MODIFYING MEMORIES: PARSING MOTIVATIONAL AND PREDICTIVE VALUE

by

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Dedication

To the loving memory of my mom: Theresa Ines Valdes Nilla

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Abstract

During memory retrieval, previously consolidated memories enter a labile state, rendering them vulnerable to disruption and/or modification. Thus, prior to reconsolidation, it is possible to manipulate or disrupt memory. Studies have demonstrated that it is possible to disrupt the reconsolidation of Pavlovian memories where a discrete stimulus (the conditioned stimulus, CS - e.g. tone) is paired with an appetitive (e.g. food) or aversive (e.g. shock) unconditioned stimulus (US). In these experiments, manipulation after memory retrieval can result in a decreased response to the CS. It has often been assumed that disrupting reconsolidation affects the entire memory. However, in a recent human Pavlovian conditioning study, researchers demonstrated that the beta-adrenergic antagonist, propranolol, can disrupt one component of a memory, without affecting other components. They suggest that propranolol does not disrupt reconsolidation by erasing memory; but rather it disrupts the affective or motivational fear response to the CS. In this dissertation, we ask if propranolol differentially affects motivational and predictive components of a CS-US association in an animal model of appetitive conditioning.

It has been shown that there is considerable individual variation in the extent to which reward-paired cues acquire motivational value. In a Pavlovian conditioned approach (PCA) task, a lever conditioned stimulus (CS) is presented and followed by the immediate delivery of a food reward. After rats learn this association, some animals will approach and interact with the lever

itself upon CS presentation (sign-trackers, STs - Hearst & Jenkins, 1974), while others will approach the location of reward delivery (goal-trackers, GTs - Boakes, 1977). We hypothesize these behavioral differences to be due to differentially attributing motivational value to reward-paired cues. That is, in GTs a CS acquires predictive value, while in STs a CS acquires predictive *and* motivational value. However, not all stimuli evoke the same behaviors, nor do they acquire motivational value to the same degree. For example, a tone CS does not evoke sign-tracking, but rather goal-tracking in all animals (even STs), and it does not appear to acquire incentive-motivational properties to the extent of a lever CS (Meyer, Cogan, & Robinson, 2014; Beckmann & Chow, 2015).

The experiments in this dissertation use the model of individual differences described above to determine whether propranolol differentially disrupts the reconsolidation of motivational or predictive components of an appetitive memory in rats. Given that others have suggested propranolol selectively disrupts motivational components of a memory, we explore whether propranolol can disrupt memory for stimuli that acquire motivational value to lesser extents, such as a tone CS. Our lab has previously found that reward-paired cues engage mesocorticolimbic or 'motive circuit' brain regions in STs and GTs. Thus, we also explore how propranolol affects the engagement of brain regions, particularly those involved in motivate by reward-paired cues, and whether a tone CS can engage the same regions as a lever CS.

In Chapter Two, I administered propranolol after retrieving a lever CS memory in STs and GTs, and found that propranolol selectively disrupts sign-tracking but not goal-tracking behavior. This suggests that propranolol disrupts reconsolidation in rats by affecting the motivational component, but not the predictive component of memory. In Chapter Three, I found that propranolol does not disrupt goal-tracking to a tone CS, suggesting that propranolol only

disrupts motivational value of cues that acquire such value. Lastly, in Chapter Four, I found that propranolol decreases the extent to which cues engage 'motive circuit' brain regions in STs. We conclude that propranolol does not erase memory, but rather degrades emotional/motivational value. Together these findings provide a preclinical model that can be used to further treatments for disorders that may be exacerbated by reward- or trauma-paired cues.

Chapter I

Introduction

Learning: Pavlovian CS-US Associations

The associations formed between rewarding or aversive stimuli and the cues that predict them influence behavior in ways that can be crucial for survival. For example, the sound of leaves rustling (e.g. cue) may indicate a nearby predator (e.g. aversive stimulus) for a field mouse, causing the mouse to run and hide. Likewise, perhaps the sound of the mouse scurrying through the forest alerted the predator to approach this potential food source. That is to say, stimuli paired with specific conditions serve as cues to trigger specific patterns of behavioral responses. Originally termed "conditional reflexes", these behavioral responses were characterized by Ivan Pavlov (1927) through his iconic classical conditioning experiments. In his initial studies, Pavlov measured salivary secretions (an unconditioned response, UR) produced by dogs in response to food (an unconditioned stimulus, US). The dogs received presentations of a neutral stimulus, in this case the sound of a metronome, paired with food. He observed that the sound of the metronome (now a conditioned stimulus, CS) began to elicit a similar salivary response as the food itself, thus forming a conditioned response (CR) to the presentation of the CS. This illustration of a CS to predict the US and acquire some properties of the US is a topic of significant interest to many researchers, and has been well studied over the past several decades.

Early discussions of Pavlovian conditioning often oversimplified the process by which CS-US associations are learned; many researchers neglected to mention that these associations only occur under certain conditions (Rescorla, 1988). Pavlov's use of the term "conditional *reflexes*" is misleading in itself, suggesting that CRs are as simple and automatic as the reflex of the knee to extend upon being tapped by a hammer. However, these associations have a number of constraints and boundaries under which they are learned. In order for a neutral stimulus to become a conditioned predictor of a reward/aversion (US), this stimulus must be relevant in some way to the US. Specifically, a CS must be presented contingently, it must be salient relative to other stimuli in the environment, and it must provide information about the US.

The first crucial factor in the conditioning of a neutral stimulus is that it must be presented contingently with the US (Bilbrey & Winokur, 1973; Blanchard & Honig, 1976). In other words, stimuli must be presented in a time-dependent, consistent manner in which a neutral stimulus predicts the US. For example, if a neutral stimulus and a US occur in close time proximity, and the neutral stimulus predicts the US with a high probability, the neutral stimulus will become a conditioned stimulus. However, if the neutral stimulus and a US are presented randomly and intermittently, the neutral stimulus does not reliably predict the occurrence of the US and thus conditioning of the neutral stimulus will not occur (Rescorla, 1968).

Second, the neutral stimulus must be more salient than other stimuli in the environment. In nature, multiple sensory cues are available at any given time; in order to become a reliable predictor, a neutral stimulus must first be distinguishable from other sensory cues that may be present. For example, in a task where a rat must learn to suppress bar-pressing in the presence of a tone signaling shock, learning will occur faster with louder (i.e. more salient) tones (Kamin, 1969). Salient stimuli are more likely to attract attention toward them, which is required for an

association to be formed. That is, if a stimulus does not stand out in an otherwise busy environment, nothing will be learned about it.

Third, a neutral stimulus must be able to predict information [relative to other cues] in order to become conditioned (Pavlov, 1927). If a CS-US association has been formed, and another neutral stimulus is presented with the CS, this neutral stimulus will not become a secondary conditioned stimulus. This is because there is nothing new to learn. The CS already predicts the US, and therefore the second stimulus does not provide any new information that would be relevant to the US (Kamin, 1969; but see Holland et al., 2014). Additionally, animals learn more (measured by CRs) about stimuli that predict a US with high probability, even in the presence of other stimuli that predict this same US at a lower probability. For instance, presentation of neutral stimulus A results in the delivery of a reward every single time, while presentation of neutral stimulus B results in the delivery of this same reward, but this only occurs half of the time. In this case, A is a better predictor than B, thus, more will be learned about A. However, if A and B equally predict the reward half of the time, subjects will respond equally to both of these cues. In other words, individuals will learn to respond to stimuli that predict a reinforcer at a high probability relative to the other stimuli that are available at the time (Wagner, 1970).

The CRs evoked by Pavlovian conditioned stimuli are quite complex because stimuli represented by a Pavlovian CS evoke complex motivational states by themselves. Most Pavlovian conditioned cues predict stimuli that are necessary for survival (i.e. food or water). This must be considered in the conditions under which Pavlovian associations are learned, as many different types of cues (i.e. auditory, visual) can predict rewards/aversions. The behaviors required for obtaining such stimuli can range across a spectrum, as they must be flexible in order

to adapt to different circumstances (see Culler, 1938; Fanselow & Wassum, 2016). That is, obtaining unconditioned rewarding stimuli (e.g. food) may require a number of different behavioral responses. Timberlake (1994) proposed a hierarchical model in which he describes strategies and subsequently generated behaviors required for approaching different situations. The model proposes five levels. First, there is an underlying motivation (system) or drive (e.g. hunger) for initiating a sequence of behaviors (e.g. seeking). Second, there is a general approach (subsystem) that must be used to satisfy this hunger state; this approach could be predatory, or it could be engaging in seeking behavior to find a food source. Third, there must be a strategy (mode) employed to find a food source once the general approach is determined. If there are no food sources in sight, this would require general search behavior. Fourth, a motor strategy (module) must be used to pursue the food source once it is localized. These can range from aggressive strategies, such as chasing a food source, to more passive strategies, such as cautiously approaching it. Fifth, this sequence will lead to a behavioral output (action), which will be specific to the circumstances determined by the preceding strategies in the model. There are multiple actions that can be generated from one initial 'system' cascade. In the example of hunger, the actions may include moving toward or approaching a food source, grabbing and manipulating the food source, chewing and swallowing, and carrying any leftover food to store for later consumption, or to bring back to a nest. The proximity of the food source at any given level can largely influence subsequent behavior (see Figure 1.1). Each one of these actions, though generated sequentially to fulfill one hunger state, requires its own subsystem, mode, and module. That is, each action requires constant updates of the circumstance in order to successfully obtain the food.

A CS can evoke different components of the behavioral strategies outlined by Timberlake partly because a CS can acquire different associations with different components of the same US. Delamater and Oakeshott (2007) suggest a schematic of the possible features of an appetitive US including sensory, hedonic, motivational, temporal, and response features; all (or some) of which can be acquired associations by a CS. These different features allow the CS to provide different information about a US, which can also have different components. This leads to a large range of characteristics that are internally represented by the CS. Thus, the CR that is expressed by a CS can be extremely variable; an observation that was noted by Pavlov in his original studies:

> "The essential feature of the highest activity of the central nervous system …consists not in the fact that innumerable signaling stimuli do initiate reflex reactions in the animal, but in the fact that under different conditions these same stimuli may initiate quite different reflex reactions"
> (Pavlov, 1927, Conditioned Reflexes, Lecture I, p. 14)

That is, the same CS paired with the same US can evoke different CRs in an animal as a function of either internal or external circumstances.

Pavlovian CRs to a CS can vary with internal changes such as motivational 'state' or 'drive' (Bindra & Palfai, 1967; Mowrer, 1940; Baumeister, Hawkins, & Cromwell, 1964). For example, Bindra and Palfai (1967) measured investigatory, approach, and grooming behavior to a CS in rats that were either water deprived (high drive), or non-water deprived (low drive). Upon CS exposure after conditioning, they found that high drive rats exhibited greater levels of approach and investigatory behavior, while their low drive counterparts exhibited more grooming behavior. These behavioral differences are likely due to the fact that hunger or states of water deprivation increase locomotion and general activity (Baumeister et al., 1964; Bindra, 1968). This study demonstrates that a CS can come to evoke motivational states to the same extent as the US itself.

The nature of the CS itself can also influence conditioning (Holland, 1977; Holland et al., 2014; Meyer et al., 2014; Beckmann & Chow, 2015; Sigmundi & Bolles, 1983; Sigmundi, Bouton, & Bolles, 1980; Linwick, Patterson, & Overmier, 1981). For example, many species will more readily learn to associate a visual stimulus with an appetitive US, while they will more readily learn to associate auditory stimuli with an aversive US (Foree & LoLordo, 1973; Jacobs & LoLordo, 1977; Shettleworth, 1972). The reasons underlying the differences in conditioning between different CS modalities can be explained by the adaptive values of such cues. As mentioned earlier, CS-US relationships are more readily formed between more relatable stimuli. The reflexive responses elicited by auditory and visual stimuli prior to conditioning are quite different. For example, a visual light CS evokes higher levels of rearing behavior than a tone CS (Holland, 1977, 1979). Holland argues that different CRs emerge as a result of these stimuli having different characteristics that require different actions in order to obtain them, much like they would for obtaining unconditioned stimuli. Additionally, rats will not readily learn an association between a neutral gustatory stimulus and an external shock (Garcia & Koelling, 1966). The likelihood of a neutral stimulus becoming a CS requires that the modality must be relatable to the US. That is to say, associations are formed in evolutionarily adaptive ways, and the probability of an external noxious stimulus predicting the internal feeling of illness is low. Thus, the acquisition of an aversive association in this case will be of low likelihood.

Stimuli in the environment can serve as valuable predictors of reward or aversion under certain boundaries, and they can elicit innumerable unconditioned and conditioned responses. Studying the psychological and neurobiological mechanisms by which cues can evoke different CRs is crucial for understanding how evolutionarily adaptive processes (e.g. learning about cues in the environment that predict the presence of stimuli that are crucial for survival) can

sometimes drive behavior in maladaptive ways. Particularly, it is important to understand the mechanisms by which these associations are formed, and how they persist.

Memory: Strengthening of Neural Associations

Consolidation

Physiological changes accompany Pavlovian learning, and play a role in the storage and maintenance of CS-US associations. Donald Hebb (1949) expanded upon the establishment and persistence of neural connections underlying Pavlovian learning in his book, "The Organization of Behavior". In referencing Pavlovian learning, he states:

> "Learning… consists of a *lasting change of facilitations* between the activities of specific neural structures. The change results when two structures (single pathways or assemblies) that have sufficient anatomical connections are active at the same time."
> (Donald Hebb, 1949, The Organization of Behavior, p.180)

These are the assumptions under which "neurons that fire together, wire together". With this assertion, Hebb discusses the "memory trace" – a concept which integrates neural activity and psychological associations into the explanation of a lasting memory. The process by which learned associations are transformed into stable traces in the brain is called 'memory consolidation' (Müller & Pilzecker, 1900).

Memory consolidation was first recognized as a concept in the early 1900's (Müller & Pilzecker, 1900). Three important components of memory consolidation are; 1) it is a time dependent process, 2) it produces stable traces, and 3) these traces are accompanied by structural changes. These ideas were presented long before any neurobiological evidence of consolidation, however many studies have subsequently provided evidence for these components (see McGaugh, 1966; Wang, Hu, & Tsien, 2006 for review).

Memory is a time-dependent process in that it takes time for a memory to undergo consolidation. Early case studies of amnesia in patients having undergone electroconvulsive shock treatment (ECST) drew attention to the fact that sometimes events or experiences are not recalled (see Burnham, 1903). Later studies began to use animal models in order to further explore this phenomenon, and found that memories were only disrupted if a manipulation was applied within a specific time frame after each trial or training session (Duncan, 1949). If this window passed without interruption, then the memory would be consolidated and available for future recall. The window for consolidation is roughly six hours (McGaugh, 1969), although there have been some reports of disrupting consolidation at later time points (Taubenfeld, Milekic, Monti, & Alberini, 2001; Bekinschtein et al., 2007).

Not all experiences are consolidated into stable, *long-lasting* traces. Ebbinghaus (1913) outlined the existence of different functional traces in memory. The dual trace hypothesis proposes that two separate traces occur at the time of simultaneous stimulation; one supporting a short term memory process (STM), and with repeated reverberation, one supporting a long term memory (LTM) (Ebbinghaus, 1913; Hebb, 1949). STMs begin to decay shortly after they are formed, and are not consolidated. Although STM starts decaying almost immediately, this does not affect the ability of STM to be consolidated into LTM. This provides support for the existence of two separate traces, rather than one trace (short-term) that is later converted into a long-term trace (Wickelgren & Berian, 1971; Wickelgren & Norman, 1966); although, at this point experimental evidence has not been sufficient to confirm this. LTMs are consolidated within hours, and these memories were initially thought to be permanent (Duncan, 1949; Agranoff, Davis, & Brink, 1965; Gold & McGaugh, 1975; McGaugh, 1966; Izquierdo et al., 1998; but see Revelle & Loftus, 1992; Revelle & Loftus, 1990)

The formation and maintenance of memories are mediated by structural changes such as an increase or decrease of activity within neuronal connections, or synapses. These observed changes, termed 'synaptic plasticity', provide a useful model for studying the physiological basis of memory. Although synaptic plasticity is involved in many different processes (i.e. motor reflexes, sensory processing) and can occur in many different forms, here, synaptic plasticity will only be discussed in relation to memory.

Mechanisms of Structural Plasticity in Consolidation

Protein Synthesis. Early on, researchers identified a role for protein synthesis in memory formation, and found that it was specific to consolidated (not short-term) memories (Flexner, Flexner, & Stellar, 1963; Agranoff et al., 1965; Goelet, Castellucci, Schacher, & Kandel, 1986; Davis & Squire, 1984). The synthesis of new proteins is required for the structural changes induced by an intracellular cascade. Thus, protein synthesis inhibition can affect memory consolidation by preventing structural changes, although the exact mechanisms by which this occurs are debatable (Radulovic & Tronson, 2008). There are a number of different effectors upstream of protein synthesis that have also been implicated in memory consolidation, detailed (in order) below.

Neurotransmitters. Glutamatergic signaling is crucial for memory consolidation (Bliss & Collingridge, 1993; Morris, Anderson, Lynch, & Baudry, 1986) and plays an important role in other downstream effectors. *N*-methyl-D-aspartate (NMDA) receptors activate protein kinases that upregulate the activity and insertion of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptors into the post-synaptic membrane. This addition of AMPA receptors increases the probability of the cell becoming active, feeding back into the activation of NMDA receptors. Both AMPA receptors and NMDA receptors are, indeed, necessary for memory consolidation

(Kim, Fanselow, DeCola, & Landeira-Fernandez, 1992; Rogan, Stäubli, & LeDoux, 1997; Rumpel, LeDoux, Zador, & Malinow, 2005).

Adrenergic signaling is also crucial in modulating consolidation, particularly for arousing or emotional events (Gold & Van Buskirk, 1975; Cahill, Prins, Weber, & McGaugh, 1994; Liang, Juler, & McGaugh, 1986; Schramm, Everitt, & Milton, 2016). This is evolutionarily beneficial because remembering stressful events can be important for survival; particularly in learning how to adapt and avoid those events in the future. Specific adrenergic alpha receptor subtypes have been found to differentially modulate memory consolidation (see Arnsten, Steere, Jentsch, & Li, 1997 for review). Beta-adrenergic activation has been demonstrated to disrupt memories that involve arousal in humans (Cahill et al., 1994; Cahill & McGaugh, 1996; Nielson & Jensen, 1994; van Stegeren, Everaerd, Cahill, McGaugh, & Gooren, 1998) and rats (Liang et al., 1986; Cahill & McGaugh, 1996; Salinas, Introini-Collison, Dalmaz, & McGaugh, 1997).

Many other neurotransmitters have also been found to modulate memory consolidation by disrupting or facilitating the formation of a memory (see McGaugh, 1973, 2015). These include dopamine (Gozzani & Izquierdo, 1976; LaLumiere, Nawar, & McGaugh, 2005), acetylcholine (Passani et al., 2001; Hasselmo, 2006), serotonin (Meneses, Terrón, & Hong, 1997; Corradetti, Ballerini, Pugliese, & Pepeu, 1992), and endocannabinoids (Yim, Hong, Ejaredar, McKenna, & McDonald, 2008).

Intracellular mechanisms. AMPA and NMDA receptor activation is required for induction of the intracellular cascades that promote structural changes. Further downstream, protein kinases subsequently activate transcription regulators, such as CREB. Memory consolidation requires protein kinase A activation (Nader, Schafe, & Le Doux, 2000; Schafe & LeDoux, 2000), protein kinase C activation (Weeber et al., 2000), and CREB (Josselyn et al.,

2001). Transcription regulators eventually promote new protein synthesis. These proteins regulate further structural modifications and responsivity of the neuron (e.g. changes in dendritic branching and spines, as discussed above). Thus, inhibiting protein kinases and transcription regulators affects disrupts protein synthesis and either modifies or eliminates any structural changes.

Long-term potentiation (A model for memory). Long-term potentiation (LTP) induces structural changes via post-stimulation activation or inhibition of a cell and can occur through different patterns of repeated activation at synapses (from neuronal inputs or experimentally induced stimulation). These fluctuations can continue to occur after the initial stimulation, and can last for seconds to minutes. With continued stimulation, these temporary fluctuations result in structural changes that promote long-term strengthening (long-term potentiation, LTP) or weakening (long-term depression, LTD) of the synapse, respectively (Bliss & Lømo, 1973; Douglas & Goddard, 1975).

The structural changes induced by LTP have been found to occur as a result of learning (see Carew, Walters, & Kandel, 1981). For example, increases in dendritic spine density have been observed after learning Pavlovian associations. The addition of dendritic spines and branching in the synapse allows for receptors on the post-synaptic cell to be in greater abundance and proximity to that of the pre-synaptic cell. Thus, the post-synaptic cell becomes more likely to be excited by the pre-synaptic cell. These kinds of changes can occur through a feedback loop of intracellular cascades that upregulate (or downregulate) receptors as a result of different patterns of activation (see Lamprecht & LeDoux, 2004 for review).

It's important to note here that a causal relationship between memory consolidation and LTP has not been determined. However, LTP has been suggested as a physiological model for

the formation and maintenance of memory (Rogan et al., 1997; Sigurdsson, Doyère, Cain, & LeDoux, 2007; Schafe, Doyère, & LeDoux, 2005; Tsien, Huerta, & Tonegawa, 1996; Kandel, 2001; Abraham & Williams, 2003; Schafe, Nader, Blair, & LeDoux, 2001), as they share many common mechanisms. For example, protein synthesis (Goelet et al., 1986; Flexner et al., 1963; Davis & Squire, 1984; McGaugh, 2000; Kandel, 2001), glutamate (see Bortolotto, Fitzjohn, & Collingridge, 1999; Murphy & Glanzman, 1997), dopamine (Li, Cullen, Anwyl, & Rowan, 2003; Floresco, Blaha, Yang, & Phillips, 2001; Frey, Matthies, Reymann, & Matthies, 1991; see Jay, 2003 for review) protein kinase A (Huang & Kandel, 1998; Abel et al., 1997) protein kinase C (Malinow, Madison, & Tsien, 1988; Ben-Ari, Aniksztejn, & Bregestovski, 1992), and CREB (Silva, Kogan, Frankland, & Kida, 1998; Tronson, Corcoran, Jovasevic, & Radulovic, 2012) have all been demonstrated to affect LTP induction and/or maintenance. The correlation between mechanisms underlying both behavioral evidence and physiological models of memory provide an opportunity to study memory from a multidisciplinary perspective.

After being consolidated, the idea of persistence and stability of a memory faces many challenges. Despite experimental demonstrations of disrupting consolidation, a debate persisted regarding the stability of a memory once it has been consolidated. One major challenge to the concept of a fixed memory trace is the proposal of a "reconsolidation" phase, suggestive that a memory can, after initial consolidation, return to flexible states and become vulnerable to disruption.

Memory: Reconsolidating Neural Connections

Reconsolidation

Misanin, Miller, and Lewis (1968) found that consolidated memories, just like new memories, can be susceptible to disruption. In this experiment, conditioned fear, measured by the

suppression of licking behavior upon CS presentation, was disrupted by electroconvulsive shock treatment (ECS). Water-deprived rats were first trained to retrieve water from a drinking tube in a chamber. On the training day, they received a single presentation of a tone CS followed by footshock. The next day, animals received a CS presentation, immediately followed by ECS treatment, or no subsequent treatment. Twenty-four hours later, animals were placed back into the chambers, and their licking behavior upon presentation of the CS was assessed. The group that received ECS treatment made significantly more licks after CS presentation compared to the group that had not received ECS, indicating that the ECS-treated group demonstrated an attenuated conditioned fear response to the CS. Additionally, they demonstrated that ECS-induced disruption was dependent upon the reactivating the memory prior to treatment, as rats that received no reactivation session followed by ECS treatment did not show this attenuation of fear. The authors of this paper proposed that disruption was occurring as a result of the state of the memory, and hypothesized that its susceptibility lies in the fact that the memory was in a state of "change" (e.g. "in transit from stored to active"). That is to suggest that stored memories (e.g. inactive) are stable, while retrieved memories (e.g. active or reactivated) are destabilized and thus vulnerable to modification or disruption.

Although several studies demonstrated that retrieved memories were susceptible to disruption following the initial findings by Misanin and colleagues (Mactutus, Riccio, & Ferek, 1979; Judge & Quartermain, 1982; Riccio & Richardson, 1984), the phenomenon of disrupting a consolidated memory was initially dismissed. This is because most of these studies only *transiently* disrupted memory. The interest in this idea was renewed with several studies that demonstrated a more permanent disruption of memories that had previously undergone consolidation (Przybyslawski & Sara, 1997; Nader et al., 2000; Sara, 2000). For example, Nader

et al. (2000) found that a protein synthesis inhibitor (PSI) disrupted a consolidated memory in Pavlovian fear conditioning. Upon reactivating a memory by presenting a fear-conditioned CS, they infused a PSI into the amygdala. Twenty-four hours later, when animals were returned to testing cages and again, presented with the CS, the animals displayed less freezing (index of fear) behavior. The authors concluded that the memory had been "eliminated". Posing a challenge to the "consolidation hypothesis", researchers began to discuss the different accounts for which memories could be disrupted upon retrieval. If memories are susceptible to disruption after initial consolidation, this implies that there must be some processes by which recalled memories are again, consolidated. This additional consolidation phase was termed 'reconsolidation' (Przybyslawski, Roullet, & Sara, 1999; Sara, 2000).

Mechanisms of Reconsolidation

Molecular mechanisms. There is a large degree of overlap between mechanisms of consolidation and reconsolidation. As previously discussed with consolidation, reconsolidation also requires protein synthesis (Schafe & LeDoux, 2000; Nader et al., 2000; Debiec, LeDoux, & Nader, 2002), other downstream effectors that regulate for intracellular gene expression (Kida et al., 2002; Kelly, Laroche, & Davis, 2003; Tronson, Wiseman, Olausson, & Taylor, 2006; Tronson, Wiseman, et al., 2012), require similar patterns of neuronal activation (Reijmers, Perkins, Matsuo, & Mayford, 2007; Tayler, Tanaka, Reijmers, & Wiltgen, 2013), and LTP induction (Fonseca, Nägerl, & Bonhoeffer, 2006; Doyère, Debiec, Monfils, Schafe, & LeDoux, 2007; Kim et al., 2010). The molecular mechanisms of reconsolidation are illustrated in Figure 1.2. The overlap in molecular mechanisms and physiological models of plasticity (e.g. LTP) between consolidation and reconsolidation provides evidence that memories enter labile states when they are "active", either during consolidation, or upon recall.

Neurotransmitters. Similar effects of been demonstrated to play a role in reconsolidation, including glutamate (NMDA receptors - Lee & Everitt, 2008a; Przybyslawski & Sara, 1997) dopamine (Blaiss & Janak, 2006), and adrenergic signaling (Dębiec & Ledoux, 2004; Przybyslawski et al., 1999; Do Monte, Souza, Wong, & de Padua Carobrez, 2013; Gazarini, Stern, Carobrez, & Bertoglio, 2013; Dębiec, Bush, & LeDoux, 2011).

The beta-adrenergic antagonist propranolol has been specifically implicated in disrupting reconsolidation of memories that involve emotional arousal. This effect has been demonstrated in appetitive and aversive conditioning across a variety of different species, including rats (Dębiec & Ledoux, 2004; Bernardi, Lattal, & Berger, 2006; Robinson & Franklin, 2007; Milton, Lee, & Everitt, 2008; Schramm et al., 2016; Muravieva & Alberini, 2010; Otis & Mueller, 2011; Abrari, Rashidy-Pour, Semnanian, & Fathollahi, 2008; Taherian et al., 2014; Salinas et al., 1997; Diergaarde, Schoffelmeer, & De Vries, 2006), mice (Vetere et al., 2013; Villain et al., 2016), and Lymnaea (Hughes, Shymansky, Sunada, & Lukowiak, 2016). Recent human studies have drawn attention to propranolol, as it has been demonstrated to disrupt both experimentally induced and pathological emotional responses to stimuli (Kindt, Soeter, & Vervliet, 2009; Soeter & Kindt, 2011, 2010; Saladin et al., 2013; Lonergan & Pitman, 2013; Lonergan et al., 2016).

Interestingly, propranolol has been demonstrated to disrupt both LTD- and LTP-related learning and maintenance (Kemp & Manahan-Vaughan, 2008; Straube, Korz, Balschun, & Frey, 2003). It has also been found to reverse structural plasticity in the form of spine density after administration (Vetere et al., 2013). Thus, it appears that propranolol may be disrupting the reconsolidation of memory by reversing the synaptic changes that occur with the formation and maintenance of memory.

Conceptualizing Reconsolidation

There is some debate surrounding the distinction between consolidation and reconsolidation. Indeed, there are many mechanistic similarities between these two processes; both require protein synthesis (Schafe & LeDoux, 2000; Nader et al., 2000; Debiec et al., 2002), other downstream effectors that regulate for intracellular gene expression (Kida et al., 2002; Kelly et al., 2003; Tronson et al., 2006; Tronson, Wiseman, et al., 2012), as well as LTP induction (Fonseca et al., 2006; Doyère et al., 2007). Thus, many researchers have argued that reconsolidation is simply another iteration of a consolidation-like process. The lingering consolidation hypothesis (Nader et al., 2000; Alberini, 2005; Przybyslawski & Sara, 1997; Litvin & Anokhin, 2000) suggests that early memories are susceptible to disruption upon recall. However, with time, the consolidation period will come to an end, and these memories will no longer be susceptible to disruption. That is to say, the true period of consolidation is much longer than 3-6 hours after the initial potentiation of a memory formation/trace, and memories are constantly being updated upon retrieval to strengthen (or weaken) this trace.

On the other hand, experiments have provided a wealth of evidence that consolidation and reconsolidation are distinct processes (Nader, 2003; Nader et al., 2000; Dudai & Eisenberg, 2004; Lee, Everitt, & Thomas, 2004). For example, different brain areas appear to be engaged during consolidation and reconsolidation (Hernandez, Sadeghian, & Kelley, 2002). Additionally, the amount of time during which memories are susceptible to disruption is much shorter during reconsolidation than initial consolidation (Gordon, 1977). This suggests that reconsolidation is a separate process from a prior terminated consolidation period. Thus, upon disrupting a memory during retrieval, this view suggests that we are affecting the storage

capacity of the memory. Given these qualitative differences, it is difficult to argue that reconsolidation is simply another iteration of initial consolidation.

Before it was termed 'reconsolidation', several researchers theorized that memories fall into active and inactive states (Misanin et al., 1968; Spear & Mueller, 1984), and suggested that disruption of a consolidated memory upon retrieval was related to the fact that a memory becomes "active" upon recall. Proponents of a retrieval-based view of reconsolidation would argue that memories are disrupted by an inability to retrieve the memory trace, or bring it back to an 'active' state (Judge & Quartermain, 1982; Millin, Moody, & Riccio, 2001; Riccio & Richardson, 1984). This idea was taken further by the suggestion that during retrieval, 'links' are formed which allow for memory retrieval and update. It is possible that disruption can occur by preventing the formation of such 'links' for later retrieval (Dudai & Eisenberg, 2004).

Alternatively, it is possible that disrupting reconsolidation affects storage, whereby the memory is being extracted like a book from a shelf, and simply not being put back. Thus, it is possible that the memory itself is being erased. In fact, many studies use phrases like "the memory was erased" or "the memory was eliminated" in discussing effects on reconsolidation (Lee, 2009). Recently, some researchers have suggested that reconsolidation serves to update memories (see Nader & Einarsson, 2010; Alberini & LeDoux, 2013; Lee, 2010). That is, upon retrieval, the strength and content of memories are susceptible to modification. This idea is different from previous conceptualizations of reconsolidation with regard to its functional significance. The idea that memories are erased versus the idea that memories are updated or modified present an interesting paradox. Are these two ideas mutually exclusive? Or is it possible to eliminate memory by disrupting an updating mechanism (e.g. reconsolidation)? Determining whether disrupting reconsolidation affects the entire memory (storage), or whether

the memory is simply being changed or updated in some way will be crucial for further understanding the process by which consolidated memories are restabilized after retrieval.

Erasing Memory?

