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Neural Representations of Reward in the Mesolimbic Circuit of Male Rats

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Neural Representations of Reward in the Mesolimbic Circuit of Male Rats

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Dedication

This dissertation is dedicated to my daughter, Madison. May you always find the strength and determination to follow your dreams.

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Neural Representations of Reward in the Mesolimbic Circuit of Male Rats

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The purpose of this dissertation was to investigate neural representations of the motivation for food and drug rewards. Through the analysis of the neural mechanisms underlying reward processing and associated cues I described translational pathways for treatment of cue related disorders. My research presented here focused on the role of the ventral basal ganglia in the mesolimbic circuitry, the main players in reward and attribution of motivational value to cues (i.e. incentive salience). I investigated individual differences in the attribution of incentive salience to reward-paired cues through modulation of dopaminergic inputs from the ventral tegmental area (VTA). Using Pavlovian conditioning (PCA) I assessed the motivational pull that cues elicit from rats (Flagel et al., 2007). In this model, an illuminated lever (conditioned stimulus, CS) was presented for 8 seconds at random intervals, after which, a food reward (unconditioned stimulus, UCS) was delivered into a magazine receptacle. All animals learned the predictive nature of the cue, but some, upon CS presentation, approached and interacted with lever (sign-trackers, STs), while others approached magazine (goal-trackers, GTs). After 5 days of training, phenotypes were well established and highly distinguishable. In

2 studies electrodes were implanted over target sites within the ventral basal ganglia (i.e. VTA, nucleus accumbens, NAcc, ventral pallidum, VP) following PCA training, and neural firing patterns in relation to behaviors during CS presentation were recorded. In the 3rd study, designer receptors (DREADDs) were utilized to inhibit neurons projecting from the VP to VTA in order to alter neural encoding of cues. First I found that dopamine neurons in the VTA encode incentive salience, evidenced by the increased and sustained firing magnitude in STs compared to GTs to CS presentation. Second, I found differential responses in the NAcc core and shell and in their projection sites, the dorsolateral and ventromedial VP, to food and cocaine cues. Third I found that DREADD activation of VTA neurons attributes greater incentive salience to Pavlovian cues as seen in greater cue approach behaviors in both STs and GTs. Overall, these findings demonstrate that STs and GTs employ different neural mechanisms in encoding incentive salience and rewards.

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Chapter 1: Introduction to Research

Cues Influence Behavior and Neural Coding

Organisms, including humans, attend to environmental cues that indicate objects and concerns essential to survival, for example, cues that signal food and water. When paired with rewarding stimuli, cues acquire predictive and motivational value and will, over time, come to elicit specific responses from individuals (Boakes, 1977; Brown & Jenkins, 1968; Hearst & Jenkins, 1974; Peterson, Ackilt, Frommer, & Hearst, 1972). Rewarding stimuli are those that have positive value to individuals although the degree of value may be dependent on their internal state (Aitken, Greenfield, & Wassum, 2016; Schultz, Dayan, & Montague, 1997). Manipulating salt appetite illustrates the power and properties of reward cues on neural representations of reward behavior. In normal homeostasis, rats express aversive reactions to tastes of concentrated salt solution and the neural activation patterns reflect these behaviors (Tindell, Smith, Berridge, & Aldridge, 2009; Tindell, Smith, Peciña, Berridge, & Aldridge, 2006). However, under a state of salt depletion, aversions to concentrated salt tastes switch to hedonic reactions and cues that predicted salt tastes gained incentive value (Tindell et al., 2009). Neural firing patterns in the reward circuit tracked these changes. In that study, it was notable that cues predicting concentrated salt elicited neural representations of positive hedonic value in the ventral pallidum even though the animals had only experienced that normally aversive tastes in normal homeostasis previously and the testing of cues was done in extinction (i.e., no actual reward was given).

The importance of learned predictive and motivational value assigned to cues has been studied extensively in behavioral investigations (Ahrens, Meyer, Ferguson,

Robinson, & Aldridge, 2016; Cleland & Davey, 1983; Meyer, Cogan, & Robinson, 2014; Stewart, de Wit, & Eikelboom, 1984; see also Tomie, Grimes, & Pohorecky, 2007; Carter & Tiffany, 1999). Over repeated pairings of neutral stimuli with rewards, the cue becomes a conditioned stimulus and may elicit specific behaviors. Studies have shown individual variation in response to reward cues between subjects and species (Cleland & Davey, 1983). The characteristics of the cue itself are important in driving responses (Holland, 1980a, 1980b). Visual localizable cues elicit greater orienting and approach behavior than localizable auditory cues, which elicit behaviors towards location of reward delivery (Hearst & Jenkins, 1974; Holland, 1980b). Cues that elicit approach behavior can do so even at the loss of reward receipt (Grastyán & Vereczkei, 1974; Wasserman, 1973). These last results are concerning and suggest that cues become more motivationally relevant than the reward itself. Indeed, others have suggested that expression of conditioned responses, specifically those directed towards cues, are analogous to addition-like behaviors (see Tomie et al., 2007). The studies presented here exploit differences in approach behavior to analyze coding of reward-predictive cues as a manner of determining differences in motivational value adhered to such cues.

By pairing cues with rewards in a Pavlovian paradigm, cues become pervaded with *incentive salience*, that is, motivation qualities that invoke approach and consumption of rewards in some individuals (Berridge & Robinson, 2003; Robinson & Berridge, 1993, 2001). The ability of rewards and their associated cues to acquire motivational value (i.e. incentive salience) varies significantly between individuals. In animals, one way this can be expressed is in differences in their motivation to approach and interact with learned reward-paired cues. The studies presented here use a Pavlovian Conditioned Approach (PCA) paradigm to detect such differences (Brown & Jenkins, 1968; Cleland & Davey, 1983). In the case where a discrete cue (e.g., a lever) is paired

with a palatable food reward (banana pellet), some animals (sign-trackers, ~1/3 of the population) approach and interact with the cue, while others (goal-trackers, ~1/3) engage in location of reward delivery during cue presentation (Boakes, 1977; Flagel, Watson, Robinson, & Akil, 2007; Hearst & Jenkins, 1974). The ability of reward-related cues to elicit these conditioned responses (CR) is due in part to the acquisition of predictive value. All individuals learn the predictive nature of the cue, but only sign-trackers place incentive value on them as indicated by the strong tendency to approach the cue and the fact that those individuals will work for the presentation of the cue itself (Meyer, Lovic, et al., 2012). Thus, for sign-trackers, the cue is a potent conditional reinforcer and can drive approach behavior. Further, following extinction training for food and drug rewards, discrete cue presentation elicits a robust reinstatement of reward seeking behaviors in sign-trackers, not goal-trackers (Saunders & Robinson, 2010; Yager & Robinson, 2010). This indicates that discrete cues play an important role in reinforcing and motivating reward-seeking behavior to a greater extent in some individuals (i.e. sign-trackers). In contrast contextual cues seem to more strongly influence behaviors of goal-trackers as seen by greater freezing behavior than sign-trackers when in contexts previously paired with foot shock (Morrow, Maren, & Robinson, 2011). These differences have implications in cravings for food and drug rewards and possibly susceptibility to addiction (Berridge, 2004, 2012; Robinson & Berridge, 2003; Versace, Kypriotakis, Basen-Engquist, & Schembre, 2016; Versace et al., 2016).

The purpose this study is to discern how differences in responses to cues such as those revealed by sign- and goal-tracking are differentially represented in the brain's reward circuits. Here the focus is on brain representations of neural mechanisms related to reward-based cues and related behaviors of individuals. Two simple types of neural coding that we will explore are rate coding and population coding. Rate coding

mechanisms are representations based on the firing rate of neurons. Besides simple firing rates, more complex patterns such as bursting patterns could co-exist although in general rate determinations will typically reflect these as well. Another potential neural representation we anticipate is population coding in which more (or fewer) activated neurons stand for the behavioral correlate.

My research goal is to discover functional neural representations of reward behavior in key mesolimbic circuits (nucleus accumbens, ventral pallidum, and ventral tegmental area). To this end, I will identify neural correlates of Pavlovian cues and reward seeking behavior. The Pavlovian Conditioned Approach paradigm used in this research allows us to parse predictive and incentive values of cues as well as actual consumption of the reward. We will then modify behavior with the new technique of viral insertion of receptors (DREADDs) in reward circuit elements in an attempt to modulate neural activity related to cue-induced reward-seeking. While this research does not directly study addiction, we hope that these findings may contribute to the development of therapeutic interventions for substance craving and other cue-related disorders. A better understanding of the mechanisms underlying motivational control of cues will allow for the design of therapeutic methods to treat addiction and other cue-related disorders.

NEURAL REPRESENTATION OF REWARD

Key areas of the reward pathway include the ventral tegmental area (VTA), nucleus accumbens (NAcc), and ventral pallidum (VP) (Figure 1.1). Neurons originating from the VTA project primarily to the NAcc (Albanese & Minciacchi, 1983; Beckstead, Domesick, & Nauta, 1979; Cragg & Greenfield, 1997), but also send signals to the

prefrontal cortex, amygdala, and hippocampus (Gasbarri, Packard, Campana, & Pacitti, 1994; Loughlin & Fallon, 1984; Swanson, 1982). The core of the nucleus accumbens projects directly to the dorsolateral region of the VP, while the shell projects almost exclusively to the ventromedial region (Brog, Salyapongse, Deutch, & Zahm, 1993; Root et al., 2013; Usuda, Tanaka, & Chiba, 1998; Zahm & Heimer, 1990; Zahm & Heimer, 1993). The ventromedial VP then projects back to the VTA to help regulate signaling of the pathway (Mahler et al., 2014; Tamiya, Hanada, Kawai, Inagaki, & Takagi, 1990), while the dorsolateral VP projects to the substantia nigra (SN) (Zahm & Heimer, 1990) and perhaps is involved in the motor response necessary to obtain rewards (Mogenson, Jones, & Yim, 1980). The shell of the NAcc, by indirectly projecting to the core via the VTA, may help regulate the output of the core in terms of how sensory input potentiates motor responses (Ghitza, Fabbricatore, Prokopenko, & West, 2004; Wyvell & Berridge, 2000; Zahm, 2000) or may be involved with the coding of incentive value of cues. Neurons of the NAcc also project back to the VTA (Kalivas, Churchill, & Klitenick, 1993) particularly from the shell (Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991; Lu, Ghasemzadeh, & Kalivas, 1998) and may modulate firing potentials through the circuit.

The ventral tegmental area is composed of dopamine and non-dopamine cells. Dopamine neurons make up roughly 60% of the VTA, γ-aminobutyric acid (GABA) neurons make up roughly 35%, and glutamate neurons make up about 5% (Sesack & Grace, 2010; Walsh & Han, 2014). The role of glutamate is not well know at this time, but studies have shown that these neurons project to both local dopamine and non-dopamine neurons (Geisler, Derst, Veh, & Zahm, 2007). Dopamine and GABA neurons project in a topographical manner to forebrain areas, specifically the nucleus accumbens (Beckstead et al., 1979; Carr & Sesack, 2000; Ferreira, Del-Fava, Hasue, & Shammah-

Lagnado, 2008). Dopamine neurons project to medium spiny neurons in the NAcc (Dichter, Damiano, & Allen, 2012). GABA neurons projecting from the VTA target cholinergic interneurons via inhibitory postsynaptic connections within the NAcc (Brown et al., 2012; Van Bockstaele & Pickel, 1995). This interaction causes long range inhibition of cholinergic firing in the NAcc (Brown et al., 2012; Creed, Ntamati, & Tan, 2014). Stimulation of GABA neurons projecting to cholinergic neurons via optogenetics enhanced the ability to discriminate cues associated with footshock and unpaired cues (Brown et al., 2012), showing an enhancement of stimulus-outcome learning. Activation of cholinergic interneurons also results in increased dopamine release at dopamine terminals within the NAcc (Cachope et al., 2012). The VTA also contains GABA interneurons that regulate firing of dopamine neurons (Creed et al., 2014; Johnson & North, 1992). This GABA-DA-NAcc microcircuitry has implications in motivated behaviors (Creed et al., 2014; Schultz et al., 1997). When dopamine neurons in the VTA were activated optogenetically, there was a resultant increase in reward-seeking behavior in an operant task when food was rewarded (Adamantidis et al., 2011). When an aversive stimulus was placed on an anesthetized subject, it caused a transient increase in VTA GABA firing and resultant decrease in dopamine activity (Brischoux, Chakraborty, Brierley, & Ungless, 2009; Tan et al., 2012; Ungless, Argilli, & Bonci, 2010). These results indicate roles for both GABA and dopamine neurons in influencing behaviors that result from stimuli that triggered their activation.

The NAcc and VP also contain hedonic hotspots, localized areas that function in hedonic enhancement, whereby μ-opioid stimulation increases hedonic impact ("liking") and motivation ("wanting") for food rewards (Peciña & Berridge, 2005; Peciña, Smith, & Berridge, 2006; Smith & Berridge, 2007). Studies have shown the NAcc and VP hotspots form a microcircuit that work together to elevate "liking" food rewards (Smith &

Berridge, 2007). Little is known about how these hotspots work for drug rewards and reinforcement cues. Studies have shown that activation of μ-opioid receptors of medium spiny neurons inhibits their firing in the NAcc shell (Hoffman & Lupica, 2001) which may result in increased firing of dopamine neurons in the VTA (through disinhibition), and may have implications as to how some drugs act to facilitate rewarding properties.

ROLE OF DOPAMINE

Dopamine is an important neurotransmitter involved in the mesocorticolimbic circuit and is integral to reward processing and motivational behaviors. Some argue that activation of dopamine neurons represents prediction-error (the ability to predict an outcome following presentation of a stimulus) (Cohen, Haesler, Vong, Lowell, & Uchida, 2012; Hollerman & Schultz, 1998; Schultz, 1998a), while others argue it attributes motivational value (incentive salience) to reward cues (Berridge, 2012; Saddoris, Cacciapaglia, Wightman, & Carelli, 2015; Saunders & Robinson, 2012).

In monkeys, dopamine neurons in both the ventral tegmental area and substantia nigra respond with a short phasic burst after receiving a sucrose solution reward (Hollerman & Schultz, 1998). When the reward is preceded with a stimulus, and the animal learns about the temporal stimulus-reward relationship, the stimulus becomes a cue and the dopamine response shifts to its presentation. At the same time, the neuron no longer fires at the time of the predicted reward delivery. When the reward is not delivered following a learned cue, however, or is delayed, then there is a depression of dopaminergic firing at the expected time of delivery, and in the case of a delay, a burst of dopamine occurs at the new time of reward delivery (Hollerman & Schultz, 1998). These findings support a role for dopamine in prediction learning. It may be that the role of the

substantia nigra is involved in prediction error to a greater extent than the VTA. Studies found greater activation (seen by FOS staining) in neural projections from the central amygdala to the substantia nigra, not VTA, following unexpected delivery or omission of food reward (Lee, Gallagher, & Holland, 2010). Alternatively, some (Saddoris et al., 2015) have suggested dopamine signals *both* predictive and incentive properties of reward cues, possibly resulting from differential roles for dopamine in the nucleus accumbens core and shell. In one study measuring dopamine release in the NAcc, rats were trained to press one lever (predictive cue) for presentation of 2nd lever that was followed by food delivery (Saddoris et al., 2015). They found a phasic dopamine release in the core to the predictive cue only, while there was continued release of dopamine in the shell to both cues and reward, consistent with incentive salience. Other studies have corroborated the role of the NAcc core response to cues predictive of food reward, and shell to reward receipt (Bassareo & Di Chiara, 1999). Differential responses of dopamine to predictive and incentive cues have also been discovered in the ventral pallidum (Ahrens, Meyer, et al., 2016; Tindell, Berridge, Zhang, Peciña, & Aldridge, 2005). These studies provide evidence that dopamine encods more than prediction error.

