), and the influence of stress on avoidance-based Pavlovian-to-instrumental transfer (Chapter 4).

Finally, a general discussion will highlight overall conclusions and implications of the dissertation studies.

1.2 Learning about affective information in the environment

1.2.1 Basic principles of Pavlovian conditioning. Pavlovian or classical conditioning occurs when a neutral stimulus (conditioned stimulus, CS) is repeatedly paired with an emotionally salient stimulus (unconditioned stimulus, US). Through these pairings, the CS comes to provide information regarding the occurrence of the US. Over time, the CS can acquire the affective properties of the US, and presentation of the CS itself begins to elicit a conditioned response (CR; Pavlov & Anrep, 1927). Traditional measurements of a CR include freezing behavior in rodents as well as autonomic nervous system activity (e.g. skin conductance responses, pupillary dilation) in humans.

Acquisition of Pavlovian CS-US associations can occur in both the appetitive and aversive domains. In both humans and non-human animals, this has been traditionally studied by pairing a neutral cue such as a tone or light with a primary reinforcer such as food reward, drug reward, or shock. Recently, studies of Pavlovian conditioning in humans have also begun using secondary reinforcers such as monetary gains and losses (e.g. Delgado et al., 2006; Delgado et al., 2011; Lewis et al., 2014), as well as instructed reinforcers, such as points in a video game (e.g. Lewis et al., 2013; Nadler et al., 2011). In many studies with both humans and non-human animals, conditioning is measured by comparing behavioral, neural or physiological responses elicited by a CS paired with an appetitive or aversive outcome (CS+) to responses elicited by a CS paired with a neutral outcome or no outcome (CS-).

1.2.2 Measuring conditioned responses. A useful tool for examining conditioned responses in humans is the skin conductance response (SCR). SCR measurements reflect activity of the sympathetic nervous system, and elevate with increased physiological arousal. Given that sweating is controlled by the sympathetic nervous system, increases in sympathetic nervous system activity enhance SCR via increases in sweat gland activity. SCR can be collected during both appetitive and aversive conditioning procedures, and is traditionally analyzed by examining the difference between the conditioned response to the CS of interest (CS+) and the conditioned response to a neutral CS (CS-). Past studies of conditioning and affective learning have had much success utilizing SCRs as a measurement of conditioned responses (e.g. Delgado et al., 2006; Delgado et al., 2008b; LaBar et al., 1998; Olsson et al., 2005). Other measurements of conditioned responses that have been used with success in humans include pupillary dilation and conditioned eyeblinks.

1.2.3 Pavlovian learning: neural and physiological correlates. The striatum is a subcortical brain region that functions as the major input unit of the basal ganglia, both in humans and some non-human animals. Evidence suggests that the striatum is involved in a host of reward-related processes (for review, see Balleine et al., 2007), including Pavlovian and instrumental conditioning. The ventral striatum, in particular, is activated during both Pavlovian and instrumental learning, while the dorsal striatum is involved primarily in instrumental learning (O'Doherty et al., 2004). Neuroimaging studies of Pavlovian conditioning report activity in the human striatum with both primary

reinforcers, such as juice (O'Doherty et al., 2002; O'Doherty et al., 2004) or pleasant odors (Gottfried et al., 2002), and secondary reinforcers, such as monetary gain (Kirsch et al., 2003; Knutson et al., 2001).

In both humans and many non-human animals, the amygdala, a subcortical component of the limbic system, has been implicated in the acquisition of aversive CS-US associations with primary reinforcers (see Phelps & LeDoux, 2005 for review). It has been suggested that the amygdala plays less of a role in aversive Pavlovian conditioning with secondary reinforcers (e.g. monetary loss, Delgado et al., 2011), though it is involved in the process of second order learning (Parkes & Westbrook, 2010; 2011). The striatum, which is traditionally associated with reward processing, has also been implicated in aversive conditioning. Many laboratory studies report activity in this region during aversive Pavlovian conditioning with both primary (e.g. Becerra et al., 2001; Büchel et al., 1998; Delgado et al., 2011; Jensen et al., 2003; LaBar et al., 1998; Phelps et al., 2004) and secondary reinforcers (e.g. Delgado et al., 2008b; Seymour et al., 2007). It should be noted, however, that striatal activity is not always seen in studies of aversive conditioning (e.g. Gottfried et al. , 2002).

In normal populations, fear conditioning tasks lead to enhanced SCR for a CS paired with an aversive outcome as compared to a CS paired with a neutral outcome (e.g. Delgado et al., 2008b). Patients with lesions to the amygdala do not show enhanced SCR in response to fearful conditioned stimuli. While these patients can explicitly report CS-US associations, they are unable to exhibit a physiological fear response (e.g. LaBar et al., 1995; Bechara et al., 1995). The skin conductance response reflects general activity of the autonomic nervous system, and therefore is not specific to aversive responses. SCR

are also enhanced in the presence of CS paired with rewarding outcomes as compared to neutral CS (Delgado et al., 2008a). Pupillary dilation has been shown to mirror SCR responses with both appetitive and aversive stimuli, also reflecting general physiological arousal (Bradley et al., 2008).

1.2.4 Instrumental learning: behavior and neural correlates. Whereas Pavlovian conditioning occurs passively, instrumental conditioning involves goal-directed behavior. During instrumental conditioning, the performance of an action leads to either the promotion or elimination of an appetitive or aversive outcome. Thus, following repeated pairings, the relationship between the behavioral response (R) and the appetitive or aversive outcome (O) is learned. When an instrumental behavior leads to a rewarding outcome, the R-O contingency is reinforced and, in turn, the behavior is more likely to be performed in the future (e.g. Skinner, 1938; Thorndike, 1911). Avoidance learning, on the other hand, involves the relationship between a behavior and subsequent avoidance of an aversive outcome. The elimination of the aversive outcome following the performance of the behavior serves to increase future instances of the behavior (Solomon & Wynne, 1953). The reverse can also be the case – in the instance that instrumental behavior leads to elimination of a rewarding outcome or the occurrence of a negative outcome, the behavior will decrease (Skinner, 1938).

Instrumental learning is dependent upon the ability to encode response-outcome associations. In rodents, inactivation of the dorsomedial striatum prevents this action-outcome learning (Yin et al., 2005). As in appetitive Pavlovian conditioning with humans, appetitive instrumental conditioning is associated with increased activity in the

human striatum. In particular, the ventral striatum functions as a "critic," utilizing available information to provide a prediction of the likelihood of upcoming reward. Operating in tandem with the ventral striatum is the dorsal striatum, which functions as an "actor," maintaining information about the outcomes associated with an instrumental response (O'Doherty et al., 2004).

Animal models of avoidance learning illustrate that the active avoidance of an aversive outcome involves a neural pathway that differs from that in Pavlovian learning. In particular, avoidance learning involves the basal nucleus of the amygdala, wherein activation of projections to the striatum allows for active avoidance responses to be made (Amorapanth et al., 2000; Killcross et al., 1997). The neural correlates of human avoidance learning mirror those in animal models, as interactions between amygdala and striatum underlie the active avoidance process (Delgado et al., 2009).

1.3 Extinction learning

1.3.1 Basic principles of extinction learning. Through Pavlovian conditioning, a previously neutral CS comes to elicit a conditioned response via repeated pairings with a US. Extinction occurs when a CS that has been previously paired with an emotionally salient US is repeatedly presented in the *absence* of any reinforcement. Over time, the presentation of the CS may no longer elicit a CR (or may elicit a diminished CR); in this instance it is said that extinction learning has occurred. Extinction learning can also occur following the acquisition of an instrumental response-outcome association. In this instance, the elimination of the reinforcing outcome following performance of an instrumental response leads to diminished or eliminated instrumental responding. As is

the case with conditioning, extinction learning occurs in both the appetitive and aversive domains.

Successful extinction does not result in an erasure of the original memory trace, but rather involves the formation of a new memory for the stimulus (Myers & Davis, 2002; although the original association may partially weaken - see Delamater & Westbrook, 2014, for review). That is, the initial CS-US contingency is not "un-learned," but is simply overridden by a new, "re-learned" CS-US association. This idea has been demonstrated in studies showing that the CR can, under certain conditions, return following extinction. In one such condition, presentation of the US after extinction, particularly when the presentation occurs in the same context as the initial CS-US learning, can lead to reinstatement of the CR. Alternatively, renewal of the CR can occur if the context is changed after extinction, either to the original learning context or to a novel context (Bouton, 2002; Bouton et al., 2006). Spontaneous recovery is the reemergence of fear post-extinction after a temporal delay (Bouton, 2002; Bouton et al., 2006; Effting & Kindt, 2007; Schiller et al., 2008). Spontaneous recovery occurs independently of any contextual factors (besides the context of time), and thus depends on the ability of the original fear memory to override the updated, extinguished CS-US association. Extinction learning is essential for preventing the recurrence of maladaptive behaviors – specifically, extinction failure for reward and fear can manifest as drug relapse (Kalivas et al., 2005; Garavan & Hester, 2007) and anxiety disorders (Delgado et al., 2006; Milad, et al., 2006), respectively.

1.3.2 Neural correlates of extinction learning. In humans, both extinction learning (Gottfried & Dolan, 2004; Linnman et al., 2012) and retention of fear extinction (Kalisch et al., 2006; Phelps et al., 2004) involve top-down regulation of subcortical areas by the ventromedial prefrontal cortex (vmPFC), a brain region implicated in cognitive processes such as emotional control. Additionally, the strength of vmPFC activation during recall has been found to correlate with the quality of extinction learning (Phelps et al., 2004). The increase in vmPFC activation during fear extinction has been found to correlate with a simultaneous decrease in amygdala activation. Specifically, activation of vmPFC leads to the excitation of GABAergic inhibitory neurons, which inhibit the amygdala and, subsequently, the aversive fear memory (see Peters et al., 2009 for review). Therefore, the updated CS-US contingency is able to override the initial conditioned association.

Research suggests that the vmPFC is crucial for the prevention of spontaneous recovery. Lesions to vmPFC in rodents prevent the recollection of extinction learning after a delay of 24 hours, leading to spontaneous recovery of fear (Quirk et al., 2000). In neuroimaging work with humans, successful memory for delayed tests of extinction relies on the recruitment of the vmPFC (Phelps et al., 2004). It has been postulated that extinction circuits for both positive and negative reinforcers activate the same regions of medial prefrontal cortex (mPFC, Peters et al., 2009). If these circuits indeed overlap, similar techniques should be effective for preventing extinction failure in both domains.

1.3.3 Consideration of affective learning brain regions in the context of large-scale neural networks. Cognitive and affective processes in the brain are regulated by a

host of large-scale neural networks. For instance, the executive control network (ECN) subserves a host of executive functions related to cognitive control (Dosenbach et al., 2007; Smith et al., 2009), while the default mode network (DMN) is thought to be involved in interoceptive, self-referential processes (Gusnard et al., 2001; Raichle et al., 2001; Shulman et al., 1997; Smith et al., 2009). Anatomical connections underlying these networks are present at any given time, though functional connectivity fluctuates as the brain moves through varying states of activation and rest (Smith et al., 2009). It is important to consider not just the role of individual brain regions in various cognitive and affective processes, but also the functional interaction of these regions with larger neural networks.

In a 2009 study, Smith et al. sought to identify the explicit large-scale networks in the human brain by carrying out a network analysis on a large database of over 1,600 brain imaging studies (BrainMap - Fox & Lancaster, 2002; Laird et al., 2005). Independent components analysis (ICA), an approach used to isolate independent patterns in multivariate data sets, was performed to identify these networks. Independently, these networks were extracted from 36 functional imaging scans of individuals at rest. 20 components were extracted from both the BrainMap and resting state data sets, and these components were subsequently compared across maps using spatial cross-correlation. Ten component maps were correlated between the two datasets ($r_{min} = 0.25$), indicating that these functional networks are utilized by the brain both while undergoing task-related activity as well as when at rest. Of particular interest to this dissertation, many medial frontal areas of the brain involved in a variety of emotion, action-inhibition and cognition tasks, are situated in the ECN. However, it remains

unknown how the strength of functional connectivity between medial frontal brain regions and the ECN varies as a function of task (e.g. during extinction learning as compared to other types of affective learning). Successful extinction learning involves top-down modulation by mPFC of subcortical brain regions and also requires cognitive control processes such as inhibition in order to suppress the initial CS-US contingency (see Peters et al., 2009, for review). Given that Pavlovian conditioning, in contrast, is thought to involve lower-level, sometimes automatic stimulus control (e.g. Esteves et al., 1994), it is possible that extinction learning may show enhanced coupling with the ECN.

1.4 Interactions between Pavlovian and instrumental learning systems

Goal-directed behaviors are often influenced by environmental cues associated with appetitive or aversive stimuli. This phenomenon, known as Pavlovian-to-instrumental transfer (PIT) can manifest as an increase in appetitive behaviors in response to a cue that, through conditioning, has attained appetitive properties (e.g., Balleine & Dickinson, 1998), or as a decrease in appetitive behaviors in the presence of an aversive conditioned cue (e.g., Estes & Skinner, 1941). It is believed that PIT can explain maladaptive behaviors maintained by positive reinforcement, such as reinstatement of drug-seeking behaviors after presentation of drug-related cues (Cardinal & Everitt, 2004). Modulation by Pavlovian cues upon instrumental responding, however, can also be assessed when that responding is maintained by negative reinforcement (e.g., avoidance learning; Rescorla & Solomon, 1967), a potentially important factor in continued or renewed drug-seeking behavior (Baker et al., 2004). In the case of drug-seeking behavior, drug-related Pavlovian cues can lead to the experience of negative withdrawal symptoms;

subsequently, these cues motivate avoidance behaviors. Little is known about the mechanisms underlying the maintenance of instrumental avoidance behavior by aversive Pavlovian stimuli.

Previous research has characterized two qualitatively distinct forms of PIT – specific and general. In *specific PIT*, reinforced instrumental responding is selectively increased in response to a conditioned Pavlovian cue with which the instrumental response once shared a reinforcing outcome. In *general PIT*, a conditioned Pavlovian cue motivates nonselective increases in reinforced instrumental responding, even when the cue and responses never shared a reinforcing outcome. Using appetitive PIT tasks, work in rodents demonstrates the necessity of the nucleus accumbens shell (Corbit & Balleine, 2011) and the basolateral amygdala (Corbit & Balleine, 2005) in specific PIT, and the nucleus accumbens core (Corbit & Balleine, 2011) and central nucleus of the amygdala (Corbit & Balleine, 2005) in general PIT. This is corroborated by human neuroimaging studies, which suggest the involvement of the striatum in specific PIT (Bray et al., 2008; Talmi, et al., 2008; Prévost et al., 2012) and the amygdala in general PIT (Prévost et al., 2012). However, the aforementioned neuroimaging studies were conducted in the appetitive domain, examining approach behaviors maintained by positive reinforcement. Furthermore, animal studies that have examined PIT in the aversive domain have rarely attempted to distinguish between specific and general PIT effects (e.g., Lolordo, 1967; (Rescorla & Lolordo, 1965). Therefore, it is unclear if similar neural mechanisms are involved when PIT occurs in an aversive context, and if the motivation to actively avoid aversive events would promote both specific and general PIT effects (as suggested by Nadler et al., 2011).

1.5 The effects of stress on learning and motivated behavior

1.5.1 The biological basis of the human stress response. Learning about the environment can take place under the influence of a host of internal and environmental factors, including stress. When a stressor is perceived, a cascade of events in two complementary biological systems is triggered. The sympathetic branch of the autonomic nervous system (ANS) responds quickly, releasing catecholamines from the adrenal medulla. Catecholamine release prepares the body for excitatory changes, promoting the "fight or flight" response (e.g. Cannon, 1915). The hypothalamic-pituitary-adrenal (HPA) axis also responds to perceived stressors, but at a much slower pace. The hypothalamus stimulates the pituitary gland, which subsequently releases adrenocorticotropic releasing hormone (ACTH). ACTH binds to receptors on the adrenal cortex, stimulating the release of the glucocorticoid cortisol (for review, see Ulrich-Lai & Herman, 2009). Catecholamine and glucocorticoid release from the adrenal cortex help to kickstart the physical response to stress.

When stress exposure occurs over a long period of time, the HPA axis remains active, and the production of cortisol is sustained. While acute stress is adaptive in the short term, chronic stress has been shown to lead to a host of negative outcomes, including impaired development of emotion regulation (Tottenham et al., 2010), slowing of wound healing (Kiecolt-Glaser et al., 1995), increased drug-seeking and drug use (e.g. Sinha, 2001), and exacerbation of symptoms in many psychiatric disorders (see Marin et al., 2011, for review). Over time, chronic stress can lead to structural changes in the brain, in regions such as mPFC and striatum (Dias-Ferreira et al., 2009; Radley et al.,

2004). Although these structural changes are not present after shorter-term acute stress, acute and chronic stress have both been found to impact components of affective learning, as will be discussed in section 1.5.2.

1.5.2 The effects of stress on Pavlovian and instrumental systems. Stress can affect many basic learning processes on both behavioral and neural levels, but efforts to understand the specific influence of stress on conditioning have produced variable results. The acquisition of a conditioned response during aversive learning in rodents, for example, has been shown to be enhanced in males under stress and depressed in females under stress (e.g. Wood et al., 2001; Wood & Shors, 1998). This is highly context dependent, however, as factors such as stressor type (e.g., swim stress, noise or restraint; Shors, 2001) and the temporal proximity of both the learning process and the experienced stress (see Joëls et al., 2006 for review) can affect the manner in which learning is altered. One prominent hypothesis suggests that there are, in fact, numerous relationships between stress and learning, and that effects of stress on learning may vary based on factors such as whether or not current resources allow for use of the multiple memory systems in the brain (see Shors, 2004 for review).

Similar variability in the effects of stress on learning has been observed in humans. For instance, acute stress has been suggested to improve performance in an eyeblink conditioning task in some studies (e.g., Duncko et al., 2007) while also impairing eyeblink conditioning in other reports (e.g., Wolf et al., 2009). One explanation for this discrepancy may be the use of alternative stressors associated with different patterns of cortisol release (e.g., Cold Pressor Test versus Trier Social Stressor Test). In

order to accurately assess the effects of stress on Pavlovian conditioning, individual differences in levels of circulating cortisol are often examined. Increased levels of cortisol during fear conditioning in males but not females, for example, correlate with elevated fear acquisition (Zorawski et al., 2006; Zorawski et al., 2005). It has been suggested that both cortisol levels (Merz, Stark, et al., 2013; Stark et al., 2006) and sex (Merz, Wolf, et al., 2013; Stark et al., 2006) may play a role in determining the specific effects of stress on the processing of CS associated with aversive primary reinforcers.

With respect to the human brain, fMRI studies have observed an influence of stress on activity in associative learning-related brain regions such as the striatum, anterior cingulate cortex, hippocampus and amygdala (Merz, Wolf, et al., 2013). The striatum, in particular, appears to be vulnerable to the effects of stress (e.g., Porcelli et al., 2012; Sinha et al., 2005), which could subsequently impact learning. Increasing stress by threat of shock, for example, has been found to increase aversive prediction errors during probabilistic learning (Robinson et al., 2013). This is consistent with observations of aversive prediction errors in the striatum during fear conditioning studies (Delgado et al., 2008b; Seymour et al., 2004). Recently, Lewis et al. (2014) found that acute stress affects the neural correlates of Pavlovian conditioning with monetary gains and losses. Stress-related differences in the ventral striatum, particularly the ventral putamen, were in part related to changes in circulating cortisol. More specifically, the stress group exhibited an increased sensitivity to magnitude in the gain domain. This effect was driven by those participants who experienced a larger increase in circulating cortisol levels in response to the stress manipulation. Taken together, these results suggest that acute stress can lead to

individual differences in circulating cortisol levels, which influence the striatum during Pavlovian conditioning with monetary reinforcers.

Little is known about the effects of stress on PIT. In rodents, chronic stress, applied prior to conditioning, does not impair Pavlovian or instrumental learning, but rather to leads to deficiencies in appetitive PIT (Morgado et al., 2012). In this same study, elimination of the chronic stress for a period of time also eliminated the behavioral PIT impairment. Acute stress has been found to reduce basal instrumental responding during an appetitive PIT task in rats, but only during certain times of day and following the application of *multiple* stressors (as opposed to a single stressor, Pielock et al., 2013). How stress affects PIT in humans and how stress affects avoidance-based PIT are two largely unanswered questions.

1.6 Clinical significance

1.6.1 Positive and negative reinforcement-based models of addiction. Many existing theories of drug dependence posit that positive reinforcement stemming from drug consumption is the primary motivating factor in continued drug-seeking and drug addiction (e.g. Robinson & Berridge, 1993; Stewart, de Wit, & Eikelboom, 1984; Stewart & Wise, 1992). These models suggest that the hedonic effects of addictive drugs increase the likelihood of drug taking (i.e. behaviors that result in the same hedonic effects). Additionally, through Pavlovian conditioning, stimuli in the environment that become associated with the reinforcing outcome of drug taking can gain incentive salience (see Everitt & Robbins, 2005, for review). A particularly influential model is the incentive sensitization theory of addiction (Robinson & Berridge, 1993). This model suggests that

because addictive drugs have the ability to enhance dopamine transmission, the dopamine system can, over time, become hypersensitive to drugs and drug-related stimuli. Consequently, the incentive salience, or motivational "wanting" associated with drug-related stimuli, becomes excessive, rendering these stimuli hyper-salient. This incentive sensitization can lead to compulsive drug seeking, as "wanting" is transformed into extreme craving.

On the other hand, negative reinforcement models of addiction posit that following drug administration, an aversive withdrawal response is felt as the amount of drug in the body decreases over time. This response then triggers drug seeking as an avoidance response, in order to combat withdrawal symptoms (Wikler, 1948). A recent model suggests that animals can detect interoceptive withdrawal symptoms as well as withdrawal and related negative affect that are experienced outside of conscious awareness. The negative affect experienced during withdrawal, which serves to rationalize drug use, biases future response options toward drug seeking and may compromise cognitive control resources (Baker et al., 2004). Self-report measures produced by drug addicted individuals suggest increases in both intentions and urges for drug seeking when experiencing aversive withdrawal symptoms (e.g. Baker et al., 1986; Wikler, 1980), supporting a negative reinforcement-based model. During withdrawal, it is believed that the dopamine system is compromised, which increases sensitivity to drug-relevant stimuli at the expense of sensitivity to non-drug stimuli (Melis et al., 2005). This is complemented by evidence suggesting that withdrawal is associated with changes in mood, motivation and fatigue, all of which may also involve changes in dopamine system activity (see Koob & Volkow, 2009, for review). Thus, changes to the dopamine system

are implicated in both positive reinforcement (incentive sensitization) and negative reinforcement (withdrawal-based) models of drug addiction.