It has often been assumed that disrupting reconsolidation affects the entire memory. This is despite the fact that memories are recognized to have different components (Lee & Everitt, 2008a) . However, in a recent human fear conditioning study, researchers demonstrated that it is possible to disrupt one component of a memory, without affecting other components. Kindt et al. (2009) presented participants with a picture of a spider (CS) paired with a shock to the wrist and a loud noise. The eyeblink reflex was used as a measure of fear-potentiated startle (FPS). FPS is a behavioral measure of fear in which a subject's reflexive behavior is potentiated by the presence of a fearful stimulus; in this case, the eyeblink reflex in the presence of an electric shock. The following day, participants were given either propranolol or a placebo pill and one presentation of the CS alone. On the final day participants received presentations of the CS alone, and FPS responses were measured. The FPS response remained intact after administration of the placebo pill. The propranolol group on the other hand, demonstrated an attenuation of the FPS response. As mentioned previously, this effect has been traditionally referred to as a "disruption of the association" or "erasure of the memory". However, in this study, prior to each trial, they asked participants to rate their expectancy of being shocked on each trial. After receiving propranolol, although there was a reduction in the FPS responses (behavioral expression of fear), there was no change in the participants' expectation of being shocked. This suggests that the memory of the association itself is not being erased; but rather, there seems to be some disruption of the affective, motivational fear response to the CS.

In animal models of aversive conditioning, it is difficult to behaviorally parse apart the predictive versus motivational value of a CS, and animal studies in the aversive learning literature have not explicitly made this distinction (see Otis, Werner, & Mueller, 2015 for discussion). Thus, it is unclear at this point whether these differential effects on memory observed in human studies can translate back to animal models.

Parsing Motivational and Predictive Value

Motivational Value

A CS paired with a US gains predictive value, in that it acquires the ability to evoke anticipatory CRs. However, the CS can also acquire motivational value, which in the case of appetitive conditioning, confers the CS with the ability to evoke complex emotional and motivational states thus acting as an incentive stimulus (Berridge, 2001; Bindra, 1978; Lajoie & Bindra, 1976; Konorski, 1967; Toates, 1986; Cardinal, Parkinson, Hall, & Everitt, 2002). Incentive-motivational stimuli[1] have three fundamental properties; 1) they elicit approach and draw individuals' attention toward them; 2) they are desired, in that individuals will learn new instrumental actions to obtain them; 3) they evoke motivational states in an individual that energize ongoing instrumental actions, or instigate seeking behaviors (Cardinal et al., 2002; Berridge, 2001; Lovibond, 1983; Milton & Everitt, 2010). Different behavioral paradigms are used to measure each of these features of an incentive stimulus, and they are neurobiologically distinct processes (Everitt et al., 1999; Cardinal et al., 2002). In Pavlovian conditioning, these behavioral measures can be utilized to dissociate cues that acquire motivational value from cues

[1] An incentive-motivational stimulus can motivate behavior by generating positive *or* aversive states in an individual. Here, our discussion of 'incentive-motivational stimuli' is confined to stimuli paired with appetitive rewards.

that do not acquire motivational value. All cues (even those that do not necessarily acquire motivational value) acquire predictive value, and are capable of evoking a CR. However, the CRs that occur in the presence of stimuli that acquire motivational value versus predictive value are different. This dissociation provides an opportunity to behaviorally distinguish between predictive and motivational components of a memory, and possibly disrupt one without the other, in animal models.

Properties of an Incentive-Motivational Stimulus

1. Conditioned Approach. The first property of an incentive-motivational stimulus is that it elicits approach behaviors, and draws attention towards it. In animal models, it has been demonstrated that a food-paired CS will evoke such responses (Williams & Williams, 1969; Breland & Breland, 1961; Bindra, 1968; Brown & Jenkins, 1968). For example, in their modified Pavlovian version of an autoshaping task, Williams and Williams (1969) placed pigeons in operant chambers with an illuminated key and a food hopper where grain pellets were dispensed. During CS-US pairings, the key was illuminated and immediately followed by the delivery of a grain pellet into the food hopper. Although key responses were not required to receive the food reward, the pigeons engaged in approach and key-pecking behavior upon key illumination. This behavior was later termed 'sign-tracking', as subjects appear to be approaching the 'sign' that predicts a reward (Hearst & Jenkins, 1974). Sign-tracking to reward-paired cues has also been found in other birds, fish, rodents, monkeys, and humans (Breland & Breland, 1961; Brown & Jenkins, 1968; Hearst & Jenkins, 1974; Nilsson, Kristiansen, Fosseidengen, Fernö, & van den Bos, 2008; Pithers, 1985; Tomie, Lincks, Nadarajah, Pohorecky, & Yu, 2012; Wilcove & Miller, 1974).

2. Conditioned Reinforcement. The second property of an incentive-motivational stimulus is that it invokes desire, in that individuals will learn novel instrumental responses to receive presentations of it (Kelleher & Gollub, 1962; Hull, 1943; Fantino, 1977; Mackintosh, 1974; Milton & Everitt, 2010). In the laboratory, this can be studied through a test of conditioned reinforcement. A typical test of conditioned reinforcement will involve prior Pavlovian conditioning of a stimulus with a reward (i.e. a light paired with a food reward). After Pavlovian training, the test of conditioned reinforcement will provide an opportunity to assess whether instrumental responses (i.e. a lever press) will be made for just presentation of the food-paired light CS. Animals will acquire novel instrumental responses for presentations of a cue that has been previously paired with a food (Hull, 1943; Kelleher & Gollub, 1962; Mackintosh, 1974; Fantino, 1977).

3. Conditioned Motivation. The third property of an incentive-motivational stimulus is that it instigates instrumental action and energizes ongoing actions. This is measured in the laboratory with tests of Pavlovian-to-Instrumental-Transfer (PIT), or conditioned motivation (Lovibond, 1983; Estes, 1948, 1943; Milton & Everitt, 2010). In a PIT procedure, subjects first receive Pavlovian training sessions where a discrete stimulus (CS) is presented and immediately followed by a reward (US). Animals then undergo instrumental training sessions where they must perform an instrumental action in order to obtain a reward. Lastly, Pavlovian cues are presented during instrumental sessions which increase the rate of instrumental responding. The ability of Pavlovian cues to energize on-going instrumental actions has been demonstrated using a CS that has been paired with the same reward (Kruse, Overmier, Konz, & Rokke, 1983; Colwill & Rescorla, 1988) or a different reward (Dickinson & Dawson, 1987; also see Corbit & Balleine, 2005).

Another way to measure conditioned motivation is the ability of a Pavlovian CS to provoke instrumental responding after extinction. Behavioral responses are extinguished after an instrumental response is no longer reinforced. Eventually, individuals will cease operant actions, because they are no longer motivated to perform them. In instrumental conditioning, non-contingent presentation of cues previously paired with reward delivery, or presenting the rewards themselves can reinstate seeking behavior (Deroche-Gamonet, Piat, Le Moal, & Piazza, 2002; Duarte, Lefebvre, Chaperon, Hamon, & Thiébot, 2003; Saunders & Robinson, 2011; Barker, Torregrossa, & Taylor, 2012). Presenting rewards or reward-paired cues generates a conditioned motivational state in the animal that can drive or renew seeking behavior.

Individual Differences in Incentive-Motivational Value

Not all stimuli acquire incentive-motivational properties, and not all animals attribute stimuli with incentive-motivational properties to the same extent. In fact, there is considerable variation in the extent to which individual animals will attribute such value to a CS. This was first noted by Zener (1937) when he conducted a similar study to Pavlov's conditioned reflex experiment in dogs (1927). The main difference between these two studies was that Zener (1937) did not restrain the dogs in harnesses, so they were able to move freely throughout the test chamber. He expanded upon Pavlov's observations by pointing out that different CRs can be observed between individual animals. He noticed that upon presentation of the bell CS, some dogs began to approach the bell over conditioning sessions, and some dogs would approach the bowl where the food was delivered. This conditioned approach, or sign-tracking, behavior to a CS is one indicator that a CS has acquired incentive-motivational value (Uslaner, Acerbo, Jones, & Robinson, 2006; Flagel, Watson, Robinson, & Akil, 2007).

There are large individual differences in the extent to which reward-paired cues affect behavior (Flagel, Akil, & Robinson, 2009; Flagel et al., 2007; Robinson & Flagel, 2009; Beaver et al., 2006; Franken & Muris, 2005; Demos, Heatherton, & Kelley, 2012). Our lab has demonstrated similar differences in rats. We have showed this using a Pavlovian conditioned approach ('autoshaping') task (similar to Williams & Williams, 1969) where animals receive repeated presentations of a lever followed by the delivery of a food reward (Flagel et al., 2007; Flagel et al., 2009; Robinson & Flagel, 2009; Meyer et al., 2012a; Fitzpatrick et al., 2013). Although the delivery of a food pellet is not contingent upon a response from the animal, two distinct behaviors emerge during the presentation of the conditioned stimulus (CS). Upon lever presentation, some animals will interact and engage with the lever itself, while other animals will approach and engage with the location of food delivery. Animals that preferentially approach the lever are called *sign-trackers* (STs – Hearst & Jenkins, 1974), and animals that preferentially approach the food cup are called *goal-trackers* (GTs – Boakes, 1977).

In the STs, the CS elicits an approach CR towards it, thus acquiring at least one property of an incentive-motivational stimulus. In the GTs, the CS does not elicit approach behavior toward the CS, but rather the location of reward delivery. Thus, the CS acts as an informational stimulus in both STs and GTs, demonstrated by the acquisition of a CR. Our lab has hypothesized that two CRs emerge because of differences in the propensity to attribute motivational value to reward paired cues (Robinson & Flagel, 2009; Meyer et al., 2012b; Flagel et al., 2009; Robinson, Yager, Cogan, & Saunders, 2014; Saunders & Robinson, 2013) . There is a wealth of evidence to support this hypothesis; STs and GTs differ in measurements of all three properties of an incentive-motivational stimulus, and these behaviors also seem to be mediated by different neural systems.

Individual Differences in Incentive-Motivational Properties Attributed to Stimuli

Conditioned Approach. Individuals are indexed as STs or GTs based on their propensity to approach a reward-paired stimulus (Meyer et al., 2012a; Flagel et al., 2009; Robinson & Flagel, 2009). Although GTs do not approach the lever, they still learn the CS-US relationship, measured by their approach toward the food cup. In subsequent tasks with different rewards, STs also have a greater propensity to approach other visual cues (e.g. light) paired with rewards (Yager & Robinson, 2013; Yager, Pitchers, Flagel, & Robinson, 2015). The propensity to approach a CS appears to be a persistent trait that can predict the extent to which individuals find different visual cue-reward combinations attractive.

Conditioned Reinforcement. A CS acts as a more effective conditioned reinforcer for STs than for GTs. For example, STs will make more nosepokes than GTs for presentation of a lever that was previously paired with a reward (Flagel et al., 2009; Lomanowska et al., 2011; Meyer et al., 2012b; Meyer et al., 2014; Beckmann & Chow, 2015). STs will also reinstate seeking behavior to a greater extent than GTs in response-contingent cue-induced reinstatement (Yager & Robinson, 2010; Saunders & Robinson, 2010). Thus, reward-paired cues acquire incentive motivational properties in STs in that they are desired.

Conditioned Motivation. The ability of a CS to spur instrumental action or to energize ongoing instrumental actions is traditionally studied using PIT (Cardinal et al., 2002). Our lab has not used PIT to study conditioned motivation, as the cues used in the Pavlovian conditioned approach (PCA) screening process may confound the influence of Pavlovian cues on instrumental responding. However, using a reinstatement procedure we have found that a reward prime will instigate seeking behavior after extinction to a greater extent in STs than GTs (Saunders & Robinson, 2011). Thus, it appears that a reward prime evokes a greater conditioned

motivational state in STs. We have also demonstrated that non-contingent presentation of a cue previously paired with a reward will motivate STs to cross an electrified grid and engage in seeking behavior to a greater extent than GTs (Saunders, Yager, & Robinson, 2013).

Influences on Motivational Value Attribution

Despite being able to localize both auditory and visual cues, rats will only approach visual stimuli (Harrison, 1979; Cleland & Davey, 1983). This is hypothesized to be because visual stimuli acquire greater motivational value than auditory stimuli. Our lab and others have demonstrated evidence to support this hypothesis, in that an auditory CS is also a less effective conditioned reinforcer than a visual CS (Meyer et al., 2014; Beckmann et al., 2015). When animals are screened and classified as STs and GTs prior to tone Pavlovian conditioning, all animals acquire a GT CR to the tone (Meyer et al., 2014; Beckmann et al., 2015). Additionally, a tone acts as a conditioned reinforcer to an equal extent in STs and GTs. Thus, even in animals that attribute greater motivational value to a CS (STs), a tone does not acquire motivational properties to the extent of a visual CS.

A recent study from our lab isolated different components of a lever CS and found that they acquire motivational value to different degrees (Singer et al., 2016). During the CS period, the lever CS visibly and audibly extends into the cage. When only the auditory component of the lever was used as a CS, it did not elicit approach behavior, and only produced GT CRs. Additionally, when STs and GTs previously trained with the lever CS underwent subsequent conditioning with only the auditory component of the lever as a CS, all animals (even STs) developed a GT CR. Compared with the a lever CS with visible and auditory features, the auditory component alone also served as a less effective conditioned reinforcer in STs and GTs.

These findings suggest even within the same CS, visual features acquire greater motivational value than auditory features.

Neurobiology of Individual Differences in Attribution of Incentive-Motivational Value

Many studies have investigated the neural mechanisms involved in incentive motivation. These studies have focused on 1) the involvement of the mesocorticolimbic circuit, a system biased toward coding sensory stimuli in the immediate environment that may be related to fight or flight responses, or natural rewards such as sex or food (LeDoux, 2000) and 2) the involvement of corticostriatal-thalamic circuitry, which is involved in regulating internal and external motivational signals for appetitive stimuli (Kelley, Baldo, Pratt, & Will, 2005). Together, these areas make up a 'motive circuit' comprised of several key brain regions including the amygdala, ventral striatum, ventral pallidum, thalamus, prefrontal cortex, and several brainstem nuclei (Cardinal et al., 2002; Kalivas & Volkow, 2005). Many of the regions in this circuit send and receive projections that regulate dopamine neurotransmission, which has been demonstrated to be crucial in the acquisition and expression of motivated behaviors. The role of dopamine, in particular, has been well-established in motivated behaviors (Cardinal & Everitt, 2004; Everitt & Robbins, 2005)

A Role for Dopamine in Attributing Motivational Value. Behavioral differences in propensity to attribute motivational value are mediated by distinct neurobiological processes. In relation to the hypothesis that STs attribute greater motivational value to a CS, one might predict that the neurobiology underlying a ST CR would overlap largely with that of motivated behavior. Indeed, the acquisition and expression of ST behavior appears to be dependent upon dopamine (Dalley et al., 2002; Danna & Elmer, 2010; Flagel, Clark, et al., 2011; Saunders & Robinson, 2012; Day, Roitman, Wightman, & Carelli, 2007; Lopez, Karlsson, & O'Donnell, 2015; Scülfort,

Bartsch, & Enkel, 2016). For example, Saunders and Robinson (2012) first trained rats in a Pavlovian conditioned approach (PCA) task for eight days and classed animals as STs or GTs. Subsequently, STs and GTs were given microinjections of a non-specific dopamine antagonist, flupenthixol (3 doses; 5, 10, and 20μg) or vehicle into the nucleus accumbens core (NAc). All animals received each of these treatments across four different test days, and were then put into chamber to assess ST and GT behavior in the PCA task. Dopamine blockade in the NAc reduced all measures of ST behavior (e.g. contacts, probability, and latency) at each of the three doses administered, compared with vehicle injections. The decreases in ST behavior were dose dependent, with the largest effect being observed after administration of the highest dose of flupenthixol. In GTs, the highest dose of flupenthixol produced a small, but significant decrease in GT behavior. However, lower doses of flupenthixol did not affect GT behavior, and other studies have reported that GT behavior does not appear to be DA-dependent (Danna & Elmer, 2010; Flagel, Clark, et al., 2011; but see Fraser, Haight, Gardner, & Flagel, 2016; Cheng, De Bruin, & Feenstra, 2003; Eyny & Horvitz, 2003; Wassum, Ostlund, Balleine, & Maidment, 2011). Despite a small decrease in one measure of GT behavior at the highest dose of flupenthixol, dopamine blockade reduced ST behavior to a much greater extent on all behavioral measures. This demonstrates overlap in the neural systems underlying ST behavior and attributing motivational value to a CS. Thus, these data support our hypothesis that a ST CR requires attribution of motivational value to a CS, while GT does not require this.

One possible interpretation of these data is that STs are unable to remember that the CS predicted a US. Our argument against this claim is conditioned orienting responses are not affected by dopamine blockade. The development of an orienting response occurs regardless of whether or not the animals will approach a cue (Cleland & Davey, 1983). For example, both STs

and GTs will develop a conditioned orienting response to a light CS or a lever CS in which they orient their head and/or body toward the CS during the CS period (Saunders & Robinson, 2012; Yager & Robinson, 2013). Thus if STs continue to perform an orienting response, even in the absence of approach behavior, this strongly suggests that the predictive value of the CS is intact.

Brain regions engaged by reward-paired cues. As mentioned previously, reward-paired cues engage a number of 'motive circuit' brain regions. Our lab has quantified c-fos expression using *in situ hybridization* and immunohistochemistry for a variety of brain regions in STs and GTs (see Figure 1.3 for illustration). They found regions in the prefrontal cortex, ventral and dorsal striatum, nuclei of the amygdala and thalamus, lateral septum, and habenula to express high levels of c-fos after exposure to food- and drug-paired cues in STs, in comparison with GTs and unpaired control animals (Flagel, Cameron, et al., 2011; Yager et al., 2015; Haight, Fuller, Fraser, & Flagel, 2016). These data are in agreement with the hypothesis that ST behavior is mediated by attributing motivational value to cues, while GT is not. Further, it suggests that a CS must acquire incentive motivational value; a CS that only acquires predictive value is not sufficient. Some regions have been found to modulate the incentive-motivational value of cues in STs, including the nucleus accumbens core and paraventricular nucleus of the thalamus (Saunders & Robinson, 2012; Haight et al., 2016). In this dissertation, a variety of brain regions will be examined in the extent to which they are engaged by reward paired cues, and further, the extent to which these regions are affected by with changes in the attribution of incentive-motivational value of cues in STs.

Summary of Current Studies

The overarching goal of my dissertation is to understand the psychological processes underlying reconsolidation of motivational and predictive components of memory. Disrupting

reconsolidation in animal models is often referred to as “disrupting the association”, or “erasing the memory” despite the fact that recent evidence suggests this does not occur in all species. Studies in humans have recently found that propranolol disrupts the *emotional* or *motivational* component of memory, while leaving the memory of the CS-US association intact. This dissociation has not been studied in non-human species, likely because it is difficult to parse the motivational and predictive value of a CS-US association in animals. Our model of individual differences in propensity to attribute motivational value to reward-paired cues provides a unique opportunity to investigate the mechanisms by which memories are disrupted during reconsolidation. In this dissertation, I have two main goals. First, I will determine whether propranolol can selectively disrupt the motivational, but not predictive components of lever-CS memory by disrupting sign- or goal-tracking behavior. As our lab has previously demonstrated, not all stimuli acquire motivational value to the extent of a lever-CS. Thus, to further explore this question, I will also examine whether propranolol disrupts behavior in response to stimuli that acquire less motivational value. Additionally, I will examine the extent to which cues that differentially acquire motivational value can engage ‘motive circuit’ brain regions. I also plan to investigate if propranolol affects the extent to which ‘motive circuit’ brain regions are engaged.

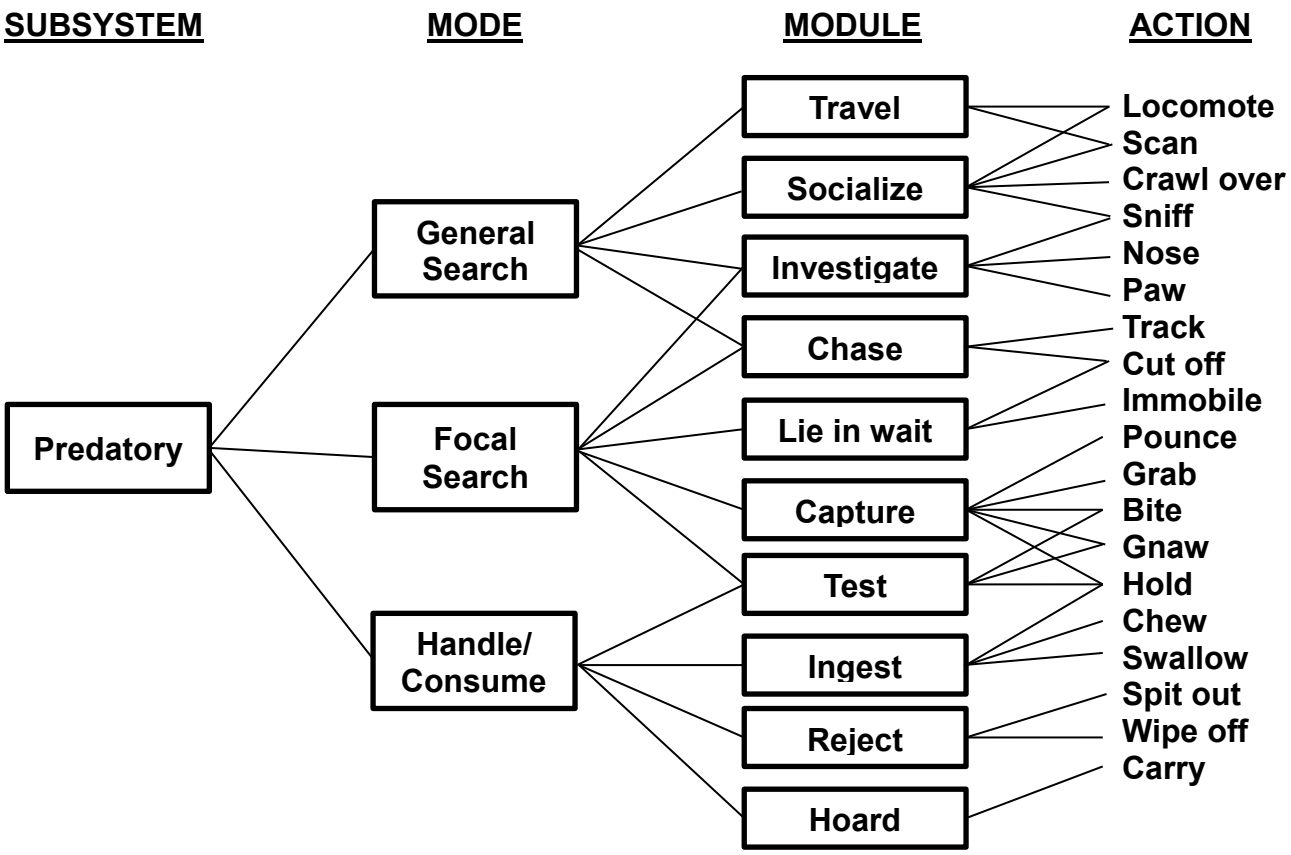


Figure 1.1. The structure of a behavior system. Adapted from Timberlake (1994). The structure of a behavior system includes four levels: system, subsystem, motivational mode, and perceptual-motor modules. This figure focuses on the motivational modes and perceptual-motor modules in the predatory subsystem of the feeding system of the rat. The far right of the figure shows the action patterns controlled by the different modules.

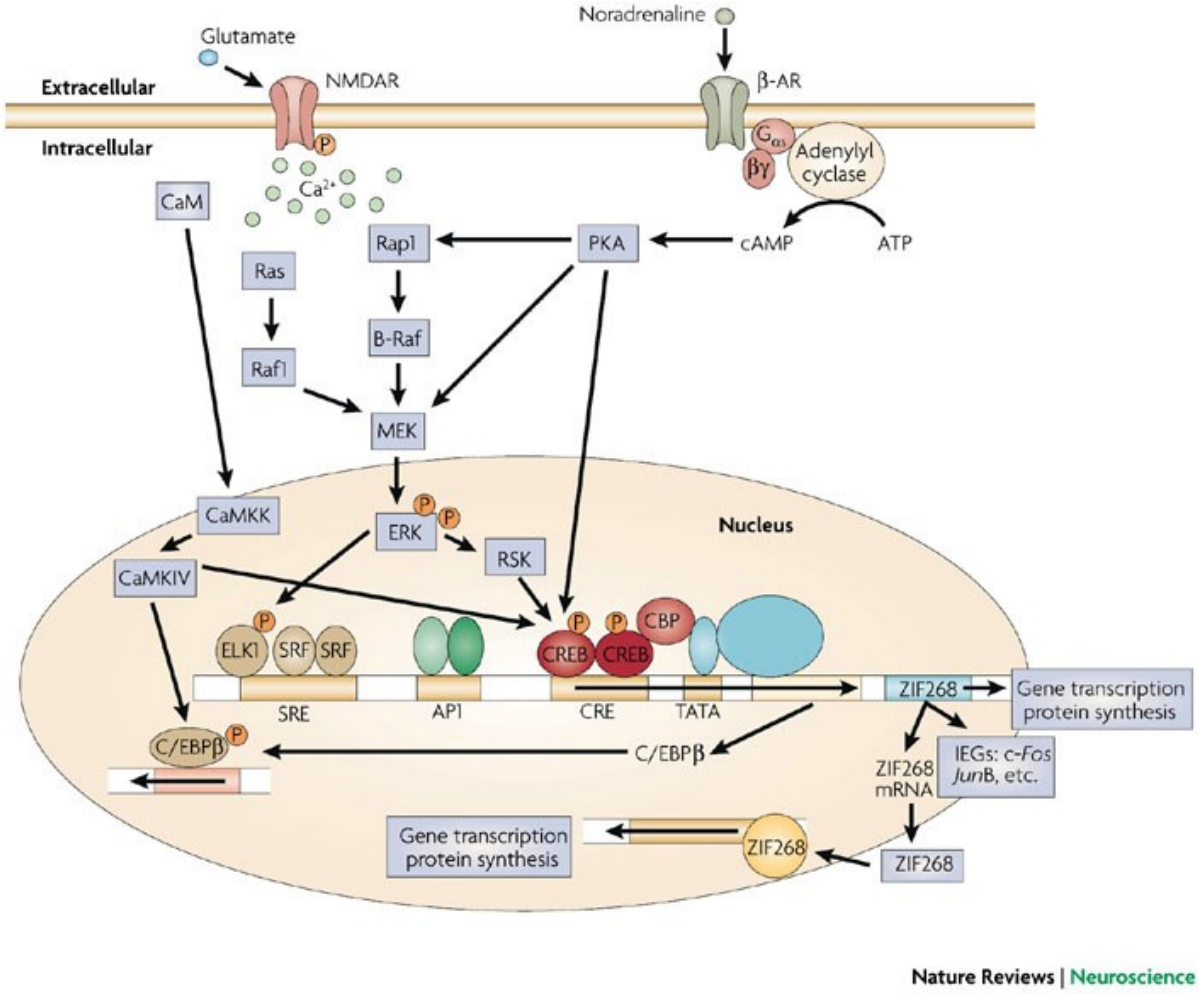


Figure 1.2. Molecular mechanisms of memory reconsolidation. From Tronson & Taylor (2007). This figure integrates findings from several studies. Of particular focus have been the molecular cascades previously demonstrated to be important in memory consolidation and those downstream of therapeutically relevant neurotransmitter targets including beta-adrenergic receptors, NMDARs (*N*-methyl-D-aspartate receptors). Molecular signaling cascades downstream of these receptors have been implicated in reconsolidation (text modified from Tronson & Taylor, 2007).

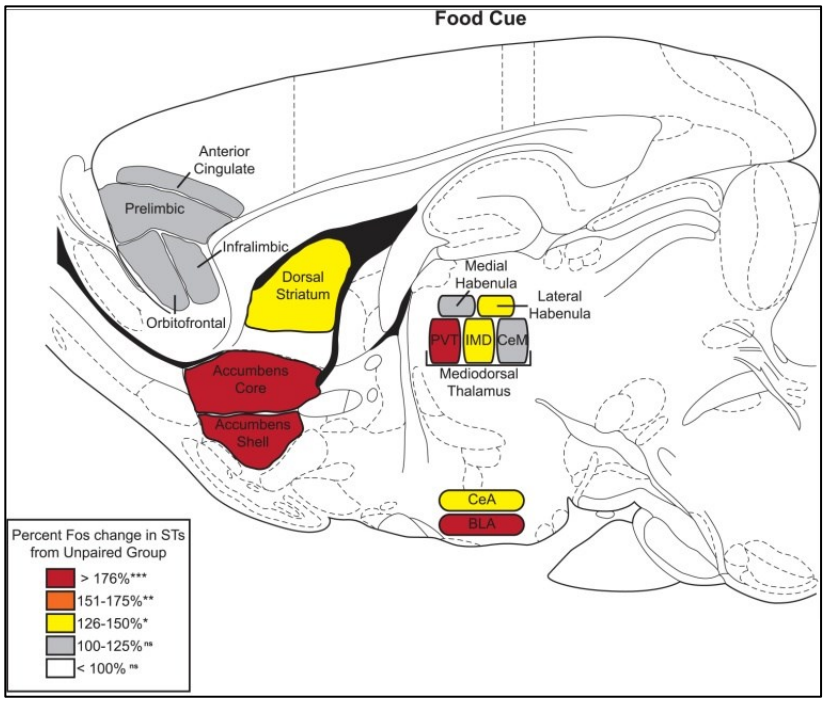


Figure 1.3. Brain regions engaged by a food-paired cue in STs. Modified and adapted from Yager et al. (2015). Summary of Fos changes after presentation of the food cue. Colors represent the percent change in Fos activation in STs compared with the Unpaired control groups. BLA, basolateral amygdala; CeA, central nucleus of the amygdala; CeM, central medial nucleus of the thalamus; IMD, intermedidorsal nucleus of the thalamus; PVT, paraventricular nucleus of the thalamus. ns, nonsignificant, $p>0.05$; *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Chapter II

Propranolol Disrupts the Reconsolidation of Sign-Tracking but not Goal-Tracking

Introduction

During memory retrieval, previously consolidated memories enter a labile state, rendering them vulnerable to disruption and modification. These memories return to stable traces once they undergo a restabilization process termed 'reconsolidation' (Misanin et al., 1968; Nader et al., 2000; Nader, 2003). Within the last couple of decades, researchers have started to dissect the neural mechanisms underlying this process. However, the conceptualization of reconsolidation is still largely debated. Some researchers have argued that reconsolidation is a storage mechanism, thus disrupting the reconsolidation of a memory will "erase" the memory completely because it is not being "re-stored". Others argue that reconsolidation uses stored information to create links that allow that memory to be retrieved. In this view, disrupting reconsolidation, would potentially act on these retrieval links, disrupting retrieval of a memory, without it being erased, per se (Dudai, 2004).

Recent human studies suggest that some memories do not fit precisely into either of these views of reconsolidation. It is possible that only some components of a memory, rather than the entire memory, are subject to modification upon retrieval. For example, Kindt et al. (2009) found that the reconsolidation of conditioned responses (CRs) to a fear conditioned stimulus (CS) was disrupted by administration propranolol. Propranolol is a beta-adrenergic antagonist that has

been previously demonstrated to specifically disrupt appetitive or aversive emotional-motivational memories (Debiec & LeDoux, 2004; Milton & Everitt, 2008). Interestingly, the participants' expectation of shock remained intact. This suggests that the emotional component (herein referred to as 'motivational' component) of a memory (e.g. fear) can be disrupted without erasing the entire memory itself.

The motivational and informational components are difficult to parse apart in animal models. However, our lab has demonstrated that rats develop different CRs upon CS presentation in an autoshaping task (Robinson & Flagel, 2009; Meyer et al., 2012); some animals will interact and engage with the lever CS itself (sign-trackers, STs – Hearst & Jenkins, 1974), while other animals will approach and engage with the location of food delivery (goal-trackers, GTs – Boakes, 1977). It is hypothesized that the development of a ST or GT phenotype is based on differential attribution of motivational properties to the CS. More specifically, that STs attribute more motivational salience to the CS than GTs.

Thus, STs and GTs can be utilized to determine whether or not propranolol disrupts all aspects of a CS-US association. That is, does propranolol disrupt reconsolidation by affecting the entire memory, or only motivational aspects of a memory? We hypothesize that propranolol will disrupt ST, but not GT behavior, thus suggesting that propranolol modifies the memory by attenuating the motivational component, while leaving the CS-US association intact. If propranolol disrupts both ST and GT behavior, this would suggest that the entire memory is being affected by disruption.

Materials and Methods

Subjects

Male Sprague-Dawley rats (Charles River, Raleigh, NC) weighing 250-275g upon arrival were

used for this study. Animals were individually housed in a climate controlled colony room with a reverse 12-h light/12 h dark cycle, where food and water were available ad libitum. Prior to experimental testing, animals were given one week to acclimate to the housing room. During this time, rats were handled several times by the experimenter. All experimental procedures were approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Apparatus

Standard (22 x 18 x 13 cm) test chambers (Med Associates Inc., St Albans, VT, USA) were used for behavioral testing. Each chamber was individually enclosed in a sound-attenuating cabinet equipped with a fan for ventilation and to impede background noise.