Both drug and food rewards stimulate the release of dopamine from the VTA into the nucleus accumbens, though in different patterns. Upon receipt of a novel food reward there is a release of dopamine in the NAcc core and shell, but this dissipates after a single exposure only in the shell (Bassareo & Di Chiara, 1999; Di Chiara et al., 1999). However, cocaine and amphetamine administration do not show the same habituation patterns as seen with food rewards (Di Chiara et al., 1999). Studies have shown that drugs, like cocaine, facilitate a surge of dopamine that is seen in the more medial core region of the nucleus accumbens (Ito, Dalley, Howes, Robbins, & Everitt, 2000; van Zessen, Phillips, Budygin, & Stuber, 2012) and the shell shows a 3-fold less uptake of

dopamine due to a lower density of binding sites (Jones, O'Dell, Marshall, & Wightman, 1996). In humans, this increased extracellular dopamine concentration in the nucleus accumbens was correlated with self-reported measures of euphoria (Di Chiara et al., 2004) which might be argued to reflect 'liking'. However this relationship is correlational and other manipulations of dopamine seem to point more clearly to a role in motivational 'wanting' rather than 'liking' (Berridge, 2007). The ability of drugs to alter dopamine transmission in the brain may lead to excessing 'wanting' associations between drug and reward-predictive cues. This property of abnormal attribution of motivational value to drug cues has been argued as the basis for drug addiction (Robinson & Berridge, 1993).

Dopamine neurons fire in 2 patterns, a tonic mode with regular spike discharge and phasic bursting mode with irregular patterns of spikes in bursts (Grace, 2000; Hyland, Reynolds, Hay, Perk, & Miller, 2002). Burst firing is the successive firing of dopamine neurons in 20-100ms intervals for short durations (200ms-1sec). Tonic firing is the spiking of dopamine neurons that lasts 3-9sec. These modes of firing result in differing release of dopamine and diffusion from synaptic space into extrasynaptic regions to influence firing of target neurons (detailed below) (Gonon, 1988). Phasic burst firing leads to release of dopamine of about 0.5-3μM immediately at the synapse (Garris, Walker, & Wightman, 1997; Mickelson, Garris, Bunin, & Wightman, 1998) while tonic firing will lead to the maximum concentration of ~250nM when all variscosities release dopamine (Gonon, 1997; Schultz, 1998b). The dopamine concentrations seen in tonic firing also leads to stimulation of autoreceptors on presynaptic varicosities (Schultz, 1998a). Due to rapid reuptake mechanisms of neurotransmitters, phasic and tonic firing patterns of dopamine neurons result in differences in dopamine diffusion and signaling patterns downstream (Grace, 2000; Schultz, 1998b). Phasic signaling of dopamine

neurons receptors may be involved in associative learning mechanisms, while tonic signaling may facilitate incentive motivation (Di Chiara et al., 2004).

Two types of dopamine receptor families have been established, the D1 receptor family (D1 and D5) and the D2 receptor family (D2, D3, D4), both of which activate G-protein coupled receptors (Baik, 2013). D1 type receptors stimulate adenylyl cyclase, which leads to activation of neural activity and gene expression (Dearry et al., 1990). D1 receptors have a low affinity for dopamine (Beaulieu & Gainetdinov, 2011) and respond to phasic neurotransmitter release (Goto & Grace, 2005). There is also evidence for the role of D1 receptors in reward seeking (Navarro et al., 2013). Studies utilizing D1 receptors agonists (SKF-82958) failed to reinstate cocaine seeking behavior in a non-reinforced task following priming with cocaine and non-selective DA agonist apomorphine further reduced self-administration of cocaine (De Vries, Schoffelmeer, Binnekade, & Vanderschuren, 1999) indicating that D1 receptors are critical for the reinforcing effects of cocaine.

D2 receptors inhibit adenylyl cyclase, which have opposing effects on neural activity and gene expression (Dearry et al., 1990). D2 receptors have a high affinity for dopamine and respond to lower tonic levels (Baik, 2013; Goto, Otani, & Grace, 2007). D2 agonists have also been implicated in drug-seeking behavior as well, and may be responsible for drug relapse (Clark & Bernstein, 2006). Mice lacking the gene for D2 receptors self-administered cocaine at higher rates as wild-type mice and treatment of wild-type mice with the D2 antagonist eticlopride also increased self-administration rates (Caine et al., 2002) indicating a role for them in modulating drug-taking behavior.

These results show evidence that phasic bursts of dopamine signal prediction error, potentially relying on D1 receptors, and tonic levels of dopamine signal, possibly through D2 receptors, reward value. Our studies presented here will attempt to address

these possibilities through the analysis of phasic and tonic firing patterns of individuals with D1/D2 receptor expression differences (Flagel et al., 2007).

The NAcc core and shell have shown differences in the expression of dopamine receptors which may influence the signaling differences seen between the regions. In the nucleus accumbens D1 receptors are typically expressed with substance P neurons and D5 neurons are present on cholinergic neurons, while D2 receptors are expressed with enkephalins (Lu et al., 1998; Nicola, Surmeier, & Malenka, 2000). Studies have shown that neurons from the shell that project both to the VTA and VP express primarily D1 receptors (Lu et al., 1998). Similarly, neurons from the core that project to the VP express primarily D2 receptors (Le Moine & Bloch, 1996; Lu et al., 1998). Further *in situ* hybridization studies have indicated that the majority of neurons in the NAcc express only D1 or D2 receptors, though a small subset have shown D3 receptors to colocalize with D1 or D2 neurons in both the core and shell (Le Moine & Bloch, 1996). There are also differences between the core and shell in response to phasic dopamine release in self-administration paradigms with the core tightly responding to reinforced response and the shell showing extended responses following responding for cocaine (Owesson-White et al., 2009), which may be due to differences in receptor expression.

INDIVIDUAL DIFFERENCES IN CUE-DRIVEN BEHAVIOR

The influential ability of cues differs between individuals (Flagel et al., 2007; Tomie, Aguado, Pohorecky, & Benjamin, 2000; Versace et al., 2016). These traits may arise from differences in neural coding patterns within the mesolimbic reward circuit. The mechanism behind this is not well known, though dopamine transmission through the mesolimbic circuit has been highly implicated over the last decade (Berridge, 2007).

Others have suggested that Pavlovian approach behaviors may in some instances become maladaptive. For example, associations between rewards and illicit drug cues may facilitate excessive motivational "wanting" and drug-seeking behavior (Robinson & Berridge, 1993, 2003). Rats expressing a sign-tracking phenotype show a cue-invoked dopamine release in the nucleus accumbens core that is not seen in goal-trackers (Flagel et al., 2011). In fact, this dopamine release increases across hundreds of trials indicating an attribution of incentive value toward the cue, vs. learning (Flagel et al., 2011). Further, blockade of dopamine through systemic antagonist (flupenthixol) administration in the nucleus accumbens core also eliminates sign-tracking conditioned response, both before and after learning cue-reward associations (Flagel et al., 2011). Similar antagonist administration in the shell does not produce behavioral changes (Nicola, 2010).

Differences in attribution of incentive salience may also relate to impulsive behavior in rats and potentially a vulnerability to addiction. The relationship between impulsivity and addiction is not well known but research has shown impulsive behavior is associated with greater vulnerability to addiction disorders (Barratt, 1994) and cocaine dependence in rats (see Tomie et al., 2007). There are multiple measures of impulsive behavior in both rats and humans that fall under the headings of "impulsive choice" and "impulsive action" (see Jentsch et al., 2014). Impulsive action relates to the inhibitory control of action and the inability to wait or inhibit motor responses; impulsive choice relates to decision making and is the preference for immediate small results at the expense of long-term outcomes (sensitivity to choice) or smaller reward depended on lowered probability of a larger reward being delivered (sensitivity to risk) (Grant & Chamberlain, 2014; Jentsch et al., 2014). High impulsive subjects have been shown to compulsively self-administer cocaine more often than low-impulsive subjects (Belin, Mar, Dalley, Robbins, & Everitt, 2008). Specifically, individuals shown to perform a

greater number of premature responses (as in serial reaction time tests) are predictive of those who will self-administer sucrose pellets (Diergaarde, Pattij, Nawijn, Schoffelmeer, & De Vries, 2009) or cocaine (Dalley et al., 2007)to a greater extent than those showing less deficits in the task. Further, subjects showing preference for an immediate small reward (as in delay discounting task) were shown to consume greater amounts of alcohol (Poulos, Le, & Parker, 1995; Tomie, Aguado, Pohorecky, & Benjamin, 1998). The latter form of impulsivity has specifically been linked to dopamine transmission, whereby injection of cocaine (effects dopamine transmission), not fluoxetine (serotonergic reuptake blocker) decreased choice for larger rewards (Logue et al., 1992).

In humans impulsive behavior also correlates with a poorer response to treatment (Poling, Kosten, & Sofuoglu, 2007) and this has been associated with dopamine transmission as well (Buckholtz et al., 2010). This study showed that humans with highly impulsive behavior expressed fewer dopamine autoreceptors (D2 and D3) in the midbrain (Buckholtz et al., 2010). Further these individuals produced greater dopamine release in response to amphetamine, which was positively correlated with magnitude of drug craving (Buckholtz et al., 2010). These results give some evidence as to the role of dopamine in the presentation of behavioral differences seen in sign-trackers and goal-trackers as it relates to human behavior. Indeed, increasing DA neurotransmission through drugs like amphetamine and cocaine increases impulsive action in rats (van Gaalen et al., 2006a). Further, administration of D1 receptor antagonists (SCH 23390) decreased premature responses in said subjects (van Gaalen et al., 2006).

Behaviorally, sign-trackers and goal-trackers differ in tests of impulsivity. One study analyzing impulsive behavior in STs and GTs found a greater number of premature responses in STs compared to GTs, but a greater propensity to choose larger rewards, especially with longer delays in reward delivery (Lovic, Saunders, Yager, & Robinson,

2011). Other studies have found that higher lever-directed responses or cue light orienting responses (similar to what we classify as sign-trackers) exhibit decreased choice for larger rewards over smaller immediate delivery (Olshavsky et al., 2014; Tomie et al., 1998). Discrepancies may result from procedural differences in the tests of impulsivity performed and/or classification of sign-trackers vs. goal-trackers. Nonetheless, findings that differences of impulsive behavior exist in STs and GTs suggest unique coding mechanisms in these groups of individuals.

The goal of this research is to provide a better understanding of the neural coding differences that may underlie some of these behavioral properties. Further, as an approach to understanding addiction, cocaine self-administration is used as a model. This research does not address impulsivity or addiction explicitly. Rather, it uses goal-tracking and sign-tracking models as a method of identifying animals with a tendency to attribute incentive salience to cues vs. animals that use alternative reward seeking strategies. Our specific aim is to determine how differences in the propensity to attribute motivational value to reward-paired cues are coded in neural firing patterns in the mesolimbic circuit. Cue-related behavioral trait differences naturally apparent in individuals are exploited to determine the extent to which patterns of neural activity characterize motivational 'wanting' (i.e. incentive salience) of reward-paired cues.

STATEMENT OF PROBLEM

Addiction is defined by the continual pursuit and consumption of drugs of abuse long after the onset of negative social, behavioral, and occupational consequences. As defined in the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013), substance use disorders are based pathological patterns of

behavior related to impaired control (use or overuse over extended periods of time), social impairment (failure to fulfill obligations at home work or school), risky use (continued use during physically hazardous situations), and pharmacological criteria (tolerance and/or withdrawal).

Addiction arises from a complex set of brain mechanisms that include sensory stimuli, reward evaluation, and motivational components (Robinson & Berridge, 2003; Volkow, Fowler, Wang, Swanson, & Telang, 2007). The net result is exaggerated pursuit and persistent consumption of substances that produce functional behavioral impairments. Discussions of addiction often relate to illicit drugs as well as socially tolerated drugs such as alcohol and nicotine.

Drug addiction is clearly a major problem worldwide, costing millions of dollars every year in medical expenses alone, and even worse, mortality. The World Drug Report estimates that 1 in 20 adults age 15-64 (250 million) used at least 1 illicit drug in 2014 and 29 million of these (12%) suffer from some drug-related disorder (World Drug Report, 2016). According to the 2015 National Survey on Drug Use and Health (NSDUH) report, 49% of all Americans have used illicit drugs in their lifetime; 10.1% showing recent use (use occurred within 30 days of survey, up from 8.3% in 2002) (Center for Behavioral Health Statistics and Quality, 2016). In the United States, the majority of such recent drug misuse is due to marijuana (82.2%), with abuse of prescription psychotherapeutics (pain relievers, tranquilizers, stimulants, sedatives) following (23.8%), and then cocaine (6.9%). The majority of the total population partaking being ages 18-25 (57.5%). These trends are similar to those reported by the 2016 European Drug Report (European Monitoring Centre for Drugs and Drug Addiction, 2016).

The population of individuals with drug problems remains a concern around the world. In 2014 there were over 200,000 drug-related deaths and 1/3-1/2 of these were due to overdose, with the highest mortality rate seen in North America (United Nations Office on Drugs and Crime, 2016) a result that is unacceptable and preventable. Drug use also accounts for 18.9% of individuals living with HIV and 46.7% of those with hepatitis C worldwide (European Monitoring Centre for Drugs and Drug Addiction, 2016).

Use of cocaine specifically represents roughly 0.4% of the world population, with use decreasing in North American and rising is Oceania (Australia and New Zealand) and some South American countries. In Europe alone, cocaine accounted for5.1% of drug use for individuals age 15-64 in 2015, the majority (85%) of which are male (European Monitoring Centre for Drugs and Drug Addiction, 2016). Of those who have used cocaine, though, 1/3 of them reside in North America, representing the 2nd largest population of cocaine users (United Nations Office on Drugs and Crime, 2016).

It is unclear what percentage of users actually seek help for their uncontrollable consumption, but global estimates indicate 1 in 6 seek treatment every year with 40-80% diagnosed for polydrug use, making treatment challenging (United Nations Office on Drugs and Crime, 2016). Further, according national statistics, relapse occurs in 40-60% of individuals (National Institute on Drug Abuse, 2012). These rates are consistent with European statistics, which show that 54% of cocaine users alone suffer relapse (European Monitoring Centre for Drugs and Drug Addiction, 2016). Although this dissertation does not examine addiction per se, it has focused attention on dopaminergic mechanisms of the brain reward system, which are a common theme related to abused substances, with a specific test of cocaine. There is currently no medication or therapy that has proven to effectively treat its use and prevent relapse. However, current behavioral therapies employed to treat cocaine include incentive-based interventions to stimulate motivation-

related brain areas to promote continued treatment (Natinal Institute on Drug Abuse, 2014; National Institute on Drug Abuse, 2012). Understanding the cause of the motivational drive to obtain cocaine and other rewards will aid the development of effective therapies to end addiction.

RESEARCH QUESTIONS AND HYPOTHESES

This project examines the neural representations of motivated behavior. Furthering the understanding of how the patterns of neural firing within the mesolimbic circuit of the brain are correlated to motivated behavior will provide clues and translational opportunities for addiction treatments. For Example: How does neural activity relate to cue-directed or goal-directed behaviors? How are individual differences reflected in coding of neural cells?