1.6.2 Avoidance-based PIT as a clinical model. The PIT task might be used in the future as a model for drug relapse, wherein drug-related stimuli motivate drug seeking through negative reinforcement (see Baker et al., 2004 for review) and/or incentive sensitization mechanisms (e.g., Everitt & Robbins, 2005; Robinson & Berridge, 2001; Stewart & Wise, 1992). It has been suggested that many clinical disorders such as addiction relate to the Pavlovian learning system (e.g. Flagel et al., 2011). Behavioral research has been successful in obtaining a specific PIT effect in nicotine-dependent individuals using smoking-related stimuli (Hogarth & Chase, 2012). Evidence suggests that negative affect leads to drug craving and increases the likelihood of relapse (see Sinha, 2007 for review). Moreover, it has been found that addictive drugs are effective at reducing many negative symptoms of withdrawal (see Baker et al., 2004 for review); thus it may be the case that attempts to avoid withdrawal symptoms can lead to relapse as well. Use of an avoidance-based PIT task would allow for an understanding of the complex relationship between both attribution of salience to drug-related CS and subsequent avoidance responses in the presence of withdrawal symptoms, as well as the combined role of these phenomena in drug-seeking behavior. Additionally, because the PIT transfer test is performed in the absence of outcomes, it has implications for understanding how encountering drug-related CSs can trigger relapse. In particular, the PIT task may be useful for distinguishing between incentive salience and avoidance-based interpretations of drug relapse.

Avoidance-based PIT may also be a useful model for gaining an understanding of other disorders involving avoidance of aversive stimuli, such as anxiety disorders and post-traumatic stress disorder (PTSD). A recent model of PTSD suggests that affected individuals have an overactive learned response to fearful CS, as well as impairments in extinguishing the relevant CS-US responses (VanElzakker et al., 2014). Additionally, greater avoidance symptoms in PTSD patients correlates with aversive CS-induced activity in brain regions involved in fear conditioning and extinction, such as amygdala and vmPFC (Sripada et al., 2013). Given the role of the fearful CS in triggering avoidance behavior in PTSD patients, PIT may shed light on the specifics of this motivated behavior. Avoidance-based PIT may also be a useful model for understanding several other anxiety-based disorders that have been shown to involve enhanced conditioning and/or extinction failure with aversive CS, such as panic disorder (Michael et al., 2007), social phobia (Hermann et al., 2002), and obsessive-compulsive disorder (Milad et al., 2013).

1.7 General description and significance of dissertation experiments

The literature reviewed in the preceding sections highlight the behavioral and neural underpinnings of Pavlovian and instrumental learning processes, as well as interactions between these two systems. While much research has begun to examine the nature of aversive Pavlovian and instrumental learning, the manner in which aversive CS-outcome contingencies are formed and updated in the brain and how these associations subsequently influence behavior are largely unanswered questions. This dissertation

addresses these gaps in the literature through three studies, using a combination of behavioral, neuroimaging, and physiological methods.

1. Evidence has shown that extinction learning involves top-down regulation by the mPFC (Gottfried & Dolan, 2004; Kalisch et al., 2006; Linnman et al., 2012; Phelps et al., 2004), a region situated, in part, in the ECN (Smith et al., 2009). While the involvement of the mPFC is evident for extinction of both appetitive and aversive CS-US contingencies separately, it is unclear how the neural circuitry underlying extinction learning with aversive CS directly compares to that of extinction learning with appetitive CS. Given that extinction failure in both appetitive and aversive domains can lead to negative outcomes (e.g. drug relapse, Kalivas et al., 2005; Garavan & Hester, 2007; and anxiety disorders, Delgado, Olsson, et al., 2006; Milad et al., 2006, respectively), it is critical to understand the overlap of the neural response to extinction learning in both domains. It is also unclear how the mPFC, as well as other brain regions involved in affective learning, function in tandem with large-scale brain networks during the extinction learning. In particular, it is important to consider the functional connectivity of the mPFC to these networks, and how this connectivity differs between extinction and other learning processes. Experiment 1 (Lewis, Smith, Manglani & Delgado, in prep) sought to answer these questions using a paradigm that examined Pavlovian conditioning and extinction with equated appetitive and aversive reinforcement. While undergoing fMRI, participants experienced gain and loss of reinforcers that were important in the context of a video game. We hypothesized that during extinction learning, the mPFC would be recruited similarly in the presence of both appetitive and aversive CS. We also

hypothesized that the mPFC would show enhanced functional connectivity with the ECN during extinction learning as compared to initial Pavlovian conditioning.

2. While the Pavlovian system is responsible for the learning and updating of CS-US associations, it often operates in tandem with – and influences – the instrumental learning system. Aversive CS, when presented in the absence of aversive outcomes, can be salient enough to motivate instrumental avoidance behaviors. This effect, known as Pavlovian-to-instrumental transfer (PIT), has been examined in the appetitive domain. In humans, the appetitive PIT effect is represented in the striatum (Bray et al., 2008; Talmi, et al., 2008; Prévost et al., 2012) as well as the amygdala (Prévost et al., 2012). Experiment 2 (Lewis, Niznikiewicz, Delamater & Delgado, 2013) examined how the control of *aversive* CS over avoidance behavior is represented in the human brain, in order to better understand the interaction between Pavlovian and instrumental systems and to quantify how this process relates to appetitive Pavlovian-instrumental interactions. Participants learned two sets of contingencies (response-outcome and stimulus-outcome), and subsequently completed an avoidance-based PIT test while undergoing fMRI. We hypothesized that the presence of an aversive Pavlovian CS would motivate instrumental avoidance behaviors in a PIT task, and that this behavioral effect would be accompanied by enhanced activity in the striatum.

3. Pavlovian extinction failure can result in negative outcomes such as drug relapse and anxiety, which highlights the strength of the influence of Pavlovian cues on instrumental behavior. This is often the case with drug relapse, as exposure to drug-related CS in the environment can lead to the experience of aversive withdrawal symptoms; this subsequently triggers a drug-seeking response as a way to avoid the

withdrawal symptoms. Given that Pavlovian-instrumental interactions underlie the basic theory of negative reinforcement models of addiction (see Baker et al., 2004 for review), it is important to understand how these interactions would occur under real-world conditions. Experiment 3 (Lewis & Delgado, in prep) examined one such condition, specifically looking at effects of acute stress on avoidance-based PIT. As in Experiment 2, participants underwent Pavlovian and instrumental conditioning as well as a PIT test. Following Pavlovian and instrumental conditioning, but prior to the PIT test, half of our participants were exposed to a socially evaluated cold-pressor stressor (Schwabe et al., 2008) while the remainder underwent a control procedure. We hypothesized that for the control group, aversive Pavlovian CS would motivate instrumental avoidance behaviors during the PIT task. We also hypothesized that participants in the acute stress group would exhibit a dampened behavioral PIT effect as compared to the control group. Within the acute stress group, it was hypothesized that the strength of the PIT effect would negatively correlate with the increase in circulating salivary cortisol resulting from stress application.

Taken together, Experiments 1, 2 and 3 sought to add to a growing literature on both the basic behavioral and neural underpinnings of aversive Pavlovian and instrumental processes, and the interaction between these two systems. The results of these three studies will be described in Chapters 2-4. Chapter 5 will discuss these results in a broader context, particularly looking at clinical implications of the dissertation studies. Limitations and future directions of this work will also be discussed.

Chapter 2: Experiment 1

Neural activation and functional connectivity during extinction learning with appetitive and aversive conditioned stimuli

2.1 Introduction

On a daily basis, appetitive and aversive events are experienced in proximity to neutral stimuli; subsequently, these stimuli acquire affective properties and come to elicit conditioned responses. This process, known as Pavlovian conditioning, is known to depend on the integrity of various subcortical regions involved in affective learning, such as the striatum (e.g. Gottfried et al., 2002; O'Doherty et al., 2004) and the amygdala (e.g. Büchel et al., 1998; LaBar et al., 1998). More recently, researchers have begun to examine the neural circuitry underlying extinction learning, wherein repeated presentation of conditioned stimuli (CS) in the absence of appetitive or aversive outcomes leads to a diminished or eliminated conditioned response (e.g. Pearce & Hall, 1980). Extinction learning does not involve the complete erasure of the original association between the CS and affective outcome. Rather, it consists of the formation of a new association, wherein the affective properties of the CS are diminished, and is essential in cases where CS elicit excessive, maladaptive emotional responses (e.g., fear in PTSD).

In contrast to Pavlovian conditioning, successful extinction learning with both humans and many non-human animals involves the recruitment of higher cortical areas. One region that is known to be involved in extinction learning is the medial prefrontal cortex (mPFC); lesions to the ventral portion of this region lead to deficits in extinction (Morgan et al., 1993; Quirk et al., 2000; Sotres-Bayon et al., 2006). In particular, the

mPFC is crucial for the retention of extinction memory (Quirk et al., 2000). Given that the mPFC projects to both the striatum, which is involved in acquisition of appetitive CS-outcome associations, as well as the amygdala, which is crucial for fear learning, it has been postulated that the mPFC regulates extinction retention in both the appetitive and aversive domains in a similar manner (Peters et al., 2009). However, it is unclear whether there is significant overlap in the neural circuitry underlying extinction learning with appetitive and aversive CS in humans, as the neural bases of these two processes have not been directly compared in the same task.

Brain regions involved in Pavlovian conditioning and extinction do not function in isolation, but rather as components of large-scale neural networks. The human brain contains multiple distinct networks that allow for cognitive processes to be carried out (Smith et al., 2009). In particular, the executive control network (ECN) is thought to support externally directed cognitive processes, as opposed to interoceptive, task-irrelevant processes (Dosenbach et al., 2007). Both Pavlovian conditioning and extinction involve not only acquisition of the initial CS-outcome contingency, but also other cognitive processes such as memory retention and retrieval. Extinction, in particular, also entails successful inhibition of other brain regions involved in formation of the initial CS-outcome association, rendering an understanding of functional connections between the mPFC and large-scale neural networks important. Nonetheless, it remains unclear how brain regions involved in appetitive and aversive conditioning and extinction function in tandem with the ECN during these learning processes.

In this study, we posed two main questions. First, does extinction learning with appetitive and aversive reinforcers of varying magnitude depend on similar or distinct

neural circuitry? Second, does the strength of functional connectivity between brain regions involved in extinction learning and the ECN vary across learning processes? To investigate these questions, we performed an experiment in which participants underwent Pavlovian conditioning and extinction with both appetitive and aversive reinforcement while undergoing functional magnetic resonance imaging (fMRI). We hypothesized that during extinction, the mPFC would exhibit differences in activity in response to CS of varying magnitude, but that this region would be recruited similarly for both appetitive and aversive stimuli. We also hypothesized that the mPFC would show enhanced connectivity with the ECN during extinction as compared to Pavlovian conditioning (acquisition), given that extinction is an active learning process requiring suppression of a previously learned CS-outcome association.

2.2 Materials and Methods

2.2.1 Participants. Twenty-four right-handed volunteers were recruited via flyers posted on the Rutgers campus (14 female, 10 male). Final analysis includes 23 participants (13 female, 10 male, mean age = 21.50, SD = 2.72) as 1 participant was excluded due to failure to meet the learning criterion (see Procedure description). All participants gave informed written consent prior to the experiment. The study was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects in Research and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2.2 Experimental procedure. Participants played a simple computer game that tested both Pavlovian conditioning and extinction. The experiment used gain and loss of desirable items as appetitive and aversive outcomes, respectively. The use of instructed reinforcement in the context of the game ensured that appetitive and aversive outcomes were equated to one another as much as possible, given that participants have no prior experience with the game. At the onset of the experiment, participants were told that the items that could be gained in the game (arrows, bombs) would protect them from goblin attacks. However, participants were warned that just as these items could be gained, they could also be taken away. In order to successfully complete the game, participants were informed that they needed to "collect" as many items as possible. Items were "collected" as participants encountered them during the task. Participants underwent two blocks of Pavlovian conditioning (Figure 2.1A) and two blocks of extinction (Figure 2.1B).

1) Pavlovian conditioning: Participants underwent four experimental blocks, each consisting of 40 trials. On each trial, participants viewed a neutral fractal image (CS) for 4s followed by an outcome (US) for 1s. Each of the four fractals was paired with one specific outcome (e.g. gain of five items) throughout the two conditioning blocks, such that there were four CS-US pairings. The outcomes varied in both valence and magnitude, such that participants could gain five items (arrows or bombs), gain one item, lose five items or lose one item. Fifty percent of trials were reinforced. Unreinforced trials consisted of CS presentation followed by a 1s blank screen. Trials were presented in random order and were separated by a 7-9s jittered inter-trial interval (ITI). At the end of each block, participants were asked to make affective ratings, which asked how much they liked or disliked each CS. CS were shown on the screen one at a time along with a

scale from 1-5, ranging from “strongly dislike” to “strongly like.” Participants responded to the affective ratings before moving on to the next block of trials.

2) Extinction: Following Pavlovian conditioning, participants underwent two blocks of extinction. The extinction blocks mirrored the Pavlovian conditioning blocks with the following changes. In the first extinction block, four of the first eight trials were reinforced in order to eliminate obviousness of a change from conditioning to extinction. Following these eight trials, trials continued on in random order, consisting of the same format as the conditioning blocks. However, all subsequent trials in the extinction blocks were unreinforced. Participants performed affective ratings following each extinction block.

2.2.3 Learning criterion. We computed a measure of learning (L) for each participant using CS ratings from the end of block 2. Learning was measured by taking the average of ratings for both the high and low magnitude positively valenced stimuli and subtracting the average of the high and low magnitude negatively valenced stimuli:

L = avg [(posHi_Block2 + posLo_Block2)] – avg [(negHi_Block2 + negLo_Block2)]

If the value of L was greater than zero, participants were reasoned to have learned and were subsequently included in all analyses. Participants with a value of L less than or equal to zero were not included in data analysis.

2.2.4 Behavioral analysis: All behavioral analyses consisting of more than two *t*-tests within a family of comparisons were corrected for multiple comparisons using the sequential Bonferroni correction (Holm, 1979; Rice, 1989).

2.2.5 fMRI acquisition: Images were acquired using a 3T Siemens TRIO scanner at the Rutgers University Brain Imaging Center (RUBIC). Structural images were collected using a T1-weighted MPRAGE sequence (256 × 256 matrix; FOV = 256 mm; 176 1 mm sagittal slices). Functional images were acquired using a single-shot gradient echo EPI sequence (TR = 2000 ms, TE = 25 ms, FOV = 192 mm, flip angle = 90°, bandwidth = 2232 Hz/Px, echo spacing = 0.51) and comprised thirty-five contiguous oblique-axial slices (3 × 3 × 3 mm voxels) parallel to the anterior commissure-posterior commissure line.

2.2.6 fMRI preprocessing and analysis: general linear model. BrainVoyager QX software (version 2.3; Brain Innovation) was used to preprocess and analyze the imaging data. Preprocessing consisted of 3D motion correction (six parameters), slice scan time correction (trilinear/sinc interpolation), spatial smoothing with a 3D Gaussian filter (4 mm FWHM), voxelwise linear detrending, and high-pass filtering of frequencies (3 cycles per time course). Structural and functional data of each participant were transformed to standard Talairach stereotaxic space (Talairach & Tournoux, 1988).

A random effects general linear model (GLM) was conducted for each of four runs using each of the four CS (high gain, low gain, high loss, low loss) and each of the four US as regressors of interest. We also included 6 regressors of no interest (6 motion parameters). Regressors were convolved with a 2-gamma hemodynamic response function and z-transformed at the single participant level. Analyses were performed at a threshold of $p < 0.005$, corrected for multiple comparisons using the Cluster Level

Statistical Threshold Estimator plugin in BrainVoyager. All post-hoc analyses consisting of more than two *t*-tests within a family of comparisons were corrected for multiple comparisons using the sequential Bonferroni correction (Holm, 1979; Rice, 1989).

2.2.7 fMRI preprocessing: functional connectivity. Given the limited functionality of BrainVoyager QX software in running complex connectivity analyses, we performed these analyses in FSL (Smith et al., 2004). Data were first preprocessed using tools from SPM (Ashburner, 2012). First, the origin of each image was shifted to the AC-PC. Each time series was realigned to its first volume in order to correct for head motion, and spatial unwarping was applied. Non-brain tissue was removed using the brain extraction tool in FSL (Smith et al., 2004). Next, intravolume slice-timing correction was applied. Coregistration of the mean functional image to the anatomical scan was then applied, and unified segmentation normalization of the anatomical was computed (Ashburner & Friston, 2005). This was then used to reslice the functional data to standard stereotaxic space (Montreal Neurological Institute template, 3mm isotropic resolution). Finally, a 6 mm full-width-half-maximum Gaussian kernel was used for spatial smoothing of normalized functional images.

Additional controls for head motion were applied, given that head motion has the potential to significantly distort brain connectivity results (see Power et al., 2015 for review). First, motion spikes were identified using the FSL-based *fsl_motion_outliers* tool. Motion spikes were assessed using two metrics: 1) root-mean-square intensity difference of each individual volume relative to a reference volume (the first time point) and 2) frame-wise displacements, or the mean root-mean-square change in rotation and

translation parameters relative to a reference volume (the first time point). A boxplot threshold (75^{th} percentile plus 1.5 times the interquartile range) was applied to both metric values within each run. This threshold determined volumes that would be classified as spikes, which were then regressed out of the data set (Power et al., 2015; (Satterthwaite et al., 2013). A high-pass filter with a 100 second cutoff was then applied to remove low frequency drift in the MR signal.

2.2.8 fMRI analysis: functional connectivity. The functional connectivity analysis consisted of four major steps. First, an independent components analysis (ICA) was used to obtain networks in the dataset. Next, a network matching the ECN, as identified in prior work (Smith et al., 2009) was obtained. Third, voxelwise connectivity was quantified with ECN for acquisition and extinction phases. Finally, the connectivity maps were contrasted between each condition (acquisition, extinction) for the ECN. Brain regions were identified in which connectivity differences were associated with differences in learning phase (e.g. regions wherein connectivity with ECN was greater during acquisition than extinction, and vice versa). These procedures are described in further detail below, and are illustrated in previous research (Smith et al., 2015).

FSL's Multivariate Exploratory Linear Decomposition into Independent Components Version 3.10 (Beckmann & Smith, 2004) was used to identify 20 large-scale neural networks. A dual regression analytical approach (e.g. Filippini et al., 2009; Leech et al., 2011; Murty et al., 2014; Smith et al., 2015; Utevsky et al., 2014) was used to determine individual differences in connectivity with networks identified by the ICA. In the first step of dual regression, spatial maps are regressed on to individual participants'

functional data. This step results in a *time points* X *components* set of beta coefficients that characterize the temporal dynamics for the spatial maps of each participant and session. In the second step, these temporal dynamics are regressed onto functional data for each participant, producing a set of spatial maps. The spatial maps quantify each voxel's connectivity with each map identified in the group ICA, for each participant and session. This temporal regression step of the dual-regression procedure estimates each voxel's connectivity with each spatial network, but importantly also controls for the influence of other networks that may reflect various artifacts. Six additional motion parameters were regressed out this step, as were volumes identified as outliers.

A spatial correlation analysis was then performed to identify maps in the ICA that corresponded to the ECN, as reported by Smith et al. (2009). The component that best matched the ECN was selected from this correlation (ECN: r_{max} = 0.620; other components: r_{mean} = 0.013; SD = 0.133). Of note, the spatial correlation analysis is identical to a conventional correlational analysis, as it examines the correlation between two sets of spatial points.

For each participant, difference images across learning sessions (i.e. extinction minus acquisition, and vice versa) for the ECN were then computed. This examination of connectivity differences between sessions is considered a between-sessions psychophysiological interaction (PPI) analysis (Friston, 2011; O'Reilly et al., 2012). A group-level GLM was then run to determine whether differences in learning phase (e.g. extinction minus acquisition) correlated with changes in connectivity for ECN. The model also included covariates to control for individual differences in head motion between sessions.

Monte Carlo permutation-based statistical testing with 10,000 permutations (Nichols & Holmes, 2002) was used to assess statistical significance of connectivity maps in a nonparametric fashion. Analyses were performed at a threshold of $p < 0.05$, corrected for multiple voxelwise comparisons across the whole brain and across the ECN for multiple network comparisons (Smith et al., 2015; Utevsky et al., 2014). Clusters of activation were estimated using threshold-free cluster enhancement (Smith & Nichols, 2009). Finally, to evaluate uncertainty, the effect sizes were bootstrapped (N = 10,000) and the 99.9% confidence interval was identified. Identifying this confidence interval is helpful for determining the likely magnitude of the true effect (Kriegeskorte et al., 2010; Vul et al., 2009; Yarkoni, 2009).

2.3 Results

2.3.1 Behavioral results: Affective ratings from the end of each of the four phases were analyzed as a subjective, implicit measure of acquisition and extinction. Behavioral ratings are presented in Figure 2.2. A three-way ANOVA examining the factors of valence (positive, negative), magnitude (high, low) and block (early acquisition, late acquisition, early extinction, late extinction) revealed a main effect of valence ($F_{(1,22)} = 74.215$; $p < 0.001$) and a main effect of magnitude ($F_{(1,22)} = 5.413$; $p < 0.05$). A significant valence X magnitude interaction was also present ($F_{(1,22)} = 17.142$; $p < 0.001$), as was a valence X block interaction ($F_{(3,20)} = 11.604$; $p < 0.001$). Post-hoc t-tests examined the change from late acquisition to late extinction for all four CS (high gain, low gain, high loss, low loss). For all CS, there was a significant shift toward a

neutral rating from late acquisition to late extinction (all p's < 0.05). Therefore, implicit subjective ratings indicated that extinction had occurred for all CS.

2.3.2 Neuroimaging results - GLM. Our primary interest in running the GLM analysis was to examine whether CS valence or magnitude modulated any affective learning regions over the course of extinction. A 2 (valence) x 2 (magnitude) x 2 (block) whole-brain repeated measures ANOVA was conducted across the two extinction blocks. Regions exhibiting a main effect of valence are displayed in Table 2.1. Additionally, a valence x block interaction revealed regions that were modulated across the course of extinction as a function of CS valence (Figure 2.3A and Table 2.2). Of note, clusters emerged in left caudate (*x, y, z* = -16, 13, 6) and right inferior frontal gyrus (IFG; *x, y, z* = 23, 34, 6). Post-hoc *t*-tests confirmed that activation increased in both of these regions over the course of extinction for negatively valenced CS (left caudate: $t_{(22)} = 2.592$; $p < 0.05$; Figure 2.3B; right IFG: $t_{(22)} = 2.701$; $p < 0.05$; Figure 2.3C), but remained stable for positively valenced CS (all p's > 0.05).