Pavlovian training chambers each had a food cup placed 3 cm above the stainless steel grid floor in the center of one wall, and a red house light on the opposite wall, which remained illuminated throughout the duration of all sessions. An illuminated retractable lever was located 6 cm above the floor and 2.5 cm away from the food cup on either the left or right side (counterbalanced across chambers). Infrared photo-beams located inside the food cup were used to record head entries. All experimental events were controlled and recorded by a MED-PC computer system.

Drugs

Propranolol (DL-Propranolol hydrochloride, 99%; Acros Organics, NJ, USA) and Nadolol (analytical standard; Fluka, St. Louis, MO) were dissolved in 0.9% sodium chloride.

Drugs were administered intraperitoneally (i.p.) at a dose of 20mg/kg/injection.

Pavlovian conditioned approach (PCA)

PCA training. Rats were trained using a Pavlovian conditioned approach (PCA) procedure described previously (Flagel et al. 2007; Meyer et al. 2012). On the two days preceding the start of the experiment, 45mg banana-flavored pellets (Bio-Serv) were placed into

home cages to habituate rats to this food. Following food habituation days, rats were trained to retrieve pellets from the food cup during a pretraining session, during which 25 pellets were dispensed into the food cup on a 30 s (0-60 s) variable time (VT) schedule. The red house light remained illuminated throughout the duration of the session. If a rat failed to consume all 25 pellets, they were given an additional pretraining session. On the day following pretraining, PCA training began. Briefly, animals were trained over five consecutive daily sessions. Each session consisted of 25 trials in which an illuminated lever (conditioned stimulus, CS) was presented for 8 seconds and followed by the delivery of a 45mg banana-flavored food pellet (unconditioned stimulus, US) into the food cup. CS-US pairings occurred on a VT 90 (30-150 s) schedule. The delivery of the food pellet was not contingent upon any response from the animal. All lever deflections, food cup entries, and latency to approach each location were recorded.

PCA index scores. Animals were classified as sign-trackers (STs) or goal-trackers (GTs) using the criteria previously described by Meyer and colleagues (2012). Briefly, PCA index scores were calculated as an average of three measures of approach behavior during the 8 s CS period; response bias to approach either the lever CS or the food cup [(#lever deflections – #food cup entries)], probability to approach either the lever CS or food cup [(P(lever) – P(food cup)], and latency to approach either the lever CS or food cup [(lever deflection latency – food cup entry latency)/8]. This average produced an index score ranging from -1.0 to +1.0, where a score of -1.0 indicated a strong bias toward approaching the food cup, and a score of +1.0 indicated a strong bias toward approaching the lever. Index scores were averaged across training days 4 and 5, and these values were then used to classify rats as STs or GTs. Animals receiving scores between +0.6 to +1.0 and >50 lever contacts were classified as STs, and animals with scores between -0.6 to -1.0 and >50 food cup entries were classified as GTs.

Experiment 1: The effect of propranolol on the reconsolidation of ST and GT CRs

PCA Retrieval and Reconsolidation

A total of 59 rats (STs *n*=30, GTs *n*=29) were used for this experiment. Animals were excluded from the analysis if they failed to retrieve all 25 pellets on any of the eight Pavlovian sessions (STs n=2, GTs n=2). STs (n=28) and GTs (n=27) continued consecutive daily training sessions for an additional three days (8 days total, including initial Pavlovian training sessions).

Retrieval sessions. Pavlovian conditioning sessions on days 6 and 7 served as retrieval sessions. Behavioral testing on these days were identical to initial Pavlovian training, with the exception that immediately after the end of the session and before returning to home cages, animals received an injection of either propranolol (20mg/kg) or saline. STs and GTs were divided into propranolol (STs *n*=14, GTs *n*=15) or saline (STs *n*=14, GTs *n*=12) injection groups, and were counterbalanced based on their index scores from Sessions 4 and 5. Rats received the same treatment (propranolol or saline) on both days.

Test session. Rats underwent a final Pavlovian conditioning session (Day 8) in order to assess the effect of post-session injections administered on the previous day.

Experiment 2: The effect of propranolol on the reconsolidation of conditioned orienting in STs and GTs

Video scoring

In addition to conditioned approach, rats also develop a conditioned orienting response to cues that predict rewards. This is not something that occurs as a startle reflex, and animal receiving unpaired CS and US presentations, will habituate to the lever presentation, and will not orient towards it (also see Yager & Robinson, 2013). A conditioned orienting response develops equally in both STs and GTs, and previous studies in our lab have found that disrupting ST

behavior does not disrupt the CS-US association, measured by an intact conditioned orienting response (Saunders & Robinson, 2012; Yager & Robinson, 2013). Since Experiment 1 sessions were not video recorded, orienting behavior was not initially quantified. Thus, we ran a separate group of animals in this experiment (STs only, n=20), and video recorded all sessions. Briefly, animals underwent PCA training for 5 days, and were assigned to propranolol or vehicle groups and counterbalanced based on their index scores from Sessions 4 and 5. All rats received i.p. post-session injections after sessions 6 and 7. An additional training session was administered on day 8, in order to assess the effect of post-session injections administered on the previous day. Conditioned orienting and conditioned approach to the lever CS were scored offline. An orientating response was scored if the rat oriented its body and/or head toward the CS during the first half (4 s) of the CS presentation, even if the rat did not approach the CS. An approach response was scored if the rat either moved toward the CS, bringing its nose within 1 cm of the lever within the first half of CS presentation, or if the rat took two or more steps toward the lever within the first half of CS presentation. Rats would sometimes take two or more steps toward the lever, thus approaching it, but not necessarily bringing its nose within 1 cm. This behavior was still scored as an approach response. If a rat approached the CS, an orienting response would be scored, as orienting responses preceded approach responses. Additionally, if a rat engaged the CS, indicated by a computer recorded contact, approach and orienting responses would be scored, as both of these behaviors preceded a contact. Thus, we were able to analyze three behaviors within the first half of the lever CS presentation: 1) an orienting response, 2) an approach response, and 3) a contact (indicated by computer-scored contact). We chose to analyze the first 4 s of the CS period because activity in response to conditioned stimuli (relative to

unpaired or non-reinforced stimuli) is more likely to occur in the first half of CS presentation (Holland, 1977).

Experiment 3: The effect of propranolol on ST and GT CRs without memory retrieval

Non-retrieval Control

Studies have previously found that to disrupt reconsolidation propranolol must be administered after retrieval, and within the reconsolidation window (2 h – Przybyslawski & Sara, 1997; Przybyslawski, Roullet & Sara, 1999). In order to determine whether the effects of propranolol could be attributed to mechanisms other than reconsolidation, a separate control group was given injections without memory retrieval.

Injections without Memory Retrieval. Rats remained in their home cages on days 6 and 7. During the same time of day as their previous conditioning sessions, animals were given injections of either propranolol (20mg/kg) or saline. Propranolol (STs n=8, GTs n=9) and saline (STs n=9, GTs n=8) groups were counterbalanced based on index scores from Sessions 4 and 5. Rats received the same treatment (propranolol or saline) on both days.

Test session. Rats underwent a final Pavlovian conditioning session in order to assess the effect of injections administered in home cages on the previous two days.

Experiment 4: The effect of nadolol on the reconsolidation of ST and GT CRs

Nadolol Control

In order to determine whether the effect of propranolol observed in Experiment 1 was due to action on the central or peripheral nervous system, a separate group of animals were given nadolol, rather than propranolol. Nadolol shares similar affinity (if not equal, then higher) for beta-adrenergic receptors as propranolol. However, nadolol is less lipophilic and does not cross the blood-brain barrier (Escoubet et al., 1986; Joseph, Lynham, Colledge, & Kaumann, 2004)

Thus, it does not easily penetrate the central nervous system, and therefore primarily exerts its effects in the peripheral nervous system.

Retrieval sessions. The procedure in this group of animals was identical to that of Experiment 1, with the exception that animals received post-session injections of either nadolol (20mg/kg) or saline. STs and GTs were divided into nadolol (STs n=19, GTs n=8) or saline (STs n=16, GTs n=9) injection groups in a counterbalanced order. Rats received the same treatment (nadolol or saline) on both days.

Test session. Rats underwent a final Pavlovian conditioning session in order to assess the effect of post-session injections administered on the previous day.

Statistics

Linear mixed models (LMM) were used to analyze all repeated measures data. The best-fitting model of the covariance structure was determined by the lowest Akaike information criterion score (Verbeke & Molenberghs, 2009). Analyses were conducted on acquisition data to confirm that STs and GTs differed in their acquisition of respective CRs (Phenotype X Session interaction), and that treatment groups did not differ during acquisition (Phenotype X Session X Treatment interaction). Separate LMM analyses were conducted for each of the following data sets: lever contacts, probability to approach the lever, latency to approach the lever, and food cup entries, probability to approach the food cup, latency to approach the food cup, video-scored orienting, video-scored approach, and computer scored probability. LMM analyses were used to analyze the main effects and interactions of treatment, session, and phenotype across Sessions 6-8 (Treatment X Session, Phenotype X Session, and Treatment X Phenotype). A priori hypotheses were tested post-hoc Bonferroni comparisons in order to examine the effect of treatment within each phenotype, between each of Days 6 through 8.

If there was a main effect of Phenotype, LMM were run on each phenotype to assess the effect of treatment on STs across sessions or GTs across sessions (Treatment X Session). Independent samples t-tests were used to compare propranolol and vehicle groups lever presses and latency on the first trial in the time course data for Session 8.

Results

Experiment 1: The effect of propranolol on the reconsolidation of ST and GT CRs

Pavlovian conditioned approach (PCA)

Figure 2.1(A-C and G-I) illustrates the acquisition of lever- and food cup-directed behavior in STs and GTs. Across training days 1-5, animals classified as STs made significantly more lever contacts than GTs (Phenotype x Session interaction, $F_{(4,109)}$=57.40, p<0.001), showed an increased probability to approach the lever (Phenotype x Session interaction, $F_{(4,51)}$=34.24, p<0.001), as well as a decrease in latency to approach the lever (Phenotype x Session interaction, $F_{(4,78)}$=44.27, p<0.001), see Figure 2.1A-C. There were no significant differences between propranolol and vehicle groups in acquisition of lever-directed behavior in the number of contacts (Phenotype X Treatment X Session interaction, $F_{(4,109)}$=1.06, p=0.38), probability to approach the lever ($F_{(4,51)}$=2.12, p=0.09), or latency to approach the lever ($F_{(4,78)}$=1.32, p=0.27). In contrast, GTs made significantly more food cup entries than STs (Phenotype x Session interaction, $F_{(4,51)}$=31.63, p<0.001), demonstrated a significant increase in probability to approach the food cup (Phenotype x Session interaction, $F_{(4,51)}$=38.84, p<0.001), and a decreased latency to approach the food cup (Phenotype x Session interaction, $F_{(4,51)}$=36.76, p<0.001), see Figure 2.1G-I. There were no significant differences in acquisition between treatment groups in the number of food cup entries (Phenotype X Treatment X Session, $F_{(4,51)}$=0.91, p=0.47),

probability to approach the food cup ($F_{(4,51)}$=0.29, p=0.88), or latency to approach the food cup ($F_{(4,51)}$=1.04, p=0.40).

PCA Retrieval and Reconsolidation

Lever and food cup-directed behavior for Sessions 6-8 can be seen in Figure 2.1D-F and I-L.

Lever Contacts. STs contacted the lever significantly more than GTs on all days (effect of Phenotype, $F_{(1,51)}$=251.65, p<0.001: Figure 2.1D). Compared with vehicle controls, propranolol differentially affected lever-directed behavior in STs and GTs across sessions 6 through 8 (Phenotype X Treatment X Session interaction, ($F_{(2,102)}$=3.82, p<0.05 Figure 2.1D). Among GTs, there were no differences in conditioned responding across sessions or treatment group (no Treatment X Session interaction, $F_{(2,25)}$=2.92, p=0.07). In the STs, propranolol significantly decreased conditioned responses across sessions, compared with vehicle controls (Treatment X Session interaction, $F_{(2,26)}$=6.03, p=0.007: Figure 2.1D). Post-hoc comparisons revealed significant differences in the ST propranolol-treated group between Days 6 and 7, Days 6 and 8, and Days 7 and 8 (*p's*<0.001).

Lever Probability. STs also contacted the lever with greater probability than the GTs on all days (effect of Phenotype, $F_{(1,54)}$=436.85, p<0.001: Figure 2.1E). Propranolol significantly decreased the probability of lever-directed behavior (Treatment X Session, $F_{(2,65)}$=9.07, p<0.001: Figure 2.1E), and this did not appear to be dependent upon phenotype (no Phenotype X Treatment interaction, $F_{(1,55)}$=0.05, p=0.82). Within the GTs, propranolol seemed to suppress an increase in probability to approach, compared with vehicle controls (Treatment X Session interaction, $F_{(2,25)}$=3.84, p<0.05: Figure 2.1E).

In the STs, propranolol significantly decreased probability to approach the lever across sessions, compared with vehicle controls (Treatment X Session interaction, $F_{(2,26)}$=7.41, p<0.005: Figure

2.1E). Similar to lever contacts, post-hoc comparisons revealed significant decreases in probability to approach the lever in propranolol-treated animals on Days 6 and 7, Days 6 and 8, and Days 7 and 8 (*p*'s<0.01). Post-hoc comparisons of the vehicle-treated GTs revealed significant increases in the probability of making a lever-directed response between Days 6 and 7, and Days 6 and 8 (*p'*s<0.01). Again, this suggests that propranolol may have had a slight suppression on lever-directed behavior in the GTs.

Lever Latency. STs approached the lever significantly faster than the GTs (effect of Phenotype, $F_{(1,52)}$=211.72, p<0.001: Figure 2.1F). Propranolol significantly increased latency to approach the lever compared with vehicle controls (Treatment X Session interaction, $F_{(2,69)}$=10.5, p<0.001: Figure 2.1F) again, irrespective of phenotype (no Phenotype X Treatment interaction, $F_{(1,52)}$=1.14, p=0.29). Within the GTs, there was again, a suppression effect on latency increase compared to the control group (Treatment X Session interaction, $F_{(2,25)}$=3.5, p<0.05: Figure 2.1F). Post hoc comparisons reveal significant differences between Days 6 and 8 in vehicle controls but not propranolol-treated animals (p=0.01). Within the STs, propranolol significantly increased latency compared to vehicle controls (Treatment X Session interaction, $F_{(2,26)}$=9.7, p=0.001: Figure 2.1F). Post hoc comparisons reveal significant differences between Sessions 6 and 7, 6 and 8, and 7 and 8 in propranolol-treated STs (*p*'s<=0.001).

Food Cup Entries. The number of entries into the food cup was significantly higher in GTs, than STs (effect of Phenotype, $F_{(1,51)}$=263.62, p<0.001: Figure 2.1J). This was expected, as GTs have a greater propensity to approach the food cup. There were no significant main effects of Treatment or Session. Thus, it appears that propranolol had no effect on the number of food cup entries made by STs or GTs.

Food cup Probability. GTs approached the food cup with a significantly higher probability than STs (effect of Phenotype, $F_{(1,51)}$=1052.24, p<0.001: Figure 2.1K). Treatment of propranolol or vehicle differentially affected lever-directed behavior in STs and GTs across sessions 6 through 8 (Phenotype X Treatment X Session interaction, $F_{(2,58)}$=3.73, p<0.05: Figure 2.1K). Post-hoc comparisons indeed, reveal a significant decrease in probability of GT approaching the food cup between Days 6 and 8, and Days 7 and 8 (*p*'s<0.001). Thus, a significant difference beginning after Day 7 in probability to approach the food cup does seem to be apparent. However, it should be noted that phenotype did not predict the effect of treatment (no Phenotype X Treatment interaction, $F_{(1,51)}$=0.193, p=0.663: Figure 2.1K).

Within the GTs, there was no main effect of treatment ($F_{(1,25)}$=3.75, p=0.06: Figure 2.1K), and the CRs made by the propranolol and vehicle treated groups did not differ across sessions (no Treatment X Session interaction, $F_{(2,25)}$=1.21, p=0.31: Figure 2.1K). Within the STs, the probability of food cup entries did differ slightly between propranolol and vehicle groups (effect of treatment, $F_{(1,26)}$=4.26, p=0.049), but the effect of treatment across sessions was not statistically significant (no Treatment X Session interaction, $F_{(2,26)}$=2.95, p=0.07: Figure 2.1K).

Food Cup Latency. GTs approached the food cup significantly faster than the STs (effect of Phenotype, $F_{(1,52)}$=697.61, p<0.001: Figure 2.1L). There was a significant effect of treatment (effect of treatment, $F_{(1,52)}$=4.21, p<0.05), primarily driven by significant difference in latency between days 6 and 8 in the propranolol group. Treatment did not differentially affect STs and GTs (Phenotype X Treatment interaction, $F_{(1,52)}$=0.26, p=0.87: Figure 2.1L).

Within GTs, there was no significant effect of treatment ($F_{(2,25)}$=1.6, p=0.22: Figure 2.1L) and no differences between vehicle and propranolol groups across sessions (Treatment X

Session, $F_{(2,25)}$=1.19, p=0.32: Figure 2.1L). In the STs, there were also no significant effects of treatment ($F_{(2,26)}$=3.30, p=0.08: Figure 2.1L).

Propranolol disrupted ST behavior as indicated by the number, probability, and latency in which animals approached the lever. GT behavior was not significantly affected across sessions by propranolol. The probability and latency of food cup approach behavior did appear to be affected significantly between Sessions 7 and 8. However, a follow-up analysis within the GTs did not reveal any significant differences, likely because the vehicle-treated group also showed a decreasing/increasing trend in probability and latency, respectively (see Figures 2.1 H and I).

Time Course Analysis of Lever-directed Behavior in STs During the Final Session

The time course data during session 8 is illustrated in Figure 2.2, beginning with the first trial, and followed by three-trial blocks of the remaining 24 trials in the session.

It is possible that the decrease in ST behavior after administration of propranolol may have been a result of behavioral deficits induced by the drug. Thus, we analyzed whether behavior on the first trial differed as a result of treatment. If propranolol were causing behavioral or locomotor deficits that were responsible for the decrease in ST behavior, we would expect to see these differences in the beginning of the session.

There were no significant differences between propranolol- and saline-treated groups in the number of lever contacts made during the first trial ($t_{(26)}$=.020, p=0.08: Figure 2.2, top), or latency to approach the lever on the first trial ($t_{(26)}$=1.02, p=0.20: Figure 2.2, bottom). Treatment did not different affect the number of contacts per trial throughout the session (no Treatment X Trial interaction, ($F_{(8,208)}$=1.9, p=0.06), or the latency to which they approach the lever on each trial throughout the session (no Treatment X Trial interaction, ($F_{(8,26)}$=1.38, p=0.25). A sign-tracking conditioned response is intact on the first trial on session 8, even in propranolol-treated

STs, indicated by no significant differences between treatment groups. This suggests that propranolol does not induce behavioral deficits that impair the ability to make a sign-tracking conditioned response.

The number of contacts and latency to approach the lever on the first trial did not differ as a result of treatment. This provides support that the CS-US association was intact, and that locomotor responses were not impaired in the propranolol group, as their responses did not differ from the control group at the beginning of the session. Linear mixed models revealed that there were no significant interactions to indicate different trends between propranolol and saline groups across trials in either contacts (Treatment X Trial interaction, $F_{(8,26)}=1.25$, $p=0.31$) or latency (Treatment X Trial interaction, $F_{(8,26)}=1.38$, $p=0.25$), as both groups showed a similar decrease in responding toward the end of the session. However, it is worth noting that the number of contacts and latency to approach per trial in the propranolol group is much lower than the saline group for trial blocks 2-5. Figure 2.2 illustrates that the propranolol group decreases responding and increases latency much earlier in the session than the saline group.

Experiment 2: The effect of propranolol on the reconsolidation of conditioned orienting in STs and GTs

The effects of propranolol were analyzed using both computer- and video-scored measurements of behavior. First, we wanted to demonstrate that we replicated effect of propranolol on ST behavior from Experiment 1. After analyzing the acquisition of a ST CR with computer recorded contacts, probability, and latency to approach the lever, we analyzed the effect of propranolol on these measurements. Second, we wanted to compare the probability of making a computer recorded contact with the probability of engaging in a behavior that resulted in video-scored orientation and/or approach. We first analyzed the acquisition of these responses, in order to

demonstrate that all of these CRs develop across training sessions. Since we did not habituate rats to the CS prior to training, we also analyzed video for an unpaired control group, to demonstrate that these orienting behaviors are learned as a result of CS-US pairings, and not elicited by unpaired stimuli. Next, we compared the effect of propranolol on the probability of animals making a computer recorded contact video-scored conditioned approach and orienting behaviors. We have previously demonstrated that STs but not GTs will acquire a conditioned approach response, while all animals will equally acquire a conditioned orienting response (Saunders & Robinson, 2012; Yager & Robinson, 2013).

Computer-Scored Contacts, Probability, and Latency

Acquisition

The acquisition of ST behavior measured by computer-scored contacts, probability, and latency to approach the lever is illustrated in Figure 2.3A-C. As in Experiment 1, animals classified as STs significantly increased lever contacts across Sessions 1-5 (effect of Session, $F_{(4,30)}=4.82$, $p<0.005$: Figure 2.3A), showed an increased probability (effect of Session, $F_{(4,23)}=6.37$, $p=0.001$: Figure 2.3B), as well as a decrease in latency to approach the lever (effect of Session, $F_{(4,30)}=18.08$, $p<0.001$: Figure 2.3C). Food-cup directed behavior data are not shown, but STs showed a decrease in number of food cup entries across Sessions 1-5 (effect of Session, $F_{(4,18)}=8.18$, $p=0.001$), decrease in probability (effect of Session, $F_{(4,29)}=17.40$, $p<0.001$), and an increase in latency to approach the food cup (effect of Session, $F_{(4,20)}=14.24$, $p<0.001$). There were no significant differences in acquisition of ST behavior between animals that were later divided into propranolol- and vehicle-treated groups in number of lever contacts (Treatment X Session interaction, $F_{(4,30)}=0.53$, $p=0.71$), probability to approach the lever ($F_{(4,24)}=1.03$, $p=0.41$), latency to approach the lever ($F_{(4,30)}=0.72$, $p=0.58$), number of food cup entries ($F_{(4,18)}=0.40$,

p=0.81), probability to approach the food cup ($F_{(4,30)}$=0.41, p=0.80), or latency to approach the food cup ($F_{(4,20)}$=0.17, p=0.95).

Retrieval and Reconsolidation

Lever-directed behavior for propranolol- and vehicle-treated STs across Sessions 6-8 are shown in Figure 2.3D-F. As in Experiment 1, propranolol significantly decreased lever contacts (Treatment X Session interaction, $F_{(2,18)}$=5.40, p=0.01: Figure 2.3D), probability to approach the lever (Treatment X Session interaction, $F_{(2,18)}$=6.61, p=0.007: Figure 2.3E), and increased latency to approach the lever (Treatment X Session interaction, $F_{(2,20)}$=14.05, p<0.001: Figure 2.3F) compared with vehicle controls, across Sessions 6-8. Post hoc comparisons revealed significant differences between Sessions 6 and 8 in increased latency, probability, and contacts (p'<0.001), and a significant difference between Sessions 7 and 8 (p=0.006). There were no significant effects of propranolol on GT behavior in the STs (data not shown).

Time Course Analysis Lever-directed Behavior in STs During the Final Session

As in Experiment 1, we analyzed the effects of propranolol on the number of lever contacts made during each trial, and latency to approach the lever on each trial. It should be noted that the time course analysis was only conducted on computer-scored data. The time course for Experiment 2 is illustrated in Figure 2.4, starting with the first trial and followed by eight 3-trial blocks for the remaining 24 trials.

There were no significant differences between propranolol and saline groups on the number of lever contacts made during the first trial ($t_{(18)}$=-0.69, p=0.50: Figure 2.4, top) or on latency to approach the lever during the first trial ($t_{(18)}$=-0.88, p=0.40: Figure 2.4, bottom). This replicates the first trial analysis in Experiment 1. In this experiment, treatment groups were significantly different in the number of contacts made across the session (Treatment X Trial

interaction, $F_{(8,41)}$=2.60, p=0.02). Post-hoc comparisons revealed statistically significant differences from trial block 1 in trial block 5 (p<0.005), blocks 6 and 7 (p's<0.05), and block 8 (p<0.001). Propranolol and saline groups were also significantly different in latency to approach the lever throughout the session (Treatment x trial effect, $F_{(8,71)}$=4.03, p=0.001). Post-hoc comparisons revealed significant differences from trial block 1 in trial blocks 5, 6, 7 ($p's$<0.05), and block 8 (p<0.001). Unlike Experiment 1, the Treatment X Trial interactions were significant for both the number of contacts per trial and latency to approach the lever on each trial. The time courses analyses in both Experiments 1 and 2 demonstrate similar trends between the first trial and trial block 5. The main difference in Experiment 2 is that the saline treated animals maintained high levels of responding until the last trial.

Video-Scored Approach, Video-Scored Orienting, and Computer-Scored Lever Contacts

Acquisition

Figure 2.5 illustrates the acquisition of contact, approach, and orienting responses on Sessions 1 and 6 during the first half (4 s) of the CS period. Data are displayed as probability of computer-scored lever contacts, video-scored approach, and video-scored orienting (calculated by # of trials engaged in behavior/25).

Computer Scored Lever Contacts. Propranolol and vehicle treated STs increased their responding compared with unpaired animals between Sessions 1 and 6 (Group X Session interaction ($F_{(1,23)}$=36.76, p<0.001: Figure 2.5A). There was no difference in acquisition between propranolol and vehicle treated groups (no Treatment X Session ($F_{(1,23)}$=0.39, p=0.54). The acquisition of approach behavior measured by computer-scored lever deflections can be seen in Figure 2.5A.

Video Scored Approach. Illustrated in Figure 2.5B, STs increased their probability to approach the lever CS compared to unpaired controls between Sessions 1 and 6 (Condition X Session interaction, $F_{(1,32)}=53.44$, $p<0.001$). There was no difference in acquisition between the propranolol and vehicle treated STs (no Treatment X Session interaction, $F_{(1,32)}=0.003$, $p=0.96$). See Figure 2.4B for acquisition of video-scored approach in STs and unpaired animals.

Video Scored Orienting. The probability to orient toward the lever for both propranolol and vehicle treated STs increased from Sessions 1 to 6 compared with unpaired controls (Condition X Session interaction, $F_{(1,41)}=60.87$,$p<0.001$: Figure 2.5C). There was no difference in acquisition between the propranolol and vehicle treated STs (Treatment X Session interaction, $F_{(1,41)}=1.38$, $p=0.25$: Figure 2.5C). Unpaired animals showed a decrease in probability to orient to the CS. However, STs in both treatment groups developed an orienting response, demonstrated by an increase their probability to orient toward the CS between Sessions 1 and 6. This is in agreement with previous evidence that an orienting response is a *conditioned* response that develops over time, specifically to a conditioned stimulus.

Retrieval and Reconsolidation

Figure 2.5 illustrates the effect of treatment on contact, approach, and orienting responses between Sessions 6 and 8 during the first half (4 s) of the CS period. Data are displayed as probability of computer-scored lever contacts, video-scored approach, and video-scored orienting (calculated by # of trials engaged in behavior/25).

Computer Scored Lever Contacts. As previously mentioned, propranolol dramatically decreased the probability of contacting the lever (Figure 2.5D). There was a main effect of treatment between the ST propranolol and vehicle treated groups ($F_{(1,18)}=4.22$, $p=0.05$). Treatment also significantly affected approach between Sessions 6 and 8 (Treatment X Session

interaction, $F_{(1,18)}$=22.08, p<0.001). Thus, as demonstrated in the computer-scored data above, the probability of approaching the lever CS during the first 4 s of presentation decreased significantly in the propranolol-treated rats between Days 6 and 8.

Video Scored Approach. Figure 2.5E illustrates that propranolol affected conditioned approach slightly more than conditioned orienting behavior. There was a main effect of treatment between the ST propranolol and vehicle treated groups in approach ($F_{(1,18)}$=8.34, p=0.01). Treatment also significantly affected approach between Sessions 6 and 8 (Treatment X Session interaction, $F_{(1,18)}$=4.87, p=0.04). Propranolol had a significant effect on conditioned approach behavior between Days and 8. Interestingly, the effect on video-scored conditioned approach behavior is not as dramatic as the effect of propranolol on computer-scored contacts.

Video Scored Orienting. In Figure 2.5F, a small, but significant difference in orienting behavior (effect of treatment, $F_{(1,18)}$=4.63, p=0.04) can be seen on Day 8 between propranolol and vehicle groups. However, the difference in orienting on Day 8 was not the result of a significant change between treatment groups between Sessions 6 and 8 (no Treatment X Session interaction, $F_{(1,18)}$=4.08, p=0.06). Although there appears to be a slight group difference in orienting responses during Session 8, propranolol-treated animals were still orienting to the lever with an average of over 95% of trials. This suggests that propranolol is not causing a deficit in the orienting response, and thus, the CS-US association is still intact, even with a dramatic decrease in probability to contact the lever.

Together with the time course analyses, these data demonstrate that propranolol has different effects on different conditioned responses to a lever CS. Excluding the first few trials of the session, propranolol significantly reduces the number of lever contacts that animals will make during a session, it has a small, but significant effect on approach behavior, and has no

effect on orienting behavior. The latency to engage in contact and approach behaviors increases throughout the session, while the probability of engaging in these behaviors decreases throughout the session. These latency analyses suggest that propranolol is decreasing the incentive motivational value of the lever CS. That is, the lever CS still acts as a reliable predictor of reward and in some aspect still acts as a motivational stimulus, in that animals will continue to sign-track to the lever CS to some extent. However, the *excitement*, *vigorous* approach, and *attraction* elicited by the lever CS are significantly decreased after propranolol treatment. Interestingly, this lack of excitement, vigorous approach, and attraction to the lever decreases across trials on Session 8, as if the lever CS is continuing to lose incentive properties throughout the final session.

Experiment 3: The effect of propranolol on ST and GT CRs without memory retrieval

Non-retrieval Control

Pavlovian conditioned approach (PCA)

The acquisition of ST and GT conditioned responses across training can be seen in Figures 2.6A-C and 2.6G-I. Across training days 1-5, animals classified as STs made significantly more lever contacts than GTs (Phenotype x Session interaction, $F_{(4,30)}=33.45$, $p<0.001$), showed an increased probability to approach the lever (Phenotype x Session interaction, $F_{(4,30)}=27.25$, $p<0.001$), as well as a decrease in latency to approach the lever (Phenotype x Session interaction, $F_{(4,30)}=22.55$, $p<0.001$). In contrast, GTs made significantly more food cup entries than STs (Phenotype x Session interaction, $F_{(4,30)}=21.99$, $p<0.001$), demonstrated a significant increase in probability to approach the food cup (Phenotype x Session interaction, $F_{(4,30)}=36.97$, $p<0.001$), and a decreased latency to approach the food cup (Phenotype x Session interaction, $F_{(4,30)}=42.31$, $p<0.001$). There were no significant differences between

propranolol or saline treated groups in the acquisition across days 1-5 measured by the number of lever contacts (Phenotype X Treatment X Session interaction, $F_{(4,30)}$=1.85, p=0.14), probability to approach the lever ($F_{(4,30)}$=1.37, p=0.27), latency to approach the lever ($F_{(4,30)}$=0.95, p=0.45), number of food cup entries ($F_{(4,30)}$=1.33, p=0.28), probability to approach the food cup ($F_{(4,30)}$=1.58,p=0.21), and latency to approach the food cup ($F_{(4,30)}$=2.17, p=0.09).

PCA Retrieval and Reconsolidation

Lever-directed behavior. Figure 2.6D-F indicates that propranolol did not significantly affect lever directed behavior in STs or GTs. STs made significantly more lever contacts than GTs (effect of Phenotype $F_{(1,30)}$=81.57, p<0.001), did so in a higher probability ($F_{(1,30)}$=355.53, p<0.001), and in a shorter latency ($F_{(1,30)}$=132.67, p<0.001). However, there were no significant effects on lever contacts as a result of treatment ($F_{(1,30)}$=0.032, p=0.86: Figure 2.6D) or session ($F_{(1,30)}$=0.83, p=0.37: Figure 2.6D), no effect on probability as a result of treatment ($F_{(1,30)}$=0.23, p=0.64: Figure 2.6E) or session ($F_{(1,30)}$=0.33, p=0.57: Figure 2.6E) and no effect on latency as a result of treatment ($F_{(1,30)}$=0.56, p=0.46: Figure 2.6F) or session ($F_{(1,30)}$=1.91, p=0.17: Figure 2.6F), indicating that propranolol or vehicle injections on Days 6 and 7 did not affect responding during Day 8.