The studies presented will exploit behavioral trait differences in the propensity to assign motivational value (i.e. incentive salience) to reward-linked cues. I will analyze neural activity during presentation of natural (food) and drug rewards, and their predictive cues. Cues that are highly valued (high incentive salience) should show an increase in explorative and interactive behaviors directed towards them over less valued cues in animals that exhibit the sign-tracking trait. Prior work in our lab has shown altered neural firing rates and changes in the sizes of active neural populations in the ventral pallidum related to food-associated Pavlovian cues as well as to cues predicting 'liked' and 'wanted' tastes (Tindell, Berridge, & Aldridge, 2004; Tindell et al., 2009, 2006). Due to the reciprocal connections of the VP, NAcc, and VTA I propose that the differences in assigning incentive salience to cues will be represented in neural coding patterns in other key areas of the mesolimbic circuit as well. Such differences may drive

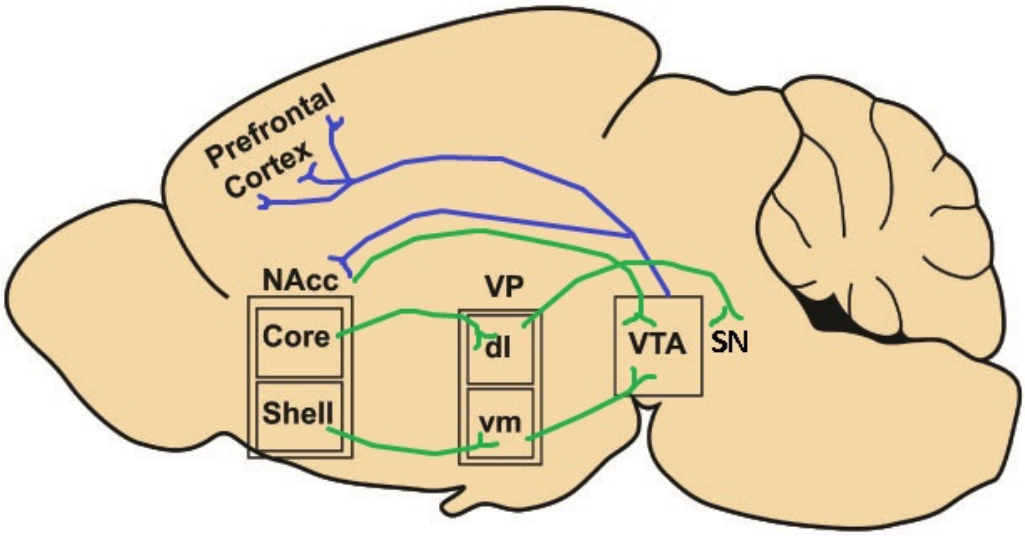
alterations in synaptic reorganization, and help explain the resulting addiction-like behavior and relapse in some individuals. The specific coding patterns of neurons in the mesolimbic circuit are of most interest due to their involvement in reward and motivation. Through a combination of novel techniques to target specific reward pathways, electrophysiological recordings at downstream sites, and behavioral analysis, the neural dynamics of the reward circuit will become better understood.

Currently, little research has been conducted exploring neural coding properties as it relates to differences in cue-driven behavior. That is, there may be greater recruitment of neurons responding to cue presentation (population coding) as well as enhanced neural activation (increased rates for excitatory neurons, decreased rates for inhibitory neurons) in some individuals. We see differences in individuals in their propensity to approach and interact with reward-paired cues, along with differences in coding patterns in the VP (Ahrens et al., 2016). How dopamine neurons code such differences has yet to be determined. By exploiting the behavioral differences in sign-trackers and goal-trackers the relationship between neural coding and cue-related disorders throughout the mesolimbic circuit can be better understood. My central hypothesis is that neural coding (i.e. neural representations) of STs is greater than GTs during cue presentation in terms of number of neurons responding (population coding) and magnitude of response due to the differences in attribution of incentive salience. Also I expect the value of the cue will be represented in all areas of the mesolimbic circuit (VTA, NAcc, VP).

Although all individuals show dependency to drugs over extended use, sign-trackers may be especially vulnerable to addiction-like behaviors due to their strong attraction to cues. The studies presented here are novel, translational, and functionally relevant. Here, I am focusing attention on the neural correlates of incentive and predictive cues in individuals who differ in their propensity to attribute incentive salience to reward-

paired cues. I will also analyze dopamine cells directly in response to incentive motivation by predictive cues and address how neuromodulation of dopamine affects behavioral motivation. A recent study in my lab has shown that individuals show differences in magnitude to reward cues in the VP. I expect to see similar magnitude differences in the VTA and NAcc. Through neuromodulation of the dopaminergic circuit, I was able to assess the effect of dopamine innervations on behavior. By analyzing how these anatomic structures are functionally connected, I can begin to determine how incentive and predictive behaviors are encoded in the mesolimbic circuit. This provides evidence to a potential novel treatment of addiction disorders, such as obesity and drug abuse.

Figure 1.1: Mesolimbic Circuit and Regulation of Dopamine



Dopamine (DA) neurons (blue) project from ventral tegmental area (VTA) to nucleus accumbens (NAcc). The NAcc then sends GABA projections (green) to the ventral pallidum (VP) in a topographical manner, with core neurons projecting to the dorsolateral (dl) region, and shell to ventromedial (vm) region. The VPvm then projects to the VTA causing downstream disinhibition. The VPdl projects to the substantia nigra (SN) and is involved in motor output.

Chapter 2: Neurons of the Ventral Tegmental Area Encode Predictive and Incentive Cues

INTRODUCTION

Individual differences in the attribution of incentive salience to environmental cues for reward may play an important role in addictive behavior and other cue-related disorders. Some individuals are highly motivated by reward-predictive cues, while others do not show interest in them (Flagel, Watson, Akil, & Robinson, 2008). Such differences can be detected through Pavlovian conditioning. In the case where a discrete cue (e.g., a lever) is paired with a palatable food reward (banana pellet), some animals (sign-trackers, STs) approach and interact with the cue, while others (goal-trackers, GTs) engage in location of reward delivery during cue presentation (Flagel et al., 2007). All individuals learn the predictive nature of the cue, but only STs place motivational value on them i.e., the attribution of incentive salience (Berridge, 2007). Thus, for STs, the cue is a potent conditional reinforcer that elicits approach behavior (Saunders & Robinson, 2010, 2011). These behavioral differences may underlie the tendencies toward obesity, addiction, and other cue-related disorders (Meyer, Ma, & Robinson, 2012; Peciña et al., 2006; Yager & Robinson, 2010) in some individuals.

The neural mechanisms of individual variability in behavioral response to reward cues is still under active investigation (Ahrens, Meyer, et al., 2016). One key component of this variability may be neural circuits that utilize the transmitter dopamine. Dopamine neurons have been implicated in many different psychological functions related to reward – learning, prediction error, reward evaluation, but the way in which it may be involved in addiction is uncertain and controversial.

The pattern of anatomical connectivity of the mesolimbic dopamine circuit supports the idea of a role in reward mechanisms clearly. Dopamine neurons in the ventral tegmental area (VTA), the main reward component of the midbrain dopamine system, project to the nucleus accumbens (NAcc) core and shell. The core in turn projects to dorsolateral ventral pallidum (VPdl) while the shell projects to the ventromedial ventral pallidum (VPvm). Interestingly, dopamine signals in the nucleus accumbens results in differing responses in the core and shell. Dopamine release in the core, not shell, is biased toward encoding prediction error of cues; specifically that phasic release of dopamine to reward-predictive cues vary as a result of reward value (Creed et al., 2014; Day, Jones, & Carelli, 2011; Saddoris et al., 2015). Alternatively, the shell, not core, region of the nucleus accumbens responds to changes in incentive value of rewards and their predictive cues (Saddoris et al., 2015; Wheeler et al., 2011). The heterogeneity of dopamine signaling indicates its role in role in reward processing is more complicated than first thought. How neurons of the VTA affect signaling through the NAcc and subsequent dopamine release is still not well known.

Sign-trackers (ST) and goal-trackers (GT) differ in dopamine release patterns (Flagel et al., 2007) and neural firing patterns observed in the ventral pallidal targets of dopamine projections (Ahrens, Meyer, et al., 2016). These differences between STs and GTs may play an important role in expressed behavioral differences. Rats with a sign-tracking phenotype show cue-invoked dopamine release patterns in the NAcc core that are not seen in goal-trackers (Flagel et al., 2011). In fact, this difference in dopamine release between phenotypes supports a role for the attribution of incentive salience to cues (Berridge, 2007; Flagel et al., 2011). In a direct comparison of incentive salience attribution to prediction-related events, STs showed a relative increase in dopamine release in response to cues in contrast to stable cue-related dopamine release in GTs

(Flagel et al., 2011). This stands in contrast to the idea that cues should show an increase in dopamine activation as suggested by the proponents of dopamine/learning mechanism perspective (Schultz et al., 1997). Although there is evidence for phenotypic variation of dopaminergic impact on ventral pallidal neurons (Ahrens, Meyer, et al., 2016) and dopamine release in the NAcc (Flagel et al., 2011), direct comparison of these trait differences on dopamine neurons themselves has not been done. The objective of this study is to investigate these differences associated with individual behavioral differences associated with attribution of incentive motivation to reward-predictive cues.

All cues that precede a reward are predictive in nature. Some, however, also have incentive value, in that they elicit approach towards them. Studies in my lab have utilized a 2-cue paradigm to pull apart predictive from incentive cues. In this method the first cue (lever presentation) is a predictive cue. The presentation contains all of the prediction power of impending reward; it may also have incentive value for some individuals (sign-trackers). We also have a second cue – a combination of lever retraction and feeder click. Because this second cue is redundant in its predictive qualities, i.e., it provides no more information about the impending reward; the second cue then is purely incentive in nature (Smith, Berridge, & Aldridge, 2011). This project serves to determine whether dopamine neurons encode differences in approach behaviors and incentive salience. We hypothesize that firing in dopamine neurons in STs will be stronger than GTs in response to incentive cues and perhaps less responsive to predictive cues.

METHODS

In this experiment dopaminergic neurons from the ventral tegmental area (VTA) were targeted. Spikes of dopaminergic neurons have unique characteristics making them distinguishable from other neural types (see Roesch, Calu, & Schoenbaum, 2007).

Dopamine neurons were identified by three defining characteristics (see Pan, Schmidt, Wickens, & Hyland, 2008): 1) a low basal firing rate (<10 Hz), 2) long spike duration (>1.2ms), and 3) a >50% decrease in firing rate following apomorphine injection.

Animals and Care:

Male Sprague Dawley rats were used with an initial weight of 200-250g (Charles River, Wilmington, MA). Males were housed in a reverse light:dark (14:10) cycle with lights off at 10:00. Upon arrival, they received 2 days to habituate to their new surroundings. They remained in pairs until electrode implantation at which point they were housed individually. Subjects were handled daily for 7-10 days before Pavlovian Conditioned Approach (PCA) training. All testing was performed during the dark cycle, between 10:00-18:00 with water and food available *ad libitum* throughout the study (except while in testing chamber). All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA) and Institutional Animal Care and Use Committee (IACUC).

Pavlovian Conditioned Approach (PCA):

Animals (n = 28) first underwent Pavlovian conditioned approach (PCA) training to determine phenotype (see below). This paradigm has been shown to effectively identify the behavior of many types of mammals (rats, mice, voles) in terms of their level of attributing incentive salience to cues. In this procedure, animals are placed in a metal and Plexiglass chamber situated with a house light and white noise speaker on one wall. Opposite that in the center, approximated 1 cm from the floor is a magazine for food delivery. To the left or right (placed randomly for each animal) roughly 6cm from the floor is an illuminated retractable lever. Session began with illumination of house light and white noise.

Training on Day 1 began with 25 trials to familiarize the animals to delivery of banana-flavored food pellets (BioServ, Frenchtown, NJ) into the magazine. During magazine trials, pellets (unconditioned stimulus, UCS) were delivered into the magazine on a variable time 30 schedule (average 30 sec, range 15-45 sec). PCA training followed magazine training for 5 days. The Pavlovian trial had a predictive cue, consisting of an illuminated lever (conditioned stimulus, CS) inserted through the wall into the cage for 8 seconds. The reward pellet was released at the moment the lever was retracted and delivered into the magazine 1.2 sec later. Note that pellet delivery required no response by subject. Trials were presented on a variable time 90 schedule (average 90 sec, range of 30-150 sec).

At the end of each training session, animals were returned to their home cage. All subjects learn the predictive nature of the cue (Flagel et al., 2007). Some direct their attention towards the lever during presentation (sign-trackers, STs, n = 6), while other direct their attention towards location of pellet receipt (goal-trackers, GTs, n = 11). Still others oscillate between both (intermediates, N=11 not studied here). On the last day of training, behavioral videos were analyzed to determine attention direction and specific behavior expressed during lever presentation and to calculate resulting PCA index.

PCA indexing:

Previous research has indicated that this PCA paradigm will elicit sign-tracking (ST) and goal-tracking (GT) phenotypes from the animals. Behavioral phenotypes are apparent and stable by 4-5 days of training (Flagel et al., 2007). Previous reports have indicated that phenotype can be determined by calculating PCA index (see Meyer et al., 2012). Value is determined by (a) latency difference [(time to approach magazine during CS – time to approach lever)/8], (b) response bias [(# lever deflections - # magazine

entries)/(# lever deflections + # magazine entries)], and (c) approach probability difference [(probability of contacting lever – probability of contacting magazine)]. A score of <-0.5 indicates a GT phenotype, >+0.5 indicates a ST phenotype and -0.5 to +0.5 indicates an intermediate phenotype (Figure 2.1).

Electrodes:

Electrodes were manufactured in the lab. Two 23AWG steel cannulae (Granger, Chicago, IL) were positioned 1.6mm apart. Into the steel, a 32AWG polyimide tubing (Small Parts, CA) was threaded for advancement. The tubing was then adhered to a threaded screw. Each full turn of the screw advanced the cannula 1/3mm. Tetrodes, 4 wires (12.5µm, California Fine Wire) wound tightly and heat fused together, were thread through the tubing, with 4 tetrodes per bundle. One end of the wires was for cortical implantation, while the other was separated and pinned individually with gold pins to create a circuit. A grounding wire was also soldered to the electrode board. The back of the electrode contained an adapter piece that connected to a signal amplifier, which transmited signals from the wires to a computer where it was recorded for analysis.

Implant Surgery:

Electrode bundles were sterilized prior to implant. Animals were anesthetized with isoflurane (3%) initially in an induction box until breathing slowed. Fur on the top of the head was shaved rostral to caudally from between the eyes to the back of the skull. The head was then fixed in a Kopf stereotax using ear bars positioned under the temporal arch, and bite guard. Isoflurane was provided via a nosepiece. Animals were maintained under 2-2.5% isoflurane. Body temperature was maintained using a temperature therapy pad. We then placed a small amount of hair removal lotion (Nair) over the shaved area and let sit for 5 minutes to completely remove hair around incision site. Animals were

then given a subcutaneous injection of lidocaine (0.5ml/kg, LidoJect, Henry Schein) as a pre-surgical analgesic and to reduce bleeding. Lubricating ointment (Puralube, Henry Schein) was applied to the eyes. The scalp was scrubbed with a disinfectant solution (e.g. sterile iodine), and rinsed with alcohol. Making sure subject did not respond to toe pinch, an incision was made (appx 2 inches in length) in a rostral-caudal direction from just behind the eyes to just behind the ears. Skull was cleared of all tissue to expose Bregma and lambda. Bleeding was controlled with cautery when necessary. Using a pointer attached to the stereotaxic apparatus the anterior-posterior (AP), medial-lateral (ML), and dorsal-ventral (DV) coordinates of Bregma and lamda were found. To ensure the head was level, the difference in DV measurement between Bregma and lamda was not more than 0.25mm. The coordinates for electrode implant was then determined, targeting VTA at: AP: 4.9mm, ML: +/- 0.7mm, DV: 8mm. Placement of bundles was marked on the skull using a sterilized pencil and a 1mm craniotomy was made. These were cleaned to remove bone fragment and dura. Following, an array of 4-6 bone screws were fixed to the skull around the implant area, ensuring they do not impede implant of electrode. Recording electrodes were then positioned over the craniotomies and lowered slowly into the brain until 1mm above the VTA. Dental acrylic (Dentsply, Henry Schein) was then used to anchor electrode to skull and to completely cover incision. After acrylic dried a topical analgesic (lidocaine hydrochloride jelly, Fisher Scientific) and Triple Antibiotic Ointment (Fisher Scientific) was applied around incision. Animals were then removed from stereotax. When subjects began to move, they were given 2.5mg/kg injection of flunixin (FlunixiJect, Henry Schein) and 0.1ml Penicillin (Henry Schein) immediately as well as for 2 days post-surgery.