We also observed a main effect of magnitude in several brain regions (Table 2.3). Regions identified by the ANOVA that exhibited a magnitude x block interaction are shown in Figure 2.4A-B and in Table 2.4. Importantly, clusters emerged in ventral caudate (*x, y, z* = -4, 4, -3) and medial prefrontal cortex (mPFC; *x, y, z* = 2, 34, 6). In both of these regions, post-hoc *t*-tests confirmed that activation increased from early to late extinction for high magnitude stimuli (ventral caudate: $t_{(22)} = 2.899$; $p < 0.05$; Figure 2.4C; mPFC: $t_{(22)} = 3.774$; $p < 0.005$; Figure 2.4D), but remained stable across the course of extinction for low magnitude stimuli (all p's > 0.05). Additional regions that displayed

a main effect of time and a valence x magnitude interaction are displayed in Table 2.5 and Table 2.6, respectively.

2.3.3 Neuroimaging results – connectivity analysis. This analysis began by using independent components analysis (ICA) to isolate and identify neural networks. Overall, we identified 20 networks, many of which matched cognitive and sensory networks identified in prior work (Smith et al., 2009). The subsequent dual regression analysis focused on a network in our data that strongly resembled the ECN (Figure 2.5A). In this analysis, we examined regions that showed greater connectivity with ECN during extinction as compared to acquisition (Figure 2.5B), and vice versa. Of note, we found that the mPFC (*x, y, z* = 0, 41, 14) exhibited enhanced functional connectivity with the ECN during extinction as compared to acquisition. This finding is in line with previous research suggesting that the mPFC plays an active role in extinction learning with both positive and negative CS, given its anatomical connections with affective learning regions such as the striatum (e.g. Peters et al., 2009). Increased functional connectivity with ECN during extinction as compared to acquisition was also apparent in ventral putamen (*x, y, z* = 18, 14, -10). Additional regions found in this contrast are reported in Table 2.7. Interestingly, no brain regions exhibited enhanced functional connectivity with the ECN during acquisition as compared to extinction.

2.4 Discussion

We investigated two key questions in this study, the first being whether differences in valence or magnitude of a stimulus affect the neural correlates underlying

extinction learning. Both valence and magnitude were found to modulate the amount of activity in affective learning brain regions. In particular, both the left caudate and the right IFG showed increased activity over the course of extinction for negatively valenced CS, and no change over time for positively valenced CS. The human striatum is a key brain region in many affective learning processes, including conditioning with both primary and secondary reinforcers in the appetitive (e.g. Gottfried et al., 2002; Kirsch et al., 2003; Knutson et al., 2001; O'Doherty et al., 2002; O'Doherty et al., 2004) and aversive domains (e.g. Becerra et al., 2001; Büchel et al., 1998; Delgado et al., 2011; Delgado et al., 2008b; Jensen et al., 2003; LaBar et al., 1998; Phelps et al., 2004; Seymour et al., 2007). During extinction learning, the ventromedial prefrontal cortex (vmPFC), through projections to the striatum and amygdala, is thought to inhibit the activity of these subcortical regions, promoting extinction (see Peters et al., 2009, for review). Thus, it is somewhat surprising to see activity in the left caudate increase over the course of extinction in the presence of negatively conditioned stimuli. One possibility is that the process of extinction is slower for aversive as compared to appetitive conditioned stimuli, in line with the principle of loss aversion (Kahneman & Tversky, 1979). Therefore the number of trials in this study may not have been sufficient to observe a potential subsequent decrease in striatal activity over the course of extinction. The right IFG also showed increased activity for negatively valenced stimuli from early to late extinction, mirroring activity in the striatum. While the right IFG is not traditionally associated with affective learning processes, it is known to play a role in inhibition of subcortical brain regions in a variety of tasks (see Aron et al., 2004, for review). One possibility here, then, is that an increase in right IFG activity over the

course of extinction is necessary to inhibit the initial aversive CS-US associations formed during acquisition.

With regard to magnitude, clusters in ventral striatum and mPFC exhibited increased activation from early to late extinction for high magnitude stimuli, and no change for low magnitude stimuli. The peak of the striatum cluster resulting from this analysis is more medial and posterior than the cluster exhibiting a main effect of valence, yet the peaks of both of these clusters are situated in the caudate. As was hypothesized with negatively valenced stimuli, it may be the case that high magnitude stimuli are slower to extinguish. Therefore, the number of trials needed to see a decrease in striatal activity in response to high magnitude stimuli may not have been reached over the course of extinction. The mPFC also showed greater engagement over the course of extinction in response to high magnitude stimuli, which may also reflect a slower time course for extinction (engagement of this region was enhanced for low magnitude as compared to high magnitude stimuli during the first block of extinction). However, the region seen here is more dorsal than the vmPFC typically associated with top-down regulation of subcortical areas during extinction learning (e.g. Gottfried & Dolan, 2004; Kalisch et al., 2006; Linnman et al., 2012; Phelps et al., 2004), and therefore may not reflect this regulation. Instead, this region may be more specifically encoding information about stimulus magnitude as the representation of the various stimuli are updated.

Given that vmPFC did not emerge in any of our analyses across extinction, it is possible that activity in this region does not significantly differ by stimulus valence or magnitude. However, one drawback of our study is that our GLM was not incredibly sensitive to the extinction time course. Extinction was broken up into two phases, “early”

and “late,” and behavioral ratings of stimulus liking were made at the end of each of these phases. An important future direction will be to consider subtle changes in brain regions involved in affective learning, particularly the vmPFC, over a more sensitive time course. This could be accomplished by examining changes in brain regions on a trial-by-trial basis, or by looking across smaller groups of trials. Collecting more frequent behavioral ratings (e.g. every 5 to 10 trials, for instance) would also help to better delineate the time course of extinction learning.

The second key question that we examined in the current study was whether or not brain regions involved in affective learning processes were more highly interconnected with the executive control network (ECN) during acquisition as compared to extinction, and vice versa. We used independent components analysis (ICA) to identify 20 neural networks, one of which strongly matched the ECN. Regions that showed significant differences in functional connectivity with ECN between acquisition and extinction were then obtained using dual regression analysis. Both the ventral putamen and the mPFC exhibited enhanced functional connectivity with the ECN during extinction learning as compared to acquisition. Given that activity in the ECN has been associated with a host of exteroceptive processes (Dosenbach et al., 2007; Smith et al., 2009), our results suggest that both the mPFC and ventral putamen are more engaged in executive functioning (i.e. cognitive control) during extinction learning than during acquisition. Extinction is thought to involve cognitive control processes such as inhibition, as suppression of the original CS-US association is necessary to promote the expression of the new, extinguished CS-US contingency (see Peters et al., 2009, for review). In contrast, evidence suggests that Pavlovian conditioning is governed by non-

executive stimulus control, sometimes occurring automatically and outside of conscious awareness (e.g. Esteves et al., 1994). Our data lend support to these notions, as affective learning brain regions exhibited greater functional connectivity with the ECN during extinction than acquisition. Complementing this result, we did not find any brain regions that showed the opposite pattern (i.e. greater functional connectivity with the ECN during acquisition as compared to extinction).

To build upon our combined ICA/dual regression approach, a full psychophysiological interaction (PPI) analysis would help to better characterize the functional connectivity patterns see in our data. Rather than examining the functional connectivity between a brain region and a neural network, PPI looks at the functional coupling between two brain regions. More specifically, this analysis seeks to determine whether the correlation in activity between two brain regions is modulated by the experimental context (Friston et al., 1997). Given that both mPFC and ventral striatum exhibit greater functional connectivity with the ECN during extinction as compared with acquisition, PPI would allow us to examine whether or not mPFC is more functionally coupled with ventral striatum during extinction as well. Thus, PPI is an important tool for future examinations of functional connectivity during affective learning processes.

One region that did not show up in any of our analyses was the amygdala, which is known to be crucial for Pavlovian learning with aversive primary reinforcers (e.g. shock, for review see Phelps and LeDoux, 2005). However, the reinforcers used in the current study were of an instructed nature, given that they gained their appetitive and aversive properties in the context of a game. Thus, these reinforcers are more akin to secondary reinforcers, such as money, which also are not inherently appetitive or

aversive. Research suggests that the amygdala is not involved in Pavlovian learning with aversive secondary reinforcers (Delgado et al., 2011). As was discussed earlier, the striatum, in contrast, is active during conditioning with both primary and secondary appetitive and aversive reinforcers. Given that modulations in striatal activity were present in all analyses in the current study, it is likely that this region is very important for affective learning with instructed reinforcers as well (supporting the results of Lewis et al., 2013). Including other categories of reinforcers (i.e. primary and secondary) in future versions of this task would be useful for determining which of the effects seen in the current study are specific to the type of reinforcement used and which are consistent across various types of appetitive and aversive outcomes.

Overall, this study adds to a growing body of both human and non-human animal studies that seek to better understand the neural basis of extinction learning. Our data help to delineate changes in cortical and subcortical activity over the course of extinction, as well as how these changes might vary as a function of stimulus valence or magnitude. Additionally, we utilized a fairly novel functional connectivity approach to show that several regions involved in affective learning are more functionally connected to the ECN during extinction as compared with acquisition. Using a combination of the traditional GLM and the more novel ICA/dual regression analysis gave a more complete picture of how affective learning brain regions are functioning not just in isolation, but in tandem with large-scale brain networks. We believe that functional connectivity approaches such as the one taken here will be very important for future neuroimaging studies, as they provide a more holistic approach to understanding the neural basis of behavior. In the current study, this combined approach helped to examine the idea that the mPFC is

common to extinction learning with both appetitive and aversive reinforcers (Peters et al., 2009). Examining other aspects of functional connectivity, such as how the mPFC directly influences both the striatum and amygdala during extinction learning, will be necessary to understand whether disorders of extinction failure in the appetitive and aversive domains (e.g. addiction and anxiety disorders, respectively) can be alleviated using similar treatment methods.

Chapter 3: Experiment 2

Avoidance-based human Pavlovian-to-instrumental transfer

3.1 Introduction

Environmental cues that gain appetitive or aversive properties through conditioning can subsequently influence goal-directed behaviors. This interaction between Pavlovian and instrumental learning systems, known as Pavlovian-to-instrumental transfer (PIT), is able to manifest as increased behavior in the presence of an appetitive conditioned cue (Balleine & Dickinson, 1998) or decreased behavior in the presence of an aversive conditioned cue (Estes & Skinner, 1941). Additionally, increases in behavior have also been examined in the presence of aversive conditioned stimuli, highlighting the ability of these stimuli to motivate avoidance learning (e.g. Rescorla & Solomon, 1967). Negative reinforcement-based models of addiction posit that avoidance of withdrawal symptoms is a major factor in drug relapse (e.g. Baker et al., 2004). Given the clinical significance of avoidance-based PIT, it is important to understand this process at both the behavioral and neural level.

The PIT paradigm has been used extensively to probe basic cognitive and motivational processes in studies of animal learning. Studies of appetitive PIT in rodents have shown that *specific PIT*, wherein instrumental responding increases selectively in the presence of a conditioned cue with which the instrumental response once shared an outcome, is dependent on the integrity of the nucleus accumbens shell (Corbit & Balleine, 2011) and the basolateral amygdala (Corbit & Balleine, 2005). Animal work also suggests that the nucleus acccumbens core (Corbit & Balleine, 2011) and the central nucleus of the amygdala (Corbit & Balleine, 2005) are necessary for *general PIT*, or

nonselective, enhanced responding in the presence of a conditioned cue. More recently, PIT and its underlying neural basis have been extended to investigations in humans. These initial neuroimaging studies of PIT have focused on the influence of appetitively conditioned stimuli on instrumental responses maintained by positive reinforcement and highlight the involvement of the striatum in specific PIT (Bray et al., 2008; Talmi et al., 2008; Prevost et al., 2012) and the amygdala in general PIT (Prevost et al., 2012). It has been suggested that in the aversive domain, aversive conditioned stimuli motivate both specific and general avoidance-based PIT (Nadler et al., 2011). However, the underlying neural representation of this phenomenon has yet to be examined in humans.

Experiment 2 sought to investigate the neural correlates of both specific and general PIT using an avoidance learning task with aversive conditioned stimuli. Specifically, we adapted a behavioral PIT paradigm (Corbit and Balleine, 2005) to examine how aversive Pavlovian stimuli motivate instrumental avoidance behavior. We expected to observe specific and general PIT effects using negative reinforcement. We further hypothesized that the striatum would be engaged during specific PIT maintained by negative reinforcement, in accordance with previous studies of PIT with positive reinforcement. Additionally, we predicted that striatal activation would correlate with general PIT, highlighting the general motivational properties of this region in an avoidance learning context.

3.2 Materials and Methods

3.2.1 Participants. Twenty-four right-handed volunteers were recruited via flyers posted on the Rutgers campus (12 female, 12 male). Final analysis included 20

participants (12 female, 8 male, mean age = 20.84, SD = 2.99) as 3 participants were excluded due to failure to meet instrumental learning criteria (see Procedure description) and 1 participant was excluded due to excessive head motion during the scanning session. All participants gave informed written consent prior to the experiment. The study was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects in Research and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

3.2.2 PIT task procedure. The current study examined both specific and general PIT using a computer game paradigm modified from that used by Nadler et al., (2011). At the start of the experiment, participants were told that they would be playing a simple computer game wherein their goal was to defend a fictional kingdom against attacks by various creatures. Participants proceeded to perform 3 phases of the PIT task: 1) instrumental phase, 2) Pavlovian phase, and 3) transfer test phase (outlined in Table 3.1).

1) Instrumental Phase: Instrumental training was modeled after a Sidman avoidance task (Sidman, 1953a,b), used extensively to study negative reinforcement processes in rodents (e.g. Mackintosh, 1974), but more rarely used with humans. In the instrumental phase, associations between two distinct instrumental responses (R1 and R2) and the avoidance of two distinct aversive outcomes (O1 and O2) were acquired. Prior to the start of the instrumental phase, participants were instructed that they would be attacked by two different creatures (e.g., goblin, troll, or ogre, counterbalanced across participants) and that they could utilize two available button presses, each of which

yielded a different type of imaginary shield. Participants were told that each shield may or may not be effective at defending against a particular type of attack, and that they had to learn which button press would engage an imaginary shield that would protect them from a specific attack (e.g., button 1 yielded an imaginary shield that was effective at protecting against goblin attacks). Participants underwent two sessions of instrumental conditioning during which they were to learn the avoidance contingency in effect. In one of these sessions the R1-O1 avoidance contingency was in effect, and during the second session the R2-O2 avoidance contingency was in effect. During a single session, only one outcome was presented (either O1 or O2). Each session lasted for 180 s and during this time an aversive outcome was scheduled to occur 1 s after the termination of the previous outcome, unless the participant made the appropriate button press response within this time period. If the correct button was pressed, this delayed the occurrence of the aversive outcome by an additional 3 s. Therefore, this schedule should favor participants learning that one R could lead them to avoid getting attacked by a particular O. To discourage participants from randomly responding at all times, any button presses that occurred while the aversive outcome was on the screen were without any consequences.

When an aversive outcome (O1 or O2) was scheduled to occur it was shown on the center of the screen for 1 s. A fixation cross was presented on the screen at other times (see Figure 3.1A). Participants were allowed to perform instrumental responses R1 and R2 at will in order to prevent the aversive outcomes (O1 and O2) in each training phase, but a different one of these responses was operational during each phase. Thus, R1 prevented O1 in the first session and R2 prevented O2 during the second. In this schedule, participants could prevent the aversive outcome from occurring by continually

performing the correct response during the fixation period. At the end of the second instrumental session, participants were asked to rate the efficacy of each R-O contingency on a scale from 1 to 10. For each outcome, the rating for the incorrect response was subtracted from the rating for the correct response. Participants were excluded from further analysis if this calculation resulted in a value less than or equal to zero for either outcome, because this would indicate that the participant had not learned both of the instrumental contingencies. Based on this criterion, three participants were excluded from the remainder of the study, given that it would have been impossible to obtain an explicit PIT effect without learning of the initial R-O contingencies. We did not collect imaging data during this phase of the study.

2) Pavlovian Phase: During the Pavlovian phase, participants were asked to learn five stimulus-outcome (S-O) contingencies. In the spirit of the game, participants were told that a wizard would teach them about various colored signals, representative of different types of attacks, and that it was necessary to pay attention in order to learn what each colored signal represented. On every trial, one of five stimulus-outcome pairings was presented, such that each visual stimulus (S1-S5) was paired with either one of the previously viewed aversive outcomes (O1 and O2; e.g., goblin attack, troll attack), a novel aversive outcome (O3; e.g., ogre attack), or one of two different neutral outcomes (O4 and O5; i.e., a screen that read "malfunction" or the presentation of a fixation dot). Therefore, O4 was representative of a neutral outcome while O5 represented no outcome. Stimuli appeared on the screen for 4 s and outcomes were subsequently presented at stimulus offset for 1 s (Figure 3.1B). A jittered ITI with a duration of either 7 s, 9 s, or 11 s separated the trials. Stimulus-outcome pairs were shown 9 times each, in random order,

for a total of 45 trials. Participants were instructed to refrain from instrumental responding during the Pavlovian phase. At the end of this phase, participants viewed S1-S5 one at a time while the text "What did this signal represent?" appeared on the screen along with a list of the five potential options. In order to check for explicit knowledge of each S-O contingency, participants were asked to respond verbally with the correctly paired outcome. This was meant to be used as an exclusionary criterion, given that the inability to learn S-O contingencies would have prevented an explicit PIT effect from being obtained in the transfer phase. However, all participants correctly reported all S-O contingencies; therefore, no participants were excluded based on the Pavlovian learning criterion.

3) Transfer Phase: Participants were instructed that the wizard would now send out the colored signals about which they had just learned, and that they would be free to utilize the available button presses (i.e. shields) as they saw fit during this phase. The transfer phase included presentation of the five previously seen visual Pavlovian stimuli (S1-S5) in the absence of reinforcement. That is, the entire transfer phase was performed under extinction conditions. During this phase, participants were free to respond using R1 and R2, or to not respond at all, in response to the presentation of S1-S5. Each trial began with a 2 s - 12 s jittered fixation period. A stimulus (S1-S5) was then presented on the screen for 4 s, followed by a jittered 2 s – 12 s screen that said "Recharging Magical Shield" during which participants were explicitly told not to make instrumental responses (Figure 3.1C). However, participants were free to make responses during either the pre-stimulus fixation period or during stimulus presentation. Both the pre-stimulus fixation period and the post-stimulus "recharge" period were included in order to have a) a

baseline measure that allowed for instrumental responding and b) a baseline measure wherein no responding occurred with which to compare the behavioral and BOLD responses from the stimulus presentation period of each trial. Stimuli S1-S5 were shown 12 times each in random order for a total of 60 trials.

3.2.3 Behavioral analysis. As in our previous study (Nadler et al., 2011), we measured specific and general forms of PIT by comparing the number of instrumental responses (R1 and R2) made a) across stimulus types (S1-S5) and b) during presentation of stimuli S1-S5, as compared to the pre-stimulus fixation and post-stimulus "recharge" period. All behavioral analyses consisting of more than two *t*-tests within a family of comparisons were corrected for multiple comparisons with the sequential Bonferroni correction (Holm, 1979; Rice, 1989).

3.2.4 fMRI acquisition and analysis. Images were acquired using a 3T Siemens TRIO scanner at the Rutgers University Brain Imaging Center (RUBIC). Structural images were collected using a T1-weighted MPRAGE sequence (256 × 256 matrix; FOV = 256 mm; 176 1 mm sagittal slices). Functional images were acquired using a single-shot gradient echo EPI sequence (TR = 2000 ms, TE = 30 ms, FOV = 192, flip angle = 90°, bandwidth = 2232 Hz/Px, echo spacing = 0.51) and comprised thirty-two contiguous oblique-axial slices (3 × 3 × 3 mm voxels) parallel to the anterior commissure-posterior commissure line. Functional images were collected during both the Pavlovian and transfer phases of the task. BrainVoyager QX software (version 2.3; Brain Innovation) was used to preprocess and analyze the imaging data. Preprocessing consisted of 3D

motion correction (six parameters), slice scan time correction (trilinear/sinc interpolation), spatial smoothing with a 3D Gaussian filter (4 mm FWHM), voxelwise linear detrending, and high-pass filtering of frequencies (3 cycles per time course). One participant was excluded from analysis due to excessive motion during functional runs. Structural and functional data of each participant were then transformed to standard Talairach stereotaxic space (Talairach and Tournoux, 1988).

In modeling the transfer phase, a random effects GLM was conducted using each of the five stimulus types (S1-S5) as regressors of interest. We also included 6 regressors of no interest (6 motion parameters). Regressors were convolved with a 2-gamma hemodynamic response function and z-transformed at the single participant level. Transfer phase analyses were performed at a threshold of $p < .001$, FDR corrected. All post-hoc analyses consisting of more than two *t*-tests within a family of comparisons were corrected for multiple comparisons with the sequential Bonferroni correction (Holm, 1979; Rice, 1989).

3.3. Results

3.3.1 Behavioral results: instrumental conditioning. To measure instrumental learning, we assessed the number of times that participants experienced aversive outcomes during the instrumental phase – a measure commonly employed to determine successful Sidman avoidance learning (e.g., Klein & Rilling, 1972; Sidman, 1962; Ulrich et al., 1964). To obtain an estimate of learning over time, we broke up each 180 s block into six 30 s bins. We observed a significant decrease in the number of experienced aversive outcomes from the first 30 s to the last 30 s of each 180 s block (one-tailed

paired *t* test, $t_{(18)} = 9.179$; $p < 0.001$), indicating that the correct R-O contingencies were learned over time. Importantly, this decrease happened irrespective of outcome (O1: $t_{(18)} = 8.286$; $p < 0.001$; O2: $t_{(18)} = 6.229$; $p < 0.001$), suggesting that both R-O contingencies were acquired successfully. To confirm, participants were asked at the end of the instrumental phase to verbally report, on a scale of 1 to 10, how effective each response (R1 and R2) was at preventing each outcome (O1 and O2). For those participants who met the instrumental learning criterion, verbal ratings were as follows: R1-O1 (correct contingency), mean = 9.684, SD = 0.749; R2-O1 (incorrect contingency), mean = 1.842, SD = 1.344; R2-O2 (correct contingency), mean = 9.684, SD = 0.820; R1-O2 (incorrect contingency), mean = 1.947, SD = 1.682.