Food Cup-directed behavior. Food cup entries, probability, and latency on Sessions 5 and 8 can be seen in Figures 2.6J-L. GTs made significantly more head entries than STs (effect of Phenotype, $F_{(1,30)}$=176.79, p<0.001) with higher probability ($F_{(1,30)}$=696.79, p<0.001), and shorter latency ($F_{(1,30)}$=524.18, p<0.001). But there were no significant differences in lever contacts as a result of treatment ($F_{(1,30)}$=0.71, p=0.41; Figure 2.6J) or across sessions ($F_{(1,30)}$=3.04, p=0.10: Figure 2.6J), no effect on probability by treatment ($F_{(1,30)}$=1.28, p=0.27:

Figure 2.6K) or session ($F_{(1,30)}$=0.76, p=0.39: Figure 2.6K), and no effect on latency by treatment ($F_{(1,30)}$=.16, p=0.69: Figure 2.6L) or session ($F_{(1,30)}$=0.61, p=0.44: Figure 2.6L).

Experiment 4: The effect of nadolol on the reconsolidation of ST and GT CRs

Nadolol Control

Pavlovian conditioned approach (PCA)

The acquisition of ST and GT conditioned responses across training can be seen in Figure 2.7A-C and G-I. Across training days 1-5, animals classified as STs made significantly more lever contacts than GTs (Phenotype x Session interaction, $F_{(4,46)}$=9.27, p<0.001), showed an increased probability to approach the lever (Phenotype x Session interaction, $F_{(4,46)}$=13.71, p<0.001), as well as a decrease in latency to approach the lever (Phenotype x Session interaction, $F_{(4,46)}$=24.24, p<0.001). In contrast, GTs made significantly more food cup entries than STs (Phenotype x Session interaction, $F_{(4,46)}$=6.10, p<0.001), demonstrated a significant increase in probability to approach the food cup (Phenotype x Session interaction, $F_{(4,46)}$=25.68, p<0.001), and a decreased latency to approach the food cup (Phenotype x Session interaction, $F_{(4,46)}$=31.45, p<0.001). There were no significant differences between acquisition in nadolol- or saline-treated groups in the number of lever contacts (Phenotype X Treatment X Session interaction, $F_{(4,179)}$=0.09, p=0.98), probability to approach the lever ($F_{(4,54)}$=0.44, p=0.78), latency to approach the lever ($F_{(4,115)}$=0.42, p=0.80), number of food cup entries ($F_{(4,65)}$=0.62, p=0.65, probability to approach the food cup ($F_{(4,82)}$=0.33, p=0.86), or latency to approach the food cup ($F_{(4,124)}$=0.43, p=0.79).

PCA Retrieval and Reconsolidation

Lever-directed behavior. Illustrated in Figure 2.9, Nadolol did not significantly affect lever-directed behavior across Sessions 6 through 8. STs made significantly more lever contacts

than GTs (effect of Phenotype $F_{(1,47)}$=85.12, p<0.001), did so in a higher probability ($F_{(1,47)}$=1056.95, p<0.001), and in a shorter latency ($F_{(1,47)}$=314.45, p<0.001). However, there were no significant effects on lever contacts by treatment ($F_{(1,56)}$=0.046, p=0.83: Figure 2.7D) or session ($F_{(2,86)}$=0.24, p=0.79: Figure 2.7D), on probability by treatment ($F_{(1,49)}$=1.11, p=0.29: Figure 2.7E) or session ($F_{(2,60)}$=0.19, p=0.83: Figure 2.7E) or latency by treatment ($F_{(1,52)}$=0.67, p=0.42: Figure 2.7F) or session ($F_{(2,67)}$=0.53, p=0.59: Figure 2.7F), indicating that propranolol or vehicle injections after Sessions 6 and 7 did not affect responding during Session 8.

Food cup-directed behavior. Figure 2.9 shows that Nadolol also did not affect food cup-directed behavior. GTs made significantly more head entries than STs (effect of Phenotype, $F_{(1,47)}$=384.08, p<0.001) with higher probability ($F_{(1,49)}$=227.94, p<0.001), and faster latency ($F_{(1,47)}$=292.80, p<0.001). There was a significant effect of treatment ($F_{(1,50)}$=4.20, p=0.04: Figure 2.7J), but no significant effect on food cup entries by session ($F_{(2,94)}$=1.74, p=0.18: Figure 2.7J), no effect on probability by treatment ($F_{(1,53)}$=0.07, p=0.80: Figure 2.7K) or session ($F_{(2,92)}$=0.48, p=0.62: Figure 2.7K), and no effect on latency by treatment ($F_{(1,50)}$=0.07, p=0.795: Figure 2.7L) or session ($F_{(2,79)}$=0.58, p=0.56: Figure 2.7L). Treatment did not differentially affect STs and GTs (no Treatment X Phenotype interaction, $F_{(1,50)}$=3.18,p=0.08). Within the GTs, propranolol and vehicle groups were not different (no effect of Treatment, $F_{(1,15)}$=2.65, p=0.12).

Discussion

In the present series of experiments, we asked whether propranolol differentially affected the reconsolidation of ST and GT CRs. In the first experiment, we found that post-session treatment with propranolol decreased subsequent ST behavior, but had no effect on GT behavior. Next, we replicated this effect in STs only, and demonstrated that propranolol decreased conditioned approach responses, and especially the vigor with which rats engaged the lever-CS,

but had a negligible on conditioned orienting responses. The video-scored observations of behavior were the most telling, in the precise effects of propranolol on behavior. We quantified two behaviors from the video recordings; orienting and approach. As illustrated in the time course analysis of computer-recorded behavior, most propranolol-treated rats still rapidly approached and contacted the lever on a majority of the trials during the final session. Thus, we describe more detailed account of the effects on propranolol below.

On the final test session, STs in the saline-treated group would orient to the lever upon presentation, and immediately approach and contact the lever. On most trials, rats approached and engaged the lever vigorously for the entire 8 s CS period. Propranolol-treated animals, on the other hand, showed a dramatic decrease in the vigor and excitability during the final test session. This was in sharp contrast to the behavior evoked by the lever CS prior to propranolol treatment during Session 6, and also relative to the saline-treated rats. Interestingly, the decrease in vigor observed in propranolol-treated animals often did not occur until after the first few lever CS trials. As mentioned previously, all sessions were reinforced, so this decrease across the session could not be the result of an extinction effect within the session. After these initial trials in which propranolol-treated rats still approached the lever with intensity and vigor, their responding to the CS decreased through the remainder of the session. On some trials, rats would orient toward the lever upon CS presentation, and stare at it until it retracted. At the time of lever retraction, most rats immediately retrieved the pellet from the food-cup. During other trials, the rats would orient toward the lever and slowly approach it. From this point, rats primarily responded in one of the following three ways for the rest of the CS period. One, they would stare at the lever for the remainder of the CS period without contacting it. Two, they would sniff around the lever and delicately investigate the lever with their front paws. Sometimes these contacts resulted in a

computer-scored lever contact, but other times, the contact was not strong enough to result in a computer-scored response. Third, the rats would approach the lever within close proximity, and they would pause before engaging with the lever. Mostly, this engagement was not with the extreme vigor that they interacted with the lever prior to propranolol treatment, except during the first few trials.

In Experiment 2, despite a dramatic decrease in approach CRs in the STs, orienting CRs indicative of the CS-US association remained intact. Another indication of an intact CS-US association can be supported by the time course effect of propranolol. That is, during the first few trials, animals are still vigorously approaching the CS, suggesting that the CS-US association is still intact. Using STs and GTs as a model of attributing motivational value to cues, these experiments suggest that propranolol disrupts the motivational component of a memory, without affecting the CS-US association. In Experiment 3, we demonstrated that this effect was contingent upon memory retrieval. Lastly, we showed that nadolol, a beta-adrenergic antagonist that only acts peripherally, does not affect ST or GT behavior. These data support our hypothesis, in that propranolol disrupted ST, but not GT behavior. This effect seems to be restricted to central nervous system beta-adrenergic blockade after a memory has been retrieved.

Alternative Explanations

One alternative explanation for a decrease in ST behavior could be due to general locomotor deficits. However, we believe that our data provide strong evidence against this possibility. First, in our procedure, animals were given injections *after* each training session. Propranolol administered intraperitoneally is cleared from the central and peripheral nervous system within 8-16 hours and has a half-life of 1.5 hours (Laverty & Taylor, 1968; Kim, Hong, Park, Kang, & Lee, 2001). Thus, it is very unlikely that propranolol would still be present 24

hours after the injection on the previous day. Additionally, as mentioned above, it appears that the decrease in ST behavior induced by propranolol occurs after the first few trials. If a motor deficit were present, we would expect that animals would show this deficit in responding from the first trial. Lastly, we conducted a control experiment during which animals received propranolol injections without retrieving the memory. Again, if the decrease in ST behavior were due to lasting effects of propranolol on locomotor activity, we would expect to see this decrease even without retrieving the memory. In Experiment 3, we found that this is not the case. Collectively, we believe these data suggest that propranolol is not acting to decrease ST behavior through locomotor impairments.

A second possibility for the decrease in ST behavior is that propranolol induced a conditioned aversion to the cues and contexts in the test chamber. Again, we believe our data suggest that this is not the case. First, all Pavlovian sessions were reinforced with food, including session 8. If propranolol resulted in a conditioned aversion to the cues or context of the chamber, we might expect that rats would stop eating the food pellets. However, none of the animals included in the analysis left food pellets at the end of the session on any of the days following propranolol or vehicle treatment. We also do not believe that propranolol induces a conditioned aversion in rats based on previous reports. Others have shown that propranolol does not produce conditioned taste or place aversions (Laverty & Taylor, 1968; Sara, Dyon-Laurent, & Hervé, 1995; Przybyslawski et al., 1999).

Reconsolidation of Motivational Value

Individual differences in approach behavior to a CS, such that those observed in STs and GTs are just one measurement of motivational attribution. By definition, motivational stimuli acquire three properties; 1) they elicit approach, and direct an individual's attention toward it; 2)

they are desired and individuals will work to obtain them; 3) they evoke motivational states in an individual that energize and instigate seeking behavior. These properties are psychologically and neurobiologically dissociable (Cardinal et al., 2002), and the reconsolidation of these properties have been previously investigated (see Milton & Everitt, 2010 for review). The second property of a motivational stimulus has been found to be dependent upon beta-adrenergic receptor activation. For example, if propranolol is administered immediately after memory reactivation, it will decrease the extent to which rats will work for presentation of a previously reward-paired CS, compared with vehicle controls (Milton & Everitt, 2008; Schramm et al., 2016). Other studies have found that this is not the case for all properties of a motivational stimulus. It has been previously reported that administering propranolol does not affect the ability of a CS to energize or instigate seeking behavior, and also does not affect conditioned approach behavior (Lee & Everitt, 2008). Thus, in appetitive learning, propranolol is currently considered to only affect one property of a motivational stimulus; the ability of a CS to act as a conditioned reinforcer (Milton & Everitt, 2010).

The current experiments do not confirm previous findings. However, there are several procedural differences between our studies and those reported by Everitt and colleagues (Lee & Everitt, 2008b; Milton et al., 2012; Milton et al., 2008) that may account for different effects of propranolol. First, our study investigated conditioned approach to a CS in STs and GTs, rather than a general population of animals. In Lee and Everitt (2008b), the probability of approach behavior after ten days of PCA training averaged between 60 and 70 percent. In Experiments 1 and 3, the STs were approaching the lever with a 90% probability or higher. It is possible that a greater degree of motivational value attribution is required for propranolol to disrupt reconsolidation. Additionally, Lee & Everitt (2008) injected animals 30 minutes prior to memory

reactivation, rather than after memory reactivation as in the present experiments. The half-life of propranolol is relatively short (1.5 h – Kim, Hong, Park, Kang & Lee, 2001), thus it is possible the effects of propranolol peaked before the memory was reactivated. In the current experiments, we also administered a higher dose of propranolol (20mg/kg) for two consecutive days. Any of these procedural differences could account for the differential effects of propranolol on conditioned approach; however, follow-up studies will be required for further exploring these parameters.

Parsing Motivational and Predictive Value

There is a substantial difference between memory erasure and memory modification. Indeed, many studies have used the term "erasure" in disrupting reconsolidation (see Sandkühler & Lee, 2013). Others have made similar implications. For example, Milton & Everitt (2008) state "systemic injections of the β-adrenergic receptor antagonist dl-propranolol can disrupt the reconsolidation of … CS–sucrose memories". However, the present studies suggest that the CS-US association remains intact, despite disrupting the motivational value of the CS. In Experiment 2, we demonstrate that disrupting ST behavior is not a result of a forgotten association. That is, the CS still evokes a conditioned response (e.g. orienting) in the STs, indicating that they still remember that the CS predicts the US. This is in agreement with human fear conditioning studies, where a CS becomes less likely to elicit motivated fear behaviors, but maintains predictive value (Kindt et al., 2009; Soeter & Kindt, 2010, 2011).

Implications for Reconsolidation

The current findings have important implications for conceptualizing reconsolidation. As mentioned, many studies discuss disrupting reconsolidation to be a disruption of a CS-US association. Here, we have demonstrated that this is not the case for all memories, in that

emotional memories are modified by propranolol, rather than disrupted. Alternatively, there may be different memories to represent associations that a CS acquires with different features of a US (Delamater & Oakeshott, 2007). Thus, propranolol may disrupt an association, but specifically an association with the motivational component of a US, which is present in STs, but not GTs. Some individuals have expressed an ethical concern for erasing the "factual" component of a memory with propranolol (Kolber, 2006; but see Kolber, 2011). However, our findings that propranolol decreases emotional/motivational value of memory without erasing it argues against any ethical concerns surrounding memory erasure. Further, the findings discussed in this chapter that disrupting reconsolidation with propranolol in animals appears to reflect the psychological processes observed in humans. Therefore, this may prove to be a valid model for investigating targeted treatments that may help to reduce the maladaptive motivational states induced by stimuli paired with rewards or aversions.

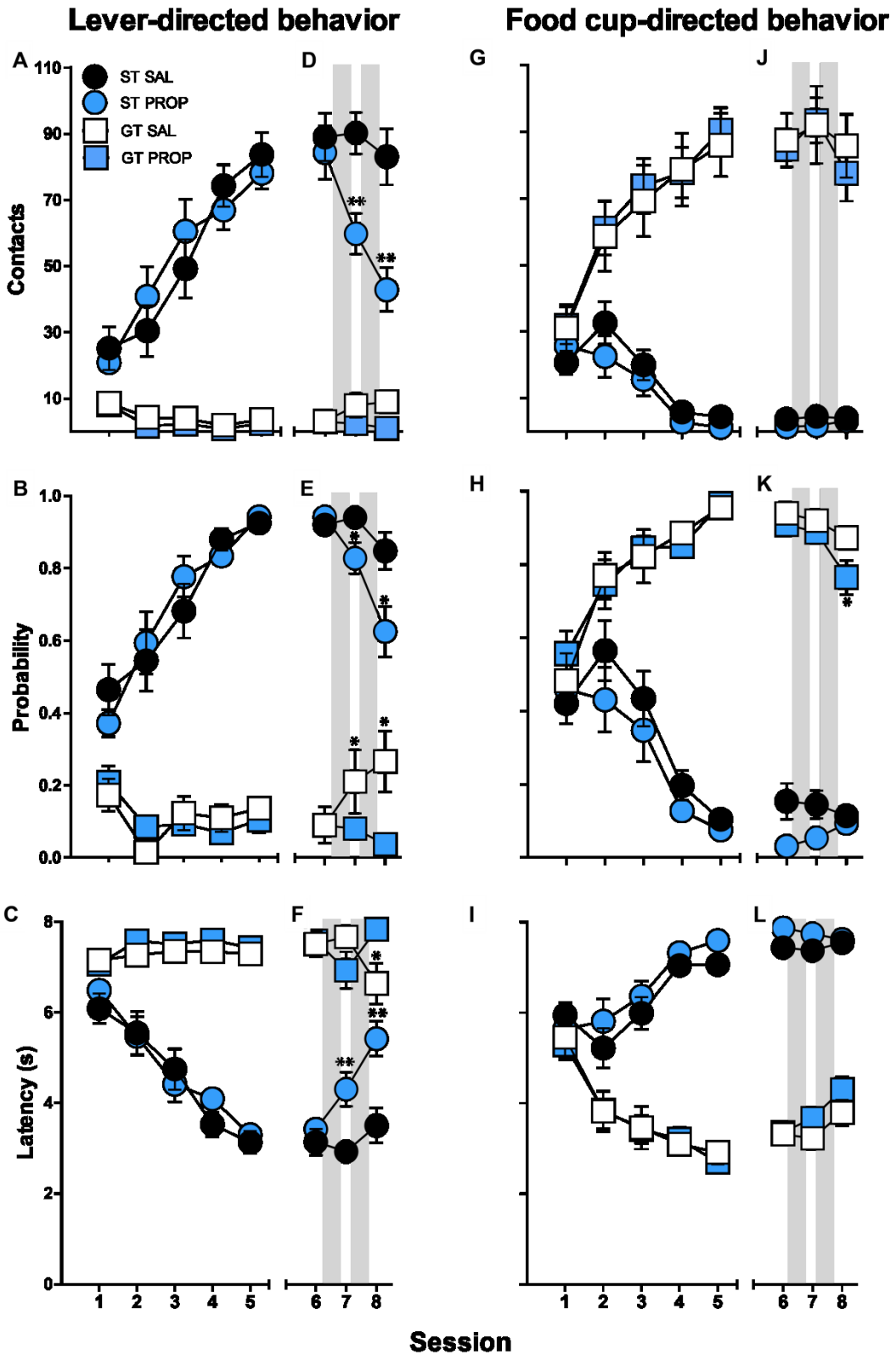

Lever-directed behavior
Food cup-directed behavior
A
D
G
J
B
E
H
K
C
F
I
L
ST SAL
ST PROP
GT SAL
GT PROP
Contacts
Probability
Latency (s)
Session

Figure 2.1. Lever- and food-cup directed behavior in sign-trackers (STs) and goal-trackers (GTs) across training sessions 1-8. Data represent mean ±SEM. The acquisition of a sign-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of lever contacts. (B) The probability of lever-directed responding. (C) The average latency to approach the lever. The acquisition of a goal-tracking conditioned response across Sessions 1-5 is illustrated in G-I. (G) The number of food cup entries. (H) The probability of food cup-directed responding. (I) The latency to approach the food cup. The effect of post-session injections on sign-tracking conditioned responses across Sessions 6-8 is shown in D-F. Grey bars indicate when animals received propranolol or saline injections (immediately after Sessions 6 and 7). (D) The number of lever contacts. (E) The probability to approach the lever. (F) The latency to approach the lever. The effect of propranolol or saline post-session injections on goal-tracking conditioned responses across Sessions 6-8 is illustrated in J-L. (J) The number of food cup entries. (K) The probability of food cup-directed responding. (L) Latency to approach the food cup. * $p<0.01$, ** $p<0.001$ (relative to Session 6).

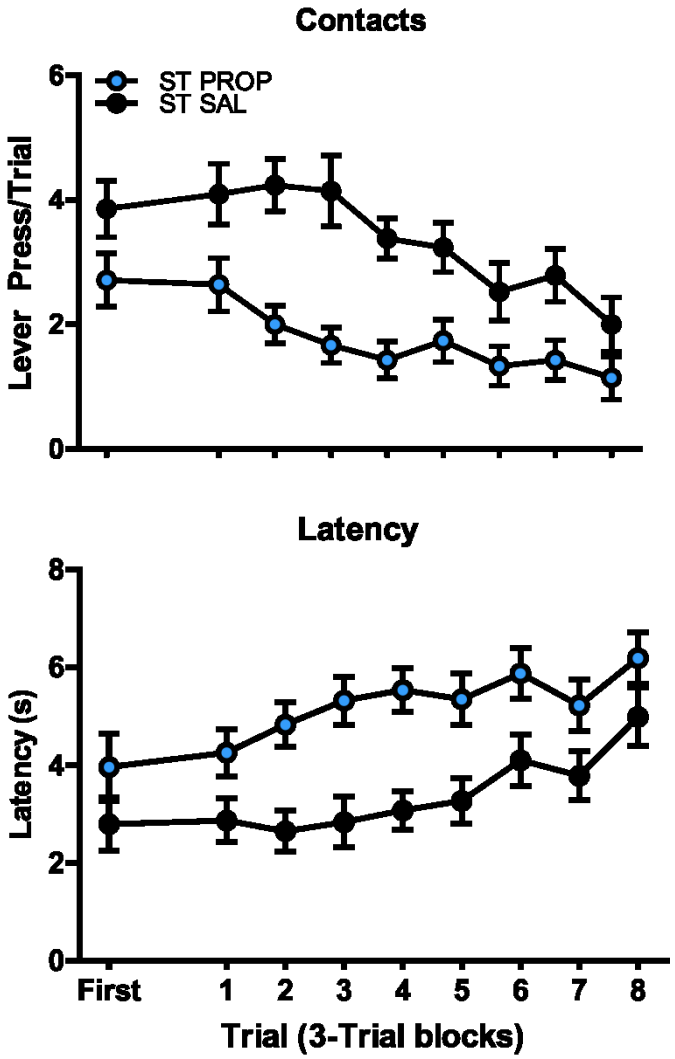


Figure 2.2. Time Course of responding during session 8 in propranolol- and saline- treated sign-trackers (STs). Data represent mean ±SEM presented in three-trial blocks (with the exception of the first trial). The time course of average lever presses per trial on Session 8 in propranolol- and saline-treated STs (top). The time course of average latency to approach the lever on each trial during Session 8 in propranolol- and saline-treated STs (bottom).

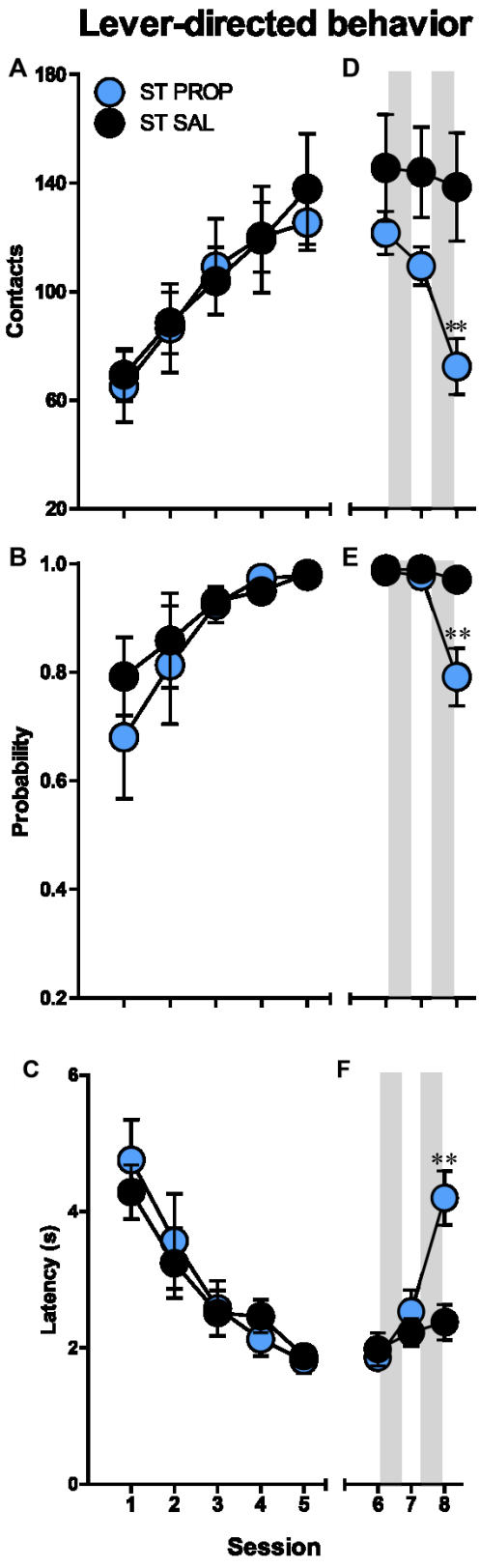

Lever-directed behavior
A
D
ST PROP
ST SAL
180
140
100
60
20
Contacts
**
B
E
1.0
0.8
0.6
0.4
0.2
Probability
**
C
F
6
4
2
0
Latency (s)
**
1
2
3
4
5
6
7
8
Session

Figure 2.3. Lever-directed behavior in propranolol- and vehicle-treated sign-trackers (STs). Data represent mean ±SEM. The acquisition of a sign-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of lever contacts. (B) The probability of lever-directed responding. (C) The average latency to approach the lever. The effect of post-session injections on sign-tracking conditioned responses across Sessions 6-8 is shown in D-F. Grey bars indicate when animals received propranolol or saline injections (immediately after Sessions 6 and 7) (D) The number of lever contacts. (E) The probability to approach the lever. (F) The latency to approach the lever. $^{**}p<0.001$ (relative to Session 6).

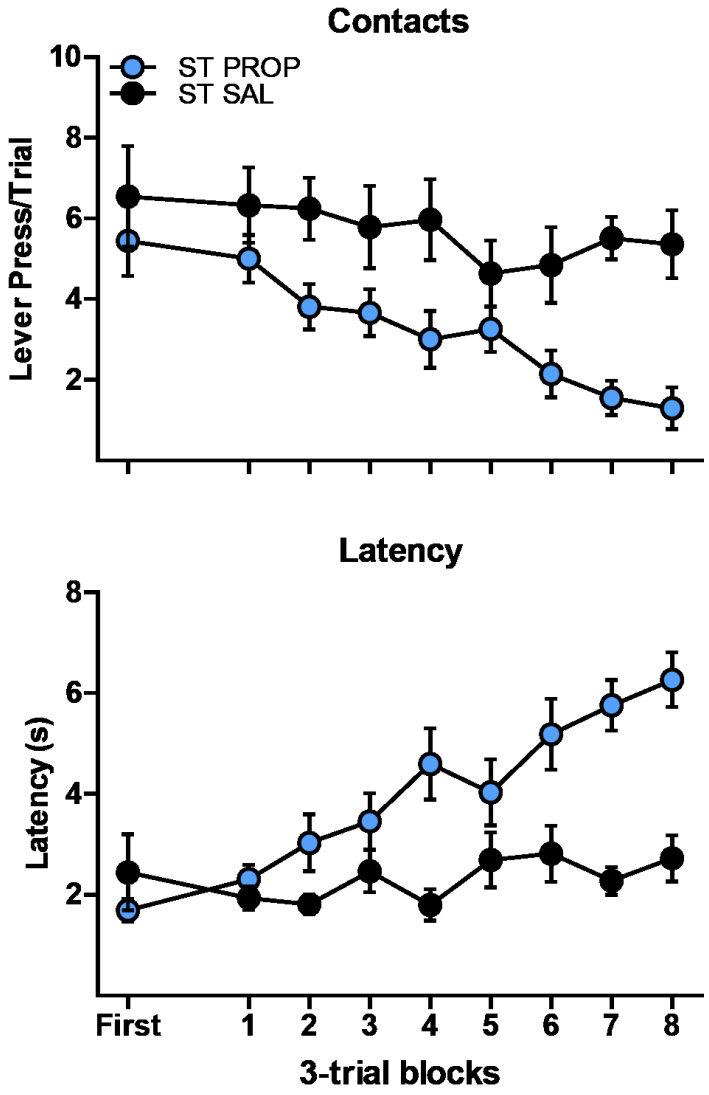


Figure 2.4. Time course of trials on session 8 in propranolol- and saline-treated sign-trackers (STs). Data represent mean ±SEM presented in three-trial blocks (with the exception of the first trial). The time course of average lever presses per trial on Session 8 in propranolol- and saline-treated STs (top). The time course of average latency to approach the lever on each trial during Session 8 in propranolol- and saline-treated STs (bottom).

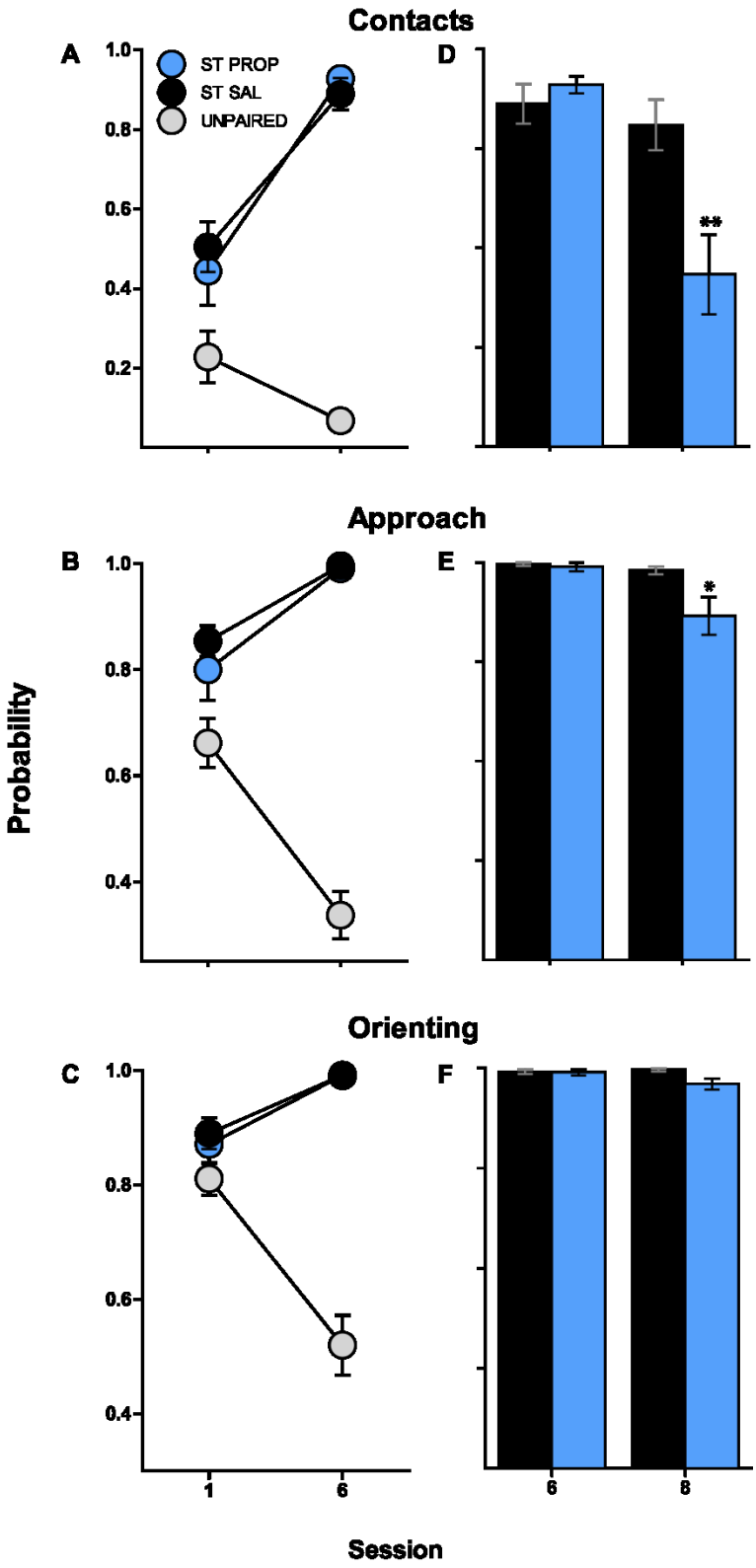
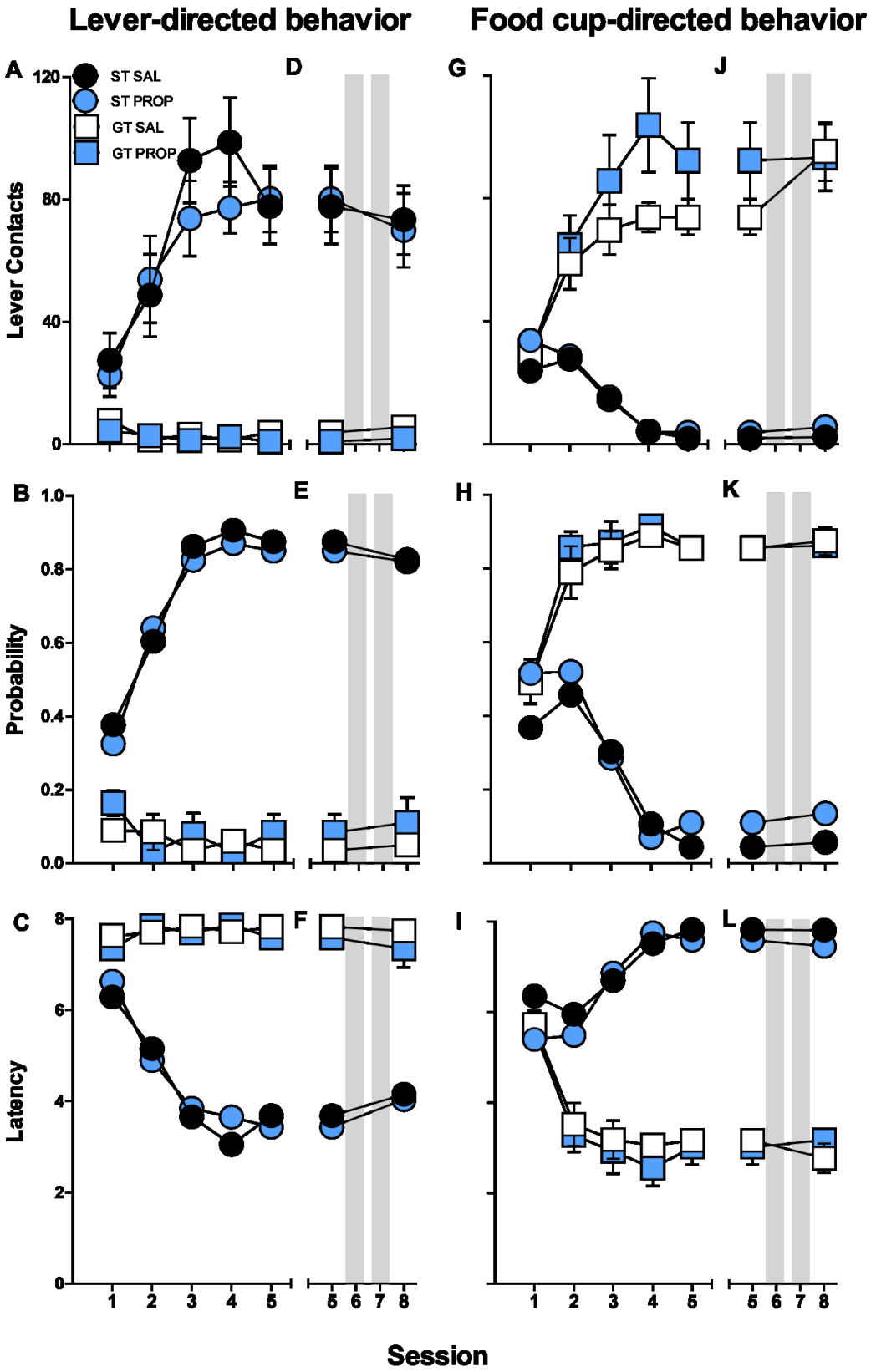

Contacts
A
1.0
0.8
0.6
0.4
0.2
ST PROP
ST SAL
UNPAIRED
D
**
Approach
B
1.0
0.8
0.6
0.4
Probability
E
*
Orienting
C
1.0
0.8
0.6
0.4
1
6
F
6
8
Session

Figure 2.5. Computer-scored contacts, video-scored orienting, and video-scored approach behavior. Data represent mean ±SEM. The acquisition of computer-scored contacts, video-scored orienting, and video-scored approach in STs and unpaired animals between Sessions 1 and 6 are illustrated in A-C. (A) Probability of computer-scored contacts. (B) Probability of video-scored approach behavior. (C) Probability of video-scored orienting behavior. The effect of post-session propranolol or saline injections between Sessions 6 and 8 are illustrated in D-F. (D) Probability of computer-scored contact. (E) Probability of video-scored approach behavior. (F) Probability of video-scored orienting behavior. *p<0.05, **p<0.001 (relative to Session 6).