Animals were singly housed from this point forward as cage mates have the tendency to chew off each other's' electrodes. Enrichment (toilet paper tubes, shredded

paper, etc.) was provided by us and animal care staff. Food was also provided at the bottom of their cage as the electrode can catch between the wires of a metal dispenser. Subjects were given 7 days to rest before neural recording began.

Verification of Dopamine-like Neurons:

Neural correlates of behavioral events during the Pavlovian task were assessed in 4 sign-trackers and 7 goal-trackers on a total of 103 dopamine-like (henceforth called "dopamine") and 141 non-dopamine-like (henceforth called "non-dopamine") neurons. Dopamine and non-dopamine neurons were discriminated by their spike width and average baseline firing rates (Figure 2.2). Dopamine neurons show wider spike lengths (> 1.2ms) than non-dopamine neurons (<1.0ms). Dopamine neurons fired at an average rate of 1.37 spikes/sec, and non-dopamine neurons at 5.13 spikes/sec. Distributions of baseline firing rates show a non-Gaussian pattern for both types. To verify the identity of dopamine neurons selected on the basis of waveform patterns (Jo, Lee, & Mizumori, 2013), some animals (n = 9) were injected with apomorphine (0.75mg/kg, s.c.) or saline immediately after PCA testing sessions were completed (n = 11). While recording from the same neurons as during the PCA session, animals were tested with 25 additional PCA trials. Apomorphine, which is a dopamine receptor agonist, produces a reduction of dopaminergic neuron firing rates (Aebischer & Schultz, 1984). A total of 59 neurons, 32 dopamine and 27 non-dopamine were analyzed for pharmacological effects of apomorphine injection (Figure 2.3). Results from these sessions show a decrease of dopamine neuron firing, consistent with others' reports (Jo et al., 2013; Pan et al., 2008). Of the dopamine neurons, 77% showed a reduction (>40%) in firing rate following apomorphine injection and were confirmed to be dopamine. These neurons showed significant differences to non-dopamine neurons for average firing rate ($t_{(57)}$=3.93,

$p<0.01$) and average length ($t_{(57)}=9.89$, $p<0.01$). These characteristics were used differentiate the remaining dopamine and non-dopamine neurons recorded.

Neural Discrimination and Analysis:

Recorded neurons were discriminated using Offline Sorter (Plexon Inc., Dallas, TX). Neural reactivity was analyzed using a laboratory-prepared custom database application (The Form, University of Michigan) and NeuroExplorer (Plexon, Dallas, TX). Cross correlations were run to ensure no redundancies in the discriminated neural waveforms. The mean rate of firing for each unit was calculated during 1) CS onset (100-400ms after lever presentation), 2) cue interaction (1-8sec after lever presentation), 3) CS offset/pellet delivery (100-400ms after lever retraction/feeder click), and 4) UCS, reward delivery/consumption (600-1600ms following CS offset) for each trial of the PCA session (Figure 2.4). The same was done for a background interval, a 5 sec period of time without any attention-directed behavior to determine baseline firing rate. The background period was used to determine population mean and standard deviation for each neuron at baseline levels. Those trials where the mean baseline was zero were deleted from the analysis. Only those neurons with at least 10 good trials (baseline rate > 0) were included in the analysis. For these neurons a Kruskal-Wallis was performed, comparing the mean firing rates during the periods specified on a trial basis to determine significant rate changes (increase or decrease) during the testing session. A Bonferroni-corrected pairwise Mann U post-hoc was performed on significant units to determine those time intervals that were significantly different ($\alpha < 0.05/15$). All neurons underwent visual inspection to corroborate statistical findings. Further, as baseline firing rates differ between cells, a Z-score was calculated as a way to normalize firing rate changes (magnitude) of individual neurons in order to perform population analyses of responsive

neurons. Excitatory and inhibitory differences were compared between neural types (dopamine and non-dopamine) of STs and GTs for each time interval using a Friedman's test and Dunn's corrected post-hoc pairwise comparisons ($\alpha < 0.05/2$). The absolute magnitude was also analyzed in the same manner.

The number of neurons recorded from each rat varied between individuals, ranging from 4 to 83 (Table 2.1). To address the possibility that recordings from one subject may be driving results, a Pearson's correlation analysis was performed on phenotypic index scores (related to the extent to which individuals express a conditioned response, towards -1 for goal-trackers or +1 for sign-trackers) and the average response magnitude of cells from that individual rat. The magnitude of all units was averaged for each subject and time periods tested, and then plotted as a function of index score. In this manner, each subject is only allotted one data point and presents the contribution that neurons from each subject provide in responses to the Pavlovian task. The results further allow us to determine how motivational output relates to firing rates of neurons.

Population analyses were performed using chi-square. We compared proportions of neurons responding to the specified events (CS onset, CS offset, etc.) between ST dopamine and GT dopamine, and ST non-dopamine and GT non-dopamine neurons. We also looked at proportions of excitatory and inhibitory responses in these groups.

Lesion and End Point:

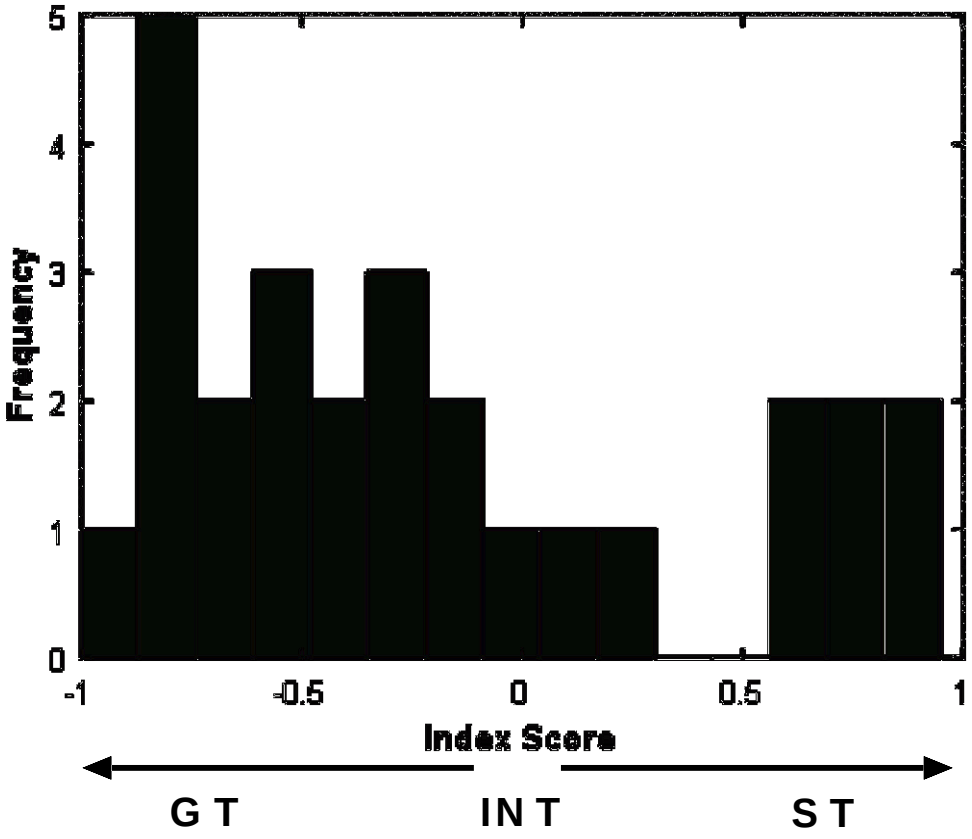
Twenty-four hours after the final testing day, subjects were lesioned to confirm placement of electrode bundles. Typically, animals were awake during the procedure. A few animals were placed in the induction box and anesthetized with isoflurane initially at 3% and then maintained via nosepiece at 2%. To make a lesion, a wire was connected to the back of the electrode and a small current (0.5mA) was passed for 30 sec through a

selected wire in each bundle. This causes a micro-injury at the tip of the electrode bundle that can be visualized through histology. Subjects were euthanized 48 hrs later using an overdose of sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals). Brains were removed and frozen fresh using dry ice. They were stored at -20^{o}C.

Histology:

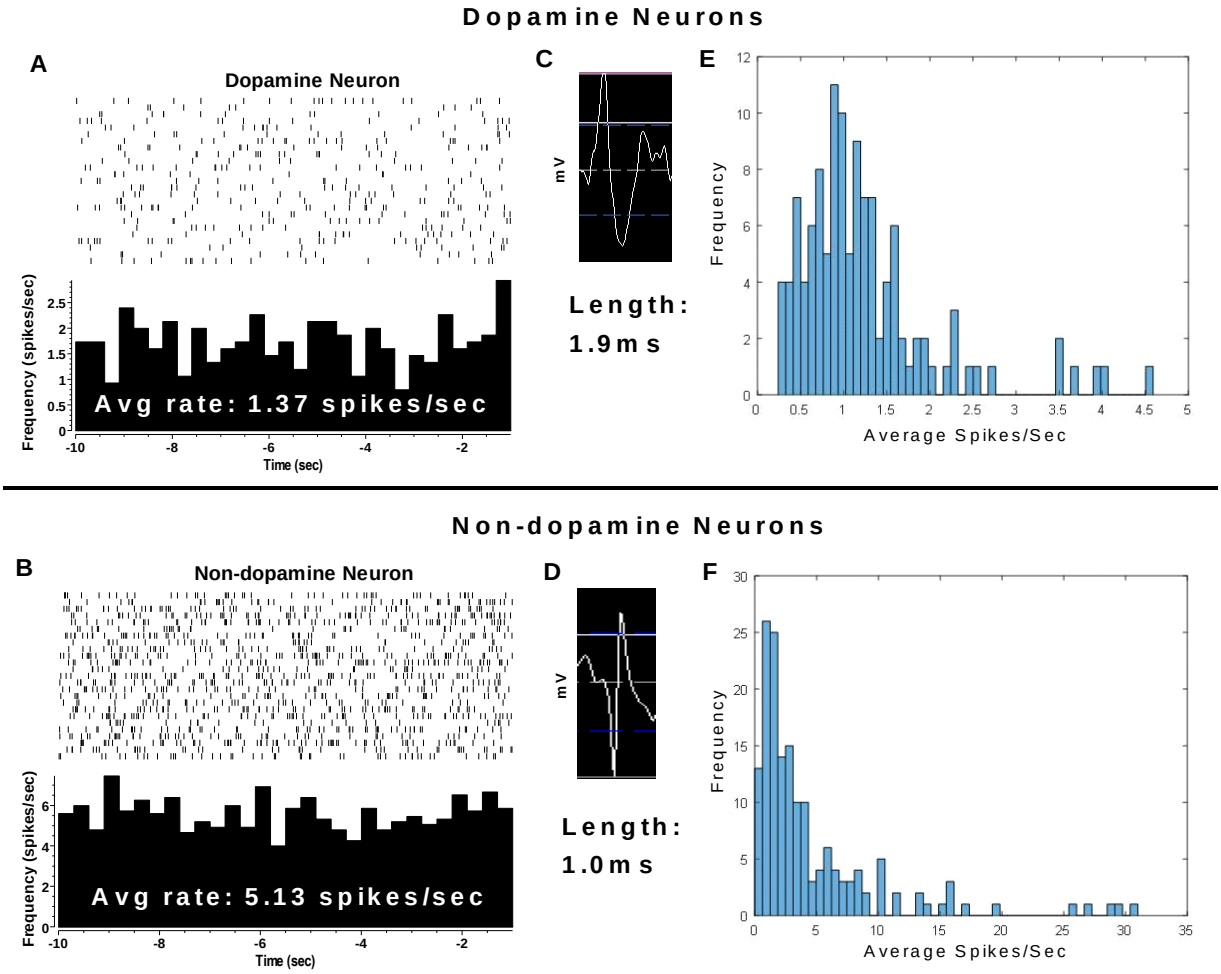
Brains were sliced coronally and stained with Cresyl Violet. Electrode bundle placement was confirmed for each testing day using the Paxinos and Watson brain atlas (1997). This allowed for the accurate assessment of the location from which neurons were recorded (Figure 2.5). Electrophysiological recordings were only used on days in which bundles were within the VTA.

Figure 2.1: Distribution of Index Scores



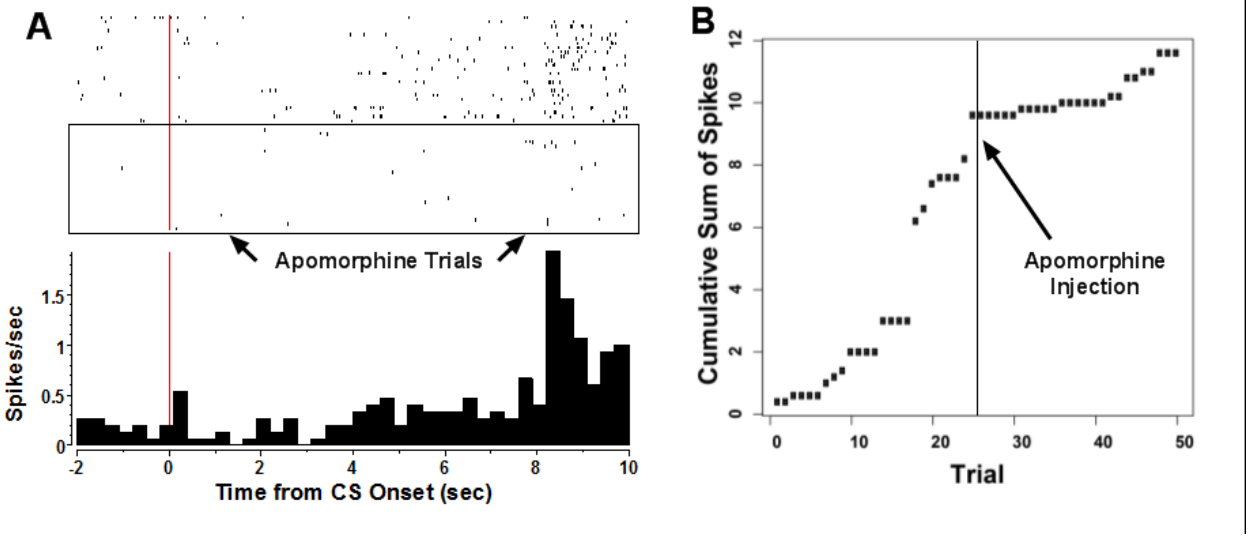
Scores were calculated from video recordings and predict probability of approaching magazine (goal-tracker, GT, -0.5 to -1.0) or lever (sign-tracker, ST, +0.5 to +1.0), or both (intermediates, INT, -0.5 to +0.5).

Figure 2.2: Dopamine and Non-Dopamine Neurons Have Different Characteristics



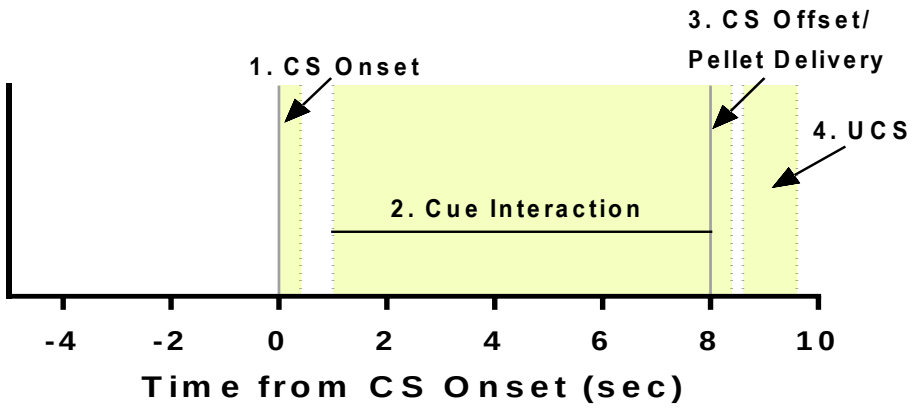
Examples of baseline firing rates (prior to cue presentation) for a Dopamine (A) and non-dopamine (B) neuron is shown. Typical spikes are presented for each neuron with dopamine (C) neurons producing spikes that are wider than those from non-dopamine (D) neurons. Frequency distributions of dopamine (E) and non-dopamine (F) neurons provide range of baseline firing rates seen in this study.