3.3.2 Behavioral results: Pavlovian conditioning. Following the Pavlovian phase, all participants were asked to explicitly verbalize the outcomes associated with S1-S5 by answering the question "What did this signal represent?" All participants had correctly learned all five S-O contingencies by the end of this phase, as indicated by success in explicitly matching each stimulus to its associated outcome.

3.3.3 Behavioral results: Pavlovian-to-instrumental transfer. To measure specific and general PIT, we compared instrumental responding (R1 and R2) made a) across all 5 stimulus types and b) during stimulus presentations, as compared to the pre-stimulus fixation period. A three-way repeated-measures ANOVA examining the effects of stimulus (S1-S5), interval (pre-stimulus and stimulus) and response (R1 and R2) revealed a significant main effect of stimulus ($F_{(4,76)} = 22.627$; $p < 0.001$) and a

significant main effect of interval ($F_{(1,19)}$ = 34.898; p < 0.001). Post hoc *t*-tests revealed that the amount of instrumental responding was elevated during presentation of S1-S3 as compared to the neutral stimulus, S5 (all p's < 0.001). No significant differences in amount of instrumental responding were present amongst S1-S3 (e.g. S1 vs S2; all p's > .05). There were also no significant differences between instrumental responding during presentation of S4 and S5 (p = 0.218). Regarding the main effect of interval, a significantly greater number of instrumental responses were made during the stimulus period as compared to the pre-stimulus period. A stimulus X response interaction was also observed ($F_{(4,76)}$ = 26.447; p < 0.001), as was a stimulus X interval interaction ($F_{(4,76)}$ = 22.982; p < 0.001). Finally, a 3-way stimulus X interval X response interaction was observed ($F_{(4,76)}$ = 25.480; p < 0.001). This 3-way interaction was further analyzed via one-way ANOVAs across the four levels of responding (pre-stimulus, stimulus, R1 and R2) for each stimulus. Significant main effects were obtained for S1-S3 (all p's < 0.01), but not for S4-S5 (all p's > 0.05). Post-hoc *t*-tests supported the finding that S1 selectively elevated R1 (p < 0.001) but not R2 responding (p > 0.05) relative to the prestimulus period, and that S2 selectively elevated R2 (p < 0.001) but not R1 (p > 0.05) responding relative to the prestimulus period. Post-hoc *t*-tests for S3 revealed that R1 and R2 responding did not significantly differ (p = 0.401) but were both significantly greater than responding during the prestimulus period (all p's < 0.001). Therefore, a specific PIT effect was found, wherein a selective increase in R1 and R2 occurred during presentation of S1 and S2, respectively, that is, when both the stimulus and response shared a learned Pavlovian outcome. Additionally, a general transfer effect was observed such that the stimulus (S3) associated with the novel aversive outcome (O3) elicited a nonselective

increase in both available responses (R1 and R2) as compared to the pre-stimulus baseline. No increases in instrumental responding from pre-stimulus period to stimulus presentation occurred for S4 or S5.

We divided the PIT test into 5 bins of 12 trials each in order to examine potential changes in number of responses made across time, as has been done in previous studies (Bray et al., 2008; Prevost et al., 2012). Importantly, the PIT test was performed in extinction and without any reinforcement, suggesting that any instrumental responding in this phase is an actual behavioral expression of PIT. We performed a two-way repeated-measures ANOVA, examining the effects of both stimulus type (S1-S5) and bin (1-5) on total number of instrumental responses made during stimulus presentation. This ANOVA revealed a main effect of stimulus ($F_{(4,28)} = 9.355$; $p < 0.001$), as expected, and no main effect of bin ($F_{(4,28)} = 0.487$; $p = 0.745$) or stimulus X bin interaction ($F_{(16,112)} = 1.006$; $p = 0.456$). Importantly, although the PIT test was performed under extinction conditions, participants continued to respond using R1 and R2 in response to the Pavlovian stimuli throughout the duration of the PIT test.

3.3.4 Neuroimaging results: Pavlovian-to-instrumental transfer. To identify brain regions involved in specific and general PIT, we performed a one-way ANOVA comparing activation during presentation of all five stimuli (S1-S5, Fig. 3A). We then examined the overall F-test, FDR corrected to a threshold of $q < 0.001$. All significant clusters are reported in Table 3.2. Of particular interest was bilateral activation in the putamen (left, *x, y, z* = -22, 4, 6; right, *x, y, z* = 17, 7, 3), cingulate cortex (*x, y, z* = 2, 13, 42) and bilateral insula (left, *x, y, z* = -43, -2, 6; right, *x, y, z* = 35, 1, 6). We focused on

these regions given their role in human conditioning, avoidance learning and PIT (e.g. Bray et al., 2008; Delgado et al., 2008b, 2011; Kim et al., 2006; Prevost et al., 2012; Talmi et al., 2008). To understand directionality, parameter estimates were extracted from these regions and post-hoc two-tailed *t*-tests were run. Bilateral putamen (Fig. 3B), cingulate cortex (Fig. 3C), and bilateral insula (Fig. 3D) all exhibited increased activation during presentation of specific transfer stimuli (S1 and S2) and the general transfer stimulus (S3) compared to the neutral stimulus, S5 (all *p*'s < 0.05). The cingulate showed increased activation in response to specific transfer stimulus S2 compared to the general transfer stimulus ($p < 0.05$), but there was no difference between S1 and the general transfer stimulus ($p > .05$). Within all of these regions, there were no differences in activation during presentation of S4 and S5 (all *p*'s > 0.131).

3.3.5 Relationship between Pavlovian striatal activation and behavioral PIT. We were interested in the relationship between striatal activation during the Pavlovian learning phase and subsequent motivated responding in the transfer phase. Specifically, we were interested in how the striatal response to S1-S3 while learning S-O contingencies would later impact R1 and R2 instrumental responses while viewing S1-S3 in extinction. We hypothesized that greater Pavlovian phase activation in striatum while viewing S1-S3, representing increased motivation during learning, would subsequently lead to increases in motivation during the transfer phase, as evidenced by more vigorous instrumental responding. Thus, the peaks of activation in both left (*x, y, z* = -22, 4, 6) and right putamen (*x, y, z* = 17, 7, 3) from the transfer phase ANOVA were used to create regions of interest (ROI) in the Pavlovian phase. Parameter estimates from the Pavlovian

phase using these ROIs were extracted and correlated with subsequent behavior in the transfer phase. Left Pavlovian putamen activation during presentation of S1 and S2 positively correlated with number of responses made during specific PIT (S1: $r = 0.523$, $p = 0.018$; S2: $r = 0.495$, $p = 0.027$). A trend for a positive correlation between left putamen activation during presentation of S3 and number of subsequent general PIT responses was observed (left, $r = 0.390$, $p = 0.089$). In right putamen, activation in response to S1, S2 and S3 was positively correlated with instrumental responding, but was only significant for S2 ($r = 0.620$, $p = 0.004$) and not for S1 ($r = 0.225$, $p = 0.340$) or S3 ($r = 0.051$, $p = 0.831$). For S4 and S5, no correlations between Pavlovian phase putamen activation and subsequent responding during the transfer phase were found (all p's > 0.216). Thus, greater putamen activation toward aversive stimuli in the Pavlovian phase was associated with greater instrumental responding toward those same stimuli in the transfer phase, but this was only significant for specific transfer stimuli (S1 and S2).

3.4 Discussion

In the current study, our aim was to understand the behavioral and neural manifestation of avoidance-based PIT in humans. Behaviorally, the ability of stimuli associated with aversive outcomes to motivate instrumental responses paralleled a prior version of this task (Nadler et al. 2011), and extended it by using a purely avoidance procedure (as opposed to the quasi-avoidance procedure previously used). A specific PIT effect was found, wherein an instrumental response that previously signaled the omission of a specific aversive outcome was selectively increased in the presence of a conditioned stimulus that signaled that same aversive outcome. A general PIT effect was also

observed, as responding for both R1 and R2 increased above baseline in the presence of a conditioned stimulus that signaled a novel aversive outcome to which participants had never learned they could avoid. Investigating avoidance-based PIT in the human brain, we observed increased activation in corticostriatal circuits including the striatum (bilateral putamen) and the cingulate cortex during specific and general forms of PIT. Furthermore, activity in the putamen ROI during Pavlovian conditioning correlated with the vigor of instrumental responding during specific PIT. Our findings support previous research suggesting that corticostriatal regions are involved in PIT in humans (e.g., Bray et al., 2008; Prevost et al., 2012; Talmi et al., 2008), and further suggest this involvement occurs when the context is aversive. That corticostriatal activation was present during motivated responding to avoid negative outcomes fits with the claim made by Dickinson and Dearing (1979) that there should be a convergence between neural circuits for "rewarding" outcomes across motivational classes.

As was pointed out by Rescorla and Solomon (1967), aversive conditioned stimuli can influence instrumental responding by either facilitating or suppressing behavior through their activation of a "central motivational state" that interacts with the motivation to respond. For instance, it has been long known in research with rats that stimuli signaling electric foot shock will suppress food-reinforced lever pressing (e.g., Estes & Skinner, 1941) but increase lever pressing maintained on a shock avoidance schedule (e.g., Rescorla & LoLordo, 1965). The first effect, conditioned suppression, is generally understood to reflect a motivational conflict between food seeking and the anticipation of danger (Rescorla & Solomon, 1967). However, the second effect, conditioned facilitation, is thought to reflect a motivational synergy between the

anticipation of danger and the knowledge of how to avoid that danger (e.g., Seligman & Johnston, 1973). It is by no means obvious that the neural substrates mediating these two effects should partially overlap. While this analysis is only in its infancy, both with humans and with non-human animals, the present data implicate corticostriatal regions in the facilitative effect of such stimuli on negatively reinforced avoidance responding. This is complimentary with recent work suggesting striatal involvement in the association between aversive stimuli and the inhibition of behavioral responses during PIT (Guerts et al., in press).

One goal of our avoidance-based PIT procedure for humans was to follow closely the methodologies of PIT studies conducted with non-human animals (e.g., Corbit and Balleine, 2005). Corbit and Balleine (2005) found that rodents selectively increased responding toward conditioned stimuli when both the specific instrumental response and the stimulus shared an outcome, an effect we replicate and extend with humans. Both humans and rodents also show a non-selective increase in behavior (general PIT) in the presence of a conditioned stimulus that was never seen during instrumental conditioning, and, therefore, did not share an outcome with any available instrumental responses. However, unlike Corbit and Balleine (2005) and other previous animal studies of PIT, the current study examined PIT with negative reinforcement, specifically, in an avoidance learning context. Rescorla and Solomon (1967) noted that while Pavlovian modulation by conditioned stimuli upon instrumental responding occurs when instrumental responding is maintained by positive reinforcement, it can also occur when responding is maintained by negative reinforcement. To our knowledge, this study is the first to examine PIT with negative reinforcement in the human brain. The current study suggests that negatively

reinforced conditioned stimuli are successful at motivating behavior that is aimed at preventing specific negative outcomes as well as increasing a more general avoidance behavior. Given that negative reinforcement yields a powerful influence on behavior (perhaps greater than positive reinforcement in some contexts, e.g. Niznikiewicz and Delgado, 2011), and given that past research suggests differences in the ability of appetitive and aversive Pavlovian stimuli to modulate active instrumental behaviors (Huys et al., 2011), the role of negative reinforcement in the maintenance of behavior, particularly under extinction conditions, is a topic of great interest for future research. In particular, direct comparisons of both the behavioral and neural manifestation of PIT when behavior is motivated by positive or negative reinforcement should be considered.

An important point about the Rescorla and Solomon (1967) approach is that it does not adequately anticipate the distinction between specific and general PIT effects. That is, it does not distinguish between the effects of different stimuli that both signal qualitatively distinct outcomes from the same motivational class. In order to explain specific PIT, then, another mechanism must be assumed, and the typical one is that such stimuli activate a specific representation of the outcome with which it was paired (e.g., Kruse et al., 1983). Our behavioral data support this distinction between general motivational and specific expectancy influences of Pavlovian stimuli upon instrumental avoidance responding. Here, we present evidence that such a distinction also applies to PIT in avoidance learning contexts.

Similar specific and general PIT effects upon instrumental behaviors in positive and negative reinforcement (avoidance) contexts do not necessarily entail similar underlying neural mechanisms. Consider how specific PIT is generally assumed to work

in a positive reinforcement setting. Separate response – outcome (R-O) and stimulus – outcome (S-O) associations are assumed to be learned in the instrumental and Pavlovian learning phases, respectively. During the PIT test, the S is assumed to activate a representation of the specific O with which it was paired, and this in turn is assumed to directly activate the particular R that was also associated with that O through a backward action on the R-O link (e.g., Mackintosh & Dickinson, 1979; Pavlov, 1932). In an avoidance learning situation, on the other hand, the instrumental response signals the absence of the aversive outcome, generating an R – No O association (Seligman & Johnston, 1973). The present data are interesting in suggesting that the neural substrates recruited in specific PIT in an avoidance learning context may be similar to that seen in appetitive positive reinforcement learning contexts (Bray et al., 2008; Prevost et al., 2012; Talmi et al., 2008). How are we to reconcile these differences in underlying learning with similar results in the two domains? If the avoidance response itself is supported by an anticipation that a specific aversive outcome will occur unless a response is made, then this could result in the formation of a direct O-R associative link during the instrumental learning phase (perhaps in addition to an R-No O link). Specific PIT can be mediated by these S-O and O-R links in avoidance learning. The main difference may be that in avoidance learning the O-R link is established directly, but in positive reinforcement the R-O link is used in the backward direction (Pavlov, 1932). Nevertheless, the present data point more to similarities than differences in the way in which specific PIT effects occur in appetitive and aversive domains, but additional work will be needed to more clearly identify underlying neural circuits.

Our imaging data do not fully capture any presumed underlying neural differences between general (central motivational state mediated) and specific (expectancy mediated) forms of PIT, for example, which have been previously reported (Prevost et al., 2012). In our study, the presence of a strong correlation between putamen activity during Pavlovian training and specific, but not general, PIT may suggest that this structure is chiefly involved in coding specific expectancy effects, rather than more general motivational effects of stimuli upon behavior. Given the known involvement of the striatum in the acquisition of aversive S-O contingencies with both primary and secondary reinforcers (e.g. Delgado et al., 2011), perhaps it is not surprising that greater striatal engagement during the acquisition of the S1 and S2 contingencies correlated with increased behavioral responding during PIT. However, it would be interesting for future research to examine in greater detail the properties of the specific and general transfer stimuli that lead to differences in the importance of striatal engagement during Pavlovian conditioning for the maintenance of a vigorous behavioral PIT response. Our results point more strongly to a role in specific PIT, though, and this is consistent with prior animal work demonstrating that specific, but not general, PIT effects were abolished by inactivation at the time of Pavlovian training of the dorsomedial or dorsolateral striatum (Corbit and Janak, 2010). These authors suggested that the dorsomedial striatum is more involved in acquisition of specific R-O associations while the dorsolateral striatum is more involved in acquisition of specific S-O associations.

Additionally, the relative contributions of specific PIT and general PIT effects will very likely differ in different settings. In one previous attempt to demonstrate PIT in rats using alcohol rewards, general, but not specific PIT, was attained (Glasner, Overmier

& Balleine, 2005). The authors concluded that the more cognitive specific PIT, which involved encoding individual stimulus-outcome and response-outcome relationships, was less influential than the nonspecific motivational arousal generated by the appetitive conditioned stimuli. It may be that if aversive stimuli are more salient than appetitive stimuli in certain contexts, these general PIT effects will dominate to an even greater extent over specific PIT effects.

Another noteworthy difference between the current study and previous investigations of PIT in humans (Bray et al., 2008; Talmi et al., 2008; Prevost et al., 2012) is that we obtained successful specific and general PIT using instructed reinforcers. Unlike more typically used primary reinforcers - such as food or shock - that are inherently appetitive or aversive, or secondary monetary reinforcers, the reinforcers used in the current study acquired their value through instruction at the onset of the task. In utilizing aversive outcomes with which participants have no real-world experience, we hoped to minimize individual variability in perception of the outcomes. While the reinforcers used in the current study were not biologically relevant, our task still mirrored Pavlovian learning with biologically relevant outcomes in that it assessed control by associative relationships among multiple stimuli. We were able to observe whether the specific sensory properties or the more general features of these reinforcers predict the manner in which such stimuli affect instrumental performance. Given that we were able to obtain both specific and general behavioral PIT effects, our data speak to the strength of this type of reinforcement in associative learning studies. It is noteworthy that a recent interpretation of specific and general PIT effects (Cohen-Hatton et al., 2013) suggests that if Pavlovian training follows instrumental training, the presentation of an O during

the Pavlovian phase can activate the associated R, and, if this R occurs contiguously with S, an S-R link can be acquired. Therefore, specific PIT may be a reflection of these learned S-R associations. We believe that this sort of mechanism is unlikely to apply to the present situation, as each O was not embedded within the corresponding S during our Pavlovian training phase. Given that the outcomes only occurred after the Pavlovian signals were turned off in the present study, this would mean that the S was more contiguous with the O than the presumed O-activated R motor program that would follow the O.

Interestingly, the use of instructed, non-primary reinforcers may explain why we did not see correlations between general PIT and amygdala activation, as has been found previously in studies with both humans (Prevost et al., 2012) and non-human animals (Corbit and Balleine, 2005). The human amygdala has been implicated in the acquisition of a conditioned response to aversive primary reinforcers (for review see Phelps and LeDoux, 2005), but its involvement in the acquisition of a conditioned response to aversive secondary reinforcers in humans is less clear (e.g. monetary loss, Delgado et al., 2011). Therefore, it may be possible that the lack of amygdala activation seen during conditioning with aversive secondary reinforcers extends to PIT. An important question for future studies, therefore, will be to directly compare the PIT phenomenon with primary, secondary and instructed reinforcers in order to delineate potential differences in the maintenance of behavior brought about by these distinct types of reinforcement.

While our study differs from previous studies of PIT in its use of both instructed reinforcement and an avoidance learning context, it is nonetheless an examination of the

same basic phenomenon. Thus, our results in some part, overlap with those obtained in past examinations of PIT. Human (Bray et al., 2008; Prevost et al., 2012; Talmi et al., 2008) and animal studies (Corbit and Balleine, 2011; Corbit and Janak, 2007) have found a correlation between PIT and the striatum. Like the current study, previous human studies of PIT (Bray et al., 2008; Prevost et al., 2012) also found activation in the putamen, a lateral region of striatum, during specific PIT. In contrast, research by Talmi et al. (2008) has implicated the more medial region of nucleus accumbens in PIT. Like the current study, Bray et al. (2008) and Prevost et al. (2012) separately examined specific and general PIT (though a general PIT effect was not found by Bray et al.). In contrast, the procedure used by Talmi et al. (2008) did not delineate between specific and general PIT, which may explain why their striatum ROI was in a more medial location than that found in the current study. Of note, we also found activation in cingulate cortex, a region with projections to striatum (see Haber and Knutson, 2010 for review), during both specific and general instances of PIT. While this region has not been found in previous human studies of PIT, it has been implicated, along with the insula, in studies of aversive conditioning (e.g. Büchel et al., 1998; Delgado et al., 2008b; Delgado et al., 2011; Jensen et al., 2003).

Understanding the basic behavioral and neural mechanisms underlying PIT in humans with both positive and negative reinforcement will allow for PIT to be used as a model for a variety of non-normative behavioral responses toward real-world stimuli. The ability of positively reinforced Pavlovian conditioned stimuli to motivate behavior can be applied to real-world maladaptive behavior, such as instances of drug addiction wherein drug-related stimuli in the environment trigger drug-seeking behavior (e.g. Cardinal and

Everitt, 2004). Behavioral research has already been successful in obtaining a specific PIT effect in nicotine-dependent individuals using smoking-related stimuli (Hogarth et al., 2007). Evidence suggests that negative affect leads to drug craving and increases the likelihood of relapse (see Sinha et al., 2007 for review). Moreover, it has been found that addictive drugs are effective at reducing many negative symptoms of withdrawal (see Baker et al., 2004 for review); thus it may be the case that attempts to avoid withdrawal symptoms can lead to relapse as well. Therefore the current study, which sheds light on PIT in an avoidance learning context, might be used in the future as a model for drug relapse, wherein drug-related stimuli seem to motivate drug seeking through negative reinforcement (see Baker et al., 2004 for review) and/or incentive sensitization mechanisms (e.g., Everitt and Robbins, 2005; Robinson and Berridge, 2001; Stewart and Wise, 1992). Avoidance-based PIT can also be a useful model for gaining an understanding of other disorders involving avoidance of aversive stimuli, such as phobias and post-traumatic stress disorder.

Chapter 4: Experiment 3

Effects of Acute Stress on Avoidance-Based Pavlovian-to-Instrumental Transfer

4.1 Introduction

Aversive conditioned stimuli have the ability to influence active avoidance behavior, as demonstrated in Chapter 3. Outside of the laboratory, there are many additional variables that can motivate or deter behavior. One such factor is stress, which is known to have varying effects on learning and decision-making processes (see Shors, 2004 for review). For instance, stress has been found to alter reinforcement learning (e.g. Cavanagh et al., 2010) and reduce the use of feedback during learning (Petzold et al., 2010).

Both Pavlovian and instrumental learning processes are modulated when individuals are under stress. In rodents, stress affects the acquisition of conditioned responses during aversive learning, yet the exact direction of the effect varies with gender and stressor type (e.g. Shors, 2001; Shors et al., 1992; Wilson et al., 1975; Wood et al., 2001; Wood & Shors, 1998). In humans, studies examining the effects of stress on Pavlovian learning processes have also produced mixed results (e.g. Merz, Stark, et al., 2013; Merz, Wolf, et al., 2013; Stark et al., 2006; Zorawski et al., 2005; Zorawski et al., 2006). In the domain of instrumental learning, stress has been found to promote a shift from goal-directed action to habit-based behavior in both rodents (Dias-Ferreira et al., 2009) and humans (Schwabe & Wolf, 2009; Schwabe & Wolf, 2011; Schwabe et al., 2012). The Pavlovian and instrumental learning systems interact when Pavlovian cues exert influence over instrumental learned behaviors, biasing motivated responding. This phenomenon is known as Pavlovian-to-instrumental transfer (PIT), and can manifest in

both the appetitive and aversive domains. While much recent research has focused on the modulation of instrumental and Pavlovian learning under stress, the effects of stress on PIT are largely unknown.