Lever-directed behavior
Food cup-directed behavior
A
B
C
D
E
F
G
H
I
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L
ST SAL
ST PROP
GT SAL
GT PROP
Lever Contacts
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Latency
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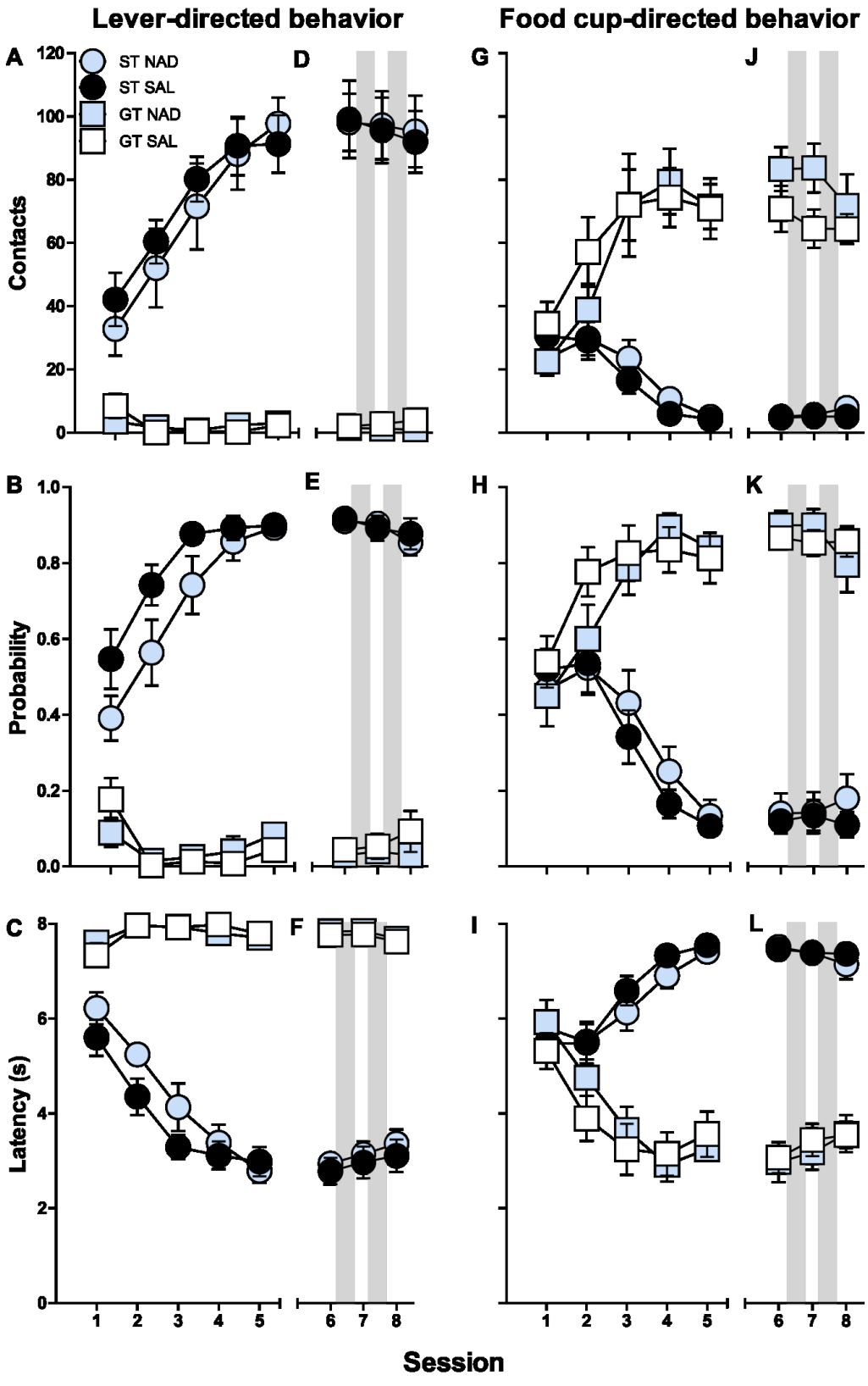
1 2 3 4 5
5 6 7 8

Figure 2.6. Lever-directed behavior and food cup-directed behavior in STs and GTs given propranolol or saline injections. Data represent mean ±SEM. The acquisition of a sign-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of lever contacts. (B) The probability of lever-directed responding. (C) The average latency to approach the lever. The acquisition of a goal-tracking conditioned response across Sessions 1-5 is illustrated in G-I. (G) The number of food cup entries. (H) The probability of food cup-directed responding. (I) The latency to approach the food cup. The effect of post-session injections on sign-tracking conditioned responses between Sessions 5 and 8 is shown in D-F. The grey bars represent saline or propranolol injections given immediately after Sessions 7 and 7. (D) The number of lever contacts. (E) The probability to approach the lever. (F) The latency to approach the lever. The effect of propranolol or saline post-session injections on goal-tracking conditioned responses across Sessions 6-8 is illustrated in J-L. (J) The number of food cup entries. (K) The probability of food cup-directed responding. (L) Latency to approach the food cup.


Lever-directed behavior
Food cup-directed behavior
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ST SAL
GT NAD
GT SAL
Contacts
Probability
Latency (s)
Session

Figure 2.7. Lever- and food cup-directed behavior in STs and GTs after post-session administration of nadolol or saline injections. Data represent mean ±SEM. The acquisition of a sign-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of lever contacts. (B) The probability of lever-directed responding. (C) The average latency to approach the lever. The acquisition of a goal-tracking conditioned response across Sessions 1-5 is illustrated in G-I. (G) The number of food cup entries. (H) The probability of food cup-directed responding. (I) The latency to approach the food cup. The effect of post-session injections on sign-tracking conditioned responses between Sessions 5 and 8 is shown in D-F. (D) The number of lever contacts. (E) The probability to approach the lever. (F) The latency to approach the lever. The effect of propranolol or saline post-session injections on goal-tracking conditioned responses across Sessions 6-8 is illustrated in J-L. (J) The number of food cup entries. (K) The probability of food cup-directed responding. (L) Latency to approach the food cup.

Chapter III

The Effect of Propranolol on the Reconsolidation of Goal-Tracking to an Auditory Stimulus

Introduction

Prior to memory reconsolidation, a retrieved memory is labile and thus subject to disruption. In Pavlovian conditioning, disrupting reconsolidation is often discussed as affecting the entire memory, i.e., degrading or erasing the association between the conditioned stimulus (CS) and unconditioned stimulus (US) (see Sandkühler & Lee, 2013). However, recent studies in humans suggest that this may not be the case. Using a fear conditioning task, Kindt et al. (2009) demonstrated that the beta-adrenergic antagonist propranolol disrupted a conditioned fear response (fear-potentiated startle) while leaving the declarative memory of the association intact. That is, propranolol decreased participants' conditioned fear responses to a CS that predicted an aversive stimulus, but this was not because they 'forgot' that the CS predicted the aversive stimulus; they were still able to describe the relationship between the CS and the aversive stimulus it predicted.

In Chapter Two, we hypothesized that propranolol may have a similarly selective effect on appetitive memories in rats, disrupting the emotional or incentive-motivational (herein referred to as "incentive-motivational") component of a memory, while leaving the core CS-US association intact. To further test this hypothesis, we examined the effect of propranolol on the

reconsolidation of memory in animals that express different conditioned responses to a CS; 1) Sign-trackers (ST - Hearst & Jenkins, 1974), animals that approach a CS, and 2) Goal-trackers (GT - Boakes, 1977), animals that approach the location of reward delivery. There is considerable evidence to suggest that although a lever-CS that predicts a food reward comes to act as a predictive CS in both STs and GTs, capable of evoking a CR in both, the lever-CS is attributed with much greater motivational value (incentive salience) in STs than GTs (Flagel et al., 2007; Robinson & Flagel, 2009; Meyer et al., 2012b). Thus, we asked whether propranolol differentially affected reconsolidation in rats that developed ST vs. GT CRs. We found that propranolol decreased ST, but not GT behavior, suggesting that propranolol had a relatively selective effect on the CR thought to reflect the motivational value of the learned association (memory). That is, the motivational value of the lever-CS was attenuated, while leaving its predictive value intact.

One question raised by the experiments in Chapter Two is whether memories are disrupted as a result of phenotype, or the specific CR evoked by the CS. That is, does propranolol disrupt motivation in animals that have a greater propensity to attribute value to a reward-paired cue (sign-track*ers*), or does it disrupt memories for cues that evoke approach behavior (sign-track*ing*)? One way to answer this question is to use a CS that does not evoke approach, or "sign-tracking" behaviors. If a *tone* CS is paired with food delivery all rats develop a GT CR (even STs) (Meyer et al., 2014; Beckmann et al., 2015), despite being able to localize it (Harrison 1979; Cleland & Davey, 1983). Thus, in the present experiment we asked if propranolol would disrupt the reconsolidation of goal-tracking evoked by a tone CS, and whether it would do so differentially in STs and GTs. Given that a tone CS is attributed with less motivational value than a lever-CS (Meyer et al., 2014; Beckmann et al., 2015), and given that

we have suggested that propranolol selectively degrades the emotional/motivational component of a lever-CS food association (Chapter 2), we predicted that propranolol given after retrieval of a tone CS-food association would not be very effective in disrupting subsequent goal-tracking behavior.

Materials and Methods

Subjects

A total of 52 male Sprague-Dawley rats (Charles River, Raleigh, NC) weighing 250-275g upon arrival were used for this study. Animals were individually housed in a climate controlled colony room with a reverse 12-h light/12 h dark cycle, where food and water were available ad libitum. Prior to experimental testing, animals were given one week to acclimate to the housing room. During this time, rats were handled several times by the experimenter. All experimental procedures were approved by the University of Michigan Committee on Use and Care of Animals.

Apparatus

Standard (22 x 18 x 13 cm) test chambers (Med Associates Inc., St Albans, VT, USA) were used for behavioral testing. Each chamber was individually enclosed in a sound-attenuating cabinet equipped with a fan for ventilation and to impede background noise. Pavlovian training chambers each had a food cup placed 3 cm above the stainless steel grid floor in the center of one wall, and a red house light on the opposite wall, which remained illuminated throughout the duration of all sessions. For Auditory Pavlovian conditioning (Experiments 1 and 2), a speaker calibrated to deliver a 2.9 kHz tone (70 dbs) was positioned directly under the house light. An illuminated retractable lever located 6 cm above the floor and 2.5 cm away from the food cup on either the left or right side (counterbalanced across chambers) was used for Pavlovian

conditioned approach (PCA) sessions (Experiment 2). Infrared photo-beams located inside the food cup were used to record head entries. All experimental events were controlled and recorded by a MED-PC computer system.

Drugs

Propranolol (DL-Propranolol hydrochloride, 99%; Acros Organics, NJ, USA) was dissolved in 0.9% sodium chloride, and was administered intraperitoneally (i.p.) at a dose of 20mg/kg/injection.

Experiment 1: Auditory Pavlovian Conditioning

Auditory Pavlovian Conditioning. Rats were trained using a Pavlovian conditioning procedure described previously (Meyer et al. 2014). On the two days preceding the start of the experiment, 45mg banana-flavored pellets (Bio-Serv) were placed into home cages to habituate rats to this food. Following food habituation days, rats were trained to retrieve pellets from the food cup during a pretraining session, during which 25 pellets were dispensed into the food cup on a 30 s (0-60 s) variable time (VT) schedule. The red house light remained illuminated throughout the duration of the session. If a rat failed to consume all 25 pellets, they were given an additional pretraining session. On the day following pretraining, Auditory Pavlovian conditioning began. Animals were trained over five consecutive daily sessions. Each session consisted of 25 trials in which a 2.9 kHz tone at 70 dbs (conditioned stimulus, CS) was presented for 8 seconds and followed by the delivery of a 45mg banana-flavored food pellet (unconditioned stimulus, US) into the food cup. CS-US pairings occurred on a variable time (VT) 90 (30-150 s) schedule. The delivery of the food pellet was not contingent upon any response from the animal. An infrared beam was used to detect head entries and latency to approach the food cup. All experimental events were controlled and recorded using a MED-PC computer system.

Rats that achieved a minimum criterion of 30 head entries into the food cup during the CS period by day 5 of training (n=23) were used for this experiment.

Retrieval sessions. Pavlovian conditioning sessions on days 6 and 7 served as retrieval sessions. Behavioral testing on these days were identical to initial Pavlovian training, with the exception that immediately following termination of the session, animals received a post-session injection of either propranolol (20mg/kg) or saline. Rats were divided into propranolol (n=12) or saline (n=11) injection groups in a counterbalanced order. Rats received the same treatment (propranolol or saline) on both days.

Test session. Rats underwent a final Pavlovian conditioning session in order to assess the effect of post-session injections administered on the previous day.

Experiment 2: Auditory Pavlovian Conditioning in STs and GTs

PCA training. Rats were trained using a Pavlovian conditioned approach (PCA) procedure described previously (Flagel et al. 2007; Meyer et al. 2012). On the two days preceding the start of the experiment, 45mg banana-flavored pellets (Bio-Serv) were placed into home cages to habituate rats to this food. Following food habituation days, rats were trained to retrieve pellets from the food cup during a pretraining session, during which 25 pellets were dispensed into the food cup on a 30 s (0-60 s) variable time (VT) schedule. The red house light remained illuminated throughout the duration of the session. If a rat failed to consume all 25 pellets, they were given an additional pretraining session. On the day following pretraining, PCA training began. Briefly, animals were trained over five consecutive daily sessions. Each session consisted of 25 trials in which an illuminated lever (CS) was presented for 8 seconds and followed by the delivery of a 45mg banana-flavored food pellet (US) into the food cup. CS-US pairings occurred on a VT 90 (30-150 s) schedule. The delivery of the food pellet was not

contingent upon any response from the animal. All lever deflections, food cup entries, and latency to approach each location were recorded.

PCA index scores. Animals were classified as sign-trackers (STs) or goal-trackers (GTs) using the criteria previously described by Meyer and colleagues (2012). Briefly, PCA index scores were calculated as an average of three measures of approach behavior during the 8 s CS period; response bias to approach either the lever CS or the food cup [(#lever deflections – #food cup entries)], probability to approach either the lever CS or food cup [(P(lever) – P(food cup)], and latency to approach either the lever CS or food cup [(lever deflection latency – food cup entry latency)/8]. This average produced an index score ranging from -1.0 to +1.0, where a score of -1.0 indicated a strong bias toward approaching the food cup, and a score of +1.0 indicated a strong bias toward approaching the lever. Index scores were averaged across training days 4 and 5, and these values were then used to classify rats as STs or GTs. Animals receiving scores between +0.6 to +1.0 and >50 lever contacts were classified as STs, and animals with scores between -0.6 to -1.0 and >50 food cup entries were classified as GTs.

Auditory Pavlovian conditioning. Immediately following PCA training and classification of STs and GTs, animals began Auditory Pavlovian conditioning sessions (days 6-10). Animals were trained over five consecutive daily sessions. Each session consisted of 25 trials in which a 2.9 kHz tone at 70 dbs (CS) was presented for 8 seconds and followed by the delivery of a 45mg banana-flavored food pellet (US) into the food cup. CS-US pairings occurred on a variable time (VT) 90 (30-150 s) schedule. The delivery of the food pellet was not contingent upon any response from the animal. An infrared beam was used in the food cup to detect head entries and latency to approach the food cup. All experimental events were controlled and recorded using a

MED-PC computer system. Rats that achieved a minimum criterion of 30 head entries into the food cup during the CS period by day 5 of training (n=22) were used for this experiment.

Retrieval sessions. Auditory Pavlovian conditioning sessions on days 11 and 12 served as retrieval sessions. Behavioral testing on these days were identical to initial Pavlovian training, with the exception that upon session termination, animals immediately received a post-session injection of propranolol (20mg/kg). STs (n = 11) and GTs (n=11) received the same treatment (propranolol) on both days.

Test session. Rats underwent a final Pavlovian conditioning session in order to assess the effect of post-session injections administered on the previous day.

Statistics

Linear mixed models (LMM) were used to examine main effects (Treatment, Session,) and interactions (Treatment X Session) on all repeated measures data. The best-fitting model of covariance structure was determined by the lowest Akaike information criterion score (Verbeke & Molenberghs, 2009). Post-hoc Bonferroni comparisons were used to test the effect of treatment within each phenotype, between each of Sessions 6 through 8 (Treatment X Session). If main effects were significant (but no significant interaction), one-way analysis of variance (ANOVA) tests were conducted within treatment or phenotype.

Results

Experiment 1

Pavlovian conditioned approach to the food cup

The acquisition of a GT CR is illustrated in Figure 3.1A-C. Across training Sessions 1-5, animals made significantly increased the number of head entries into the food cup during CS presentation (effect of Session ($F_{(4,31)}$=33.66, p<0.001: Figure 3.1A), showed an increased

probability to approach the food cup (effect of Session interaction, $F_{(4,52)}$=25.73, p<0.001: Figure 3.1B), as well as a decrease in latency to approach the food cup (effect of Session, $F_{(4,56)}$=34.56, p<0.001: Figure 3.1C). There were no significant differences between treatment groups in the acquisition of a goal-tracking response to the CS in head entries (Treatment X Session interaction, $F_{(4,31)}$=0.49, p=0.74), probability ($F_{(4,52)}$=1.38, p=0.25), or latency ($F_{(4,56)}$=0.35, p=0.85).

PCA Retrieval and Reconsolidation

Food cup entries, probability, and latency across Session 6 through 8 are illustrated in Figure 3.1D-F. There were no significant differences between food cup entries between propranolol and vehicle-treated groups (no effect of Treatment, $F_{(1,21)}$=0.000, p=0.10: Figure 3.1D), and no significant changes in the number of food cup entries across sessions (no effect of Session, $F_{(2,28)}$=1.28, p=0.29: Figure 3.1D). There was a significant change in probability to approach the food cup across sessions (effect of session, $F_{(2,42)}$=5.09, p=0.01: Figure 3.1E), but there were no differences between propranolol and saline-treated groups in the probability to approach the food cup (no effect of treatment, $F_{(1,19)}$=1.98, p=0.17: Figure 3.1E), and no effect of treatment across sessions on probability (no Treatment X Session interaction, $F_{(2,42)}$=2.59, p=0.09: Figure 3.1E). There were no significant effects of treatment ($F_{(1,21)}$=0.61, p=0.44: Figure 3.1F) or session ($F_{(2,42)}$=1.36, 0.27: Figure 3.1F) on latency to approach the food cup.

Experiment 2: Auditory Pavlovian Conditioning in STs and GTs

PCA training

ST and GT conditioned responses across training can be seen in Figure 3.2. Across training Sessions 1-5, animals classified as STs made significantly more lever contacts than GTs (Phenotype x Session interaction, $F_{(4,34)}$=14.64, p<0.001), showed an increased probability to

approach the lever (Phenotype x Session interaction, $F_{(4,44)}$=25.49, p<0.001), as well as a decrease in latency to approach the lever (Phenotype x Session interaction, $F_{(4,80)}$=20.99, p<0.001). In contrast, GTs made significantly more food cup entries than STs (Phenotype x Session interaction, $F_{(4,20)}$=16.73, p<0.001), demonstrated a significant increase in probability to approach the food cup (Phenotype x Session interaction, $F_{(4,52)}$=51.34, p<0.001), and a decreased latency to approach the food cup (Phenotype x Session interaction, $F_{(4,30)}$=3.27, p<0.001).

Auditory conditioning.

Figure 3.3A-C illustrates the acquisition of a GT CR to a tone in STs and GTs. Animals demonstrated significant increases in the number of food cup entries during the CS period (effect of Session ($F_{(4,23)}$=9.84, p<0.001), a significant increased probability to approach the food cup (effect of Session, $F_{(4,29)}$=29.96, p<0.001), and a decrease in latency to approach the food cup (effect of Session, $F_{(4,45)}$=50.79, p<0.001). There were no significant differences between STs and GTs in the acquisition of a goal-tracking response to the CS in head entries (Phenotype X Session interaction ($F_{(4,23)}$=0.82, p=0.52), probability ($F_{(4,29)}$=1.95, p=0.13), or latency ($F_{(4,46)}$=1.3, p=0.30).

Auditory conditioning and Retrieval.

The number of food cup entries significantly decreased across Sessions 6-8 (effect of Session $F_{(2,31)}$=4.23, p=0.02: Figure 3.3D), however, there were no differences between STs and GTs (effect of phenotype, $F_{(1,20)}$=0.03, p=0.87). As a follow up statistical analyses of the main effect on Session, separate one-way ANOVAs were conducted within STs and within GTs to determine if responding significantly decreased across Sessions 6-8 in either of these phenotypes. There were no significant decreases in either STs ($F_{(2,30)}$=0.34, p=0.71) or GTs ($F_{(2,30)}$=0.14, p=0.87).

There was a significant effect of Session on probability to approach the food cup ($F_{(2,40)}$=5.35, p=0.009: Figure 3.3E),). A follow-up one way ANOVA within the STs revealed a significant decrease across Sessions 6-8 ($F_{(2,30)}$=3.93, p=0.03). Post-hoc comparisons demonstrate a significant difference between Sessions 6 and 8 ($p<0.05$). Within GTs, there were no significant effects of propranolol on probability to approach the food cup. However, it should be noted that there were no significant differences between the two phenotypes (effect of Phenotype, $F_{(1,23)}$=.022,p=0.88), and no differences between decreases in phenotypes across sessions (Phenotype X Session interaction, $F_{(2,40)}$=0.18, p=0.83).

There was a significant main effect of session of food cup latency ($F_{(2,40)}$=7.56, p=0.002: Figure 3.3F). Follow up one-way ANOVAs were conducted on each phenotype. Within the STs there was a significant difference across Sessions 6-8 ($F_{(2,30)}$=4.33, p=0.02: Figure 3.3F), and post-hoc comparisons found significant differences in latency to approach the food cup between Sessions 6 and 8. Within the GTs, propranolol did not significantly affect latency. Again, as observed with probability to approach the food cup, there were no significant differences between STs and GTs (effect of Phenotype, ($F_{(1,20)}$=0.51, p=0.48), and no differences between phenotype across sessions (Phenotype X Session interaction ($F_{(2,40)}$=1.30, p=0.29).

Discussion

In the present studies, we asked if the reconsolidation of a goal-tracking CR evoked by a tone CS would be disrupted by propranolol. Given that a tone acquires less motivational value than a lever CS, we hypothesized that propranolol would not disrupt reconsolidation of goal-tracking to a tone CS. In Experiment 1, we found that propranolol did not have any effect on goal-tracking behavior to a tone CS. Next, we investigated administration of propranolol would differentially disrupt the reconsolidation of goal-tracking CRs evoked by a tone CS in STs and

GTs. Again, we found no effect of propranolol on the reconsolidation of goal-tracking to a tone CS in either STs or GTs. Here, we extend the findings from Chapter Two by demonstrating that propranolol does not disrupt goal-tracking behavior to a tone CS. This failure to disrupt GT occurred even in animals that have a propensity to attribute greater motivational value to other reward-paired lever CS (STs). This suggests that propranolol selectively disrupts sign-track*ing* (the behavior), and not necessarily appetitive memories in sign-track*ers* (the phenotype).

Motivational Value Attribution Depends on Stimulus Modality

We and others have demonstrated that a tone CS acquires less motivational value than a visual or tactile CS (Meyer et al., 2014; Beckmann et al., 2015; Singer et al., 2016). Incentive-motivational stimuli acquire three properties; 1) they elicit approach, and direct an individual's attention toward it; 2) they are desired and individuals will work to obtain them; 3) they evoke motivational states in an individual that energize and instigate seeking behavior (Cardinal et al., 2002). Despite being able to localize auditory cues, rats will not approach a tone CS (Cleland & Davey, 1983; Harrison 1979). This demonstrates that a tone CS does not appear to acquire the first property of an incentive-motivational stimulus. There are several reasons why this may be the case. First, relative to a lever CS, a tone CS is rather simple. The lever CS used in Chapter Two has multiple attributes; it moves, illuminates, and makes an audible sound upon extending into the chamber (see Singer et al., 2016 for discussion). A tone, on the other hand, produces a single auditory signal of reward availability. It is possible that the difference in salience or number of features between these stimuli can contribute to differences in motivational attribution.

This is not to imply that a tone CS does not acquire *any* incentive-motivational value. Rats will work to obtain presentation of a tone CS (Meyer et al., 2014; Beckmann et al., 2015);

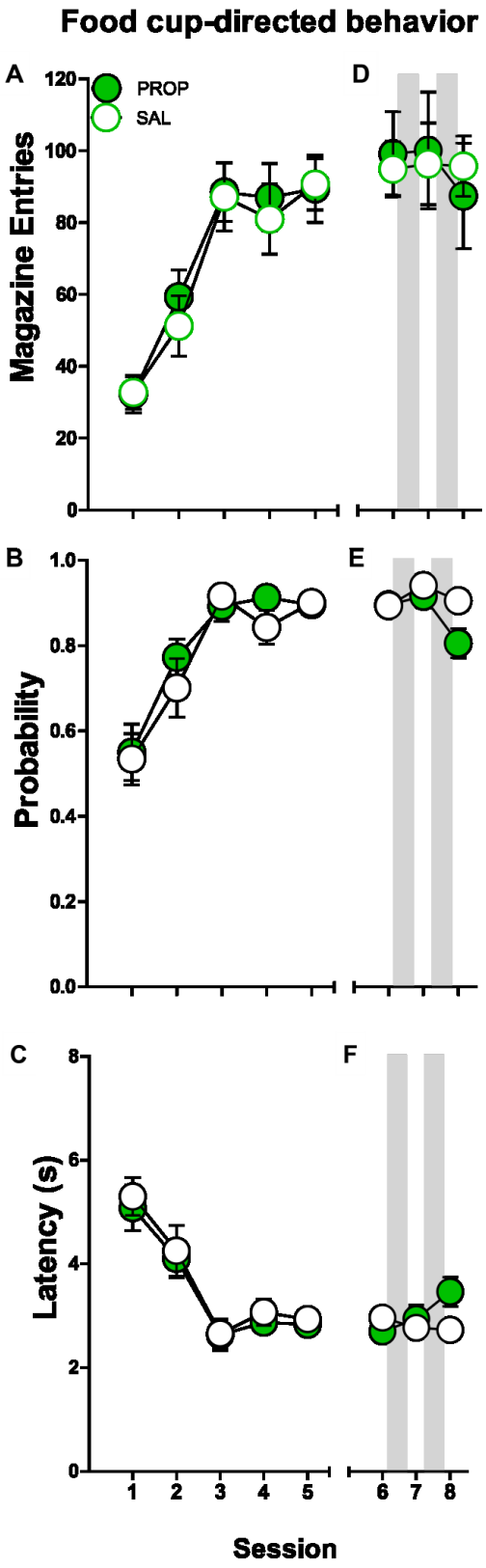
they will just work *less* than they would for a lever CS. That is, they will perform more instrumental responses to obtain presentations of a previously paired lever CS, than they will for a tone CS. Thus, it is not the case that propranolol disrupts incentive-motivational value in all cases; there appear to be boundaries - perhaps a certain threshold - under which propranolol affects incentive-motivation. It is possible that propranolol can only affect the incentive-motivational properties of stimuli to a certain extent, and that a tone CS simply is below this threshold resulting in a floor effect. However, there is evidence to suggest that ST and GT behaviors are mediated by separate neurobiological mechanisms (Flagel et al., 2011; Saunders & Robinson, 2012; Yager & Robinson, 2015). Thus, it is likely that propranolol differentially affects these systems the neural systems underlying ST and GT behaviors. Indeed, propranolol has been found to disrupt reconsolidation with intra-amygdala infusions, which is one region correlated with engagement by cues in STs (Yager & Robinson, 2015). However, the extent to which propranolol affects different brain regions in disrupting appetitive memory, and the overlap between neural systems engaged by appetitive tone and lever CSs have yet to be investigated.

Implications for Reconsolidation

The present studies extend the implications from Chapter Two for conceptualizing reconsolidation. Our findings from Chapter Two suggest that propranolol selectively affects motivational, but not predictive, components of memory in both humans and animals. Here, we found that this selectivity is a result of behavior, not phenotype. That is, propranolol will disrupt the incentive-motivation of cues that gain value to the extent of acting as powerful motivators of behavior; this is different from propranolol disrupting incentive-motivation in some individuals (STs), but not others (GTs). Thus, if GTs attribute greater motivational value to different kinds of

cues (e.g. contexts - see Saunders, O'Donnell, Aurbach, & Robinson, 2014) it is possible that propranolol could effectively disrupt incentive-motivation in those circumstances.

The clinical interest in propranolol is to decrease incentive-motivational value of cues that motivate pathological behaviors in addiction that may be driven by different environmental stimuli. If propranolol specifically targets the motivational component of a memory, irrespective of an individual propensity to attribute value to certain kinds of stimuli, it may prove to be a promising treatment option for many individuals suffering from pathological behaviors.