Figure 2.3: Dopamine Cell Firing Changes in Response to Apomorphine



A) Raster and histogram shows firing rate of dopamine cell during Pavlovian conditioning. Box outlines trials following injection of apomorphine (0.75mg/kg, i.p.). B) Total spike accumulation before and after apomorphine injection.

Figure 2.4: Time Periods Analyzed During Pavlovian Conditioning



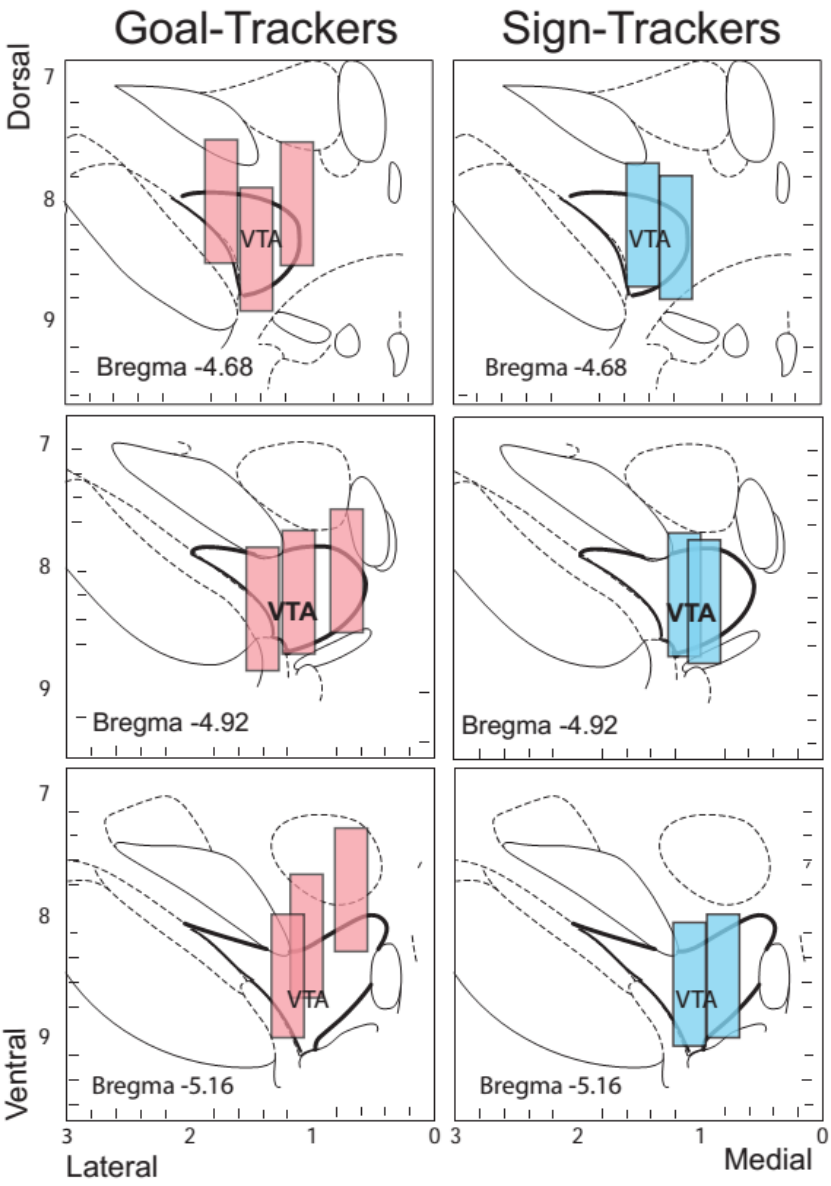
Trials were broken into a (1) 300ms CS Onset, (2) 7s Cue interaction, (3) 300ms CS Offset, and (4) 1s UCS period for analysis.

Table 2.1: Number of Neurons Recorded per Subject

Goal-Trackers:			
Subject	**No. Dopamine**	**No. Non-Dopamine**	**Total**
33	7	10	17
48	7	21	28
50	2	2	4
51	3	11	14
53	6	7	13
61	13	11	24
74	8	11	19
Total	46	73	119
Sign-Trackers:			
Subject	**No. Dopamine**	**No. Non-Dopamine**	**Total**
36	13	10	23
63	14	2	16
72	29	54	83
75	3	2	5
Total	59	68	127

Dopamine and non-dopamine neurons were analyzed for spike shape, baseline firing rate, and effects of apomorphine in each sign-trackers and goal-trackers. Individuals varied in the number of cells observed.

Figure 2.5: Electrode Locations



Placement of electrodes for goal-trackers (red) and sign-trackers (blue) were confirmed with Cresyl Violet staining of coronal brain slices. Electrodes were lowered 40-80µm each day. Bars represent total advancement of neurons.

RESULTS

Exposure to a Pavlovian food-paired CS (the lever) elicited responses in both dopaminergic and non-dopaminergic VTA neurons. These responses were observed at four distinct time points during cue exposure and reward consumption. At three of these time points (CS onset, CS offset, and UCS), neural responses were considered to be phasic events as they typically lasted from 200ms to 1s. Responses to the cue interaction period were considered tonic events, as these were sustained increases or decreases in firing that lasted several seconds, and corresponded to the interval when rats typically engaged in sign- or goal-tracking behavior. A high percentage of VTA neurons responded to one or more of these events (71% overall). There were no differences between STs and GTs in proportion of neurons that responded to at least one event. This was true for dopaminergic cells (STs 76%, GTs 77%; χ^2=0.01, ns) and non-dopaminergic cells (STs 73%, GTs 60%; χ^2=2.40, ns). There were no differences between STs and GTs in whether neurons fired to a single stimulus or were more integrative, responding to multiple events. Dopamine neurons were less responsive to a single stimulus (42%) as to multiple (58%) (χ^2=7.91, $p<0.01$). Non-dopamine neurons were slightly more responsive to multiple stimuli (56%) compared to a single stimulus (44%), but this difference was not significant (χ^2=1.53, ns). Notably, response differences between STs and GTs indicate that not a single dopamine neuron from GTs respond to anything related to cue interaction. This suggests that GTs do not code the incentive value of the lever as STs do.

Dopamine Neurons:

Mean firing rates for all neurons were calculated during a background time period (baseline) and for specific events of the Pavlovian task: CS Onset, CS Offset, Cue Interaction, and Pellet. Baseline firing rates of dopamine neurons were similar between STs and GTs, ranging from 0.37-4.58 spikes/sec for STs and 0.3-2.29 spikes/sec for GTs.

STs and GTs also showed similar firing rates to CS onset (Bonferroni-corrected Mann U test, t=0.62, ns). STs, however, showed significantly higher firing rates to CS offset (t=2.72, p<0.01), Cue Interaction (t=3.04, p<0.01), and UCS (t=2.46, p<0.01) compared to dopamine neurons of GTs (Figure 2.6).

Population activity was also examined by calculating the average magnitude of phasic and tonic firing changes (absolute value of z-scores), regardless of whether cells were categorized as responsive or non-responsive. There were no significant group differences in responses to CS onset (p = 0.27); however, the other three events (the interaction period, CS offset, and the UCS) elicited greater firing changes in STs than GTs (post hoc tests, *p*s < 0.01) (Figure 2.7). In fact, the z-score results show a ramping up of firing in dopamine neurons in STs, beginning at cue onset and peaking at UCS delivery, a pattern not seen in GTs.

Phasic Responses:

Among dopamine cells in the VTA, STs showed stronger cue responses than GTs at time points hypothesized to encode incentive motivation rather than predictive value. The percentage of cells responsive to the three phasic events, CS Onset, CS Offset, and the UCS, did not differ between STs and GTs. At these time points, there were no significant differences in excitatory responses (χ^2s=0.14-1.96, ns) or inhibitory responses (χ^2s=0.02-1.96, ns) (Figure 2.8). STs did show significantly more neurons responsive to both CS onset and CS offset compared to GTs (χ^2=5.33, p<0.05) (Figure 2.8D).

Magnitude of responses to CS onset also did not differ between STs and GTs (Friedman's test, p=0.27) (Figure 2.9). The majority of cells responding to CS onset were excitatory in nature for both STs and GTs. The excitatory (Dunn's multiple comparisons, p=1.0) and inhibitory (p=1.0) magnitude changes were similar in both STs and GTs, and

found no correlation between normalized rates compared to PCA Index (R^2=0.08, ns). These results indicate that STs and GTs are coding the predictive properties of lever presentation equally.

Dopamine neurons from STs showed a significantly higher magnitude to CS Offset compared to GTs (Friedman's test, $p<0.01$) (Figure 2.10). Both showed a gradual increase in firing rate during lever retraction that peaks at pellet delivery (UCS). In STs the response to pellet delivery is sustained and is significantly higher than response magnitude of GTs ($p<0.01$). Few cells from STs (16%) and GTs (6%) responded to both CS offset and UCS indicated that dopamine neurons are coding these events separately. The magnitude change to CS offset is also not related to PCA index (R^2=0.19, ns). However, there was a correlation to UCS (R^2=0.70, $p<0.01$). This indicates that those subjects who have the propensity to place incentive value on cues also have greater firing rate changes to reward. Of the neurons responding to UCS, 90% of them were excitatory in both STs and GTs (Figure 2.11). On the other hand, 2/3 of the neurons responding to CS offset were excitatory in STs, but inhibitory in GTs. These results indicate that STs and GTs differ in the way they respond to incentive cues.

In the current Pavlovian conditioning paradigm, we made comparisons between the initial lever presentation (CS Onset, predictive cue), and lever retraction/pellet delivery (CS Offset, incentive cue). Magnitude of response was significantly higher to CS offset than CS Onset only in sign-trackers ($p<0.01$) not goal-trackers ($p=0.44$) (Figure 2.12). These results provide evidence that dopamine cells from sign-trackers are encoding incentive value of cues.

Tonic Responses:

I also looked at tonic responses of neurons over the cue interaction interval, i.e. the time period spent engaged in a conditioned response, either directed at the lever or food cup (Figure 2.13). Only dopamine cells from sign-trackers, not goal-trackers, show a significant response to cue interaction (Friedman's, $p<0.001$). In fact, none of the responsive dopamine cells from GTs were responsive to lever interaction. Most of the ST responses (77%) were excitatory in nature. The proportion of tonic responses during the lever interaction period was significantly higher in STs than GTs for excitatory ($\chi^2=20$, $p<0.05$) but not inhibitory responses ($\chi^2=3$, ns) (Figure 2.13B).

The relationship between phenotypic index and magnitude of firing was assessed to cue interaction and found a significant positive correlation ($R^2=0.80$, $p<0.05$). This indicates that the more a subject is engaged with the cue, the greater the change in firing of dopamine neurons. These results together strongly suggest that even though both sign-trackers and goal-trackers are actively engaged in their respective conditioned response, only sign-trackers cells are coding for it.

Non-dopamine Neurons:

The baseline rate of non-dopamine neurons ranged from 0.57-30.87 spikes/sec and was significantly less in STs than GTs (average = 3.95 vs. 6.56, $p<0.05$). Non-dopamine neurons in STs also showed significantly lower firing rates than GTs during CS onset and UCS (Bonferroni-corrected t-tests, $p<0.01$), but not to CS offset and cue interaction (Figure 2.14).

In contrast to dopamine neurons, non-dopamine neurons did not encode group differences in the incentive salience attributed to the lever, as STs did not show stronger neural responses than GTs as any four time points (CS onset, the interaction period, CS offset, and the UCS). The average magnitude of responsive neurons was plotted around

the time of lever presentation (CS Onset) for STs and GTs (Figure 2.15) and analyzed for responses to the time intervals of interest. These magnitudes were presented as an absolute value so that inhibitions and excitations are represented in the same direction. ST and GT neurons showed similar patterns of phasic and tonic responses to the Pavlovian task.

Phasic Responses:

The percentage of cells responsive to the three phasic events, CS Onset, CS offset, and UCS only differed between STs and GTs to UCS. At the time points for CS Onset and Offset, there were no significant differences in excitatory responses (χ^2s=0.30-2.37, ns) or inhibitory responses (χ^2s=0.01-2.49, ns) (Figure 2.16). To UCS there were no differences in excitatory responses (χ^2=0.52, ns), but there were significantly more inhibitory responses in STs compared to GTs (χ^2=8.28, $p<0.01$). The percentage of neurons responding to CS onset, CS offset, or both were similar in STs and GTs (χ^2s=1-3.27, ns) (Figure 2.16D).

GT neurons showed greater magnitude responses to CS Onset than STs (Dunn's multiple comparison, $p<0.05$) (Figure 2.17). For GTs, 2/3 of the responses were excitatory, whereas it was only 1/2 for ST neurons. These differences could contribute to the significant differences in magnitude seen between STs and GTs to CS Onset. There were no statistical significance between STs and GTs for excitatory (Dunn's comparisons, p=0.82) responses nor for inhibitory (p=0.82) responses. There was also no correlation between magnitude of response and propensity to approach lever (PCA index; R^2=0, ns).

Despite the strong response peak in GTs to lever retraction/pellet delivery (CS Offset), there was no difference in magnitude between STs and GTs (Dunn's multiple

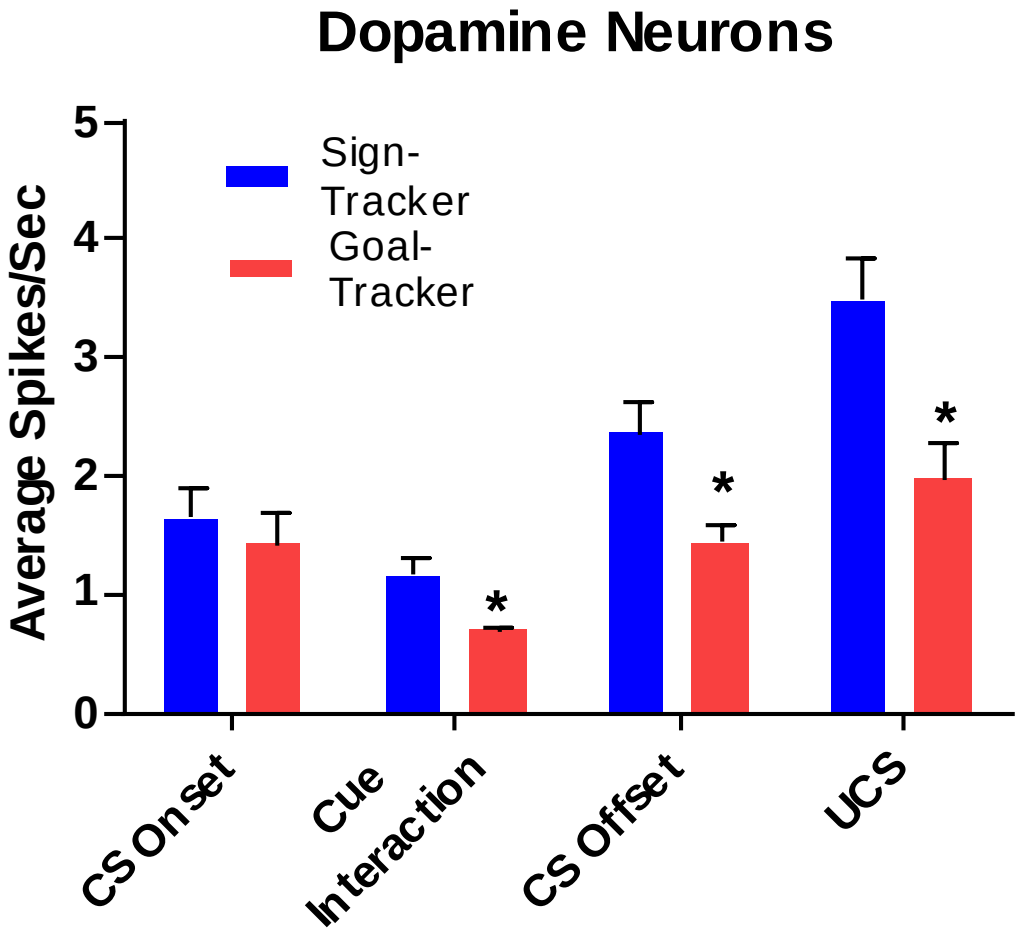
comparison test, p=0.08) (Figure 2.18). Response magnitude to pellet delivery (UCS) was also similar between STs and GTs (p=0.06). Again, there was no correlation with PCA index scores and either CS Offset (R^2=0.001, ns) or UCS (R^2=0.15, ns).