A recent study with rodents examined the effects of chronic stress on PIT with food rewards (Morgado et al., 2012). In this study, chronically stressed rats underwent Pavlovian and instrumental conditioning, wherein two distinct auditory stimuli and lever presses, respectively, were paired with food outcomes. Learning was followed by a PIT test wherein auditory stimuli were presented and both levers were available for free responding. While control animals exhibited a specific PIT effect, chronically stressed animals showed no differences in responding between the two available lever presses in response to presentation of the auditory stimuli. That is, under stress, rats were unable to exhibit specific PIT, although non-selective instrumental responding persisted. This result relates to findings in humans, showing that stress can lead to decreased explicit knowledge of response-outcome contingencies in an instrumental learning task (Schwabe & Wolf, 2009). Given that the neural correlates underlying specific and general PIT with both appetitive and aversive conditioned stimuli overlap to a significant extent (Bray et al., 2008; Lewis et al., 2013; Prevost et al., 2012; Talmi et al., 2008), it is possible that stress affects PIT similarly in both of these domains. Nonetheless, research has yet to examine how stress affects PIT in humans, or how the effects of stress on avoidance-based PIT compare to those exhibited in the appetitive domain.

In this study, we sought to understand how acute stress influences motivated avoidance behavior in a PIT task. To investigate this, we used a modification of an avoidance-based PIT task that has been successfully used with human subjects (Lewis et

al., 2013; Nadler et al., 2011). A between-subjects design was employed, wherein participants underwent either an acute stress or control procedure following initial Pavlovian and instrumental conditioning, but prior to the transfer test. We expected that participants in the control group would exhibit both specific and general PIT effects, in accordance with previous results using this paradigm. We also hypothesized that participants exposed to acute stress would exhibit some amount of motivated avoidance behavior (i.e. above baseline) in the presence of aversive conditioned stimuli, as stress did not completely diminish motivated behavior in a PIT task with rodents (Morgado et al., 2012). However, we expected that the ability to selectively respond using a key press that once shared an outcome with a given stimulus (i.e. specific PIT effect) would be impaired in line with previous research suggesting that stress impairs knowledge of response-outcome contingencies (Schwabe & Wolf, 2009). Given the comorbidity of stress with clinical disorders such as anxiety and drug addiction, understanding the role of stress in avoidance-based PIT will aid in comprehension of effective treatment for these and other negatively-reinforced disorders.

4.2 Materials and Methods

4.2.1 Participants. Fifty-five participants were recruited from the Rutgers University-Newark subject pool. A total of 10 participants were excluded from analysis due to inability to correctly learn instrumental contingencies during the instrumental learning phase of the task (9 participants) or failure to comply with task requirements (1 participant), specifically calling the experimenter into the testing room multiple times during the task and using a cell phone during task. Analyses were conducted on the

remaining 45 participants (35 female, mean age = 20.15, SD = 2.42). All participants were given informed written consent prior to the experiment. The study was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects in Research and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

4.2.2 Timeline of experimental procedures. Experimental sessions were conducted between the hours of 1:00 pm and 5:00 pm to account for circadian fluctuations in cortisol (Kirschbaum & Hellhammer, 1994). The experimenter notified participants that they could withdraw from the study at any time. Participants first performed the Pavlovian and instrumental learning phases of the PIT task. The first (baseline) cortisol sample was acquired immediately following the Pavlovian learning phase. Participants then underwent either the stress or control procedure, followed by a 15 minute break which allowed for the cortisol response to peak. During this time, participants filled out task-relevant questionnaires. The second cortisol sample was acquired at the end of the 15 minute break. Participants then underwent the transfer phase of the PIT task. The third cortisol sample was acquired at the end of the experiment (Figure 4.1).

4.2.3 PIT task procedure. The PIT task procedure consisted of a Pavlovian learning, instrumental learning, and PIT test phase, and is outlined in Table 4.1. *1) Pavlovian phase:* At the start of this phase, participants rated three aversive noises, presented at 90 dB, on a scale of 1 (not at all aversive) to 5 (extremely aversive). These

tones consisted of a high frequency beep, the sound of a boat horn, and the sound of nails on a chalkboard, and were used as the aversive outcomes throughout the experiment. During the Pavlovian phase, participants were asked to learn four stimulus-outcome (S-O) contingencies (Figure 4.2A). On every trial, one of four stimulus-outcome pairings was presented, such that each visual stimulus (S1-S4) was paired with either one of the aversive outcomes (O1-O3) or no outcome (O4, a fixation dot). Stimuli consisted of four colored squares and were counterbalanced across participants. Stimuli appeared on the screen for 4 s and terminated with the onset of the outcome, which was subsequently presented for 1 s. A jittered ITI of 5-7s separated the trials. Stimulus-outcome pairs were shown 10 times each, in random order, for a total of 40 trials. Participants were instructed to refrain from instrumental responding during the Pavlovian phase.

2) Instrumental phase: In the instrumental phase, associations between two distinct instrumental responses (R1 and R2) and the avoidance of two distinct aversive outcomes (O1 and O2) were acquired (Figure 4.2B). R1 and R2 consisted of key presses on a computer keyboard. O1 and O2 consist of aversive high frequency tone presented at 90 dB. Participants underwent two sessions of instrumental conditioning during which they learned the avoidance contingency in effect. In one of these sessions the R1-O1 avoidance contingency was in effect, and during the second session the R2-O2 avoidance contingency was in effect. During a single session, only one outcome was presented (either O1 or O2). Each session lasted for 180 s, and during this time an aversive outcome was scheduled to occur 1 s after the termination of the previous outcome, unless the participant made the appropriate instrumental button press response within this time period. If the correct button was pressed, the aversive outcome was delayed by an

additional 2 s. To discourage participants from randomly responding at all times, any button presses that occurred while the aversive outcome was on the screen were without consequence.

When an aversive outcome (O1 or O2) was scheduled to occur, participants viewed a blank screen while the aversive tone was simultaneously played for a length of 1 s. A fixation cross was presented on the screen at all other times. Participants were allowed to perform instrumental responses R1 and R2 at will in order to prevent the aversive outcomes (O1 and O2) in each training phase, but a different one of these responses will be operational during each phase. Thus, R1 prevented O1 in the first session and R2 prevented O2 during the second. In this schedule, participants could prevent the aversive outcome from occurring by continually performing the correct response during the fixation period.

For each outcome, the rating for the incorrect response was subtracted from the rating for the correct response. Participants were excluded from further analysis if this calculation resulted in a value less than or equal to zero for either outcome, because this would indicate that the participant had not learned both of the instrumental contingencies. Based on this criterion, nine participants were excluded from the remainder of the study, given that it may not have been possible to obtain an explicit PIT effect if R-O contingencies were never learned.

3) Transfer test: Participants were instructed that they would now view the colored squares from the Pavlovian phase (S1-S4), and that they would be free to utilize the available button presses (R1 and R2) as they saw fit during this phase. The transfer phase included presentation of S1-S4 in the absence of reinforcement (Figure 4.2C). That

is, the entire transfer phase was performed under extinction conditions. During this phase, participants were free to respond using R1 and R2, or to not respond at all, in response to the presentation of S1-S4. Each trial began with a 2s-6s jittered fixation period. A stimulus (S1-S4) was then presented on the screen for 4 s, followed by a jittered 2s–6s screen that stated "Reloading" during which participants were explicitly told not to make instrumental responses. However, participants were free to make responses during either the pre-stimulus fixation period or during stimulus presentation. Stimuli S1-S4 were shown 15 times each in random order for a total of 60 trials.

4.2.4 Stress application. Participants assigned to the stress group underwent a socially evaluated cold-pressor task (Schwabe et al., 2008). In this task, participants immersed their right hand into ice water (1-3°C) for 2 minutes while being videotaped by an experimenter wearing a while lab coat. Participants in the control group immersed their dominant hand in room temperature water (23-25°C) for 2 minutes. Additionally, the experimenter did not wear a white lab coat and the videotape procedure was not used with the control group.

4.2.5 Cortisol collection and analysis. To acquire salivary cortisol data, participants were asked to moisten a Salimetrics Oral Swab (SOS) in their mouths for 1 min by placing the SOS underneath their tongue. Upon completion of this procedure, participants withdrew the SOS and placed it in an individual centrifuge tube. Three samples were acquired for each participant. Samples were frozen in cold storage at −10°C, packed with dry ice and sent to Salimetrics Laboratory (State College, PA) for

duplicate biochemical assay analysis. To examine changes in cortisol as a result of the stress or control procedure, we computed the percent change in salivary cortisol from time 1 (baseline) to time 2 (15 minutes following the stress or control procedure).

4.2.6 SCR acquisition and analysis. SCR was collected with a BIOPAC MP 150 system skin conductance module, using Ag-AgCl electrodes attached to the second and third finger of each participant's non-dominant hand. SCR data was analyzed with AcqKnowledge software (BIOPAC Systems Inc.). Preprocessing included use of a low pass filter (cutoff frequency of 25 Hz) and mean value smoothing (3 samples). Responses were square root transformed prior to analysis, and were considered valid if they began between 0.5 and 4.0s after the onset of the stimulus and if the base-to-peak difference of the response was at least 0.01 microsiemens (μS). All other SCR was scored as zero.

4.2.7 Behavioral analysis. All behavioral analyses consisting of more than two *t*-tests within a family of comparisons were corrected for multiple comparisons using the sequential Bonferroni correction (Holm, 1979; Rice, 1989).

4.3 Results

4.3.1 Cortisol results. Cortisol percent change from t1 (baseline) to t2 (15 minutes following the stress or control procedure) was computed for both the stress and control groups. We observed a mean cortisol percent change of 19.583% in the stress group, and a mean cortisol percent change of -16.398% in the control group. Importantly, the change in cortisol was significantly greater for the stress group than the control group ($t_{(21)} = 2.280$; $p = 0.033$).

4.3.2 Behavioral results: tone ratings. Participants rated each of the three noise outcomes (O1-O3) at the onset of the experiment. Ratings were made on a scale of 1 ("not at all aversive") to 5 ("extremely aversive"). Ratings were as follows: beep, mean = 3.330, SD = 1.243; boat horn, mean = 3.070, SD = 1.053; chalkboard, mean = 3.160, SD = 1.224. A one-way ANOVA across the three outcomes revealed no main effect of outcome type ($F_{(2,43)} = 0.759$; $p = 0.474$), indicating that no one outcome was experienced as significantly more aversive than any other outcome. Separate ANOVAs for the stress and control groups confirmed that this result held regardless of group placement (stress group: $F_{(2,20)} = 2.445$; $p = 0.112$; control group: $F_{(2,21)} = 0.428$; $p = 0.655$).

4.3.3 Behavioral results: Pavlovian conditioning. At the end of the Pavlovian phase, participants were shown each of the four conditioned stimuli in random order, along with the question "How do you feel about this square?" Participants rated each stimulus on a scale of 1 ("strongly dislike") to 5 ("strongly like"). A one-way ANOVA across the four stimulus revealed a main effect of stimulus ($F_{(3,42)} = 47.184$; $p < 0.001$). Post-hoc *t*-tests showed that all of the stimuli paired with aversive outcomes (S1-S3) were rated significantly worse than the neutral stimulus (S4, all p's < 0.001). Importantly, these results were found irrespective of stress condition.

4.3.4 Behavioral results: instrumental conditioning. We measured instrumental learning by examining the number of times that participants experienced aversive outcomes during the instrumental phase. Each 180 s block was broken up into

six 30 s bins in order to obtain an estimate of learning across time. From the first 30 s to the last 30 s of each 180 s block, there was a significant decrease in the number of experienced aversive outcomes (one-tailed paired *t* test, $t_{(44)} = 9.350$; $p < 0.001$), indicating that the correct R-O contingencies were learned over time (Figure 4.3). This decrease was observed irrespective of outcome (O1: $t_{(44)} = 8.010$; $p < 0.001$; O2: $t_{(44)} = 5.196$; $p < 0.001$). Additionally, a 2 x 2 ANOVA examining the factors of stress group (stress, control) and bin (1, 6) showed no main effect of stress group ($F_{(1,44)} = 1.377$; $p = 0.247$), suggesting that R-O contingencies were acquired successfully in both groups. As an explicit measure of instrumental learning, participants were asked at the end of the instrumental phase to rate, on a scale of 1 to 5, how effective each response (R1 and R2) was at preventing each outcome (O1 and O2). For those participants who met the instrumental learning criterion, ratings were as follows: R1-O1 (correct contingency), mean = 3.273, SD = 1.471; R2-O1 (incorrect contingency), mean = 1.709, SD = 1.272; R2-O2 (correct contingency), mean = 4.564, SD = 1.050; R1-O2 (incorrect contingency), mean = 1.891, SD = 1.315.

4.3.5 Behavioral results: stress or control procedure ratings. Following application of the stress or control procedure, participants were asked to rate, on a scale of 1-100, how unpleasant, stressful, and painful they found the procedure. As expected, participants in the stress group found the procedure to be significantly more unpleasant ($t_{(43)} = 13.373$; $p < 0.001$), stressful ($t_{(43)} = 8.625$; $p < 0.001$), and painful ($t_{(43)} = 15.549$; $p < 0.001$) than did participants in the control group.

4.3.6 Behavioral results: Pavlovian-to-instrumental transfer - control group. To measure specific and general PIT, we compared instrumental responding (R1 and R2) made a) across all 4 stimulus types and b) during stimulus presentations, as compared to the pre-stimulus fixation period. A three-way repeated-measures ANOVA examining the effects of stimulus (S1-S4), interval (pre-stimulus and stimulus) and response (R1 and R2) was performed separately for the stress and control groups. For the control group, this ANOVA revealed a significant main effect of stimulus ($F_{(3,20)} = 4.630$; $p < 0.05$) Post hoc *t*-tests revealed that the amount of instrumental responding was elevated during presentation of S1-S3 as compared to the neutral stimulus, S4 (all *p*'s < 0.05). The ANOVA also revealed a significant main effect of interval ($F_{(1,22)} = 4.980$; $p < 0.05$). In particular, a significantly greater number of instrumental responses were made during the stimulus period as compared to the pre-stimulus period. A stimulus X response interaction was also observed ($F_{(3,20)} = 3.991$; $p < 0.05$), as was a stimulus X interval interaction ($F_{(3,20)} = 3.765$; $p < 0.05$). Finally, a 3-way stimulus X interval X response interaction was observed ($F_{(3,20)} = 3.480$; $p < 0.05$). This 3-way interaction was further analyzed via one-way ANOVAs across the four levels of responding (pre-stimulus, stimulus, R1 and R2) for each stimulus. Significant main effects were obtained for S1-S3 (all *p*'s < 0.05), but not for S4 ($p > 0.05$). Post-hoc *t*-tests supported the finding that S1 selectively elevated R1 ($p < 0.05$) but not R2 responding ($p > 0.05$) relative to the prestimulus period, indicating a specific transfer effect. S2 selectively elevated both R1 and R2 (all *p*'s < 0.05) responding relative to the prestimulus period. However, R2 responding was significantly higher than R1 responding ($t_{(22)} = 2.990$; $p < 0.01$). Given that previous work with rodents on stress and PIT (Morgado et al., 2012) classified a

specific transfer effect as a significant elevation of R2 over R1 responding in the presence of S2, we argue that we also obtained a specific transfer effect in the presence of S2. Post-hoc *t*-tests for S3 revealed that R1 and R2 responding did not significantly differ ($p = 0.729$) but were both significantly greater than responding during the prestimulus period (all p's < 0.05). Therefore, a general transfer effect was also observed, wherein the stimulus (S3) associated with the novel aversive outcome (O3) elicited a nonselective increase in both available responses (R1 and R2) as compared to the pre-stimulus baseline. No increases in instrumental responding from pre-stimulus period to stimulus presentation occurred for S4 (all p's > 0.05). These data are presented in Figure 4.4A. Taken together, these results support prior findings that aversive conditioned stimuli motivate both specific and general avoidance behaviors in humans, and extend these findings by demonstrating similar effects with a primary reinforcer.

4.3.7 Behavioral results: Pavlovian-to-instrumental transfer - stress group. A three-way repeated-measures ANOVA examining the effects of stimulus (S1-S4), interval (pre-stimulus and stimulus) and response (R1 and R2) revealed a significant main effect of stimulus ($F_{(3,19)} = 4.797$; $p < 0.05$) Post hoc *t*-tests examining responding during the stimulus period revealed that the amount of instrumental responding was elevated during presentation of S1-S3 as compared to the neutral stimulus, S4 (all p's < 0.05). The ANOVA also revealed a significant main effect of interval ($F_{(1,21)} = 14.090$; $p = 0.001$), wherein a significantly greater number of instrumental responses were made during the stimulus period as compared to the pre-stimulus period. A stimulus X interval interaction was observed ($F_{(3,19)} = 3.566$; $p < 0.05$). Contrary to results from the control group, we

observed no stimulus X response interaction with participants in the stress group ($F_{(3,19)}$ = 1.875; $p > 0.05$). However, there was a significant 3-way stimulus X interval X response interaction ($F_{(3,19)}$ = 1.401; $p < 0.05$). This 3-way interaction was further analyzed via one-way ANOVAs across the four levels of responding (pre-stimulus, stimulus, R1 and R2) for each stimulus. Significant main effects were obtained for S1-S3 (all p's < 0.05), but not for S4 ($p > 0.05$). Post-hoc *t*-tests showed that for both S1 and S2, R1 and R2 responding was elevated relative to the prestimulus period (all p's < 0.05). Importantly, for both S1 and S2, R1 responding did not significantly differ from R2 responding (all p's > 0.05). Therefore, stress group participants did not exhibit specific transfer, although a general enhancement in motivated responding in the presence of both S1 and S2 occurred. Post-hoc *t*-tests for S3 revealed that R1 and R2 responding were both significantly greater than responding during the prestimulus period (all p's < 0.05), and that R1 and R2 responding in the presence of S3 did not significantly differ ($p = 0.192$). Therefore, a general transfer effect was observed within the stress group. There were no significant increases in either R1 or R2 responding in the presence of S4 as compared to the prestimulus period (all p's > 0.05). These data are presented in Figure 4.4B. Taken together, these results support our initial hypothesis that stress would impair the specific PIT effect, given that response-outcome knowledge may have been impaired following stress application. Furthermore, our results demonstrate that general PIT effect remains in tact under acute stress.

4.3.8 Behavioral results: Pavlovian-to-instrumental transfer - combined. A three-way repeated-measures ANOVA with the factors of stimulus (S1-S4), response (R1

and R2) and stress condition (stress or control) was performed to examine overall differences in responding between the stress and control groups. A main effect of stimulus was observed ($F_{(3,41)}$ = 8.074; p < 0.001), as was a stimulus x response interaction ($F_{(3,41)}$ = 1.401; p < 0.005). However, there was no main effect of stress condition and no interactions of stress condition with either stimulus or response factors (all p's > 0.05).

As in previous studies (Bray et al., 2008; Lewis et al., 2013; Prevost et al., 2012), we divided the PIT test phase into 5 bins of 12 trials each in order to examine potential changes in number of responses made across time. Importantly, the PIT test was performed in extinction and without any reinforcement, suggesting that any instrumental responding in this phase is an actual behavioral expression of PIT. We performed a three-way repeated-measures ANOVA, examining the effects of stimulus type (S1-S4), bin (1-5) and stress group on total number of instrumental responses made during stimulus presentation. This ANOVA revealed a main effect of stimulus ($F_{(3,41)}$ = 8.047; p < 0.001), as expected, but no main effect of bin ($F_{(4,40)}$ = 1.702; p = 0.169), no stimulus X bin interaction ($F_{(12,32)}$ = 1.101; p = 0.392), and no stimulus X bin X stress condition interaction ($F_{(12,32)}$ = 0.772; p = 0.720). These results show that although the PIT test was performed under extinction conditions, participants continued to respond using R1 and R2 in response to the Pavlovian stimuli throughout the duration of the PIT test. There was also no main effect of stress condition, indicating that changes in responding across time did not signficantly differ between stress and control groups ($F_{(1,43)}$ = 0.242; p = 0.625).

Following the PIT test, participants made ratings identical to those following the Pavlovian phase for each stimulus. A two-way ANOVA with the factors of stimulus

type and stress group showed a significant main effect of trial type ($F_{(3,41)} = 8.633$; $p < 0.001$), but no main effect of group and no trial type X group interaction (all p's > 0.05). Post-hoc *t*-tests showed that all aversive stimuli (S1-S3) continued to be rated as significantly more aversive than the neutral stimulus, S4 (all p's < .005). These results compliment the sustained responding over the course of the transfer phase, indicating that even under extinction conditions, perhaps participants did not fully extinguish the aversive S-O associations. However, no differences between groups were observed.

4.3.9 Physiological Results: Stress or control procedure. The global mean SCR across the duration of the stress or control procedure was calculated in order to measure potential differences in physiological responses to the two tasks. Mean skin conductance levels were elevated during the stress procedure as compared to the control procedure ($t_{(43)} = 2.325$; $p < 0.05$).

4.3.10 Physiological Results: Pavlovian-to-instrumental transfer. A two-way ANOVA was performed on SCR data, examining the factors of stimulus type and stress group (Figure 4.5). No significant main effects of trial type or stress group emerged (all p's > 0.05), however there was a significant trial type X stress group interaction. Post-hoc independent *t*-tests showed that SCR was elevated in the control group as compared to the stress group during the presentation of S2 ($t_{(43)} = 2.250$; $p < 0.05$), but SCR levels for the stress and control groups did not significantly differ during presentation of any of the other stimuli (S1, S3, S4; all p's > 0.05).

4.4 Discussion

In the current study, our aim was to understand the effects of an acute stressor on both specific and general avoidance-based PIT in humans. In control participants who were not exposed to acute stress, the ability of aversive Pavlovian stimuli to motivate avoidance behaviors paralleled that seen in prior versions of this task (Lewis et al., 2013; Nadler et al., 2011). Control group participants exhibited a specific PIT effect, wherein instrumental responses that prevent a specific aversive outcome (e.g. R1 or R2) are selectively increased in the presence of Pavlovian stimuli (S1 and S2) that once predicted the same aversive outcome. A general PIT effect was also observed, wherein the presence of a Pavlovian stimulus that once predicted an aversive outcome for which no avoidance response was learned (S3) led to general enhancement of both R1 and R2 responding. Participants who underwent acute stress exposure also exhibited a general PIT effect. However, acute stress abolished specific PIT, as selective responding with R1 or R2 was not observed in the presence of S1 and S2. Nonetheless, a general enhancement in motivation was observed in the presence of S1 and S2, wherein R1 and R2 responding increased non-selectively above baseline. Across the course of the PIT test, instrumental responding persisted with both R1 and R2, and the amount of instrumental responding did not significantly differ between the acute stress and control groups.