Food cup-directed behavior
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Magazine Entries
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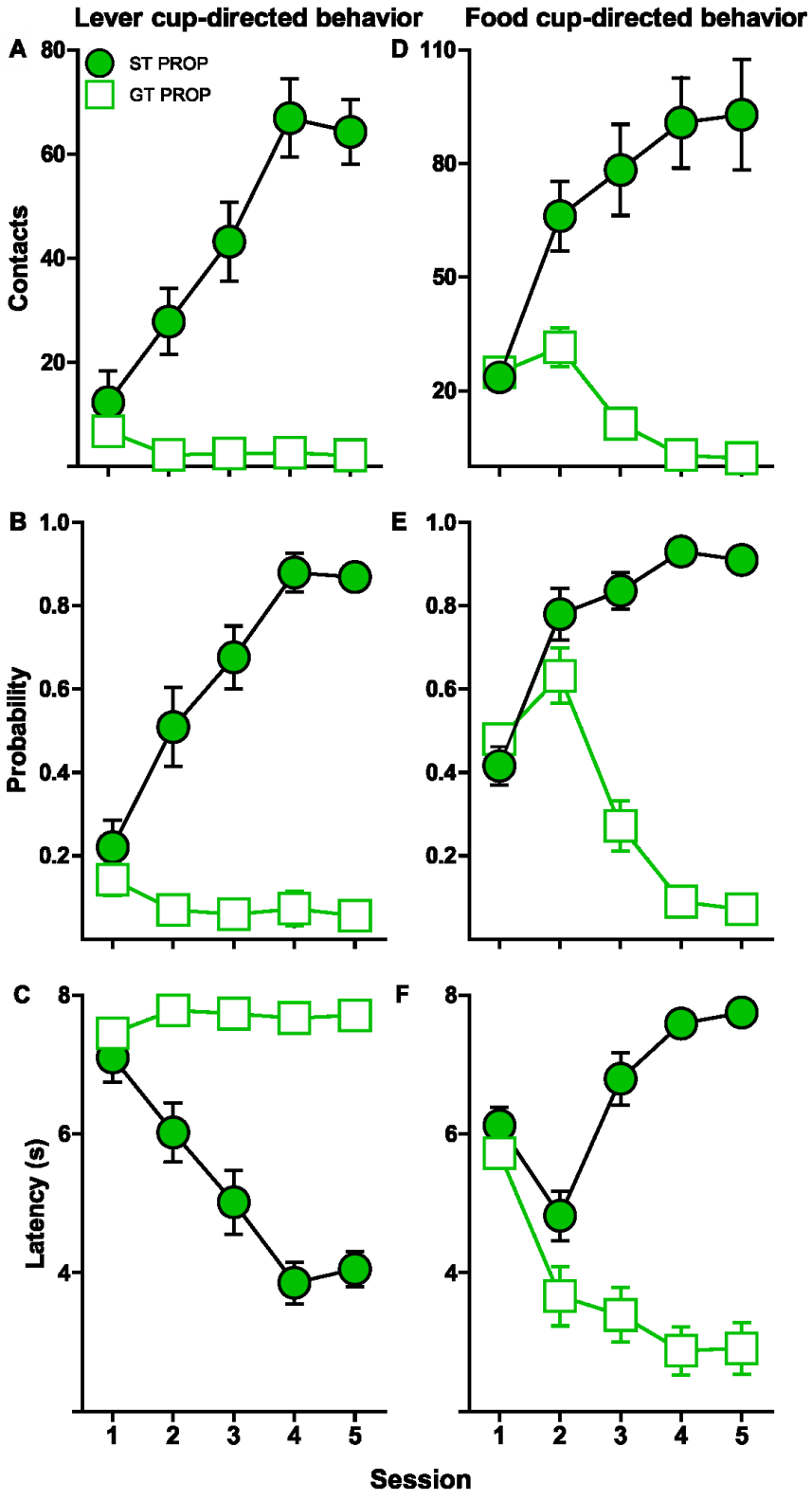
Figure 3.1. The effect of propranolol and saline injections on goal-tracking to a tone conditioned stimulus. Data represent mean ±SEM. The acquisition of a goal-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of food cup entries. (B) The probability of food cup-directed responding. (C) The average latency to approach the food cup. The effect of post-session injections on goal-tracking conditioned responses across Sessions 6-8 is shown in D-F. The grey bars represent when injections were administered (immediately after Sessions 6 and 7). (D) The number of food cup entries. (E) The probability to approach the food cup. (F) The latency to approach the food cup.


Lever cup-directed behavior
Food cup-directed behavior
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GT PROP
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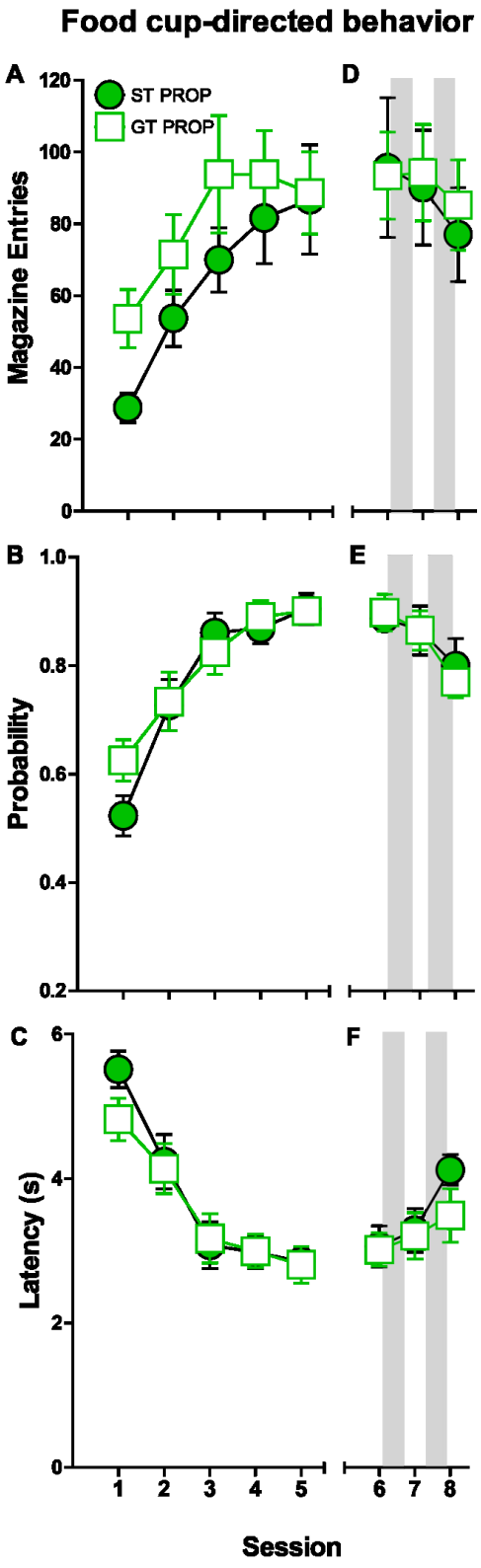
Probability
C
F
Latency (s)
Session

Figure 3.2. Acquisition of sign- and goal-tracking conditioned responses. Data represent mean ±SEM. The acquisition of a sign- and goal-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of lever contacts. (B) The probability of lever-directed responding. (C) The average latency to approach the lever. The acquisition of a goal-tracking conditioned response across Sessions 1-5 is illustrated in D-F. (D) The number of food cup entries. (E) The probability of food cup-directed responding. (F) The latency to approach the food cup.


Food cup-directed behavior
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Magazine Entries
120
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0.8
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8
Session

Figure 3.3. Goal-tracking to a tone conditioned stimulus in sign-trackers (STs) and goal-trackers (GTs). Data represent mean ±SEM. The acquisition of a goal-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of food cup entries. (B) The probability of food cup-directed responding. (C) The average latency to approach the food cup. The effect of propranolol on goal-tracking conditioned responses in STs and GTs are illustrated in D-F. (D) The number of food cup entries. (E) The probability of food cup-directed responding. (F) The latency to approach the food cup.

Chapter IV

The Neurobiology Underlying Sign- and Goal-tracking Conditioned Responses to Different Conditioned Stimuli

Introduction

When a conditioned stimulus (CS) that has been paired with a food reward acquires motivational value, the presentation of these cues can act as powerful motivators of behavior. A food-paired CS that acquires motivational properties is capable of inducing feelings of desire and craving for food, even when individuals are sated (Berridge, 2009; Schmitz, Naumann, Trentowska, & Svaldi, 2014). The brain regions engaged by presentation of these stimuli may provide insight toward the mechanisms that underlie the cravings and desire elicited by reward-paired cues that drive pathological behaviors such as eating disorders or addiction. Several key brain regions, including the ventral striatum, amygdala nuclei, thalamic nuclei, and prefrontal cortical regions comprise a so-called 'motive circuit'. In both humans (Schienle, Schäfer, Hermann, & Vaitl, 2009; Tang, Fellows, Small, & Dagher, 2012; Tomasi et al., 2015) and animals (Kelley, Schiltz, & Landry, 2005), this circuit appears to be engaged by the presentation of reward-paired cues that can drive maladaptive behaviors.

As discussed in the previous chapters, there is variation in the extent to which individuals attribute motivational value to cues. In goal-trackers (GTs - Boakes, 1977), a lever CS acquires predictive value, and thus GTs will demonstrate a conditioned response in which they approach

the location of reward delivery. In sign-trackers (STs – Hearst & Jenkins, 1974), a lever CS acquires both predictive value, and motivational value, thus, STs will vigorously approach and engage with the lever itself during the CS period. Studies using c-fos as a marker of neuronal activation have found that a lever CS engages 'motive' circuit regions discussed above in STs, but not GTs (Flagel et al. 2011; Yager et al. 2015).

In Chapter Two, we hypothesized that propranolol, a beta-adrenergic antagonist, selectively disrupts the reconsolidation of emotional or incentive-motivational associations, such as those acquired in STs. We found that propranolol disrupts ST, but not GT behavior (Chapter Two). In Chapter Three, we demonstrated that cues attributed with less motivational value (e.g. tone) are not affected by propranolol. Here, we ask how these differences in incentive-motivational value attribution are reflected in the brain. We asked two main questions: 1) Is the decrease in the incentive motivational properties of a lever CS produced by propranolol accompanied by a decrease in the ability of the lever CS to engage brain reward circuitry? and 2) Does a tone CS engage motive circuit brain regions, despite the fact that it does not appear to acquire incentive-motivational properties to the same extent of a lever CS? We hypothesized that propranolol would reduce engagement of brain regions that have been previously demonstrated to be engaged by cues in STs. Since we believe the engagement of brain reward circuitry in STs to require attribution of motivational value to a CS, we predicted that a tone CS would engage these regions to a lesser extent.

Materials and Methods

Subjects

A total of 53 male Sprague-Dawley rats (Charles River, Raleigh, NC) weighing 250-275g upon arrival were used for this study. Animals were individually housed in a climate controlled colony

room with a reverse 12-h light/12 h dark cycle, where food and water were available ad libitum. Prior to experimental testing, animals were given one week to acclimate to the housing room. During this time, rats were handled several times by the experimenter. All experimental procedures were approved by the University of Michigan Committee on Use and Care of Animals.

Apparatus

Standard (22 x 18 x 13 cm) test chambers (Med Associates Inc., St Albans, VT, USA) were used for behavioral testing. Each chamber was individually enclosed in a sound-attenuating cabinet equipped with a fan for ventilation and to impede background noise. Pavlovian training chambers each had a food cup placed 3 cm above the stainless steel grid floor in the center of one wall, and a red house light on the opposite wall, which remained illuminated throughout the duration of all sessions. For Auditory Pavlovian conditioning, a speaker calibrated to deliver a 2.9 kHz tone (70 dbs) was positioned directly under the house light. An illuminated retractable lever located 6 cm above the floor and 2.5 cm away from the food cup on either the left or right side (counterbalanced across chambers) was used for Pavlovian conditioned approach (PCA) training. Infrared photo-beams located inside the food cup were used to record head entries. All experimental events were controlled and recorded by a MED-PC computer system.

Pavlovian training

Prior to the start of Pavlovian conditioning sessions, animals were randomly assigned to the following experimental conditions; 'Lever paired' (n=28), 'Lever unpaired' (n=7), 'Tone paired' (n=7), 'Tone unpaired' (n=7), or 'Transport control' (n=4). Pavlovian training for lever-CS groups ('Lever paired' and 'Lever unpaired') and tone-CS groups ('Tone paired' and 'Tone

unpaired') is described below. Transport control animals were placed into chambers daily with the house light illuminated for the same duration as animals in the experimental groups.

Pavlovian training procedures for lever-CS groups. On the two days preceding the start of the experiment, 45mg banana-flavored pellets (Bio-Serv) were placed into home cages to habituate rats to this food. Following food habituation days, rats were trained to retrieve pellets from the food cup during a pretraining session, during which 25 pellets were dispensed into the food cup on a 30 s (0-60 s) variable time (VT) schedule. The red house light remained illuminated throughout the duration of the session. If a rat failed to consume all 25 pellets, they were given an additional pretraining session. On the day following pretraining, Pavlovian training began. Briefly, animals were trained over eight consecutive daily sessions. For animals in the paired group, each session consisted of 25 trials in which an illuminated lever (CS) was presented for 8 seconds and followed by the delivery of a 45mg banana-flavored food pellet (US) into the food cup. CS-US pairings occurred on a VT 90 (30-150 s) schedule. The delivery of the food pellet was not contingent upon any response from the animal. All lever deflections, food cup entries, and latency to approach each location were recorded.

Procedures for rats in the unpaired group were identical to the paired group with the exception that each session consisted of 25 lever-CS presentations and 25 US presentations occurring independently (on separate VT 90-s schedules).

After Session 5, animals in the paired group were classified as sign-trackers (STs, n=20) or goal-trackers (GTs, n=8) using the criteria previously described by Meyer and colleagues (2012). Unpaired animals were not classified. Briefly, PCA index scores were calculated as an average of three measures of approach behavior during the 8 s CS period; response bias to approach either the lever CS or the food cup [(#lever deflections – #food cup entries)],

probability to approach either the lever CS or food cup [(P(lever) – P(food cup)], and latency to approach either the lever CS or food cup [(lever deflection latency – food cup entry latency)/8]. This average produced an index score ranging from -1.0 to +1.0, where a score of -1.0 indicated a strong bias toward approaching the food cup, and a score of +1.0 indicated a strong bias toward approaching the lever. Index scores were averaged across training days 4 and 5, and these values were then used to classify rats as STs or GTs. Animals receiving scores between +0.6 to +1.0 and >50 lever contacts were classified as STs, and animals with scores between -0.6 to -1.0 and >50 food cup entries were classified as GTs. Only STs and GTs were used for this experiment.

Retrieval sessions. Pavlovian conditioning Sessions 6, 7, and 8 served as retrieval sessions. Behavioral testing on these days were identical to initial Pavlovian training, with the exception that immediately after the end of the session and before returning to home cages, animals received an injection of either propranolol (20mg/kg) or saline. STs and unpaired animals were divided into propranolol (STs n=9, unpaired n=3) or saline (STs n=11, unpaired n=4) injection groups, and were counterbalanced based on their index scores from Sessions 4 and 5. All GTs (n=8) received saline injections. Rats received the same treatment (propranolol or saline) on both days.

Pavlovian training procedures for tone-CS groups. Rats were trained using a Pavlovian conditioning procedure described previously (Meyer et al. 2014). On the two days preceding the start of the experiment, 45mg banana-flavored pellets (Bio-Serv) were placed into home cages to habituate rats to this food. Following food habituation days, rats were trained to retrieve pellets from the food cup during a pretraining session, during which 25 pellets were dispensed into the food cup on a 30 s (0-60 s) variable time (VT) schedule. The red house light remained

illuminated throughout the duration of the session. If a rat failed to consume all 25 pellets, they were given an additional pretraining session. On the day following pretraining, Auditory Pavlovian conditioning began. Animals were trained over eight consecutive daily sessions. For animals in the paired group (*n*=7), each session consisted of 25 trials in which a 2.9 kHz tone at 70 dbs (tone-CS) was presented for 8 seconds and followed by the delivery of a 45mg banana-flavored food pellet (US) into the food cup. CS-US pairings occurred on a variable time (VT) 90 (30-150 s) schedule. The delivery of the food pellet was not contingent upon any response from the animal. An infrared beam was used to detect head entries and latency to approach the food cup. All experimental events were controlled and recorded using a MED-PC computer system. Procedures for rats in the unpaired group (*n*=7) were identical to the paired group with the exception that each session consisted of 25 lever-CS presentations and 25 US presentations occurring independently (on separate VT 90-s schedules).

Context exposure sessions

Following Day 8 of Pavlovian training, animals received four days of context exposure sessions (Days 9-12). During these 30 minute daily sessions, the house light turned on and remained illuminated for the duration of the session. The configuration of the chamber remained the same as Pavlovian training sessions, however, no CS or US presentations occurred.

Test day: CS presentations

On day 13, following context exposure sessions, animals were placed into chambers for the final test day. The chambers were configured in the same way as Pavlovian training and context exposure sessions. During this session, the house light was illuminated, and following a 5 minute habituation period to the chamber, animals received 10 CS (4 s each) presentations once per minute. Animals in the lever-CS groups received lever-CS presentations, and animals in the

tone-CS groups received tone-CS presentations. Food (US) was not delivered at any point during this test session.

Tissue preparation

After the test session, animals were returned to their home cages. Sixty minutes later, rats were deeply anesthetized with pentobarbital sodium (390 mg/kg, i.p.) and perfused transcardially with 25 ml of 0.9% saline followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were harvested and post- fixed for 24 h at room temperature in the same fixative, then stored in 20% sucrose and 0.01% sodium azide in 0.1 M PB at 4ºC. Coronal sections (35 μm) were cut on a freezing microtome (SM 2000R, Leica) and stored in a cryoprotectant solution (30% sucrose, 30% ethylene glycol in 0.1 M PB). Sections were obtained through the brain in three parallel series. Tissue was stored at -20ºC until further processing.

Immunohistochemistry

All incubations were performed at room temperature with gentle agitation. Free- floating sections were washed three times (5 min) with 0.1 M phosphate-buffered saline (PBS) between incubations. Sections were incubated in 1% H_2O_2 for 10 min and then blocked in an incubation solution (PBS containing 2.5% normal donkey serum, Jackson Immuno; and 0.4% Triton X-100, Sigma-Aldrich) for 1 hr at room temperature. Next, tissue was incubated overnight with a rabbit polyclonal antibody against c-Fos (1:1000; sc52, lot L0115; Santa Cruz, Dallas, TX USA) in 0.1 M PBS containing 1% normal donkey serum, Jackson Immuno; and 0.4% Triton X-100, Sigma-Aldrich . Sections were then incubated in biotinylated donkey anti-rabbit IgG (1:500 in PBS containing 1% normal donkey serum and 0.4% Triton X-100) for 1 hr at room temperature followed by a 1 hr incubation in avidin-biotin-horseradish peroxidase (1:1000 in PBS; ABC elite; Vector Laboratories) at room temperature, mixed 30 minutes before use. This was

visualized using 0.02% 3,3´-diaminobenzidine tetrahydrochloride (10 min; Sigma-Aldrich) with 0.02% nickel sulfate in 0.1 M PB with hydrogen peroxide (0.015%). Sections were mounted onto Superfrost plus glass slides (Fisher) and coverslipped with dibutyl phthalate xylene.

c-Fos immunoreactivity analysis

Digital images were captured with a CCD digital camera (Retica-SRV Fast 1394,Q Imaging, British Columbia, Canada) attached to a Leica microscope (DM6000B, Leica, Wetzlar, Germany) with fixed camera settings for all subjects (using 10x objectives). Fos immunoreactive cells were counted by an individual blind to treatment conditions and were identified by black oval-shaped nuclei. Using National Institutes of Health ImageJ software, areas of analysis were defined based on landmarks (Paxinos & Watson, 1998) unique for each brain region (i.e., shape of anterior commissure, location of lateral ventricles, location and shape of optic tract, etc.). The total number of c-Fos immunoreactive cells was quantified from the left and right hemispheres from one section of each animal for each region of interest and counts were averaged per animal. The nucleus accumbens (NAc) core and shell subregions were sampled at +1.6 mm with a sampling area of 400 x 600 µm. The dorsolateral (DL), dorsomedial (DM), ventrolateral (VM) and ventromedial (VM) striatum were sampled at +0.8 mm from bregma with a sampling area of 700 x 725 µm. The lateral septum was also sampled at +0.8 mm from bregma with a sampling area of 1500 x 400 µm The AP coordinates for each brain region were selected based on previous work looking at the induction of c-Fos mRNA in STs and GTs in response to presentation of a food cue (Flagel et al., 2011; Yager et al., 2015).

Statistics

Linear mixed-models (LMM) analysis was used for all repeated measures data. The covariance structure was explored and modeled for each dependent variable and chosen based on the lowest

Akaike information criterion score (Verbeke, 2009). One-way ANOVAs were used to compare group differences in behavior upon re-exposure to the CS on test day and the average amount of Fos expression for each region of interest. Post-hoc Fisher's LSD comparisons were used to test a priori hypotheses. Lever-CS and tone-CS trained animals were analyzed separately.

Results

Lever CS: No effect of propranolol on unpaired animals

Figures 4.1A and Figure 4.1B illustrate the probability of engaging in lever- and food cup-directed behavior in unpaired animals across Sessions 1-8.

Acquisition

Lever-directed behavior. Animals significantly decreased lever-directed behavior across Sessions 1-5 (effect of Session, $F_{(4,5)}$=8.73, p=0.02), decreased probability to approach the lever (effect of Session, $F_{(4,10)}$=3.69, p=0.04: Figure 4.1A), and increased latency to approach the lever (effect of Session, $F_{(4,8)}$=4.40, p=0.04). However, there were no significant group differences between animals to be treated with propranolol or saline in lever contacts (no effect of treatment, $F_{(1,5)}$=0.62, p=0.47) across sessions (no Treatment X Session interaction, ($F_{(4,5)}$=1.60, p=0.31), probability to approach the lever (no effect of Treatment, $F_{(1,5)}$=1.00, p=0.36: Figure 4.1A) across sessions (no Treatment X Session interaction, $F_{(4,10)}$=1.26, p=0.35: Figure 4.1A), or latency (no effect of Treatment, $F_{(1,19)}$=0.78, p=0.39) across sessions (no Treatment X Session interaction, $F_{(4,8)}$=1.68, p=0.25). Both groups decreased engagement with the lever across sessions, however, there were no differences between groups in acquisition.

Food cup-directed behavior. There were no significant changes in food cup-directed behavior across sessions 1-5 in food cup entries (no effect of Session, ($F_{(4,20)}$=0.36, p=0.83), probability (no effect of Session, $F_{(4,20)}$=2.57, p=0.07: Figure 4.1B) or latency to approach the

food cup (no effect of Session, $F_{(4,20)}$=2.07, p=0.12). Treatment groups also did not differ significantly in the number of food cup entries ($F_{(1,5)}$=5.55, p=0.06) across sessions (no Treatment X Session interaction, $F_{(4,20)}$=2.46, p=0.08), probability ($F_{(1,4)}$=0.55, p=0.50: Figure 4.1B) across sessions (no Treatment X Session interaction, $F_{(4,20)}$=0.84, p=0.52: Figure 4.1B), or latency ($F_{(1,5)}$=2.59, p=0.17) across session (no Treatment X Session interaction ($F_{(4,20)}$=1.20, p=0.34).

Retrieval and Reconsolidation

Here, we compared whether treatment affected lever- or food cup-directed behavior in animals that received unpaired presentations of a lever CS and food US.

Lever-directed behavior. There were no significant effects on lever-directed behavior across sessions for contacts ($F_{(2,15)}$=0.31, p=0.73), probability ($F_{(2,15)}$=0.06, p=0.934: Figure 4.1A), or latency to approach the lever ($F_{(2,15)}$=0.13, p=0.88). There were also no significant differences between treatment groups for lever contacts ($F_{(1,15)}$=0.56, p=0.47) across sessions (no Treatment X Session interaction, ($F_{(2,15)}$=1.02, p=0.38), probability ($F_{(1,15)}$=0.04, p=0.84: Figure 4.1A) across sessions (no Treatment X Session interaction ($F_{(2,15)}$=0.68, p=0.52), or latency ($F_{(1,15)}$=0.59, p=0.45) across sessions (no Treatment X Session interaction, ($F_{(2,15)}$=1.94, p=0.18). Thus, propranolol had no effect on lever-directed behavior across Sessions 6-8 in unpaired animals.

Food cup-directed behavior. Propranolol also did not affect food cup-directed behavior across Sessions 6-8. There was a main effect of session for food cup entries, (effect of Session, $F_{(2,7)}$=8.26, p=0.01), indicating that both groups decreased, but there were no significant differences between propranolol and vehicle groups (no effect of Treatment, $F_{(1,5)}$=0.06, p=0.82) across sessions (no Treatment X Session interaction, $F_{(2,7)}$=2.57, p=0.15). There was also a main

effect of session for probability to approach the food cup, ($F_{(2,10)}$=5.46, p=0.02: Figure 4.1B), again, indicating a decrease in probability across Sessions 6-8, but propranolol and vehicle groups did not differ (no effect of Treatment, $F_{(1,5)}$=1.27, p=0.31: Figure 4.1B). There was, however, a significant interaction between treatment and session ($F_{(2,10)}$=4.18, p<0.05). The latency to approach the food cup also decreased between Sessions 6 and 8 (effect of Session, $F_{(2,9)}$=4.34, p=0.05), but there was no effect of treatment ($F_{(1,5)}$=0.66, p=0.45) across sessions (no Treatment X Session interaction, ($F_{(2,9)}$=2.87, p=.11).

c-Fos Quantification. There were no significant differences increases in c-Fos expression in propranolol- and saline-treated *unpaired* animals in the NAc core or shell, DM, DL, VM, VL subregions of the striatum, or the lateral septum. These data are presented in Figure 4.1C.

We did not find significant behavioral differences between unpaired animals that received post-session injections of propranolol or saline, nor did we see differences in c-Fos expression in any of the brain areas analyzed between these groups. Thus, for the remaining analyses, we have pooled the unpaired propranolol and saline-treated animals into one 'unpaired' group.

Lever CS: Acquisition

The acquisition of lever- and food cup-directed behavior in STs and GTs is illustrated in Figure 4.2A-C and Figure 4.2G-I. As with previous experiments, STs increased lever-directed responding compared with GTs, demonstrated by an increase in the number of contacts, probability to approach the lever, and decrease in latency to approach the lever. There were no significant differences in acquisition of ST conditioned responses between animals that were later divided into propranolol or vehicle treated groups. In contrast, GTs showed an increase in

food cup-directed behavior measured by an increase in the number of food cup entries, increase in probability to approach the food cup, and decrease in latency to approach the food cup.

Retrieval and Reconsolidation

Lever-directed behavior across Sessions 6-8 is shown in Figures 4.2D-F, and food cup-directed responses are illustrated in Figures 4.2J-L.

Lever-directed behavior. As seen in Experiments 1 and 2 from Chapter Two, propranolol significantly decreased lever contacts compared with saline-treated animals ($F_{(1,25)}$=5.34 p=0.03: Figure 4.2D), across Sessions 6-8 (Treatment X Session interaction ($F_{(2,25)}$=6.21, p=.006: Figure 4.2D). Post hoc comparisons revealed significant differences between Sessions 6 and 8 (p<0.001). There was no main effect of treatment on probability to approach the lever ($F_{(1,25)}$=2.11, p=0.16: Figure 4.2E), however groups significantly decreased across sessions (effect of Session, $F_{(2,26)}$=8.80, p=0.001: Figure 4.2E). Rats in the propranolol group showed a decreased probability to approach the lever compared with vehicle-treated rats across Sessions 6-8 (Treatment X Session interaction, $F_{(2,26)}$=4.69, p=0.02: Figure 4.2E). Post-hoc comparisons revealed significant differences between Sessions 6 and 7 (p<0.05) and Sessions 6 and 8 (p<0.001). Latency to approach the lever significantly changed across sessions ($F_{(2,34)}$=16.66, p<0.001: Figure 4.2F), although there was no main effect of treatment ($F_{(1,25)}$=3.93, p=0.06: Figure 4.2F). However, propranolol-treated rats demonstrated an increased latency across Sessions 6-8 compared with vehicle-treated rats (Treatment X Session interaction, ($F_{(2,34)}$=10.29, p<0.001: Figure 4.2F). Propranolol significantly decreased lever-directed behavior, compared with vehicle treatments.

Food cup-directed behavior. There were no significant changes across sessions in the number of food cup entries (effect of Session, $F_{(2,25)}$=1.22, p=0.30: Figure 4.2J), probability to

approach the food cup (effect of Session $F_{(2,41)}$=1.13, p=0.33: Figure 4.2K), or latency to approach the food cup (effect of Session, $F_{(2,51)}$=2.80, p=0.07; Figure 4.2L).

Lever CS: Test day behavior

During the final test session (Day 13), rats received 10 four second CS presentations once per minute. Lever-directed and food cup-directed behavior during the 10 four second CS presentations are illustrated in Figures 4.3 and 4.4.

Lever-directed behavior. A one-way ANOVA revealed significant group differences ($F_{(3,31)}$=8.93, p<0.001: Figure 4.3A). Post hoc planned comparisons show that GTs did not differ from unpaired animals. However, propranolol (p<0.05) and saline-treated (p<0.001) STs made significantly more lever contacts than unpaired animals; propranolol (p<0.05) and saline-treated (p<0.001) STs also made significantly more lever contacts than GTs; and ST propranolol-treated animals made significantly more responses than saline-treated STs (p's<0.05).

There were also significant group differences in probability to approach the lever ($F_{(3,31)}$=18.21, p<0.001: Figure 4.3B). Post hoc comparisons demonstrate that there were no differences between GTs and unpaired animals. However again, the propranolol- and saline-treated STs performed significantly more lever contacts than unpaired animals (p's<0.001), and GTs ($p's$<0.001). Propranolol-treated STs approached the lever with a higher probability than unpaired animals and GTs ($p's$<0.001), but were not different than saline-treated STs.

Lastly, there were also significant group differences in latency to approach the lever ($F_{(3,31)}$=15.271, p<0.001: Figure 4.3C). Post hoc comparisons revealed that GT and unpaired groups were not different; ST propranolol and saline groups approached the lever significantly faster than unpaired animals (p's<0.001), and the ST propranolol group approached the lever

significantly faster than unpaired animals and GTs (*p*'s<0.001), but did not approach the lever with a slower latency than ST saline-treated rats.

Food cup-directed behavior. A one-way ANOVA revealed significant differences between groups in the number of food cup entries performed on test day ($F_{(3,31)}$=19.13, p<0.001: Figure 4.4A). Post-hoc Bonferroni comparisons revealed that GTs made more food cup entries than unpaired groups (p<0.005). Additionally, ST saline and propranolol groups made significantly less food cup entries than GTs (*p's*<0.001). ST saline-treated animals made less food cup entries than unpaired animals (p<0.05). There were no differences between the remaining groups.

The probability of approaching the food cup on test day was also significantly different between groups ($F_{(3,31)}$=31.71, p<0.001: Figure 4.4B). Post-hoc comparisons show that GTs made more food cup entries than unpaired animals and ST saline-treated animals (*p's*<0.001). ST saline- and propranolol-treated animals made significantly less responses than GTs (*p's*<0.001). ST-saline treated animals also made fewer food cup entries than unpaired animals (*p*<0.01), but there were no differences between ST propranolol-treated animals and unpaired animals.

There were also significant group differences in the latency to approach the food cup on test day ($F_{(3,31)}$=13.19, p<0.001: Figure 4.4C). Post-hoc comparisons reveal that ST saline- and propranolol-treated animals approached the food cup with greater latencies than unpaired animals and GTs (*p's*<0.005).

Lever CS: c-Fos Quantification

c-Fos expression in the ventral striatum illustrated in Figure 4.5, and c-Fos expression in the dorsal striatum and lateral septum is illustrated in Figure 4.6.

Ventral Striatum. In the NAc Core, a one-way ANOVA demonstrated that there were significant group differences ($F_{(3,30)}$=10.61, p<0.001: Figure 4.5). Post-hoc comparisons were performed. Relative to the unpaired group, ST saline group expressed significantly higher levels of c-Fos (p<0.05), and GT expressed significantly lower levels of c-Fos (p<0.05). ST saline-treated animals also expressed significantly higher levels of c-Fos than GTs (p<0.001). The propranolol-treated STs expressed significantly higher levels of c-Fos expression than animals in the GT saline group (p<0.005), and lower levels of c-Fos expression than saline-treated STs (p<0.05). There were no differences between ST propranolol-treated animals and unpaired animals.

C-Fos expression in the NAc Shell also differed across groups ($F_{(3,30)}$=14.02, p<0.001: Figure 4.5). Post-hoc comparisons revealed no significant differences between unpaired animals and GTs, or ST propranolol-treated animals. There were, however, significant differences between ST saline-treated animals and unpaired animals, GTs, and ST propranolol-treated animals (*p's*<0.001). Propranolol-treated STs expressed significantly lower levels of c-Fos than ST saline-treated animals (p<0.001). Thus, propranolol decreases the extent to which a lever-CS engages the NAc Core and Shell, indicated by a decrease in c-Fos expression relative to saline-treated rats, and no differences between propranolol-treated STs and unpaired animals.