Magnitudes to CS Offset were similar between STs and GTs for excitatory (p=1.0) and inhibitory (p=1.0) responses. Magnitude to UCS was also similar between STs vs. GTs to both excitatory (p=0.14) and inhibitory (p=0.49) (Figure 2.19). Inhibitions represented roughly 2/3 of the responses seen in STs and GTs to CS Offset. To UCS, STs show an even number of excitatory and inhibitory responses, while they were mostly excitatory in GTs.

Tonic Responses:

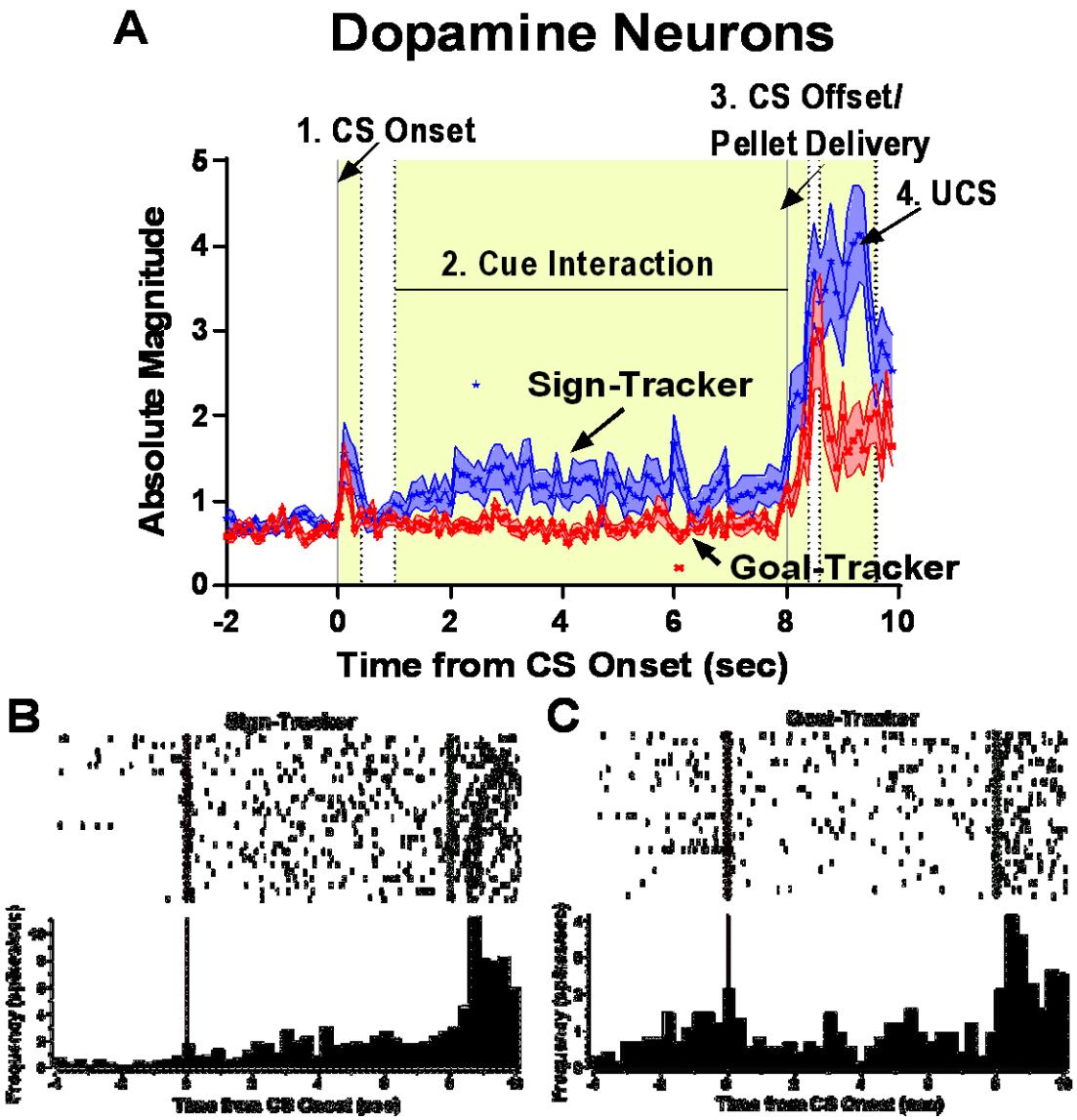
Neurons were analyzed to the cue interaction, a time period during which a subject is either engaged in the lever (sign-trackers) or magazine (goal-trackers). Magnitude of ST neurons was slightly higher than GTs (Dunn's multiple comparisons, p<0.01) (Figure 2.20). These responses were equally excitatory and inhibitory for both STs and GTs and the percentage of each did not differ (χ^2s=1.24-1.54, ns). Further, we found no correlation of magnitude with PCA index (R^2=0.15, ns). Surprisingly, these results indicate that non-dopamine neurons do not encode differences in incentive motivation, which stands in contrast to the findings of dopamine neurons.

Figure 2.6: Firing Rates to Specified Events in a Pavlovian Task



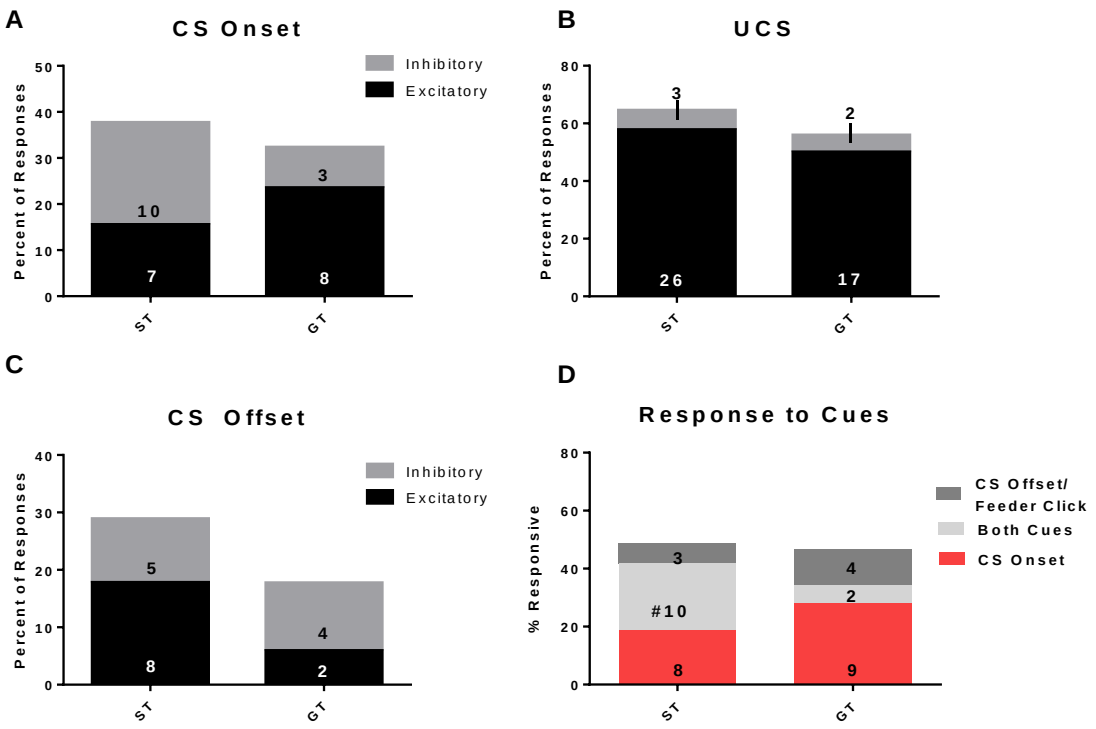
Firing rates of dopamine neurons were averaged across all neurons regardless if they were responsive to a given event or not. A Bonferroni-corrected Mann U test was performed to test differences in firing rates between STs and GTs (*$p<0.01$).

Figure 2.7: Normalized Response Magnitude to Pavlovian Cue Presentation



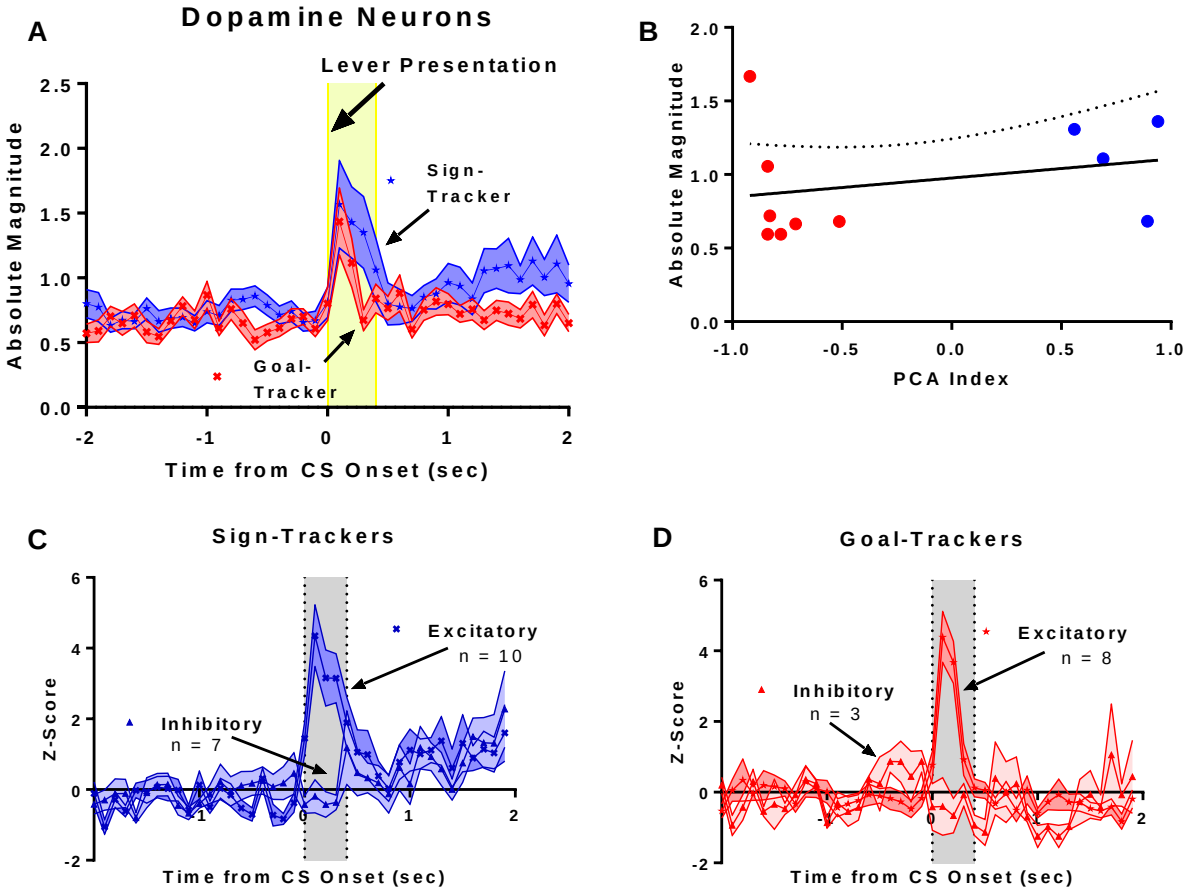
(A) Responsive cells were normalized to a background interval using a Z-score and averaged across the time period just before and after lever presentation. Absolute Z-score are presented to allow equal contributions of inhibitory and excitatory responses. Example of a dopamine neuron from a (B) sign-tracker and (C) goal-tracker. Red square – lever presentation, green symbol – lever retraction/pellet delivery

Figure 2.8: Inhibitory and Excitatory Responses in Dopamine Neurons During Pavlovian Conditioning



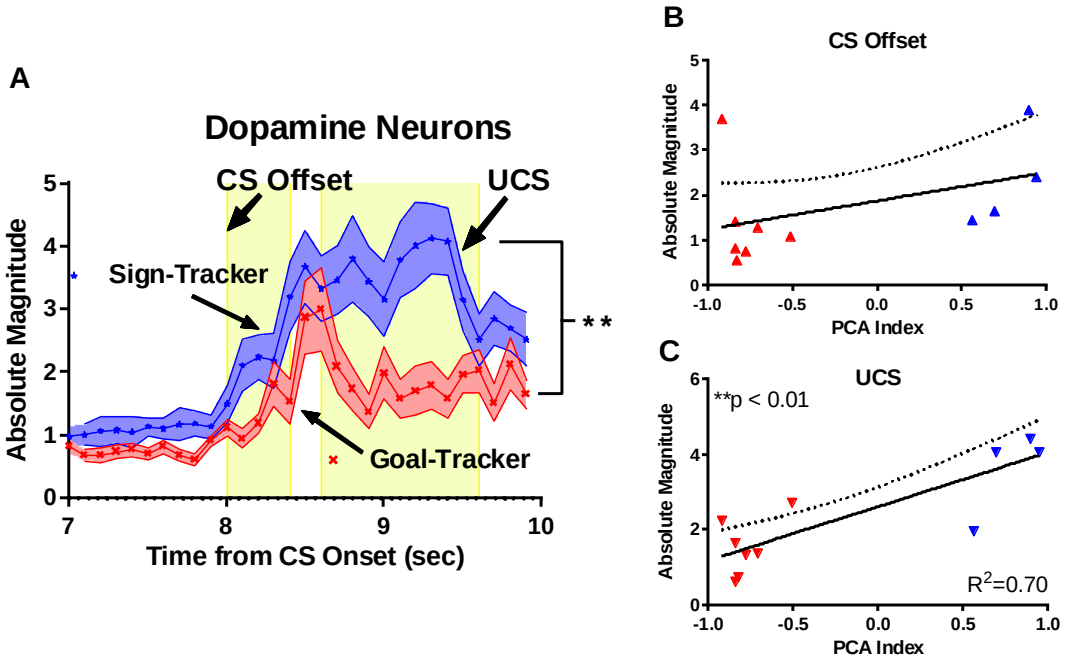
Mean firing rates of each neuron were calculated for events related to the Pavlovian task and analyzed for significant changes from baseline rates. Only responsive neurons to A) CS Onset, B) CS Offset, and C) UCS were included. Proportions of excitatory (significant increase from baseline) and inhibitory (significant decreases from baseline) were compared between ST and GT populations. D) We also analyzed the proportion of neurons responding to CS Onset alone, CS offset alone, and both. #$p<0.05$ ST vs. GT

Figure 2.9: Magnitude Differences of Dopamine Neurons to Cue Onset



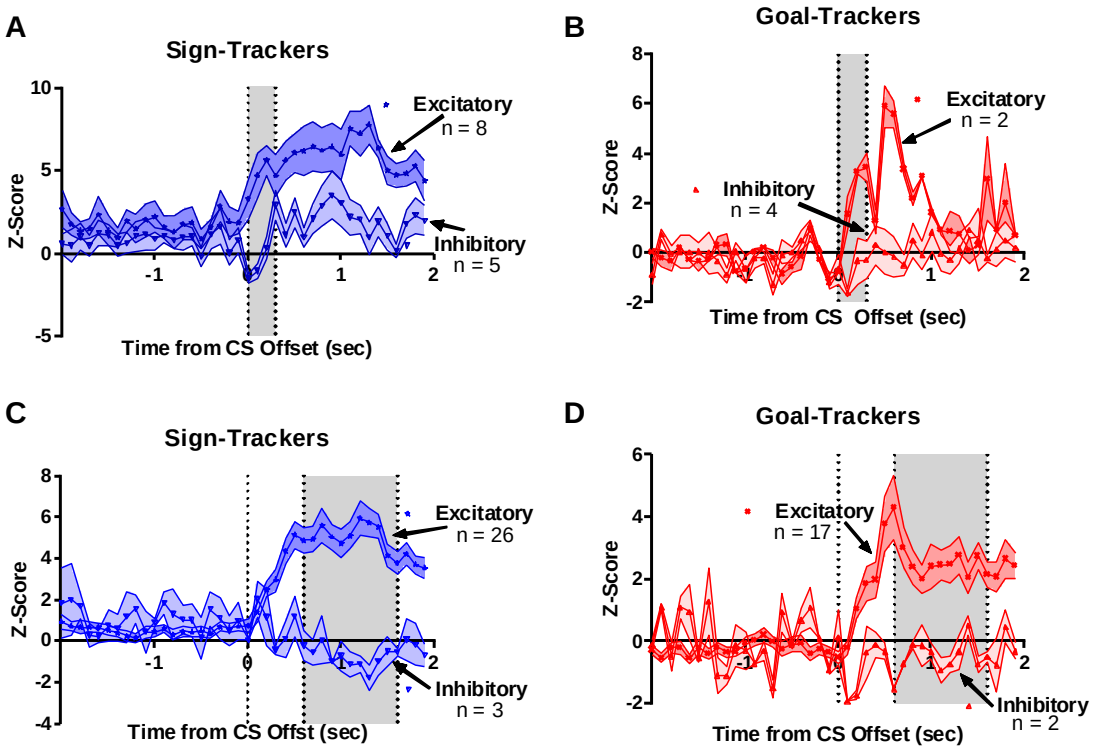
Firing rates of responsive neurons were normalized for sign-trackers (blue) and goal-trackers (red) to determine magnitude of change to cue onset (lever presentation, shaded region). The absolute change in firing rate is represented in (A) for all responsive neurons. The magnitude of responsive cells was plotted against the response bias for each subject (B). The excitatory and inhibitory of (C) sign-trackers and (D) goal-trackers show only those units responsive to CS Onset.