These results suggest that acute stress exposure does not diminish the general motivation to avoid aversive stimuli, but rather impairs some facet of directed responding toward specific PIT cues. This corresponds with evidence from the animal literature, which showed that rats under chronic stress (Morgado et al., 2012) were impaired in specific PIT. However, there are several fundamental differences between the study

performed by Morgado et al. and our current study. Importantly, in the current study we applied acute stress after Pavlovian S-O and instrumental R-O contingencies were learned, but prior to the PIT test. This differs from the study performed by Morgado and colleagues (2012), as their chronically stressed rats performed all phases of the PIT task while under stress. While Morgado et al. did not observe behavioral impairments in either Pavlovian (tested with an outcome devaluation) or instrumental learning, it is possible that chronic stress altered a non-behavioral facet of learning (e.g. physiological responses to Pavlovian cues). Thus, it is difficult to disentangle whether impairments in specific PIT did or did not stem from effects of stress during learning. In the current study, however, acute stress was not applied until after Pavlovian and instrumental learning occurred. We can therefore say with certainty that any differences exhibited during the PIT test were a result of acute stress exposure, and not due to differences in learning S-O and R-O contingencies.

Additionally, the current study examined PIT using aversive stimuli, while the experiment by Morgado et al. was performed in the appetitive domain, examining approach behaviors toward rewarding stimuli. Neuroimaging research suggests that the striatum is involved in motivated behavior toward conditioned stimuli both in the appetitive (Bray et al., 2008; Prevost et al., 2012; Talmi et al., 2008) and aversive domains (Lewis et al., 2013), suggesting that similar neural mechanisms underlie both processes. However, it has been suggested that stress facilitates the dopamine response to aversive stimuli, but reduces stimulatory dopamine responses to appetitive stimuli (Di Chiara et al., 1999). Thus, it is unclear whether the effects of stress on PIT in the appetitive and aversive domains are identical at a neural level.

Interestingly, physiological responses to the general transfer stimulus (S3) during the PIT test, measured with SCR, did not significantly differ between the acute stress and control groups. This mirrors the behavioral data, which suggested that acute stress neither enhanced nor diminished general PIT. One of the two specific PIT stimuli (S1) did not yield differential GSR responses between the acute stress and control groups, while the other specific PIT stimulus (S2) did. In particular, GSR responses in the presence of S2 were larger for the control group than the acute stress group. Post-hoc, we examined whether this difference between groups was a reflection of enhanced responding toward S2 in the control group or dampened responding toward S2 in the acute stress group. T-tests examining GSR responses in the presence of S2 versus the total average GSR response for the other three stimuli showed that there was a dampened response in the presence of S2 for the acute stress group during the PIT test ($t_{(21)} = 2.941$, $p < 0.01$). Perhaps, then, the stress group is exhibiting a primacy effect in response-outcome retention at the physiological level, given that O1-R1 associations were learned prior to O2-R2 associations during instrumental learning.

While physiological responses during the PIT test mirrored between-group differences in motivated avoidance behavior, behavioral ratings at the end of the PIT test did not significantly differ between acute stress and control groups. In particular, both groups rated both the specific and general transfer PIT stimuli (S1-S3) as significantly more aversive than the neutral stimulus (S4). These ratings indicate that while the PIT test was performed in extinction, participants may not have fully extinguished the aversive S-O associations. Lending some clarity to the between-group differences exhibited during the PIT test, behavioral ratings suggest that the lack of specific PIT in

the acute stress group was not a result of a stress-related change in subjective feelings about the stimuli.

The current study was modeled off of an avoidance-based PIT paradigm that has been used successfully in the lab (Lewis et al., 2013; Nadler et al., 2011), yet we utilized aversive noise as reinforcement, which was novel to this particular task. Prior versions of this task obtained specific and general PIT using instructed reinforcers in the context of a video game. Given that we obtained both specific and general PIT in the current version of the task, it is clear that stimuli associated with both primary and non-primary aversive reinforcers are salient enough to motivate avoidance behaviors with humans. While previously used instructed reinforcers aimed to minimize individual differences in outcome perception (given that the value of these outcomes was instructed in the context of a game), the primary reinforcement used in the current study may have been more salient. Nonetheless, the current study confirms that specific and general PIT effects can be obtained with a variety of aversive reinforcers.

One potential drawback of our task was the need to include a 15 minute temporal gap in between the two learning phases and the PIT test, so that the PIT test could be performed when cortisol was expected to peak (15 to 30 minutes following acute stress application, (Schwabe et al., 2008). For consistency, this 15 minute break was undertaken by both the acute stress and control groups. Nonetheless, prior versions of this task have not included any gap between learning and the PIT test. To ensure that the temporal gap was not biasing our data in any way, we performed a separate, between-subjects behavioral experiment (N = 40) wherein half of the participants experienced a 15 minute break between learning and the PIT test, and the other half proceeded

immediately to the PIT test following learning. Importantly, the two groups did not significantly differ in the amount of motivated behaviors performed in the presence of any of the conditioned stimuli (e.g. R1 responding toward S1, R2 responding toward S1, etc.; all p's > 0.05), indicating that a 15 minute gap prior to the PIT test does not affect specific or general avoidance-based PIT responding.

The current study had several additional limitations. First, our sample was unbalanced with regard to the sex of participants. Given the relatively small sample size, our data did not contain enough power to examine differential effects of stress on PIT in males versus females. Nonetheless, this is an important topic for future work, given that existing research has found differential effects of stress on males and females during affective learning tasks (e.g. Duncko et al., 2007; Wood & Shors, 1998; Wood et al., 2001). Consideration of other individual differences that may influence the stress response should also be taken. For instance, oral contraceptives have been shown to potentially diminish the cortisol response following stress exposure (Kirschbaum et al., 1995), and exposure to nicotine may diminish the sensitivity of the cortisol response (Kirschbaum & Hellhammer, 1994). While information on smoking status and current medications was collected in the current study, we did not, for instance, exclude individuals who smoke or take oral contraceptives.

In sum, the current study found that acute stress exposure impairs the ability to transfer specific information about S-O and R-O contingencies between the Pavlovian and instrumental systems, while preserving the general motivation to avoid aversive stimuli. Avoidance-based PIT may be useful as a model of drug relapse, given the prevalence of negative reinforcement-based models of addiction (e.g. Baker et al., 2004),

wherein avoidance of withdrawal symptoms serves as a powerful motivator of behavior. Stress is known to enhance drug seeking and drug use, as well as increasing the risk of drug abuse and relapse (Sinha, 2001). Thus, understanding the interaction of stress and motivated behavior will better allow for paradigms such as PIT to serve as effective models of clinical disorders.

Chapter 5: General Discussion

5.1 Purpose and Summary of Dissertation Studies

Negative experiences with stimuli in one's environment can in turn cause these stimuli to themselves become aversive eliciting a conditioned response. Over time, an individual's representation of a conditioned stimulus may or may not change – the aversive properties of the conditioned stimulus have the potential to extinguish, diminishing or eliminating the conditioned response. However, if extinction does not occur, the conditioned affective properties of the stimulus may begin to influence behavior. In particular, individuals might avoid a stimulus that was once associated with an aversive outcome. This interaction between Pavlovian and instrumental learning systems is very powerful, and, while adaptive in the short term, can over time become maladaptive. Avoidance of withdrawal symptoms, for instance, has been implicated in drug relapse (e.g. Wikler, 1948).

The purpose of the studies comprising this dissertation was to gain a better understanding of how aversive Pavlovian conditioned stimuli are represented and updated in the brain, and how these stimuli can subsequently motivate avoidance behaviors. We had two primary sets of hypotheses. The first set of hypotheses pertained to the neural circuitry underlying extinction learning. We hypothesized that corticostriatal brain regions underlying extinction learning with negatively valenced conditioned stimuli would overlap with brain regions involved in extinction learning with positively valenced stimuli, given that the mPFC projects to both the striatum and the amygdala, which are involved in the expression of drug-seeking and fear, respectively. We subsequently predicted that corticostriatal regions would show enhanced connectivity with the ECN

during extinction as compared to Pavlovian acquisition (Experiment 1). These hypotheses were largely supported by our data. We found that during extinction learning, regions of striatum were modulated by both stimulus valence and magnitude, and that a dorsal region of mPFC was modulated by magnitude as well. Interestingly, however, the vmPFC, which is known to play a role in extinction learning with both appetitive and aversive conditioned stimuli (Peters et al., 2009), was not modulated by either stimulus valence or magnitude. Using a combined ICA/dual regression analysis, we also found that clusters in ventral striatum and mPFC showed enhanced connectivity with the ECN during extinction as compared to acquisition. Overall, these results help to delineate how neural responses during extinction learning with aversive conditioned stimuli differ from those during extinction learning with appetitive conditioned stimuli, and show how regions involved in extinction learning fluctuate in functional connectivity with large-scale brain networks during different affective learning processes.

Our second set of hypotheses concerned Pavlovian-instrumental interactions in the avoidance domain. First, we predicted that both specific and general PIT would be elicited by aversive conditioned stimuli, and that the striatum would be engaged during this manifestation of both specific and general avoidance-based PIT (Experiment 2). We also hypothesized that acute stress would impair avoidance-based specific PIT (Experiment 3), given that it has been found to reduce knowledge of action-outcome contingencies (Schwabe & Wolf, 2009). This set of hypotheses was also largely supported by our data. Behaviorally, we were able to elicit both specific and general avoidance-based PIT using aversive conditioned stimuli, and found that the striatum, which underlies the appetitive PIT effect in humans (Bray et al., 2008; Prevost et al.,

2008; Talmi et al., 2008), is also activated during specific and general avoidance-based PIT (Experiment 2). Acute stress was found to impair the specific PIT effect, but did not decrease general PIT. Interestingly, in the presence of specific PIT cues, participants under acute stress showed a global enhancement in responding above baseline, similarly to the enhancement shown in the presence of the general PIT cue (Experiment 3). Taken together, these results shed light on the neural basis of avoidance-based PIT (and how it relates to PIT in the appetitive domain), as well as how stress, a commonly encountered environmental factor that is known to impact affective learning processes, modulates the ability of aversive stimuli to motivate avoidance behaviors.

5.2 Limitations

In addition to the limitations discussed in Chapters 2-4 of this dissertation, this research program as a whole contains additional limitations. First, the type of reinforcement used across Experiments 1, 2, and 3 was not consistent, and therefore it is difficult to directly compare the results of these studies. In particular, we utilized a primary aversive reinforcer in Experiment 3, while reinforcement in Experiments 1 and 2 consisted of outcomes that were rendered appetitive and/or aversive in the context of a video game. Given recent evidence suggesting that the neural basis underlying aversive Pavlovian processes differs with various categories of reinforcement (Delgado et al., 2011), it is difficult to universally extend our results to the real world. While the video game setup used in Experiments 1 and 2 attempted to minimize individual variability, it is possible that the instructed reinforcers used in these studies are not as motivationally salient as are primary reinforcers such as aversive noise.

Likewise, Experiments 1 and 2 were performed while participants underwent fMRI, but Experiment 3 did not contain a functional neuroimaging component. Thus, we are limited in understanding the effects of stress on avoidance-based PIT, given that Experiments 2 and 3 can only be compared in the behavioral domain. Additionally, we employed a relatively novel functional connectivity approach in Experiment 1 that we did not attempt with our neuroimaging data in Experiment 2. In the future, examining connectivity between brain regions and large-scale neural networks in our avoidance-based PIT paradigm will lend further insight into the similarities and differences underlying various Pavlovian and instrumental processes.

Across all three of our studies, we collected behavioral ratings related to the Pavlovian conditioned stimuli. There are two limitations to the behavioral ratings we collected. First, these ratings were collected only at the end of each relevant experimental phase (e.g. following each acquisition and extinction block in Experiment 1; following Pavlovian learning and the PIT test in Experiments 2 and 3. Thus, updates in how these stimuli are perceived over the course of an experimental block, which could help to explain the time course of learning, are unable to be probed. Additionally, the rating system used was inconsistent across the three studies. In Experiments 1 and 3, we used an implicit, subjective rating system, asking participants how they felt (i.e. how much they "liked" or "disliked" each conditioned stimulus). Experiment 2, in contrast, utilized explicit ratings that probed whether or not participants explicitly learned the CS-US contingencies. Follow-up studies could be improved by probing behavioral ratings at multiple time points throughout an experimental block, and also by consistently utilizing the same affective rating system.

In our neuroimaging analyses in Experiments 1 and 2, we examined brain activity collapsed across entire functional runs. As with the behavioral ratings, this approach did not allow us to examine how neural activity fluctuates on a trial-by-trial basis, or within small subsets of trials over time (as has been done in past work, e.g. Gottfried et al., 2002). This is a key limitation of our neuroimaging analyses, as we are simply examining the average neural activity over the course of several minutes during various affective learning processes, rather than examining changes in the neural basis of these processes on the order of seconds. Future analyses should model fluctuations in neural activity in key corticostriatal regions, allowing us to gain a better understanding of the time course of Pavlovian and instrumental learning processes. In Experiment 1, for instance, examining changes in neural activity within both the early and late extinction blocks would allow us to better understand whether or not appetitive and aversive conditioned CS-US associations extinguish at the same rate. This type of analysis would also lend insight into how brain activity changes over the course of the PIT test in Experiment 2. With regard to PIT, this type of analysis might also help to tease apart potential temporal dissociations in brain activity underlying specific and general PIT.

5.3 Future Directions and Implications

The studies presented in this dissertation are some of the first to look at the behavioral and neural correlates of avoidance-based interactions between the Pavlovian and instrumental systems. Therefore, a number of interesting questions remain that might be addressed with future research. In a simple extension of our PIT paradigm, it would be useful to examine motivated approach and avoidance behaviors in the same task. While

Experiment 2 found that the neural basis of avoidance-based PIT involves activation of corticostriatal regions that overlap with those seen in fMRI studies of appetitive PIT (Bray et al., 2008; Prévost et al., 2012; Talmi et al., 2008), these two phenomena have not been directly compared in the same task. In Experiment 1, we were able to directly compare the neural basis of extinction learning with appetitive and aversive conditioned stimuli, which helps to better understand the neural mechanisms underlying extinction failure in both domains. By using a similar manipulation in our PIT paradigm, we may be able to gain insight into whether maladaptive instances of motivated behavior in both the appetitive and aversive domains (e.g. drug addiction, anxiety disorders) would be receptive to similar treatment methods.

Given the relevance of our work to drug addiction, a clear offshoot of these initial studies is to examine Pavlovian-instrumental interactions in addicted populations. Recent work suggests that cigarette cues are able to motivate approach behaviors in a PIT task with smokers (Hogarth & Chase, 2012). Following up on this work, we have begun to examine the ability of smoking-related and non-smoking-related conditioned stimuli to motivate behavior in daily smokers. Deprivation from nicotine enhances the attentional bias toward smoking cues (Gross et al., 1993; Rosenblatt, 1996; Sayette & Hufford, 1994) which may subsequently influence the ability of these cues to motivate behavior. Therefore, in an initial behavioral study (Manglani, Lewis & Delgado, in prep), we sought to examine the influence of smoking deprivation on approach behavior toward cigarette cues. Participants were asked to refrain from both smoking and eating for 12 hours prior to the experiment. Importantly, we used a willingness to pay (WTP) task to equate the value of appetitive cigarette and food outcomes. In particular, participants

stated how much they would be willing to pay for one cigarette puff. They then chose from a variety of snack foods (e.g. goldfish crackers, M&M candies), and were asked how much they would be willing to pay for varying quantities of this food item. By using the WTP task, we were able to choose a quantity of the preferred snack item that each participant valued most similarly to a single cigarette puff. Participants then underwent Pavlovian conditioning, wherein three colored squares (S) represented cigarette (i.e. one puff), snack (i.e. quantity of preferred snack item with the same WTP as one puff), and neutral (i.e. paperclip) outcomes (O). Instrumental responses (R) that allowed for successful acquisition of these outcomes were then learned. In the PIT test, the conditioned colored squares (S) were presented in extinction, and instrumental responding was probed. We found that both cigarette and snack cues successfully elicited specific transfer effects. However, participants overall made a significantly greater number of cigarette-seeking as compared to food-seeking responses, even though these outcomes were similarly valued (as measured by WTP). Notably, a stronger preference for cigarettes over food at the end of the study correlated with greater cigarette-seeking behavior during the PIT test. Together, these results suggest that drug and non-drug cues may motivate approach behaviors to different extents, even when cues are similarly valued and participants have been deprived of both reward categories. Currently, we are running a follow-up study wherein a group of smokers, deprived of both smoking and eating, performs this PIT task while undergoing fMRI. In this study, we expect to see that increased motivated behavior in the presence of cigarette cues will correlate with enhanced striatal activity during the PIT test. An additional future direction of this work

would involve examining motivated avoidance behavior in the presence of cigarette cues, which may trigger withdrawal symptoms, in a population of recovered cigarette smokers.

Finally, it will be important for future research to probe the relationship between stress and addiction as it relates to Pavlovian-instrumental interactions. Stress is known to increase drug-seeking behavior, and also increases the risk of drug abuse and relapse (Sinha, 2001); therefore, it is important to understand how stress may promote both approach and avoidance behaviors in the presence of drug-relevant stimuli. One way to examine this relationship is by running a between-subjects study in a group of deprived daily smokers, wherein one group is exposed to acute stress and the other is not. Essentially, this study would follow the timeline of Experiment 3, but would feature smoking and food-relevant stimuli. Given that intial behavioral data suggests enhanced specific PIT for smoking cues as compared to food cues in daily smokers deprived of both smoking and eating, this proposed study would seek to examine whether this increased specific PIT in the presence of smoking cues is modulated when smokers are under stress.

5.4 Overall Conclusions

The goal of this dissertation was to gain a better comprehension of the interaction between the human Pavlovian and instrumental learning systems, and to understand various factors that play a role in aversive Pavlovian and instrumental learning processes. Our data shed light on neural underpinnings of these processes, highlighting the role of corticostriatal regions in both extinction learning and Pavlovian-to-instrumental transfer. Importantly, we also show that various properties of a conditioned stimulus (e.g. valence,

magnitude) as well as environmental influences such as stress modulate aspects of affective learning and motivated behavior. Understanding how aversive stimuli are represented and updated in the brain and how we are motivated to avoid said stimuli will allow for a better comprehension of how to counteract the maladaptive use of avoidance behavior implicated in both drug relapse and anxiety disorders.

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Figure 2.1 Task Schematic for Experiment 1

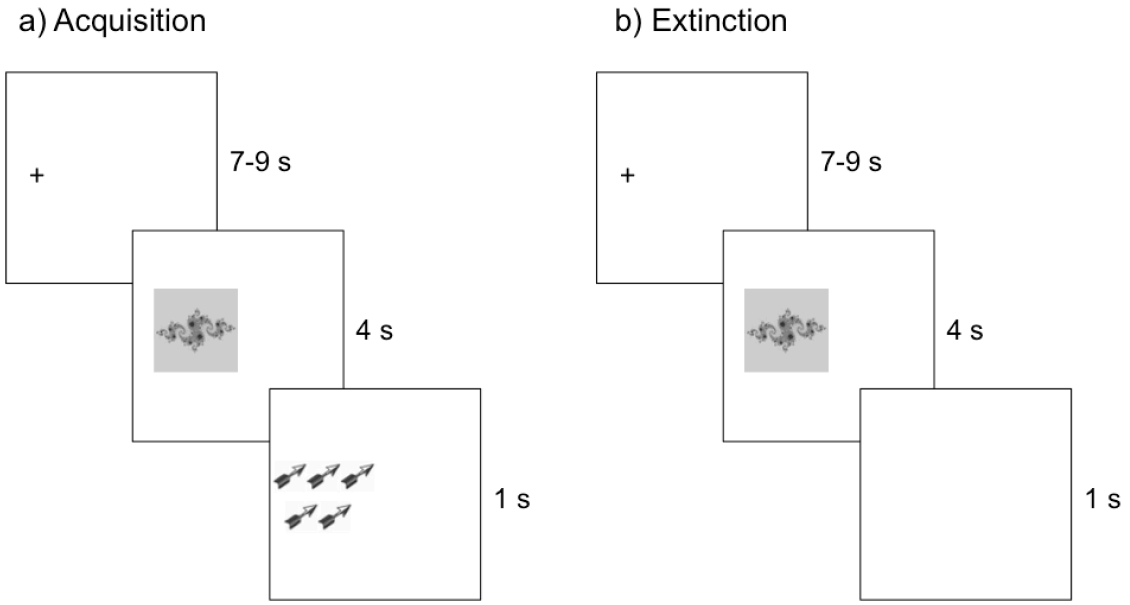

Figure 2.1. Task Schematic for Experiment 1. Participants underwent two blocks of Pavlovian acquisition and two blocks of extinction; each block consisted of 40 total trials. (A) In the Pavlovian conditioning phase, participants viewed one of four fractals for 4s, followed by either a high magnitude gain or loss of items (1 or 5 arrows or bombs), or by no outcome. Fifty percent of trials were reinforced. (B) The extinction phase mirrored the Pavlovian acquisition phase, though all trials were unreinforced.

Figure 2.2 Implicit Affective Ratings for Experiment 1

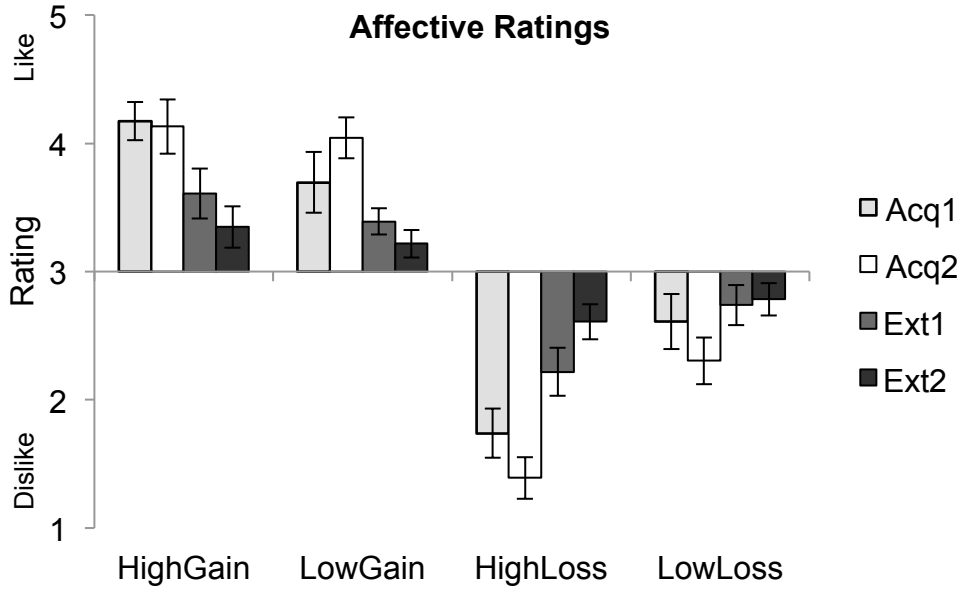

Figure 2.2. Implicit Affective Ratings for Experiment 1. For all four stimulus types, there was a significant shift toward neutral (neutral = 3) from late acquisition to late extinction, indicating that, on a subjective level, behavioral extinction had occurred (all p's < 0.05). Error bars represent standard error of the mean (SEM).