Dorsal Striatum. There were significant group differences in the dorsolateral (DL) striatum ($F_{(3,27)}$=103.71, p<0.001: Figure 4.6). Post-hoc comparisons revealed that there were no significant differences between unpaired animals and GTs or ST propranolol-treated animals. ST saline-treated animals exhibited significantly higher levels of c-fos than unpaired, GTs, and ST propranolol-treated animals (*p's*<0.001). ST propranolol-treated animals expressed significantly lower levels of c-Fos compared to ST saline-treated animals (p<0.001).

In the dorsomedial (DM) striatum c-Fos expression differed between groups ($F_{(3,27)}$=36.34, p<0.001: Figure 4.6). Post-hoc comparisons revealed that unpaired animals expressed lower levels of c-Fos than GTs (p<0.05), ST saline and propranolol-treated rats (*p's*<0.001). ST saline-treated animals expressed significantly higher levels of c-fos compared with all groups (*p's*<0.001). ST propranolol-treated animals expressed significantly higher levels of c-fos compared with unpaired animals (p<0.001) and GTs (p<0.05), but expressed lower levels of c-fos than ST-saline treated animals (p<0.001).

There were also significant group differences in the ventrolateral (VL) striatum (F(3,27)=5.95, p<0.005: Figure 4.6). Post-hoc comparisons indicate that there were no differences between unpaired animals and GTs or unpaired animals and ST-propranolol treated animals. c-Fos expression in the ST saline group (p<0.05) was significantly higher than unpaired, ST –propranolol (*p's*<0.05) and GTs (p<0.001). Additionally, ST propranolol-treated rats expressed lower levels c-Fos in this region compared with ST saline-treated animals (p<0.05).

The ventromedial (VM) striatum expressed group differences in c-Fos as well (F(3,27)=6.47, p<0.005: Figure 4.6). Relative to unpaired animals, there were no differences in c-fos expression in GTs or ST propranolol-treated animals. ST saline animals express significantly higher levels of c-Fos in this region than unpaired animals (p<0.05) and GTs (p<0.001). Propranolol-treated animals expressed significantly higher levels of c-Fos than GTs (p<0.01), but did not express lower levels than ST saline-treated animals.

Thus, propranolol significantly decreased the extent to which a lever CS engages c-fos expression in the DL, DM, and VL Striatum.

Lateral Septum. Group differences in c-fos expression were also found in the lateral septum ($F(3,30)=9.29$, $p<0.001$: Figure 4.6). Post-hoc comparisons revealed no significant differences between unpaired and GTs, or unpaired and ST propranolol-treated animals. ST-saline-treated animals expressed significantly higher levels of c-Fos than all groups (*p's*<0.001). There were no significant differences between propranolol-treated STs and unpaired or GTs, however, propranolol-treated STs expressed significantly lower levels of c-Fos than saline-treated STs ($p<0.01$). Propranolol also decreases the engagement of the lateral septum by a lever CS. The expression of c-Fos within the Striatum and Lateral Septum is illustrated in Figure 4.5.

Tone CS: Acquisition

The acquisition of a goal-tracking conditioned response to a tone CS is illustrated in Figure 4.7A-C. Across Sessions 1-8, animals increased food-cup directed responding demonstrated by an increase in the number of food cup entries, probability to approach the food cup, and decrease in latency to approach the food cup relative to unpaired animals.

Tone: Test Day Behavior

Most animals did not engaged in food cup-directed behavior during CS presentation on the test day. Thus, these data are not shown here. Despite minimal food-cup responding, LMM were conducted on these data. There were no significant differences between paired and unpaired animals on food cup entries, probability, or latency.

Tone CS: c-Fos Quantification

There were no significant differences increases in fos expression in paired animals relative to unpaired animals in the NAc core or shell, DM, DL and VL subregions of the striatum, or the lateral septum. In the VL striatum, unpaired animals expressed significantly

higher levels of c-fos than paired animals ($F(1,11)=10.55$, $p<0.01$). These data are presented in Figure 4.8.

Discussion

Here, we first asked if decreases in incentive-motivational value produced by propranolol also decrease in the extent to which these cues engage brain regions in the 'motive circuit'. In saline-treated STs, we replicated previous findings in that a CS induced higher levels of c-fos expression in the nucleus accumbens core and shell, subregions of the striatum, and the lateral septum, relative to GTs and unpaired animals (Flagel, Cameron, et al., 2011; Yager et al., 2015). In agreement with our hypothesis, we also found c-fos expression in propranolol-treated STs to be significantly lower in comparison to saline-treated STs in a majority of these brain regions. These results suggest that a decrease in the incentive-motivational value of a CS produced by propranolol is associated with a reduction in the extent to which it engages at least some regions in the 'motive circuit'. Other regions have yet to be quantified. We also asked whether or not a tone CS engages 'motive circuit' brain regions. Given that a tone acquires less incentive-motivational value, we hypothesized that a tone CS would engage these brain regions less than a lever CS. Interestingly we did not find any significant differences between paired and unpaired groups trained with a tone CS in any of the regions quantified (same as above). These results suggest that a tone CS paired with a food reward is not sufficient to engage reward circuitry and thus, does not act as incentive-motivational stimulus to the extent of a lever CS in STs.

Propranolol Decreases Engagement of Brain Reward Circuitry by a Lever Conditioned Stimulus

The amygdala and striatum are two crucial structures implicated in appetitive Pavlovian learning (see Cardinal et al., 2002). Propranolol has been found to modulate adrenergic and dopaminergic signaling in the amygdala and striatum (Peters & Mazurkiewicz-Kwilecki, 1975;

Fludder & Leonard, 1979; Tuinstra & Cools, 2000; Cools & Tuinstra, 2002), even when administered systemically (Buffalari & Grace, 2007). The amygdala, in particular, has been found to play a crucial role in disrupting reconsolidation with post-reactivation infusions of propranolol in rats (Dębiec & Ledoux, 2004; Debiec & LeDoux, 2006) and humans (van Stegeren et al., 2005; Mahabir, Tucholka, Shin, Etienne, & Brunet, 2015; Schwabe, Nader, Wolf, Beaudry, & Pruessner, 2012; Hurlemann et al., 2010). It sends dense projections to the nucleus accumbens and dorsal striatum (Kelley, Domesick, & Nauta, 1982). Thus, our finding that c-fos expression is significantly lower in striatal regions after disruption of reconsolidation with propranolol is not particularly surprising. We do not believe the decreases in engagement of brain regions to be a non-specific effect of propranolol administration, as c-fos levels in the unpaired animals did not differ between treatment groups. Additionally, it has been previously reported that propranolol injections by themselves, do not appear to affect baseline c-fos expression any more than saline injections in the nucleus accumbens core, and regions of the caudate (Ohashi, Hamamura, Lee, Fujiwara, & Kuroda, 1998).

The lateral septum has been demonstrated to play a role in the consolidation of appetitive memories (Bertaina‐Anglade, Tramu, & Destrade, 2000) although its role in reconsolidation has not been well characterized. This structure receives dense noradrenergic input from brain stem nuclei (Moore, 1978; Swanson & Hartman, 1975). Antagonism of beta-adrenergic receptors in the lateral septum have been found to modulate aggressive behaviors (Gulia, Kumar, & Mallick, 2002; Gammie, Lee, Scotti, Stevenson, & Gessay, 2012). This suggests that propranolol may modulate emotional behaviors by antagonizing beta-adrenergic receptors in this region. Previous studies have reported a slight suppression in c-fos activity after systemic administration

of propranolol (Ohashi et al., 1998). However, in the present experiment we did not observe differences between propranolol and saline-treated animals in the unpaired group.

Additional brain regions including areas in the prefrontal cortex, amygdala, locus coeruleus, and thalamus have yet to be analyzed. It is possible that we will observe decreases in propranolol in all regions that have been previously found to be engaged by reward-paired cues in STs. However, it will also be interesting to find any areas that do not change, or even areas that show an increase in c-fos activation. Increases in c-fos activity could imply that a region is modulating or 'gating' activity in other regions. In particular, there are strong suggestions that a decrease in amygdala regions will be observed, due to its established role in appetitive learning, reconsolidation, and as a site of action for propranolol, even with systemic injections. It is also possible that some areas may express a decrease in c-fos activation as a result of propranolol administration alone. Studies have not extensively studied the effects of acute versus chronic propranolol administration. However, it has been reported that 'acute' propranolol injections do not affect norepinephrine (NE) content and may even result in *increases* NE content (Laverty & Taylor, 1968; Fludder & Leonard, 1979). On the other hand, 'chronic' propranolol injections have been found to decrease NE content in the amygdala (Fludder & Leonard, 1979). In these studies, 'acute' and 'chronic' administration treatments refer to one or fourteen successive days, respectively. Thus, at this point it is unclear when [between 1 and 14 days] propranolol may begin to exert effects that are more reminiscent of 'chronic' treatment.

As mentioned in the introduction, structural changes that accompany long-term potentiation (LTP) can occur with memory formation, and a reversal of these changes can occur with disrupting the reconsolidation of such memories. For example, propranolol has been found to reverse the addition of dendritic spines in the basolateral amygdala (Vetere et al., 2013).

Therefore, it is possible that propranolol may be disrupting the reconsolidation of incentive-motivation by targeting very specific plastic changes (related to incentive-motivation/emotion) that have been found to occur with memory formation. The present study only begins to unravel the neural mechanisms by which propranolol can disrupt incentive motivation. The areas identified here, and those that will be identified with remaining analysis of regions will be important for guiding future experiments.

A Tone Conditioned Stimulus Does Not Engage Brain Reward Circuitry

We hypothesized that a tone CS would not induce Fos expression in any brain regions to the extent a lever CS induces c-Fos expression in STs. In the regions analyzed, a tone CS was not sufficient to engage brain reward circuitry. It still remains to be seen whether or not this is also the case for other regions in the 'motive circuit'. This is consistent with reports that a tone CS does not acquire incentive motivational value to the extent of a lever CS. We also do not observe engagement of regions in GTs. Based on our previous experiments, our data suggest that goal-tracking to a lever CS, and goal-tracking to a tone CS may require similar mechanisms. Our observations agree with the results from Chapter Three. In Chapter Three, we demonstrated that propranolol does not disrupt conditioned responding to a stimulus that does not acquire motivational value to the extent of a lever CS. Here, we show that a tone CS also does not appear to engage 'motive circuit' brain regions. Together, these data suggest that a tone CS is not affected by propranolol because it does not acquire sufficient motivational value such that it acts as an incentive stimulus, and thus engages brain reward circuitry.

Conclusions

Measuring the engagement of reward-paired cues by examining c-fos activation is useful for identifying potential targets. Future studies can use these data as a guide for investigating

specific mechanisms and circuits by which motivational value can be disrupted. Identifying specific targets in which propranolol decreases the motivational value of cues without disrupting memory *per se* will be useful in guiding the research and development of novel therapeutics that alleviate the negative emotions or cravings induced by aversive and reward-associated stimuli.

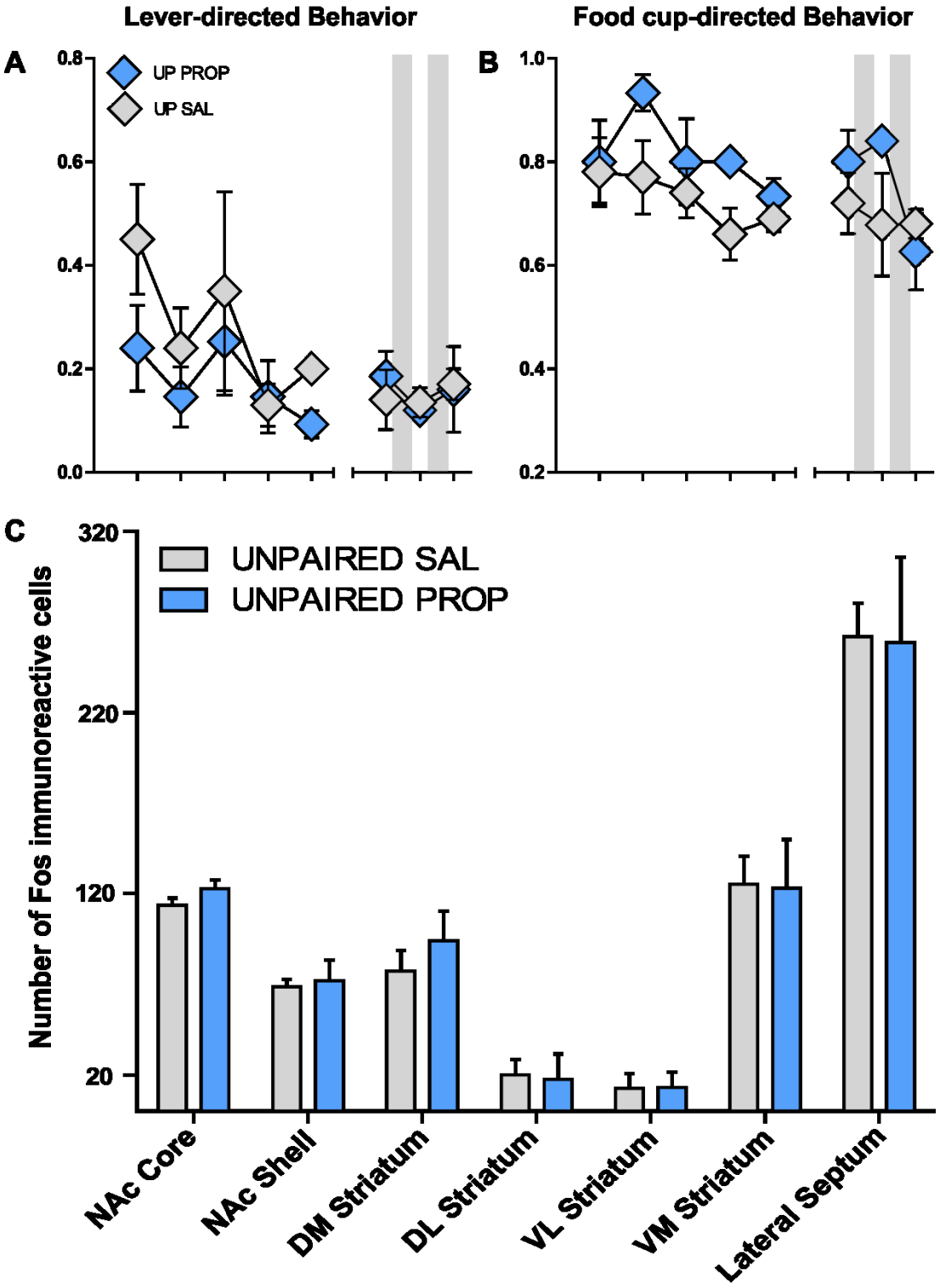
Lever-directed Behavior
Food cup-directed Behavior
A
B
C
UP PROP
UP SAL
0.8
0.6
0.4
0.2
0.0
1.0
0.8
0.6
0.4
0.2
UNPAIRED SAL
UNPAIRED PROP
Number of Fos immunoreactive cells
320
220
120
20
NAc Core
NAc Shell
DM Striatum
DL Striatum
VL Striatum
VM Striatum
Lateral Septum

Figure 4.1. The effect of propranolol and saline post-session injections in unpaired animals. Data represent mean ±SEM. The *top* of this figure illustrates the probability of sign- and goal-tracking conditioned responses across Sessions 1-8 is illustrated in A-B. The grey bars represent when propranolol and saline injections were administered (immediately after Sessions 6 and 7). (A) Probability of lever contacts. (B) Probability of food cup-directed responding. The *bottom* of this figure shows the effect of propranolol on engagement of 'motive circuit' regions by a lever CS in unpaired animals. (C) Number of Fos positive cells across seven brain regions quantified.

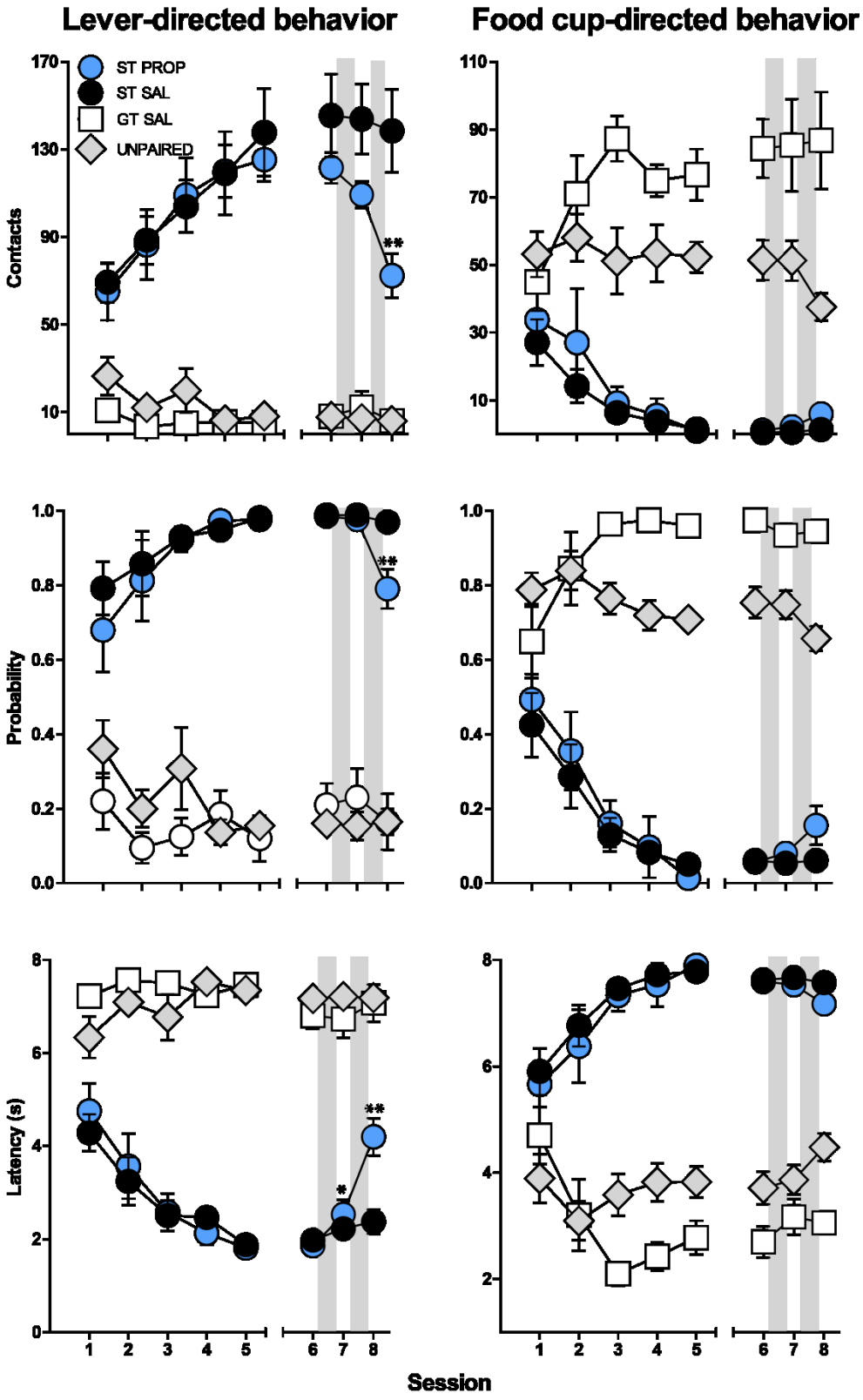
Lever-directed behavior
Food cup-directed behavior
ST PROP
ST SAL
GT SAL
UNPAIRED
Contacts
Probability
Latency (s)
Session
**
*

Figure 4.2. Acquisition of sign- and goal-tracking conditioned responses in STs, GTs, and unpaired animals. Data represent mean ±SEM. The acquisition of a sign-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of lever contacts. (B) The probability of lever-directed responding. (C) The average latency to approach the lever. The acquisition of a goal-tracking conditioned response across Sessions 1-5 is illustrated in G-I. (G) The number of food cup entries. (H) The probability of food cup-directed responding. (I) The latency to approach the food cup. The effect of post-session injections on sign-tracking conditioned responses across Sessions 6-8 is shown in D-F. The grey bars represent when administration of post-session propranolol or saline injections (after Sessions 6 and 7). (D) The number of lever contacts. (E) The probability to approach the lever. (F) The latency to approach the lever. The effect of propranolol or saline post-session injections on goal-tracking conditioned responses across Sessions 6-8 is illustrated in J-L. (J) The number of food cup entries. (K) The probability of food cup-directed responding. (L) Latency to approach the food cup. * $p<0.01$, ** $p<0.001$ (relative to Session 6).

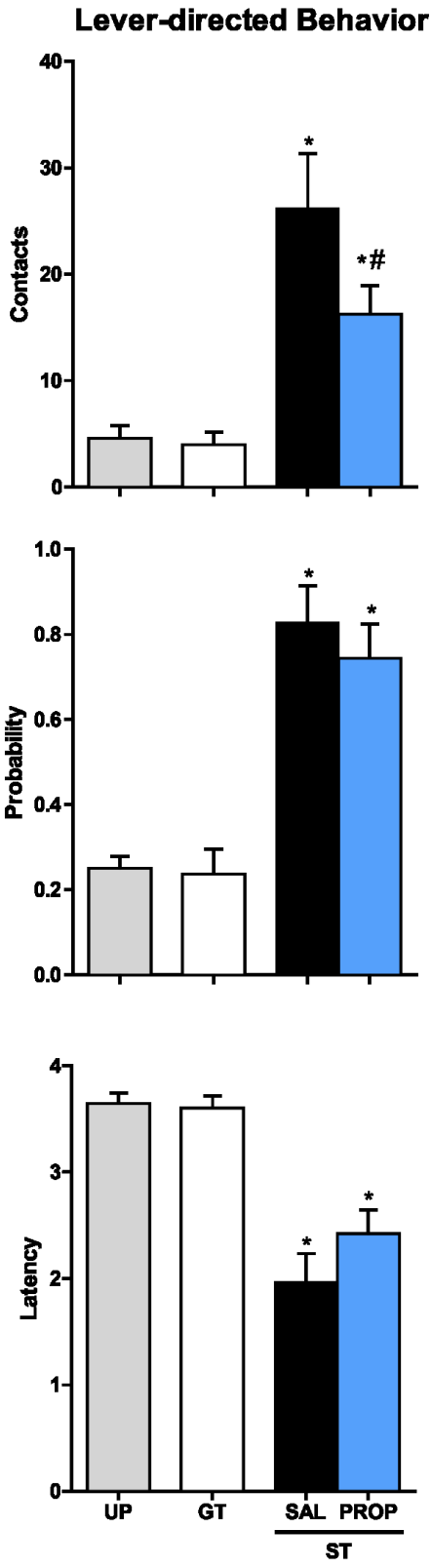
Lever-directed Behavior
Contacts
40
30
20
10
0
*
*#
Probability
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Latency
4
3
2
1
0
*
*
UP
GT
SAL
PROP
ST

Figure 4.3 Sign-tracking behavior during 4 s CS periods on the final test session in STs, GTs, and unpaired animals. Data represent mean ±SEM. The acquisition of a sign-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of lever contacts. (B) The probability of lever-directed responding. (C) The average latency to approach the lever. *$p<0.05$ (relative to unpaired) #$p<0.05$ (relative to ST saline-treated animals)

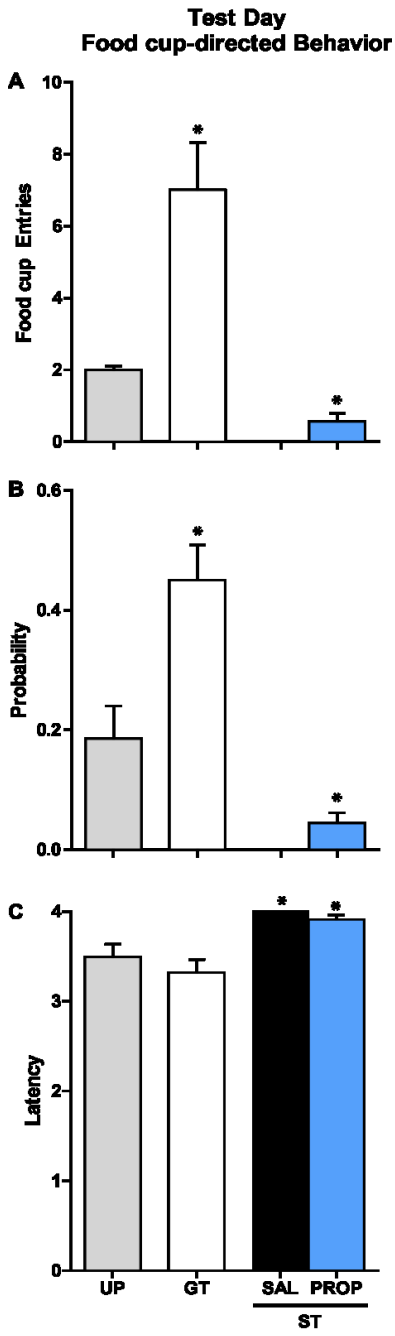

Figure 4.4. Goal-tracking behavior during 4 s CS periods on the final test session in STs, GTs, and unpaired animals. Data represent mean ±SEM. The acquisition of a goal-tracking conditioned response across Sessions 1-5 is illustrated in A-C. (A) Number of food cup entries. (B) The probability of food cup-directed responding. (C) The average latency to approach the food cup. *p<0.05 (relative to unpaired) #p<0.05 (relative to ST saline-treated animals)

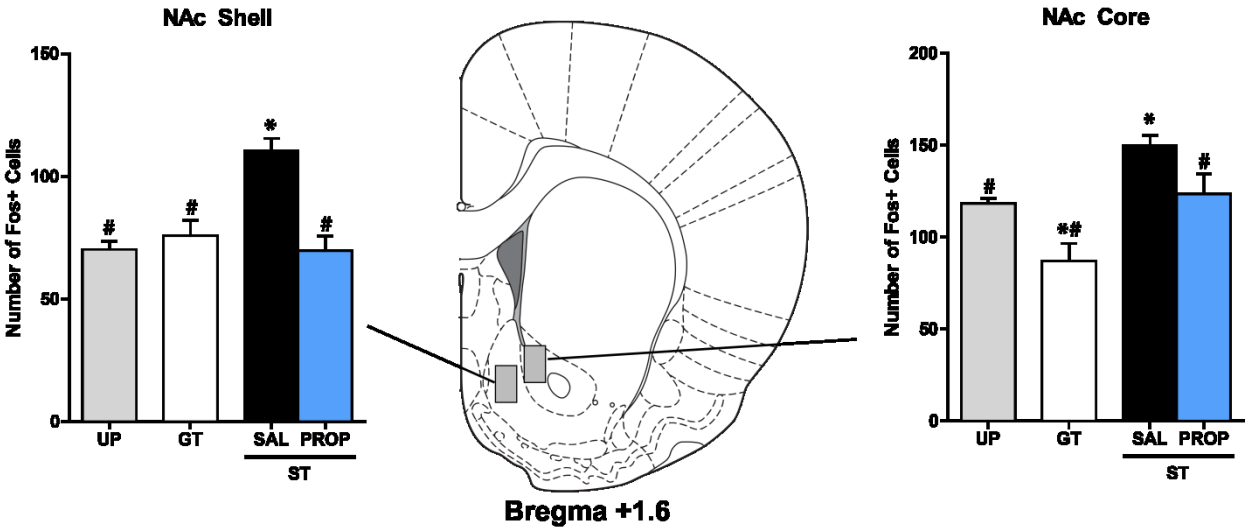

Figure 4.5. c-Fos expression engaged by a lever-CS in the ventral striatum. Data represent mean ±SEM. The number of Fos positive cells in saline- and propranolol-treated STs, saline-treated GTs, and unpaired animals.*$p<0.05$ (relative to unpaired) #$p<0.05$ (relative to ST saline-treated animals)

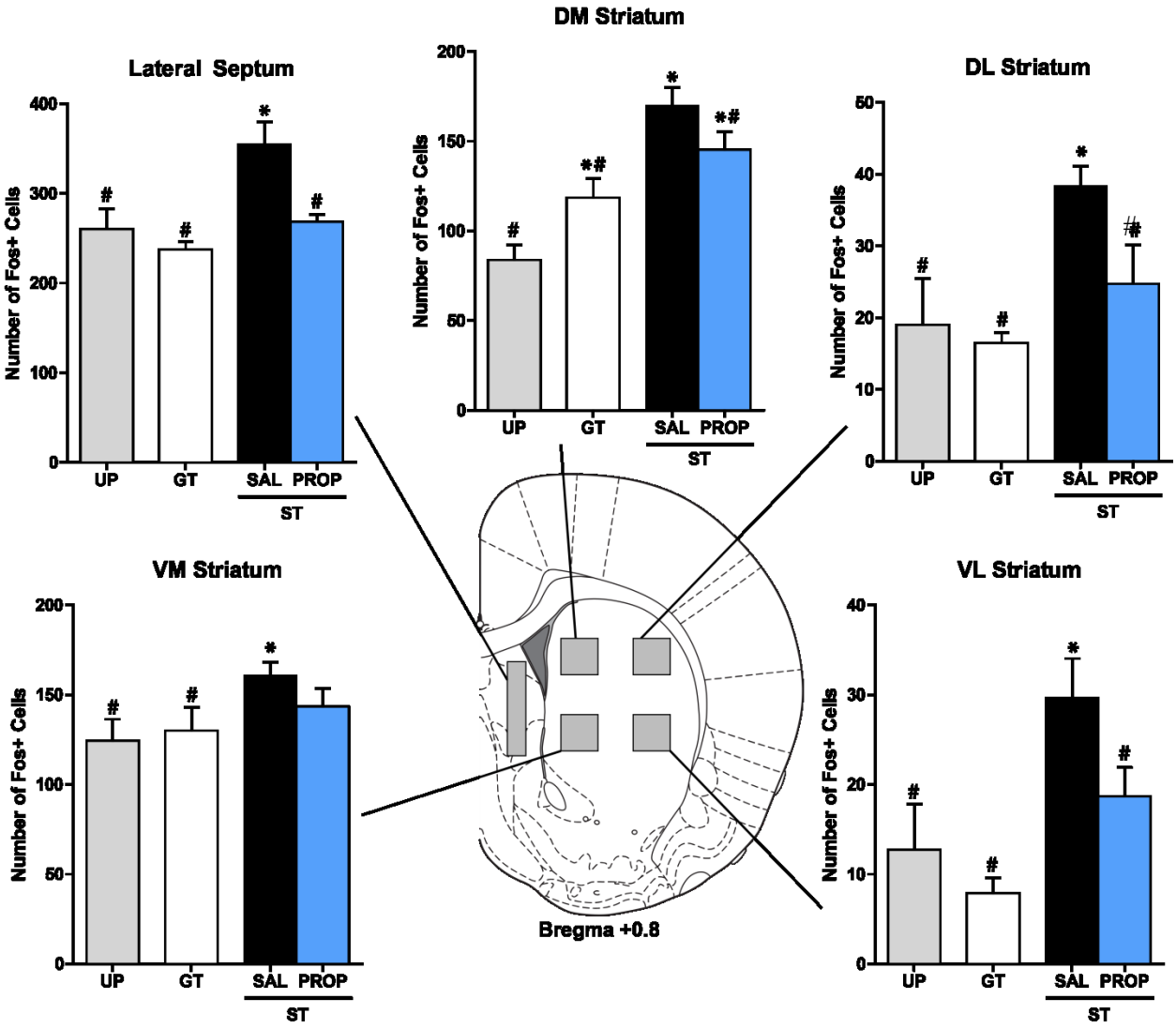

Figure 4.6. c-Fos expression engaged by a lever-CS in the dorsal striatum and lateral septum. Data represent mean ±SEM. The number of Fos positive cells in saline- and propranolol-treated STs, saline-treated GTs, and unpaired animals.*p<0.05 (relative to unpaired) #p<0.05 (relative to ST saline-treated animals)

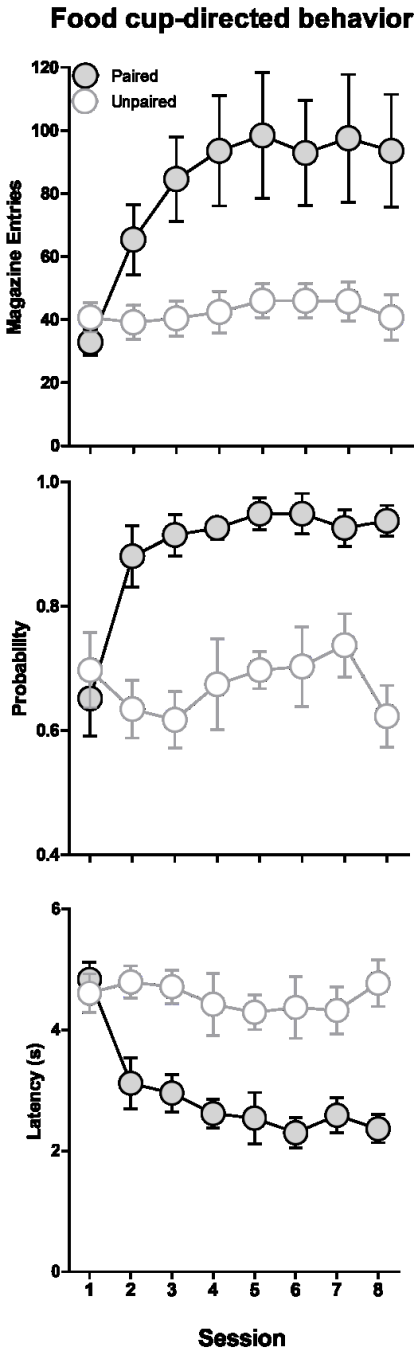

Figure 4.7. Goal-tracking to a tone CS. Data represent mean ±SEM. The acquisition of a goal-tracking conditioned response across Sessions 1-8 is illustrated in A-C. (A) Number of food cup entries. (B) The probability of food cup-directed responding. (C) The average latency to approach the food cup. The effect of propranolol on goal-tracking conditioned responses in STs and GTs are illustrated in D-F. (D) The number of food cup entries. (E) The probability of food cup-directed responding. (F) The latency to approach the food cup.