Figure 2.10: Magnitude of Dopamine Neurons to Cue Offset and Reward Delivery



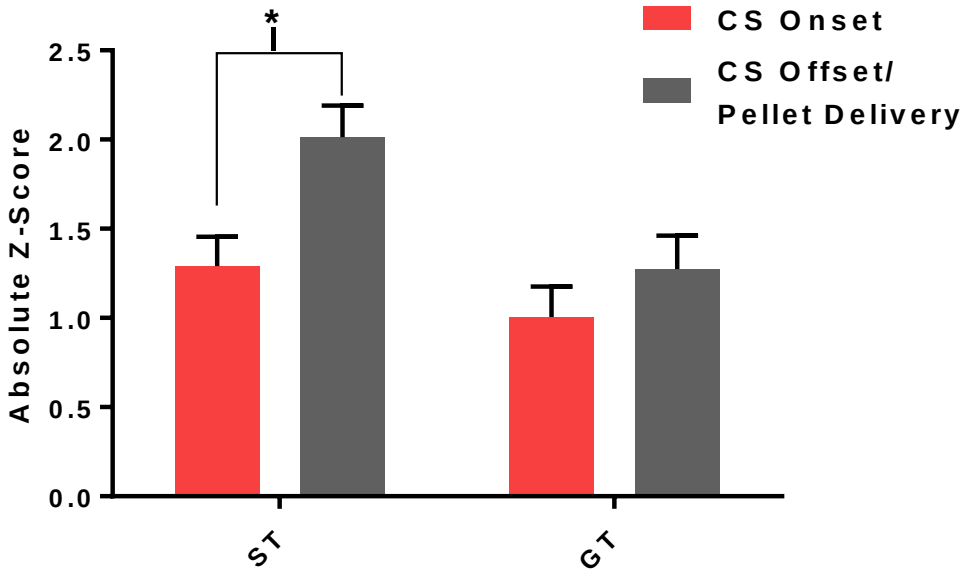
(LEFT) The magnitude of all responsive dopamine neurons were averaged during the time period of cue offset (lever retraction and feeder click) and delivery of reward (UCS) for sign-trackers (blue) and goal-trackers (red). Neural firing magnitudes were greater in STs compared to GTs. For each subject, the response magnitude was averaged across all responsive neurons to CS offset (Top Right) and UCS (Bottom right) and compared to their phenotypic index. There was a significant correlation to UCS. **p<0.01

Figure 2.11: Excitatory and Inhibitory Responses to Cue Offset and Pellet Delivery



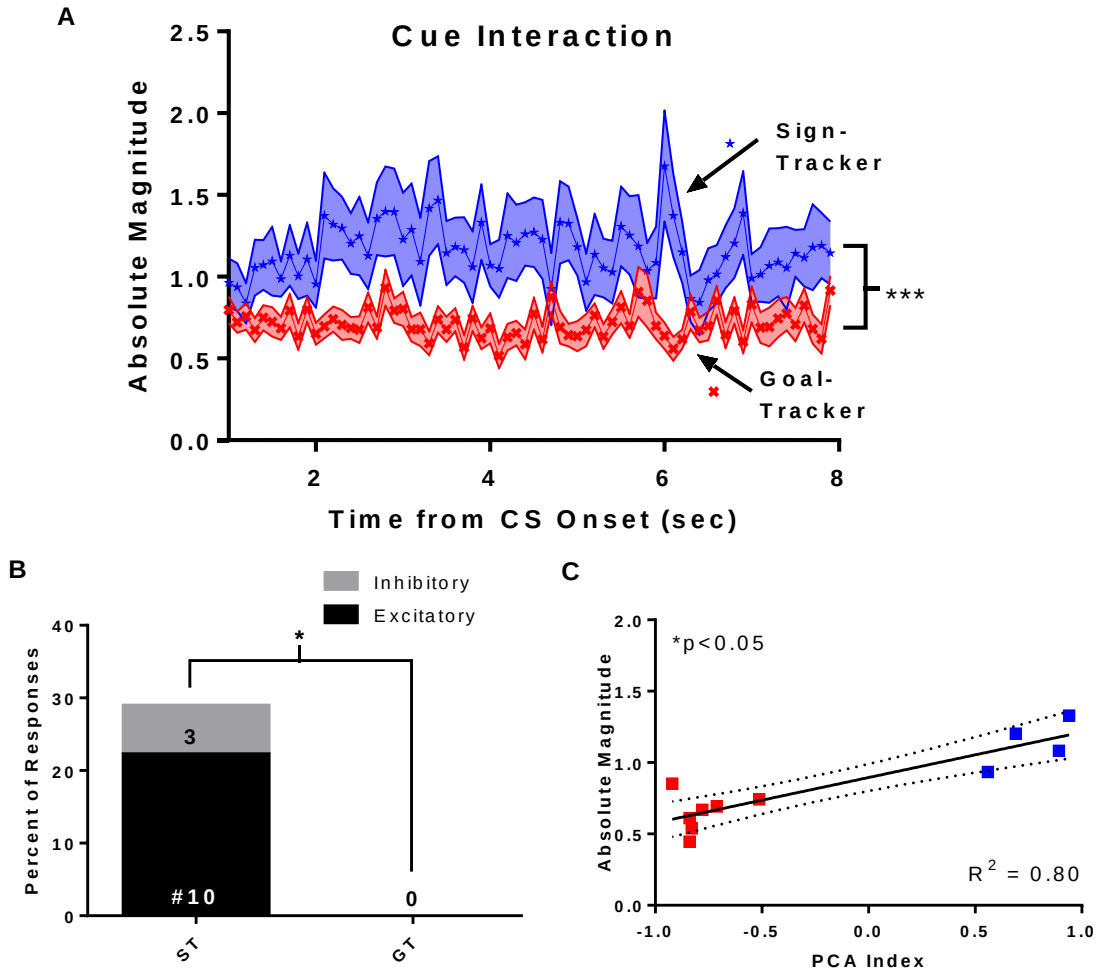
Neurons from sign-trackers (blue) and goal-trackers (red) were normalized (Z-Score) and aligned to lever retraction (CS Offset). The excitatory (A,B) and inhibitory (C, D) responses are plotted for those neurons responsive to lever retraction/feeder click (CS Offset, top) and pellet delivery, (UCS, bottom). Dotted line represents moment of lever retraction. Shaded regions represent UCS delivery and receipt.

Figure 2.12: Magnitude Changes Between CS Onset and CS Offset



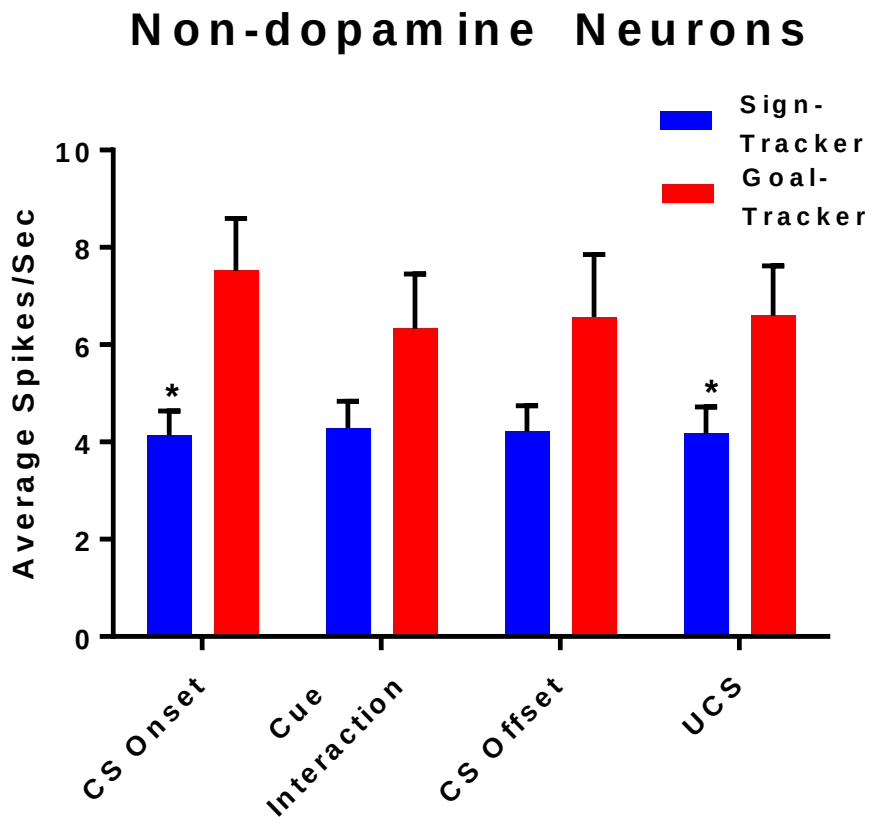
The magnitude of firing to CS Onset ("predictive" cue) and CS offset ("incentive" cue) were compared between sign-trackers (ST) and goal-trackers (GT). *$p<0.05$

Figure 2.13: Magnitude Response of Dopamine Neurons to Cue Interaction



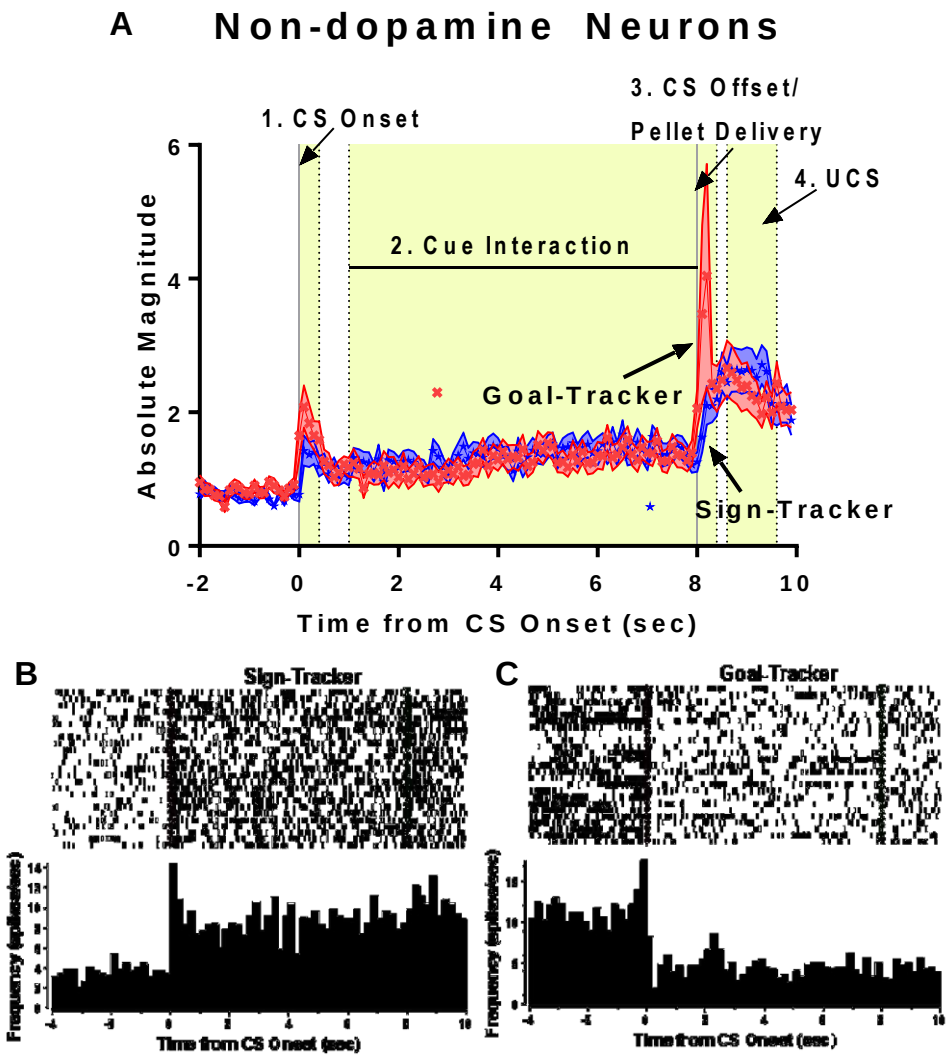
Neurons were analyzed for their response to cue interaction, the last 7 sec of lever presentation during which subjects are engaged in a conditioned response. Responses are either directed toward location of pellet receipt (goal-trackers, red) or towards lever (sign-trackers, blue). A) Magnitude of response (Z-score) was calculated during this time and presented as absolute value to account for both excitatory and inhibitory responses.(***p<0.001, STs compared to GTs). B) Most of the responses from sign-trackers were excitatory. None of the dopamine neurons from goal-trackers responded during this event. C) Correlation of magnitude with PCA index, i.e. the propensity to interact with either food cup (towards -1.0) or lever (towards +1.0). *p<0.05