Figure 2.3 Valence x Block Interaction During Extinction Learning for Experiment 1

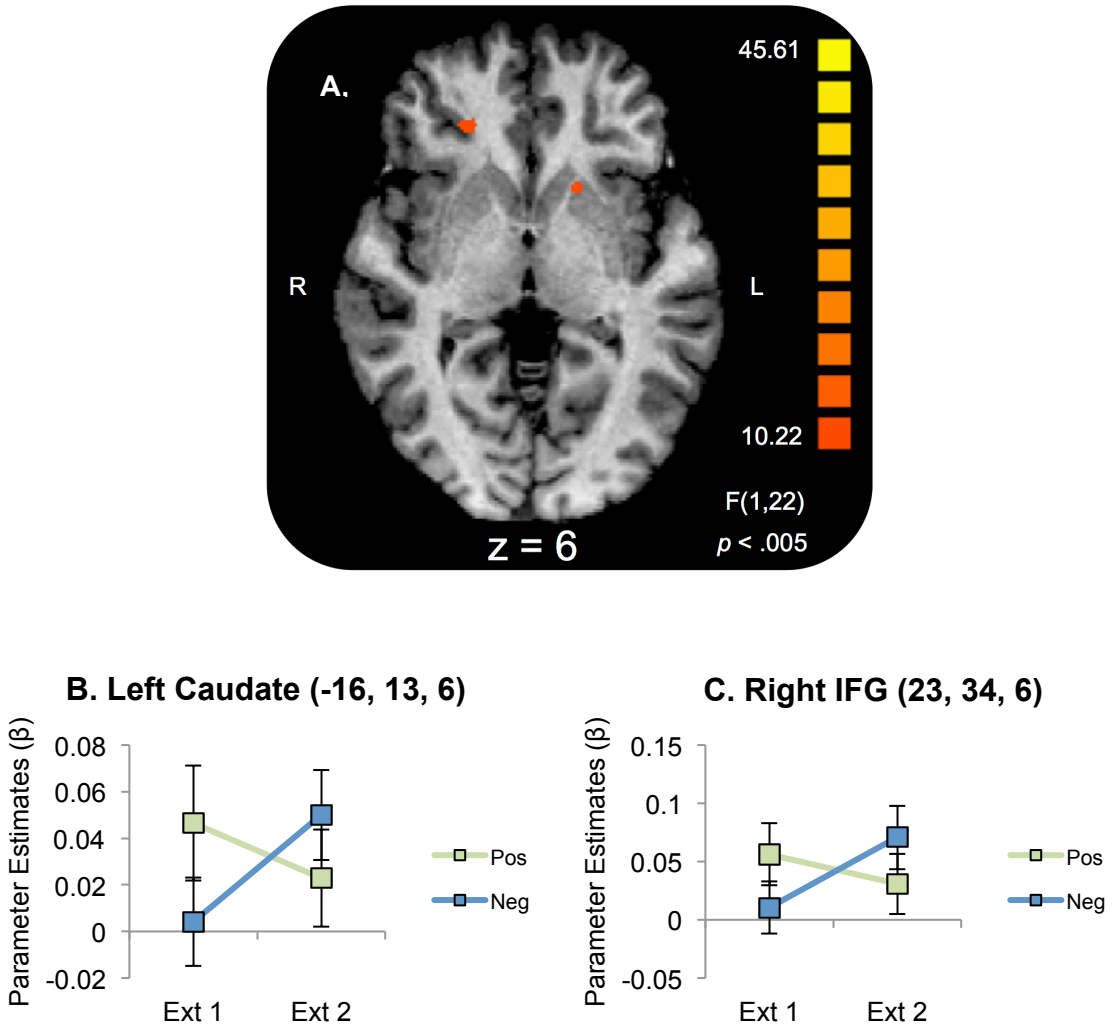

Figure 2.3. Valence x Block Interaction During Extinction Learning for Experiment 1. (A) A 2 (valence) x 2 (magnitude) x 2 (block) ANOVA across the extinction phase revealed brain regions that were modulated across extinction as a function of CS valence (z = 6). Graphs depict mean parameter estimates (β) for ROIs in (B) left caudate and (C) right IFG, both of which exhibited an increase in activation for negative CS over the course of extinction (all *p*'s < .05), and no change for positive CS over the course of extinction (all *p*'s > .05). Error bars represent SEM.

Figure 2.4 Magnitude x Block Interaction During Extinction Learning for Experiment 1

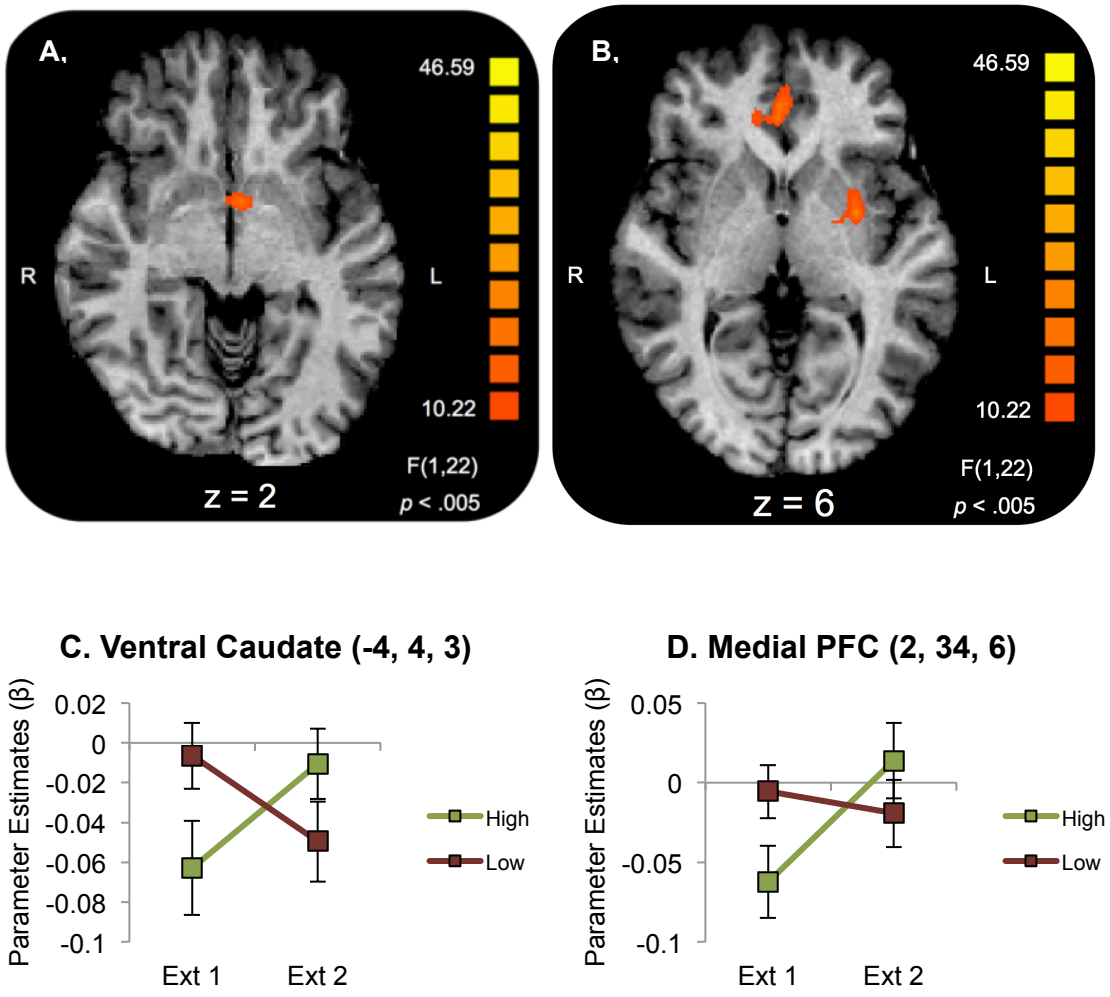

Figure 2.4. Magnitude x Time Interaction During Extinction Learning for Experiment 1. (A, B) A 2 (valence) x 2 (magnitude) x 2 (block) ANOVA across the extinction phase revealed brain regions that were modulated across extinction as a function of CS magnitude (z = 6). Graphs depict mean parameter estimates (β) for ROIs in (C) ventral caudate and (D) mPFC, both of which exhibited an increase in activation from early to late extinction for high magnitude CS (all *p*'s < .05), and no change for low magnitude CS (all *p*'s > .05). Error bars represent SEM.

Figure 2.5 Map of the ECN and Regions Showing Enhanced Connectivity with ECN During Extinction as Compared to Acquisition in Experiment 1

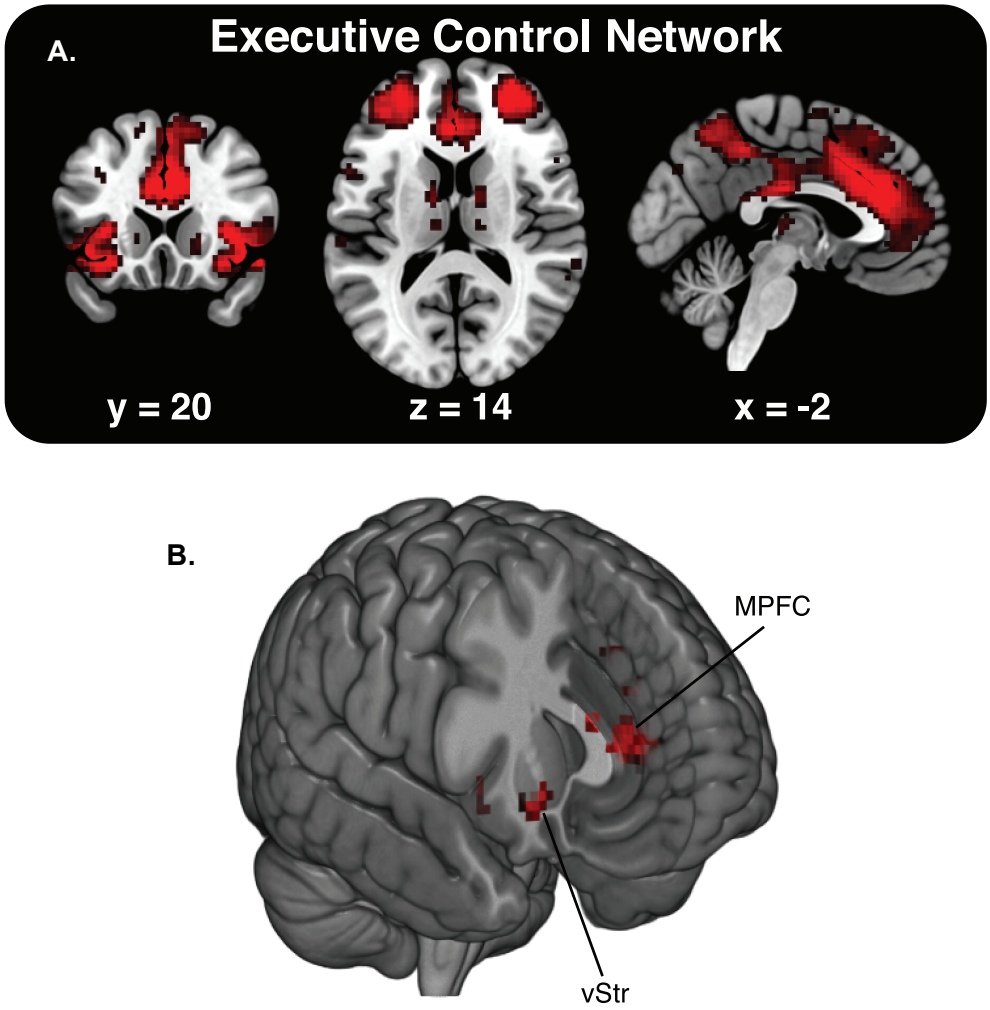

Figure 2.5. Map of the ECN and Regions Showing Enhanced Connectivity with ECN During Extinction as Compared to Acquisition in Experiment 1. (A) We used independent component analysis (ICA) to identify 20 large-scale neural networks, many of which correlated with cognitive and sensory networks from previous literature (Smith et al., 2009). In particular, we focused on one network in our data that strongly resembled the executive control network (ECN), visualized in red. (B) Our analysis identified several regions, including medial prefrontal cortex (mPFC) and ventral putamen (vStr) that showed enhanced functional connectivity with the ECN during extinction as compared to acquisition.

Figure 3.1 Task Schematic for Experiment 2

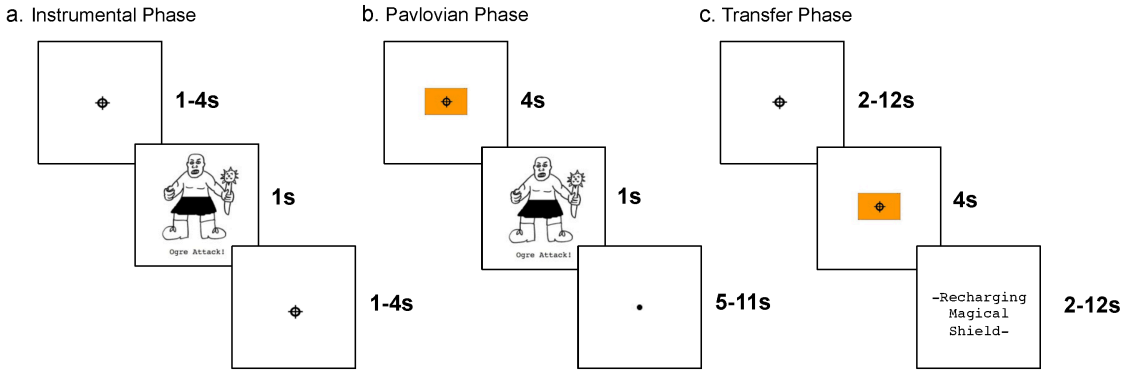

Figure 3.1. Task Schematic for Experiment 2. (A) Instrumental phase. An aversive outcome, with a duration of 1s, occurred after each 1s fixation. Participants were free to respond using R1 and R2. The correct instrumental response, when made during the fixation period, prolonged the onset of the subsequent aversive event by an additional 3s. Participants underwent two blocks of instrumental conditioning, each with a separate R-O contingency. (B) Pavlovian phase. Participants passively viewed 5 S-O contingencies, in random order, and were explicitly told to remember the contingencies presented. (C) PIT test. Participants were shown S1-S5, in random order, each preceded by a fixation and followed by a "recharge" period. Participants were explicitly told to not perform instrumental responses during the recharge period, but were free to perform R1 and R2 as they saw fit at any other period in time.

Figure 3.2 Behavioral Results for Experiment 2

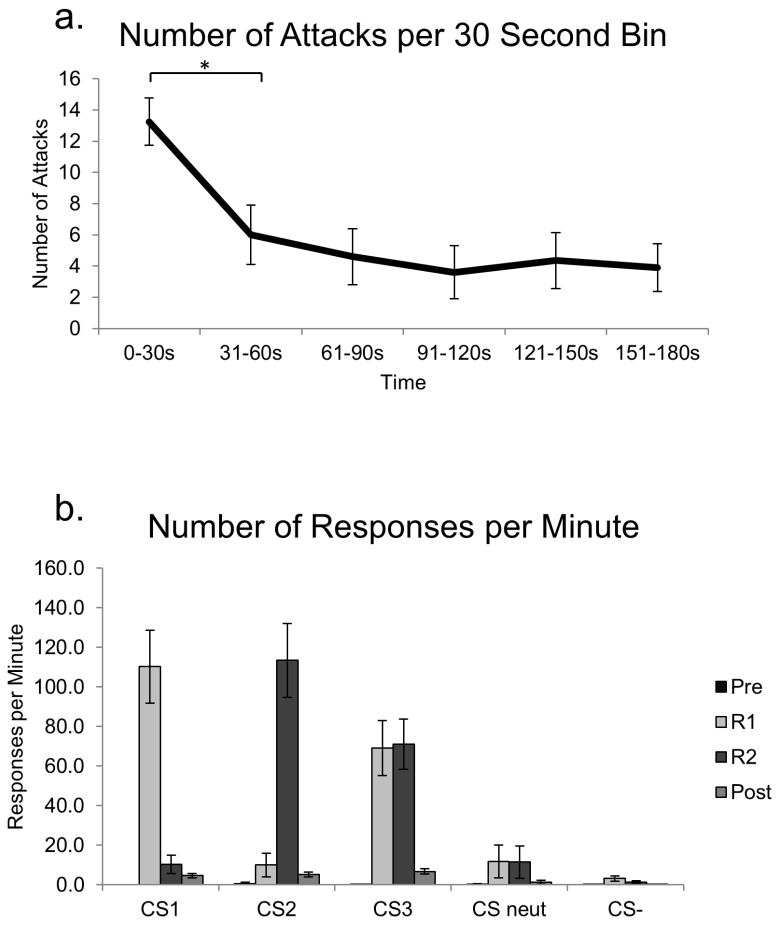

Figure 3.2. Behavioral Results for Experiment 2. (A) Number of attacks per 30 second bin during the instrumental phase. Participants experienced significantly fewer attacks during the last 30 seconds as compared to the first 30 seconds, indicating that learning of the correct R-O contingencies had occurred ($p < .001$). (B) Number of responses per minute, by trial type, during the PIT test. Specific transfer effects occurred in response to S1 and S2, wherein responding increased selectively for one of the instrumental responses (R1 or R2), but not the other, as compared to the prestimulus period (all p's < .001). In contrast, a general transfer effect was seen in response to S3, wherein responding with R1 and R2 increased nonselectively as compared to the prestimulus period (all p's <.001). The post-stimulus "recharge" period is also graphed. Error bars represent standard error of the mean (SEM).

Figure 3.3 PIT Test Neuroimaging Results for Experiment 2

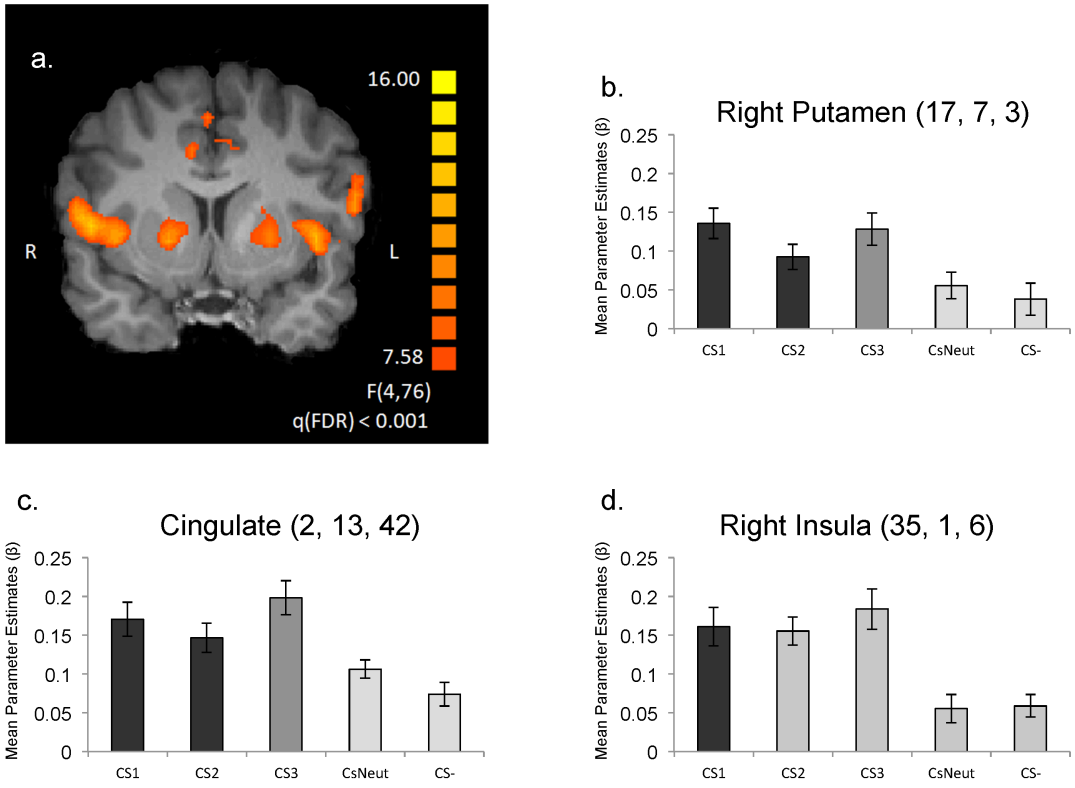

Figure 3.3. PIT Test Neuroimaging Results for Experiment 2. (A) A one-way ANOVA during the PIT test examined potential differences across S1-S5 and identified regions of interest (ROIs) in bilateral putamen, cingulate cortex and bilateral insula (y = 8). Graphs depict mean parameter estimates (β) for ROIs in (B) right putamen, (C) cingulate cortex and (D) right insula, all of which exhibited increased activation during presentation of specific and general transfer stimuli (S1-S3) as compared to the neutral stimulus, S5 (all *p*'s < .05). Error bars represent SEM.