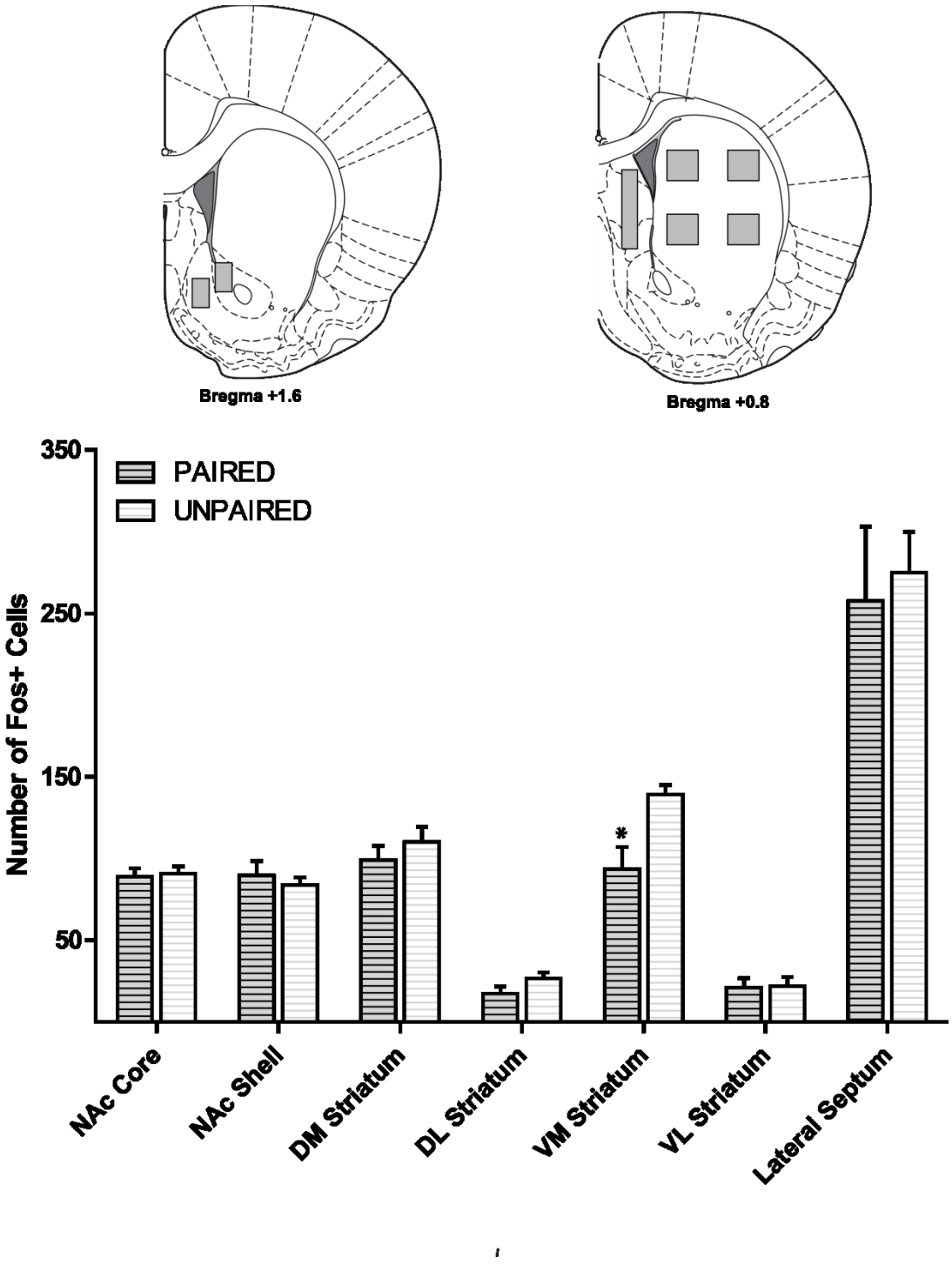

Figure 4.8. c-Fos expression engaged by a tone-CS. Data represent mean ±SEM. The number of Fos positive cells in paired and unpaired animals. *****$p<0.05$ (relative to unpaired animals)

Chapter V

General Discussion

The series of experiments described in this dissertation explored 1) how propranolol affects the reconsolidation of the incentive-motivational and predictive components of a CS-US association, and 2) the brain regions engaged by cues that differentially acquire incentive-motivational value, and how propranolol affects the engagement of these regions. We found that propranolol selectively disrupts the incentive-motivational component of a memory, while leaving the predictive component intact. Additionally, disrupting incentive-motivation decreases the extent to which brain regions are engaged by reward-paired cues.

Propranolol Selectively Disrupts Reconsolidation of Incentive-Motivational Value

Recent studies in human fear conditioning suggest that disrupting reconsolidation of CS-US associations with propranolol does not erase memory, but rather modifies it to reduce the emotional fear expression measured by conditioned responding. That is, the memory of the CS and the aversive stimulus it predicted remained intact, despite a reduction in fear responses (Kindt et al., 2009; Soeter & Kindt, 2011, 2010). There is, indeed, evidence in animals to suggest that propranolol specifically disrupts the reconsolidation of emotional memories (Dębiec & Ledoux, 2004; Debiec & LeDoux, 2006; Diergaarde et al., 2006; Robinson & Franklin, 2007; Robinson, Ross, & Franklin, 2011; Milton et al., 2008; Schramm et al., 2016). How exactly do we define *emotional* and *nonemotional* learning tasks in non-human animals? In humans, it is

relatively easy to measure emotion, because participants can effectively communicate feelings and thoughts. However, in non-human animals, we cannot measure this directly. Thus, we rely on behavioral measurements that indicate that a stimulus has acquired incentive-motivational or emotional value (as discussed in the Introduction). The dictionary defines 'incentive' as: Something that arouses feeling, or incites to action; an exciting cause or motivate; an incitement, provocation, 'spur' (Merriam-Webster). The implication with incentive-motivational stimuli is that they evoke emotional states (e.g. arousal) that motivate behavior. Though we primarily use the term 'incentive-motivation' in discussing appetitive conditioned stimuli, this term can also apply to aversive stimuli. The response induced by aversive stimuli may be opposite to that of appetitive, but these stimuli still evoke a state of arousal and spur motivation to avoid situations that predict aversive states (Hearst & Jenkins, 1974; Leclerc & Reberg, 1980; Hearst, Bottjer, & Walker, 1980; Leclerc, 1985). Although they require somewhat different psychological processes (i.e. positive versus negative), we use terms like 'emotion, and 'incentive-motivational' somewhat interchangeably, because ultimately they both generate approach or avoidance behaviors by creating a central state of motivational arousal. Thus, although much of the existing literature uses the term 'emotional' in the selective action of propranolol on memory in aversive learning, we believe this process to be similar in appetitive learning. In appetitive conditioning, we can use sign-tracking as an index of attribution of incentive-motivational value to a stimulus. However, in aversive conditioning it is unclear which features of a stimulus or context are evoking different behaviors, and whether those behaviors are indicative of different relationships with a CS (Moscarello & LeDoux, 2014) Thus, in the experiments discussed in this dissertation, we use a model of individual differences in appetitive learning (STs and GTs) to

allow us to study different component of a CS-US association (motivational/emotional versus predictive).

Propranolol Disrupts the Reconsolidation of Sign-Tracking, but not Goal-Tracking to a Lever Conditioned Stimulus

In Chapter Two, we hypothesized that propranolol would selectively disrupt the incentive-motivational properties of an appetitive association in rats. To test this hypothesis, we needed a method for parsing the incentive-motivational and predictive components of a CS-US association in rats. STs and GTs develop different conditioned responses as a result of differentially attributing motivational value to reward-paired cues. While all animals learn the predictive value of a CS, it acquires greater motivational value in STs (Flagel et al., 2009; Robinson & Flagel, 2009; Meyer et al., 2012a). Thus, the behaviors in STs and GTs allowed us to individually examine how propranolol affects a CS-US association that acquires only predictive value (in GTs), versus one that acquires predictive *and* motivational value (in STs). That is, we can ask whether reconsolidation truly erases memory or degrades the emotional motivational component of memory. To assess the effect of propranolol on the reconsolidation of an appetitive CS-US relationship, we first trained animals in a Pavlovian Conditioned Approach (PCA) task, and classed animals as STs or GTs based on their propensity to approach the CS and location of reward delivery, respectively. Across the next two days, we reactivated the memory in an additional (reinforced) PCA session on each day, and administered propranolol or saline injections immediately after each of the two sessions. On Day 8, animals were returned to the test chambers to assess the effect of treatment on the previous two days. We found that propranolol decreased ST behavior, but did not affect GT behavior. This suggests that propranolol selectively disrupts the motivational component but not the associative component

of a CS-US association. In addition to ST and GT behavior, animals also develop conditioned orienting responses with learning in a CS-US association (Saunders & Robinson, 2012; Yager & Robinson, 2013). Orienting is a learned response that develops as a CR to a conditioned stimulus (Sokolov, 1963). It has been demonstrated in humans (Verschuere, Kindt, Meijer, & Ben‐Shakhar, 2015) and rats (Lee, Wheeler, & Holland, 2011; Saunders & Robinson, 2012) that conditioned orienting responses are both psychologically and physiologically dissociable from each other (Lee et al., 2005). These responses remained intact in the STs after propranolol administration. Importantly, we have not only provided evidence that motivational and predictive components of appetitive memory are dissociable, but we also provide evidence explicitly against the claim that CS-US associations are being disrupted.

An in-depth analyses of video-scored behavior demonstrated that propranolol does not eliminate ST behavior completely, but rather, it specifically disrupts the *vigor* in which STs will approach and interact with a lever CS. A detailed account of video-scored behavior on the final test days in STs is reiterated here: STs in the saline-treated group would orient to the lever upon presentation, and immediately approach and contact the lever. On most trials, rats approached and engaged the lever vigorously for the entire 8 s CS period. Propranolol-treated animals, on the other hand, showed a dramatic decrease in the vigor and excitability during the final test session. This was in sharp contrast to the behavior evoked by the lever CS prior to propranolol treatment during Session 6, and also relative to the saline-treated rats. Interestingly, on the first few trials, the decrease in vigor observed in propranolol-treated animals often did not occur until after the first few lever CS trials. As mentioned previously, all sessions were reinforced, so this decrease across the session could not be the result of an extinction effect within the session. After these initial trials in which propranolol-treated rats still approached the lever with intensity and vigor,

their responding to the CS decreased through the remainder of the session. On some trials, rats would orient toward the lever upon CS presentation, and stare at it until it retracted. At the time of lever retraction, most rats immediately retrieved the pellet from the food-cup. During other trials, the rats would orient toward the lever and slowly approach it. From this point, rats primarily responded in one of the following three ways for the rest of the CS period. One, they would stare at the lever for the remainder of the CS period without contacting it. Two, they would sniff around the lever and delicately investigate the lever with their front paws. Sometimes these contacts resulted in a computer-scored lever contact, but other times, the contact was not strong enough to result in a computer-scored response. Third, the rats would approach the lever within close proximity, and they would pause before engaging with the lever. Mostly, this engagement was not with the extreme vigor that they interacted with the lever prior to propranolol treatment, except during the first few trials. In the description above, it is important to note that STs are still approaching and/or contacting the lever CS on many of the trials throughout the session. The decreases in ST behavior after administration of propranolol are not evident during the very first trial of the session, and primarily occur as a result of latency to engage with the lever CS. That is, propranolol appears to decrease the ability of a lever CS to induce immediate excitement and vigorous approach behavior.

In previous discussions of propranolol disrupting reconsolidation, concerns have been raised that propranolol is potentially "weakening the ability of apparatus cues to evoke memory of a reinforcing event" (Robinson & Franklin, 2007). Here, we demonstrate that GTs are capable of retaining the learned relationship between the CS and US. Additionally, within the STs, we demonstrate that predictive value of the CS is *not* being disrupted, demonstrated by an intact orienting conditioned response. These effects are not due to locomotor deficits induced by

propranolol, as we demonstrated in a separate control experiment, that propranolol administration without memory reactivation does not disrupt ST behavior (Chapter 2). Additionally, previous studies have found that propranolol does not affect general locomotor activity (Sara et al., 1995). Other studies in both humans (van Stegeren et al., 1998) and rats (Franklin & Robinson, 2007) have found that propranolol affects memory through central, but not peripheral beta-adrenergic antagonism. To study this we administered nadolol (which does not cross the blood brain barrier) instead of propranolol using the same experimental design. We found that the effects of propranolol require central nervous system action, as nadolol does not affect ST behavior (Chapter 2).

Our findings that propranolol disrupts the reconsolidation of incentive-motivation are consistent with reports in human studies of propranolol selectively disrupting emotional or incentive-motivational components of memory in Pavlovian conditioning. Between groups (STs versus GTs) and within individual animals (approach versus orienting in STs), we show that propranolol decreases motivational value without affecting the predictive value of a CS. A recent mouse study has reported a similar dissociation with propranolol disrupting reconsolidation using two different object recognition tasks; a classic object recognition task and an aquatic object recognition task (Villain et al., 2016). In a classic object recognition task, two objects are placed in a chamber, and typically mice will spend more time exploring the new object. Exploration of the new object is indicative of remembering the old or 'familiar' object. Villain and colleagues (2016) found that propranolol-treated animals spent an equal amount of time exploring an old and new object, suggesting their memory of the old object was disrupted. In their 'aquatic' object recognition task, the authors decided to time spent near the old object as a measure of 'familiar object recognition'. In this task, an object was suspended in the air above a

submerged platform in a pool of water. On the reactivation day, animals were given an additional session under the same conditions. The following day, platforms were removed and the old object was suspended from a different location. A new object was also suspended from the opposite corner. All animals (even propranolol-treated animals) spent a significant amount of time swimming under the familiar pattern, indicating recognition of the familiar object. The authors concluded that these results indicated propranolol disrupts 'emotional' (aquatic object recognition task), but not declarative (classic object recognition task) memories. This study is a unique and creative attempt to parse apart two different components of a memory in mice. However, the two tasks used to differentiation between 'emotion' and 'declarative' memory are completely different. The aquatic version of the task is significantly more stressful, as their survival (finding the submerged platform) depends on being able to find the familiar object. In contrast to their conclusions, this seems as though it should be the more 'emotional' task. Thus their argument that intact 'declarative' memory is indicated by the fact that propranolol does not affect a stressful aquatic task is not logical. In this dissertation, we present a series of experiments that 1) demonstrate that a dissociation between motivational and predictive components of memory persist in appetitive conditioning in animal models within the same task and 2) demonstrate that we can parse apart these components of memory within the same animal.

There is a wealth of evidence to support our assumption that STs and GTs differentially attribute motivational value to reward-paired cues. We have demonstrated that STs attribute greater motivational value to a lever CS than GTs across different properties of an incentive stimulus: 1) A lever CS elicits approach behavior to a greater extent in STs than GTs (Robinson & Flagel, 2009; Meyer et al., 2012), 2) STs will work significantly harder for presentation of a previously food-paired lever CS than GTs (Robinson & Flagel, 2009; Lomanowska et al., 2011;

Meyer et al., 2012; Meyer et al., 2014; Beckmann et al., 2015) , and 3) STs will reinstate seeking behavior to a greater extent than GTs when a central motivational state is evoked by a non-contingent presentation of a reward (Saunders & Robinson, 2011). Given this evidence, the studies in Chapter Two utilize STs and GTs as a model to investigate motivational versus predictive components of memory. Our findings provide further support for our initial assumptions about differences in incentive-motivation in STs and GTs.

Propranolol Does Not Disrupt the Reconsolidation of Goal-Tracking Behavior to a Tone Conditioned Stimulus

In Chapter Two we demonstrated that propranolol interferes with the motivational value attributed to a lever-CS. In the studies described in Chapter Three, we asked if this effect is unique to sign-tracking behavior, or if propranolol specifically disrupts conditioned responding in the animals that preferentially engage in sign-tracking behavior. One way to approach this question is to use a tone CS which only evokes a GT CR, even in animals that have been screened and classed as STs (Meyer et al., 2014; Beckmann et al., 2015). Our lab and others have found evidence to suggest that a tone CS acquires motivational value to a lesser extent than a lever CS (Meyer et al., 2014; Beckmann et al., 2015). A tone CS does not evoke approach behavior despite rats being able to localize it (Cleland & Davey, 1983; Harrison, 1979), and it acts as a less effective conditioned reinforcer than a lever CS. Given that we have suggested GT CRs do not require attributing incentive-motivational properties to a CS, *and* that a tone acquires less incentive-motivational properties than a lever CS, we asked if propranolol would disrupt the reconsolidation of GT evoked by a tone CS. First, we trained animals in an auditory Pavlovian conditioning task. As in Chapter Two, we then reactivated the memory with an additional conditioning session immediately followed by propranolol or saline injections. Reactivation and

injections were administered for two consecutive days, followed by a final Pavlovian conditioning session to assess the effects of injections from the previous day. We found that propranolol had no effect on GT behavior evoked by a tone CS. This suggests that propranolol specifically affects sign-tracking behavior for cues that are capable of acquiring greater degrees of motivational value. Next we asked if propranolol would differentially affect reconsolidation of a GT CR in STs and GTs. To do this, we screened animals in a PCA task, and classed them as STs or GTs. After subsequent Pavlovian conditioning with an auditory CS, and an identical reactivation and injection procedure to those in the experiment previously described, we found that propranolol did not differentially affect STs and GTs. Together these experiments suggest that propranolol disrupts the sign-track*ing* behavior, rather than conditioned responding in animals that attribute greater motivational value to cues sign-track*ers*).

The differences in attributing motivational value to a lever versus tone CS in rats may be due, in part, to the complexity of the stimulus. Our lab has recently demonstrated that features of a stimulus differentially acquire motivational value (Singer et al., 2016). The lever CS in the experiments described in Chapter Two moves, illuminates, and makes an audible sound upon extending into the chamber. It is possible that the lever CS simply acquires greater motivational value because of its saliency and multiple features. Future studies specifically comparing different components of a lever CS with a separate tone CS will be required to investigate which features of a lever CS might be more comparable to a tone CS.

These experiments provide further evidence that propranolol selectively disrupts motivational components of a memory. A tone CS does not acquire motivational value to the extent of a lever CS, and thus, the conditioned response to a tone may not necessarily have enough of a motivational component to disrupt. That is not to say that memory cannot be

disrupted in GTs, or that it is not possible to induce conditioned motivational states in GTs. In fact, Saunders et al. (2014) found that reward-paired (drug) contexts induce conditioned motivational states to a greater extent in GTs. Their findings suggest that perhaps GTs do attribute motivational value to environmental cues; however, they do so for contextual rather than discrete cues. Future studies should investigate whether propranolol disrupts the reconsolidation of conditioned motivation induced by a reward-paired context in GTs.

The experiments described in Chapter Three demonstrate that there are large individual differences in the extent to which different sensory stimuli enter into a predictive association with rewards. There are also differences in species in the extent to which these stimuli acquire motivational value. While there is a wealth of evidence to support that a tone acquires less incentive motivational properties in rats, this is not true for all species. For example, cats will readily localize and approach tones to obtain a food reward (Casseday & Neff, 1973; Grastyán & Vereczkei, 1974). Additionally, a tone conditioned stimulus will elicit approach and increase heart rate in horses, suggesting that a tone CS may evoke a conditioned motivational state in these animals (Christensen, Keeling, & Nielsen, 2005). Thus, the focus of the present series of studies should not be the effect of propranolol on the specific stimuli investigated (e.g. tone CS, lever CS), but rather the general idea that propranolol disrupts incentive-motivational or emotional memory components without affecting associations.

We have previously demonstrated that STs will develop a goal-tracking conditioned response to a tone CS (Meyer et al., 2014). This study provides support for the fact that these two behaviors, which are thought to be mediated by separate psychological and neurobiological systems, can be flexibly engaged within the same animal. We show here that when STs respond by goal-tracking a tone CS, they are not engaging the same neural systems as when they sign-

track to a lever CS. Although we have assumed that these are separate, there has not been any evidence to explicitly suggest that a pharmacological manipulation can affect sign-track*ing* to a lever CS, but not affect sign-track*ers* in their ability to respond to stimuli.

Propranolol Decreases Cue-Evoked Engagement of Brain Regions in STs

In the studies described in Chapters Two and Three, propranolol selectively disrupted the incentive-motivational component of a memory. Propranolol had no effect on memory in individuals that attribute less motivational value to cues, or involving stimuli that acquire less motivational value (tone CS). In Chapter Four, we explored how these differences in incentive-motivational value attribution are reflected in the brain.

Incentive-motivational stimuli engage several key regions in the brain that comprise a so-called 'motive circuit'. These areas include the striatum, nuclei within the amygdala and thalamus, and prefrontal cortical areas. Our lab has examined the extent to which reward-paired cues engage these brain regions in STs and GTs. Flagel et al. (2011) used *in situ* hybridization to quantify c-fos mRNA expression after exposing animals to a lever CS that was previously paired with a food reward. They found that a lever CS induces greater levels of c-Fos mRNA throughout the 'motive circuit' in STs, relative to GTs and animals that received unpaired lever presentations (not paired with food). Recently, Yager et al. (2015) found a similar effect quantifying Fos protein expression, rather than mRNA, and extended these findings to an opioid cue. Based on these data, we hypothesized that a CS would induce Fos expression in these regions only if it is attributed with motivational-value. Thus, we asked whether decreases in incentive motivation by propranolol also decrease the extent to which reward-paired cues will engage 'motive circuit' brain regions in STs. After replicating the behavioral effects from Chapter Two, animals were given a final test session, during which the lever CS was presented

10 times for 4 seconds each minute. Fos expression was significantly higher in STs, compared to GTs in subregions of the dorsal and ventral striatum, and the lateral septum, replicating previous findings (Flagel et al., 2011; Yager et al., 2015). In agreement with our hypothesis, we also found c-fos expression in propranolol-treated STs to be significantly lower in comparison to saline-treated STs in a majority of these brain regions. These results suggest that disrupting incentive motivation also decreases the extent to which cues engage 'motive circuit' brain regions.

In general, propranolol is primarily discussed as an antagonist for beta-adrenergic receptors. Norepinephrine exerts opposing actions on alpha- and beta-adrenergic receptors; alpha-adrenergic receptors exert inhibitory effects, while beta-adrenergic receptors exert excitatory effects (Buffalari & Grace, 2007). Propranolol acts to decrease neurotransmission by blocking beta-adrenergic receptors and therefore decreasing the excitatory input. Assuming there may still be norepinephrine in the synapse these neurotransmitters are now restricted to binding with alpha-adrenergic receptors, thus increasing inhibitory input (Buffalari & Grace, 2007). Propranolol also has non-specific effects on serotonin receptors (Middlemiss, 1984; Sprouse & Aghajanian, 1986). It is possible that propranolol's action on serotonin receptors may play some role in disrupting reconsolidation. However, enhancement of memory reconsolidation with a beta-adrenergic agonist, isoproterenol (structurally similar to epinephrine) infused into the amygdala is blocked by concurrent administration of propranolol (Dębiec et al., 2011). This strongly suggests that adrenergic modulation of memory is dependent upon beta-adrenergic activation or inhibition. Thus it is unlikely that non-specific serotonergic effects of propranolol modulate of memory reconsolidation.

Propranolol has been demonstrated to disrupt reconsolidation of memory in the amygdala in both aversive (Debiec & LeDoux, 2004, 2006) and appetitive (Bernardi, Ryabinin, Berger, & Lattal, 2009; Wu, Li, Yang, & Sui, 2014) tasks. For example, micro-injections of propranolol into the basolateral amygdala but not disrupt reconsolidation of morphine conditioned place preference (Wu et al., 2014). This study also investigated the role of the nucleus accumbens (NAc), and found that micro-injections of propranolol into this region had no effect on reconsolidation. These results are interesting, given that we saw a decrease in the NAc core and shell in Fos expression engaged by a reward-paired cue in propranolol-treated animals (Chapter 4). Indeed, the NAc is an important structure in the reconsolidation of appetitive memory, as protein synthesis inhibitors infused into the NAc disrupt the reconsolidation of drug conditioned place preference (Miller & Marshall, 2005; Milekic, Brown, Castellini, & Alberini, 2006). It is possible that the NAc is an immediate downstream effector of the amygdala in propranolol disrupting reconsolidation. The NAc does receive dense projections from the amygdala (Kelley et al., 1982). Additionally, the reconsolidation of striatal-dependent memories is disrupted by intra-basolateral amygdala infusions of propranolol in aversive learning (Goode, Leong, Goodman, Maren, & Packard, 2016). Thus, propranolol may be decreasing the extent to which a CS engages 'motive circuit' brain regions by affecting synaptic plasticity in the amygdala → NAc projections, which subsequently decrease the engagement of this entire circuit. That is, while the entire circuit may show decreases in activity in brain regions engaged by a lever CS, it is possible that this decreases is a result of plasticity in a subset of these regions.

This hypothesized mechanism of action fits in with the current literature on memory reconsolidation. In general, the amygdala has been largely implicated as a modulatory structure in reconsolidation of emotional or arousing memories (McGaugh, Cahill, & Roozendaal, 1996;

LeDoux, 2000; McGaugh, 2004; Blundell, Hall, & Killcross, 2001). The lateral and basolateral nuclei of the amygdala, in particular, have been found to exhibit synaptic plasticity (e.g. changes in LTP/LTD) with Pavlovian learning (Blair, Schafe, Bauer, Rodrigues, & LeDoux, 2001; Rodrigues, Schafe, & LeDoux, 2004; Samson & Paré, 2005; Samson, Duvarci, & Pare, 2005; Koo, Han, & Kim, 2004; Li et al., 2013). Using LTP as a physiological model of memory, researchers have also demonstrated that synapses within the lateral amygdala, like memories, enter a labile state during retrieval (Kim et al., 2010; Lee, Kim, & Choi, 2011). That is, LTP is differentially susceptible to pharmacological manipulation when given in correspondence with consolidated versus reactivated memories. Additionally, upon disrupting reconsolidation, studies have found a reduction in synaptic potentiation at synapses within the amygdala, compared to intact memory (Doyère et al., 2007). These data provide a solid foundation for the hypothesis that propranolol may disrupt or modify memory by acting on synapses within the amygdala to modify the extent to which the presentation of a CS will excite or inhibit cells (measured by changes in LTP/LTD). Electrophysiological studies investigating the timeline of neural activation of different regions within this circuit may provide insight toward this mechanism.

A Tone CS Does Not Engage Brain Reward Circuitry

The second question explored in the experiments described in Chapter Two was whether a tone CS engages regions in the 'motive circuit'. We have evidence to suggest that a tone CS does not acquire motivational properties to the same extent as a lever CS (Meyer et al., 2014; Beckmann et al., 2015). Thus, we hypothesized that a tone CS would not engage this circuit to the extent of a lever CS. To test this hypothesis, animals underwent Pavlovian conditioning with a tone CS. During the final test session, the tone CS was presented ten times for 4 seconds, once per minute. We found that Fos expression did not differ between animals that received paired

and unpaired presentations of the tone CS. These data suggest that a tone CS is not sufficient to engage 'motive circuit' brain regions, and supports existing data that a tone CS does not acquire incentive-motivational properties to the extent of a lever CS. So far, we have analyzed the striatum and lateral septum. It is possible that we may find differences in the other regions we plan to analyze. However, based on the data so far, it appears that goal-tracking behavior to a tone CS and goal-tracking behavior to a lever CS may be mediated by similar neural circuits.

In our analyses of engagement by reward-paired cues, we have not found any areas in which c-Fos expression is induced to a greater extent in GTs than STs (Flagel et al., 2011; Yager et al., 2015; Chapter 4). Recently, the paraventricular nucleus of the thalamus (PVT) has been found to modulate sign-tracking and goal-tracking behavior (Haight, Fraser, Akil, & Flagel, 2015). Inactivation of the PVT appears to attenuate the propensity to sign-track. However, lesioning the PVT appears to increase sign-tracking behavior in GTs. It appears that the PVT may act to modulate ST and GT behavior by acting specifically as a 'brake' on ST behavior. Although tracing studies of PVT afferents and efferents reveal differences in engagement by reward-paired cues between STs and GTs (Haight et al., 2016), regional c-fos mRNA and protein analyses have only found this area to be engaged by reward-paired cues in STs (Flagel et al., 2011; Yager, 2015). Again, based on the similarities observed thus far between goal-tracking to a lever and goal-tracking to a tone CS, it is unlikely that we will find a food-paired tone CS to engage the PVT.

It is important to mention that the differences in attributing motivational value to different kinds of cues are species-specific. Thus, the fact that a tone CS does not engage 'motive circuit' brain regions should not be generalized. Rather, an important consideration of the

findings described here is that cues that acquire less motivational value may not engage 'motive circuit' brain regions.

Clinical Relevance

The neurobiological action of propranolol in reconsolidation has been primarily studied with aversive conditioning in both rats (Debiec & LeDoux, 2004, 2006) and humans (Mahabir et al., 2015; Schwabe et al., 2012, Hurlemann et al., 2010). One main difference between these experiments is the route of administration. In rodents, propranolol is primarily administrated intraperitoneally (i.p) and in humans, it is administrated orally. Oral, but not i.p. administration of propranolol produces a metabolite called 4-hydroxy propranolol. However, this should have substantial differences in pharmacological action, as the plasma circulation of propranolol outlives that of its metabolite (Cleaveland & Shand, 1972)

Propranolol has been investigated in a number of different human studies in disrupting the reconsolidation of memory for cues in aversive tasks including fear conditioning (Kindt & Soeter, 2013; Kindt, Soeter, & Sevenster, 2014; Kindt et al., 2009; Soeter & Kindt, 2010, 2011, 2012b, 2012a), generalization of fear conditioning (Vervliet, Kindt, 2010), and imagined threats (Soeter & Kindt, 2012a). Studies have also recently investigated propranolol as a treatment to relieve pathological effects of aversive cues in phobias (Soeter & Kindt, 2015) and post-traumatic stress-disorder (PTSD - Brunet et al., 2008; Brunet et al., 2011; Mahabir et al., 2015) as well as cravings elicited by stimuli in drug and alcohol addiction (Saladin et al., 2014; Saladin et al., 2013; Lonergan et al., 2016; Lonergan & Pitman, 2013). Together, these data suggest that propranolol can be an affective therapeutic for reducing craving elicited by cues in food or drug addiction, as well as negative emotions induced by cues related to trauma. The experiments in this dissertation provide a useful animal model for guiding preclinical studies that may find a

more selective and efficient targeting of beta-adrenergic receptors to decrease emotional memories.

Conclusions

The experiments described in this dissertation show, for the first time in non-human animals, that beta-adrenergic antagonism does not disrupt emotional memories by 'erasing' them, but rather disrupting the emotional component. Our findings also raise questions about motivational and predictive components of memory. Are they stored as two separate traces? Do they exist as one trace with an emotional component that can be modified? Is it possible to 'erase' memory? Whether or not it is possible to 'erase' other kinds of memories remains to be seen.

Our studies have significant implications for animal models of reconsolidation. We demonstrate that propranolol differentially affects reconsolidation of motivational and predictive components of memory in rodents. We also provide an animal model to separate these memory components that can be adapted to a variety of different tasks. Future studies of memory reconsolidation should determine whether components of memory are differentially affected by pharmacological manipulations other than antagonism of beta-adrenergic receptors.

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