Figure 3.4 Correlation Between Putamen Activity and Specific PIT for Experiment 2

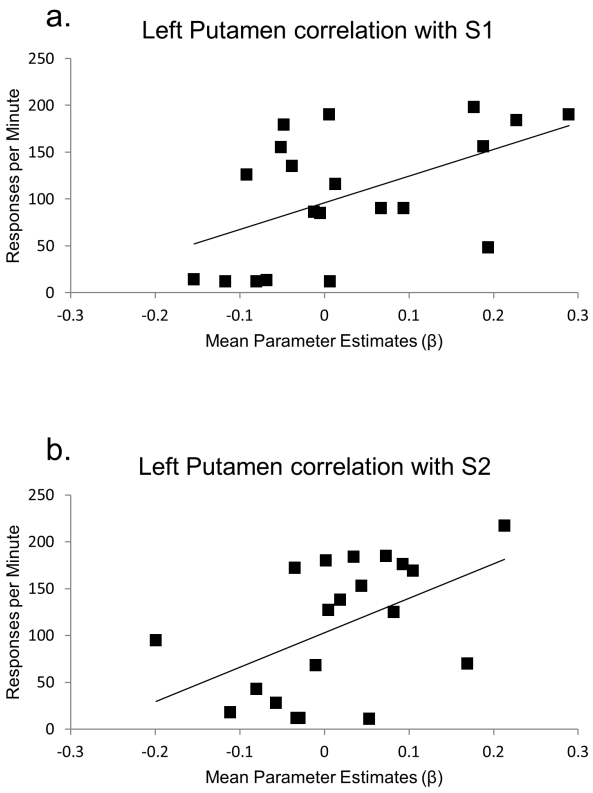

Figure 3.4. Correlation Between Putamen Activity and Specific PIT for Experiment 2. We observed a correlation between left putamen activation in the Pavlovian phase and number of specific transfer instrumental responses made during the transfer phase. Significant correlations were present between left putamen activation (*x, y,* z = -22, 4, 3) in the Pavlovian phase and subsequent number of instrumental responses made during the PIT test in the presence of both specific stimuli: (A) S1 and (B) S2. A trend for a positive correlation was present between left putamen activation in the Pavlovian phase and subsequent number of instrumental responses made in the presence of the general stimulus (C) S3 during the PIT test.

Figure 4.1 Experimental Timeline for Experiment 3

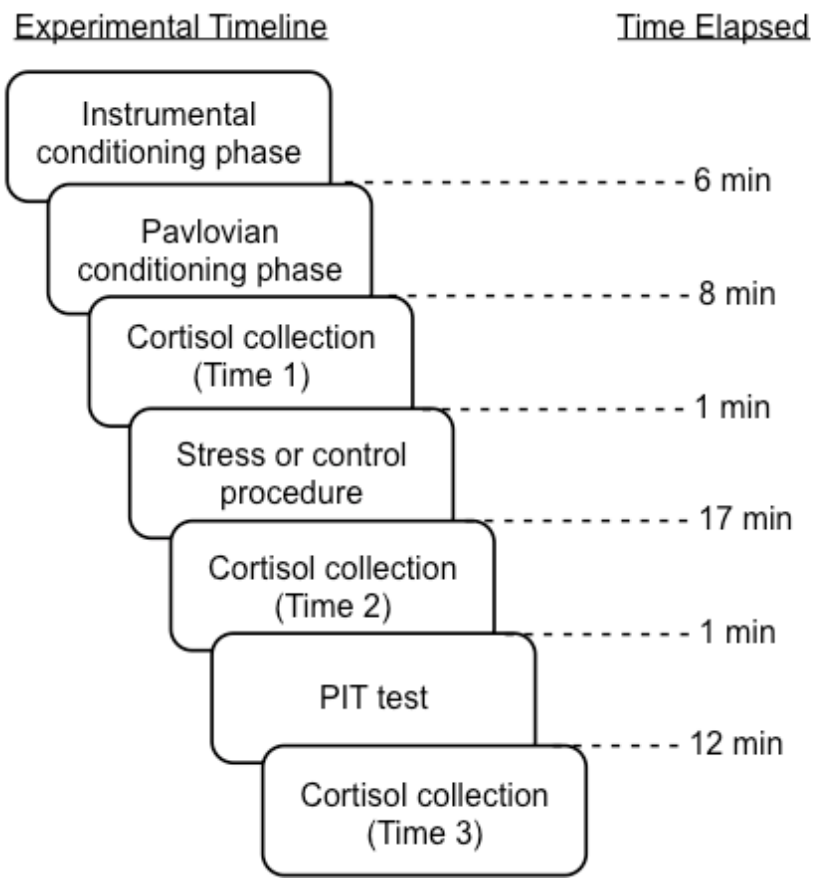

Figure 4.1. Experimental Timeline for Experiment 3. All procedures are listed, as well as the time elapsed between each procedure. Of note, the stress or control procedure lasted a total of 2 minutes, but was followed by a 15 minute break. This allowed the cortisol response to peak in the acute stress group.

Figure 4.2 Task Schematic for Experiment 3

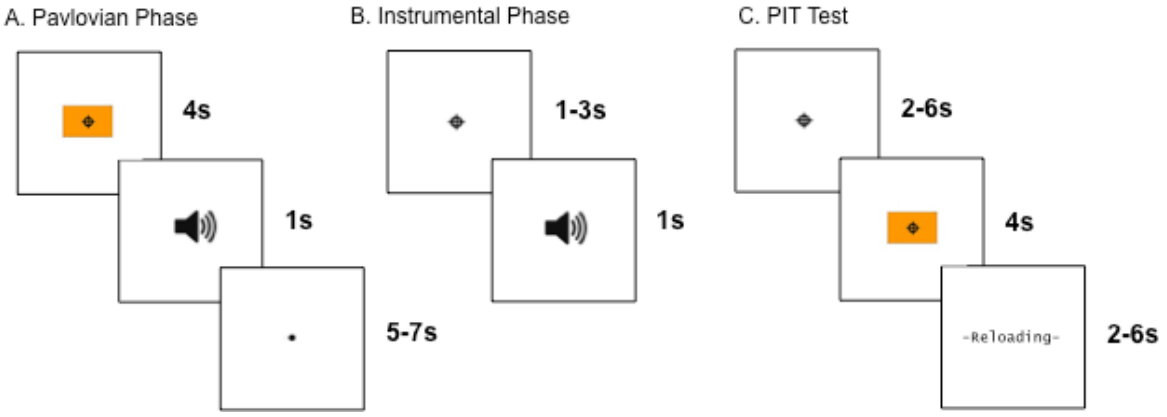

Figure 4.2. Task Schematic for Experiment 3. (A) Pavlovian phase. Participants passively viewed 4 S-O contingencies, in random order, and were explicitly told to remember the contingencies presented. (B) Instrumental phase. An aversive outcome, with a duration of 1s, occurred after each 1s fixation. Participants were free to respond using R1 and R2. The correct instrumental response, when made during the fixation period, prolonged the onset of the subsequent aversive event by an additional 2s. Participants underwent two blocks of instrumental conditioning; in each block a separate R-O contingency was learned. (C) PIT test. Participants were shown S1-S4, in random order, each preceded by a fixation and followed by a "reloading" period. Participants were explicitly told to not perform instrumental responses during the reloading period, but were free to perform R1 and R2 as they saw fit at any other period in time.

Figure 4.3 Behavioral Instrumental Conditioning Results for Experiment 3

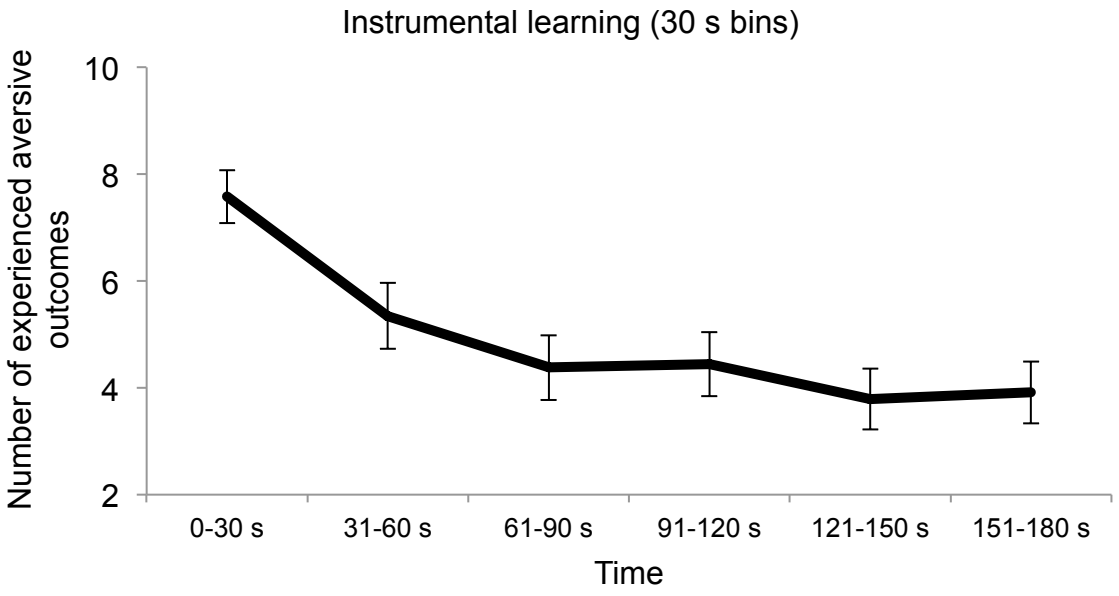

Figure 4.3. Behavioral Instrumental Conditioning Results for Experiment 3. Number of attacks (per 30 second bin) during the instrumental phase are displayed. Participants experienced significantly fewer aversive outcomes during the last 30 seconds as compared to the first 30 seconds, indicating that the correct R-O contingencies were learned ($p < 0.001$). Importantly, this decrease happened irrespective of outcome type or stress condition (all p's < 0.001). Error bars represent standard error of the mean (SEM).

Figure 4.4 Behavioral PIT Test Results

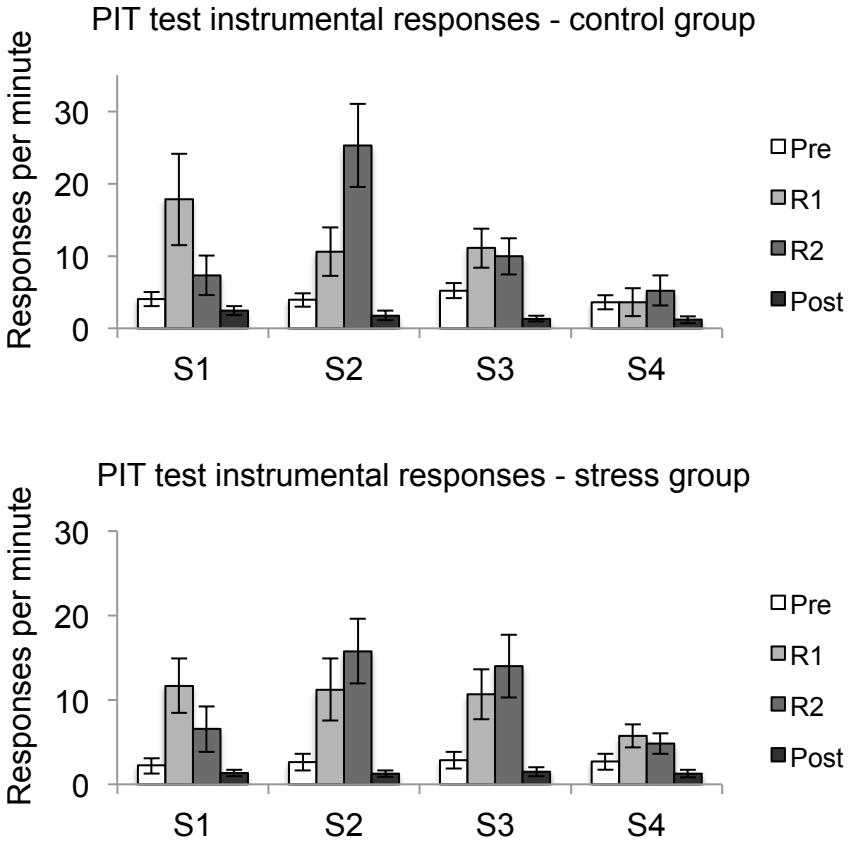

Figure 4.4. Behavioral PIT Test Results. Number of responses per minute, by trial type, are displayed. (A) Control group results. A specific transfer effect occurred in response to S1, wherein responding increased selectively R1, but not R2, as compared to the prestimulus period ($p < .05$). In the presence of S2, responding with both R1 and R2 was significantly elevated as compared to the prestimulus period (all p's <0.05). However, R2 responding was significantly higher than R1 responding ($p < 0.01$), which we consider here to be a manifestation of specific PIT. A general transfer effect was seen in response to S3, wherein responding both with R1 and R2 increased nonselectively as compared to the prestimulus period (all p's < 0.05). In the presence of S4, there was no increase for either R1 or R2 responding as compared to the prestimulus period. The post-stimulus "recharge" period is also graphed. (B) Acute stress group results. For S1, S2, and S3, R1 and R2 responding was elevated above responding during the prestimulus period (all p's < 0.05). For all three of these stimuli, the number of responses made with R1 and R2 did not significantly differ (all p's > 0.05). Therefore, a general enhancement in motivated behavior was made in the presence of S1-S3, but specific PIT did not occur for S1 and S2. There were no significant increases in R1 or R2 responding above the prestimulus period for S4 (all p's > 0.05). Error bars represent SEM.

Figure 4.5 Physiological PIT Test Results

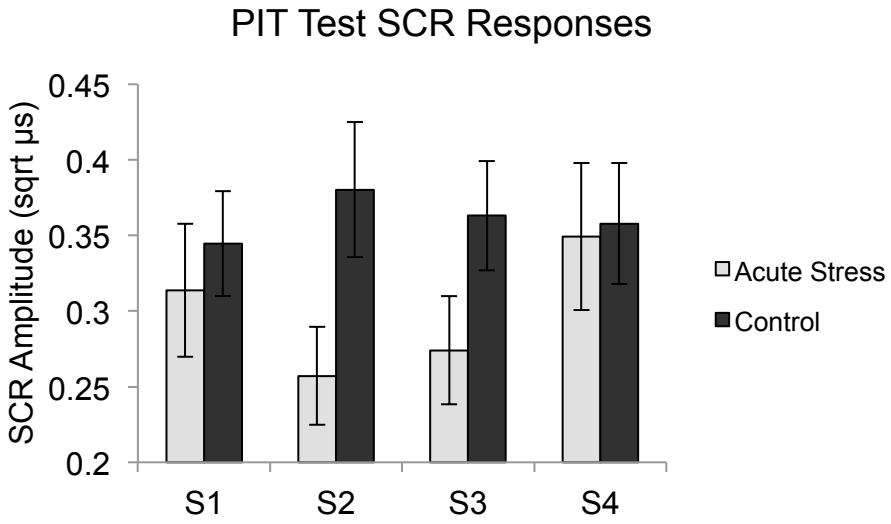

Figure 4.5. Physiological PIT Test Results. A 2 x 2 ANOVA examined the effects of stimulus type and stress group on SCR amplitude during the PIT test. SCR were elevated in the control group as compared to the acute stress group for S2 ($p < 0.05$), but did not significantly differ across groups for any of the other stimuli.

Table 2.1 Extinction Phase 2 x 2 x 2 ANOVA: Regions showing a Main Effect of Valence, $p < 0.005$, Cluster Threshold Corrected

			Talairach Coordinates				
Region of Activation	*BA*	*Laterality*	*x*	*y*	*z*	*Voxels (mm^3)*	*F*
Occipital Cortex	19	L	-4	-83	36	218	15.74
Precuneus	31	R	20	-53	24	429	23.38
Posterior Cingulate	30	R	23	-62	9	268	19.36
Parahippocampal Gyrus	30	R	17	-44	3	262	28.42
Parahippocampal Gyrus	36	R	29	-38	-9	546	22.32
Middle Frontal Gyrus	11	L	-25	35	-12	1301	28.45

BA = Brodmann's area; L = left; R = right

Table 2.2 Extinction Phase 2 x 2 x 2 ANOVA: Regions showing a Valence x Block Interaction, $p < 0.005$, Cluster Threshold Corrected

			Talairach Coordinates				
Region of Activation	*BA*	*Laterality*	*x*	*y*	*z*	*Voxels (mm^3)*	*F*
Middle Frontal Gyrus	6	R	23	-2	39	236	28.76
Medial Frontal Gyrus	10	L	-16	49	15	422	44.61
Inferior Frontal Gyrus	32	R	23	34	6	167	16.37
Caudate		L	-16	13	6	98	24.11

BA = Brodmann's area; L = left; R = right

Table 2.3 Extinction Phase 2 x 2 x 2 ANOVA: Regions showing a Main Effect of Magnitude, $p < 0.005$, Cluster Threshold Corrected

			Talairach Coordinates				
Region of Activation	*BA*	*Laterality*	*x*	*y*	*z*	*Voxels (mm^3)*	*F*
Postcentral Gyrus	3	R	11	-35	63	7738	42.47
Parietal Cortex	7	L	-16	-44	60	8543	83.55
Inferior Parietal Cortex	40	R	47	-35	30	796	24.47
Middle Temporal Gyrus	22	L	-55	-41	3	426	21.61

BA = Brodmann's area; L = left; R = right

Table 2.4 Extinction Phase 2 x 2 x 2 ANOVA: Regions showing a Magnitude x Block Interaction, $p < 0.005$, Cluster Threshold Corrected

			Talairach Coordinates				
Region of Activation	*BA*	*Laterality*	*x*	*y*	*z*	*Voxels (mm^3)*	*F*
Caudate		R	11	10	12	268	23.41
Medial Prefrontal Cortex	24	R	2	34	6	639	20.71
Putamen		L	-28	-2	6	629	25.32
Ventral Caudate	25	L	-4	4	-3	503	22.94
Superior Temporal Gyrus	38	L	-37	1	-18	272	22.97
Cerebellum		R	17	-68	-18	224	45.57

BA = Brodmann's area; L = left; R = right

Table 2.5 Extinction Phase 2 x 2 x 2 ANOVA: Regions showing a Main Effect of Time, $p < 0.005$, Cluster Threshold Corrected

			Talairach Coordinates				
Region of Activation	*BA*	*Laterality*	*x*	*y*	*z*	*Voxels (mm^3)*	*F*
Precentral Gyrus	4	R	32	-26	60	360	21.52
Superior Frontal Gyrus	8	L	-10	55	45	967	21.82
Inferior Parietal Lobe	40	R	59	-35	30	398	30.27
Middle Temporal Gyrus	39	R	-40	-62	21	422	27.31
Superior Temporal Gyrus	13	L	-46	-17	9	896	34.66
Precentral Gyrus	43	R	56	-5	9	697	29.24
Putamen		L	-25	-5	9	528	25.77
Insula	13	R	41	-8	9	1136	29.91
Occipital Lobe	18	R	5	-71	3	733	20.75
Occipital Lobe	17	L	-19	-83	0	484	24.71
Cerebellum		L	-4	-68	-6	607	23.55
Putamen		R	20	4	-9	1496	31.46

BA = Brodmann's area; L = left; R = right

Table 2.6 Extinction Phase 2 x 2 x 2 ANOVA: Regions showing a Valence x Magnitude Interaction, $p < 0.005$, Cluster Threshold Corrected

			Talairach Coordinates				
Region of Activation	*BA*	*Laterality*	*x*	*y*	*z*	*Voxels (mm^3)*	*F*
Medial Frontal Gyrus	6	L	-4	-17	63	288	19.49
Superior Parietal Lobule	7	R	11	-62	57	320	21.69
Postcentral Gyrus	3	R	41	-20	51	2074	45.48
Cingulate Gyrus	31	L	-13	-41	42	664	23.75
Superior Temporal Gyrus	42	R	62	-29	15	415	21.31
Occipital Cortex	17	L	-13	-95	6	8721	45.97

BA = Brodmann's area; L = left; R = right

Table 2.7 Regions showing enhanced functional connectivity with ECN during Extinction as Compared to Acquisition, $p < 0.05$, Whole-Brain Corrected for Multiple Voxelwise Comparisons

			Talairach Coordinates			
Region of Activation	*BA*	*Laterality*	*x*	*y*	*z*	*Voxels (mm^3)*
Frontal Pole	10	L	-27	47	35	72
Precuneus	7	R	15	-64	32	31
Precuneus	7	L	-15	-67	29	32
Paracingulate Gyrus	9		0	35	29	1
Medial Prefrontal Cortex	32		0	41	14	67
Putamen		R	18	14	-10	3

BA = Brodmann's area; L = left; R = right

Table 3.1 Contingencies Present in Experimental PIT Paradigm

Instrumental phase	*Pavlovian phase*	*Transfer test*
R1-O1	S1-O1	S1: R1 vs. R2
R2-O2	S2-O2	S2: R1 vs. R2
	S3-O3	S3: R1 vs. R2
	S4-O4	S4: R1 vs. R2
	S5-O5	S5: R1 vs. R2

Table 3.2 Regions of Activation in a One-way ANOVA During the PIT Test, *q*(FDR) < .001

			Talairach Coordinates				
Region of Activation	*BA*	*Laterality*	*x*	*y*	*z*	*Voxels (mm^3)*	*F*
Medial Frontal Gyrus	6	L	-4	-8	51	3041	15.43
Cingulate Cortex	32	R	2	13	42	679	11.75
Inferior Parietal Cortex	40	L	-49	-32	42	20493	32.45
Inferior Parietal Cortex	40	R	44	-35	42	3234	15.94
Occipital Cortex	19	R	17	-83	30	481	16.40
Superior Frontal Gyrus	9	L	-37	37	30	150	10.10
Occipital Cortex	18	L	-16	-83	27	1083	12.00
Postcentral Gyrus	3	L	56	-17	24	140	9.71
Inferior Frontal Gyrus	44	L	-52	4	21	276	21.01
Thalamus		R	11	-14	9	525	12.77
Thalamus		L	-16	-17	9	917	27.70
Insula		R	35	1	6	4404	19.00
Putamen		L	-22	4	6	842	16.95
Putamen		R	17	7	3	1116	12.77
Putamen		L	-31	-11	3	811	16.15
Insula		L	-40	3	3	787	20.15
Inferior Frontal Gyrus	47	L	-40	31	-3	474	13.86
Cerebellum		R	11	-47	-21	6280	29.47
Cerebellum		L	-40	-47	-27	319	11.55

BA = Brodmann's area; L = left; R = right; FDR = false discovery rate.

Table 4.1 Contingencies Present in Experimental PIT Paradigm

Pavlovian phase	*Instrumental phase*	*Transfer test*
S1-O1	R1-O1	S1: R1 vs. R2
S2-O2	R2-O2	S2: R1 vs. R2
S3-O3		S3: R1 vs. R2
S4-O4		S4: R1 vs. R2