Figure 2.14: Firing Rates to Pavlovian Events



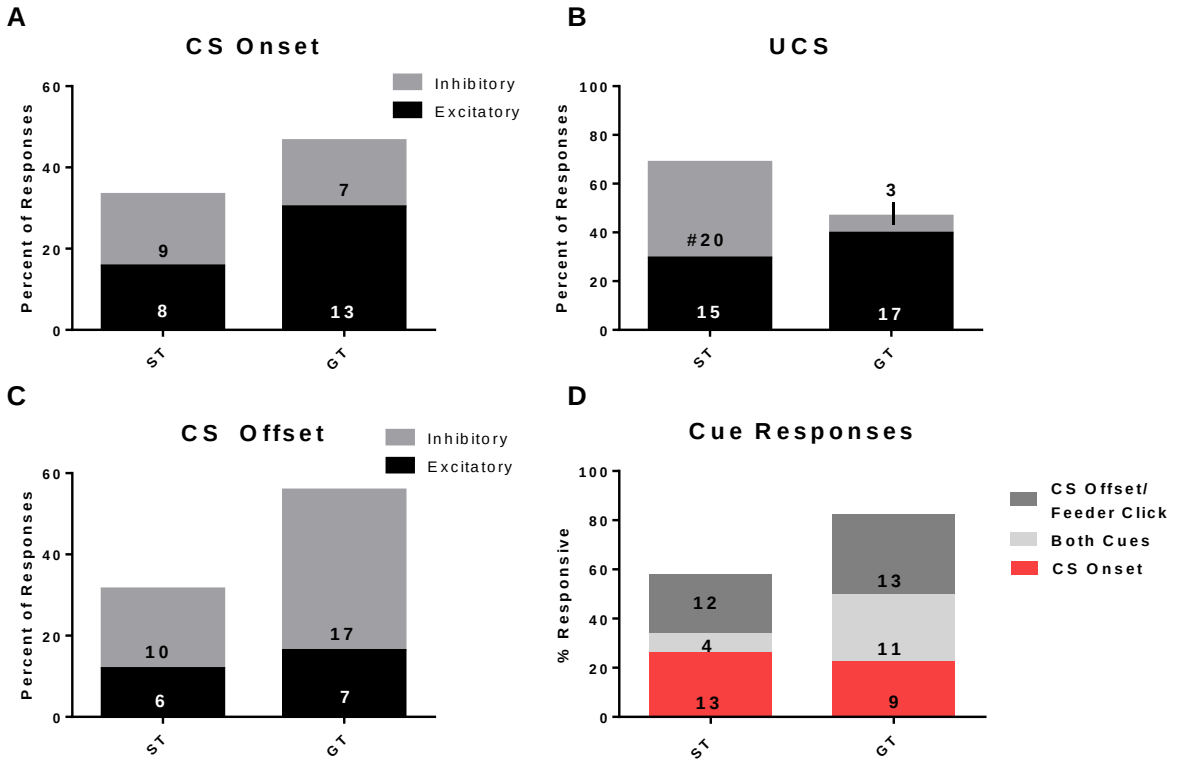
Firing rates of all non-dopamine neurons from sign-trackers (blue) and goal-trackers (red) were calculated during the time periods for CS onset (1-400ms after lever presentation), cue interaction (1-8sec after lever presentation), CS offset (1-400ms following lever retraction/pellet delivery), and UCS (600-1600msec following release of pellet). Rates of ST compared to GT, *$p<0.05$

Figure 2.15: Patterns of Non-Dopamine Neuron Firing



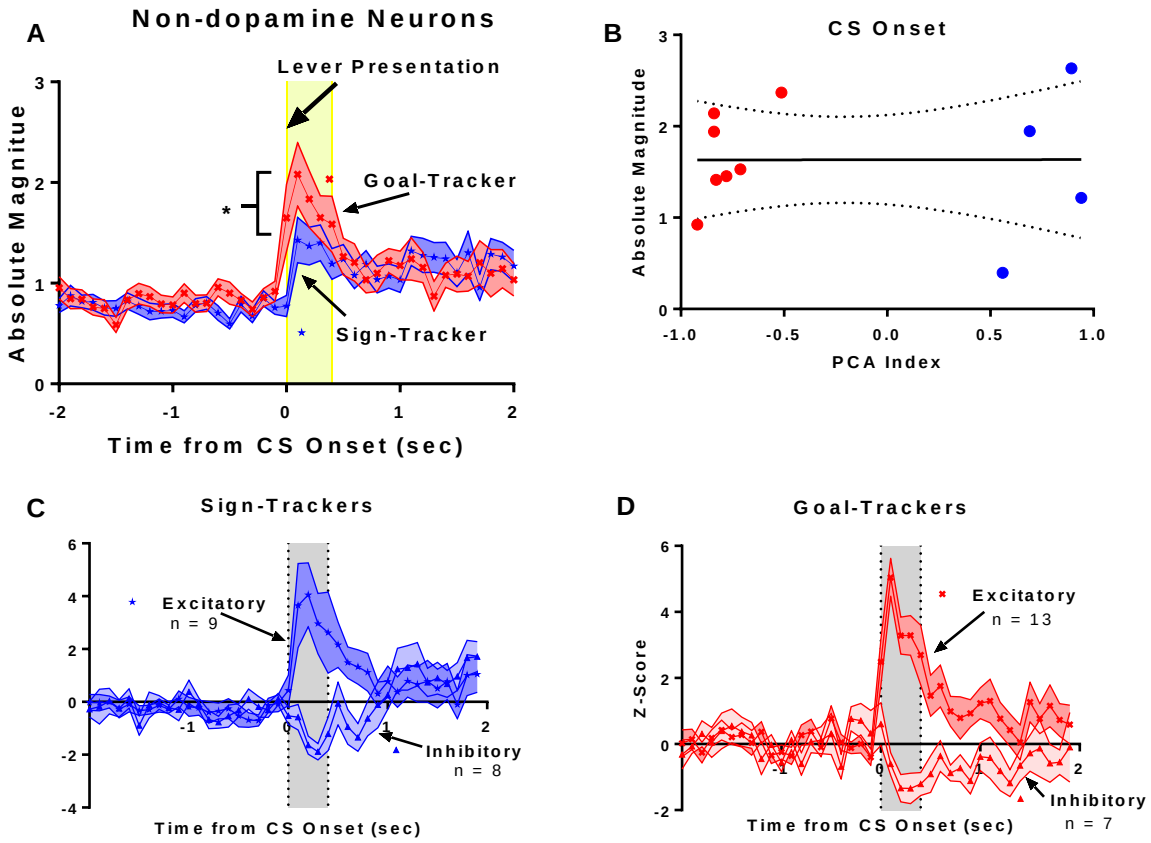
A) Magnitude of response was calculated (Z-score) for responsive non-dopamine neurons of sign-trackers (blue) and goal-trackers (red) during a Pavlovian task. A lever is presented (CS onset) for 8sec, after which it is retracted (CS Offset) and a food reward (UCS) is delivered. B) Example of non-dopamine neurons from a sign-tracker. C) Example of non-dopamine neuron from goal-tracker. Data presented as average +/- SEM. Red square – lever presentation, green symbol – lever retraction/pellet delivery

Figure 2.16: Inhibitory and Excitatory Responses During Pavlovian Conditioning



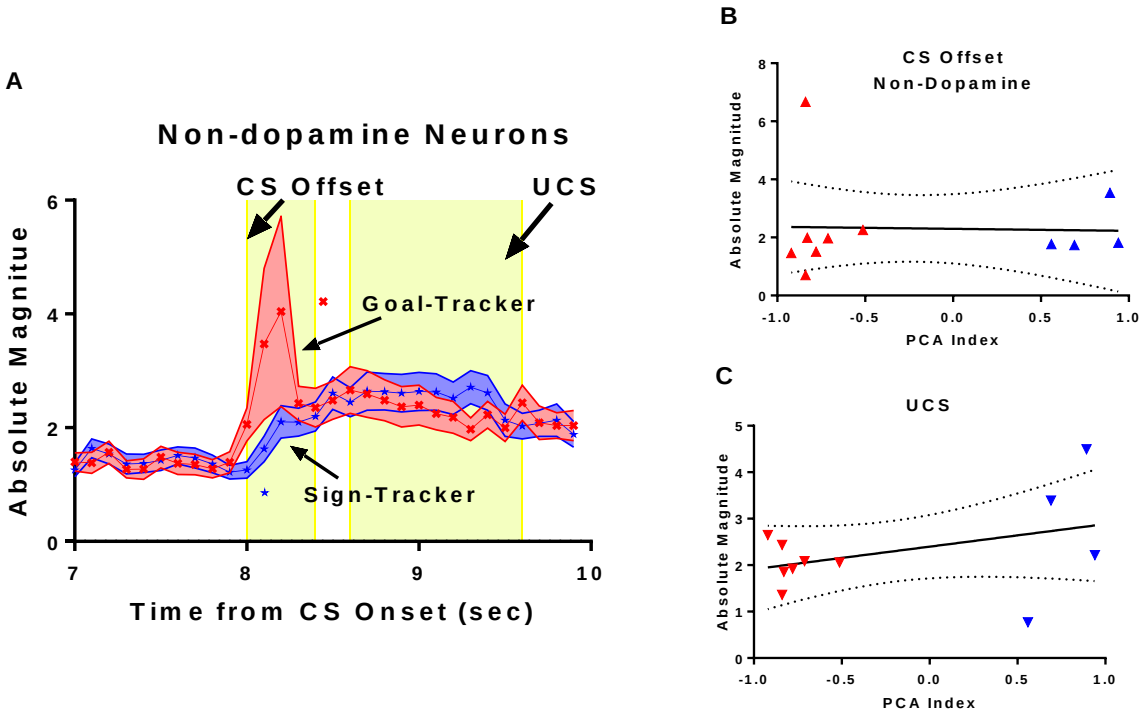
Mean firing rates of non-dopamine neurons were calculated for events related to the Pavlovian task, and analyzed for significant changes from baseline rates. Proportions of excitatory (significant increase from baseline) and inhibitory (significant decreases from baseline) were compared within ST and GT populations (not significant) as well as between ST and GTs (#$p<0.01$).

Figure 2.17: Coding Properties to CS Onset (Lever Presentation)



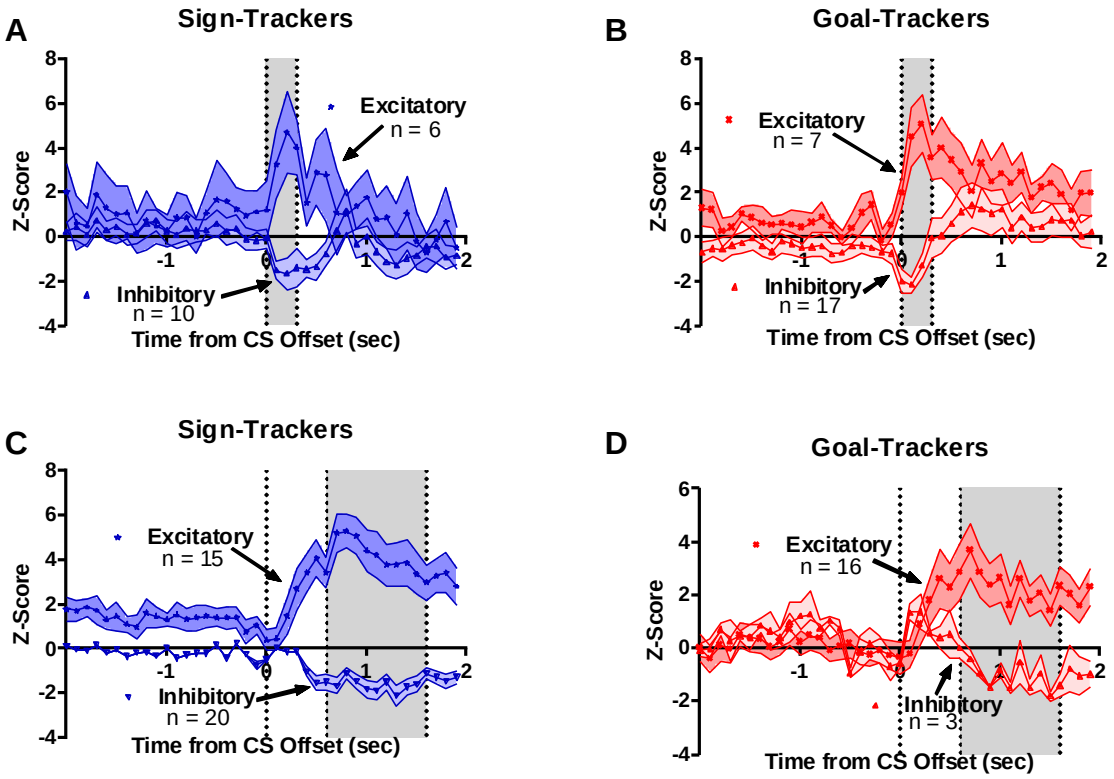
Only responsive neurons are presented. A) Magnitude of individual neuron firing was calculated by taking the absolute value o the Z-score and averaged for animal phenotype. B) Magnitude of firing was compared to PCA Index. An index of -1 to -0.5 indicates GTs, while an index of +0.5 to +1 indicates STs. Neurons were analyzed for inhibitory (firing less than background rate) and excitatory (firing greater than background) C) in sign-trackers and D) goal-trackers. Data presented as average +/- SEM, *p<0.05 ST vs. GT

Figure 2.18: Coding Properties to CS Offset and Reward Delivery



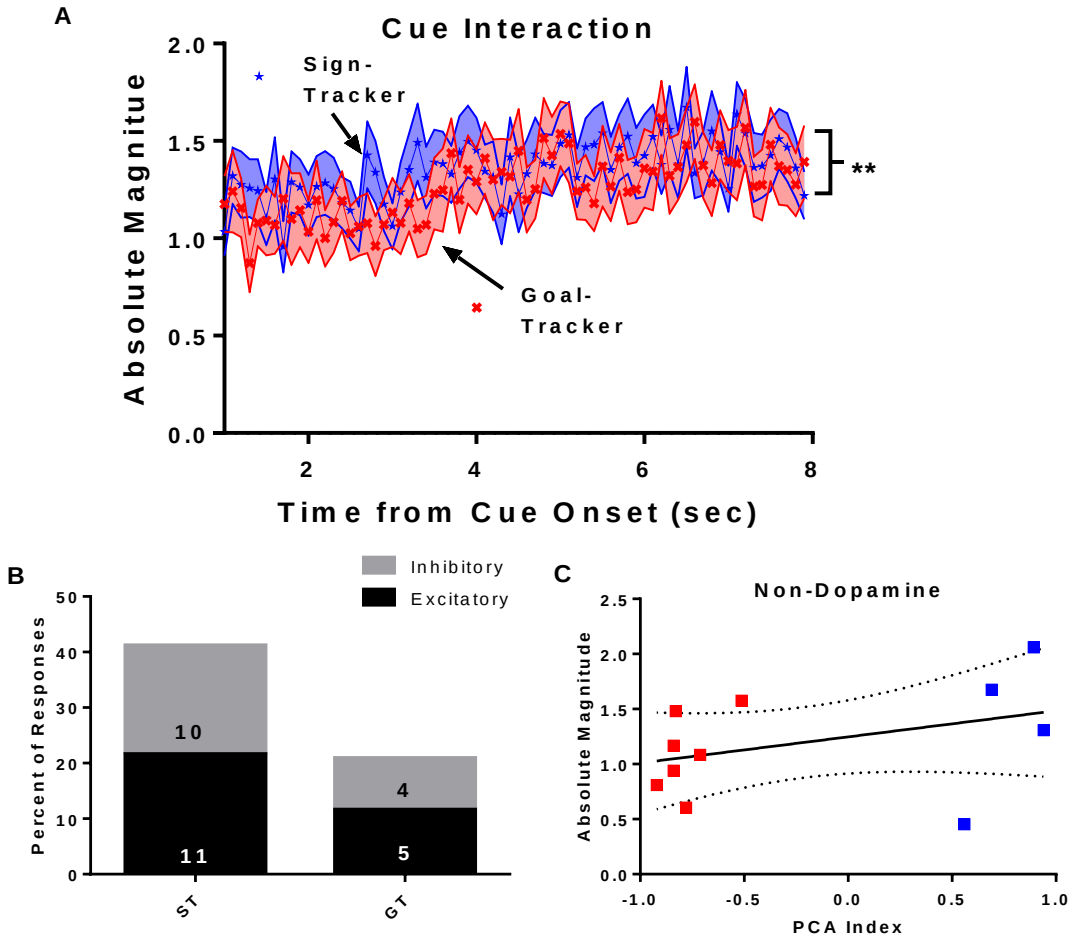
Non-dopamine neurons responsive to Pavlovian task were normalized (Z-score) to baseline. A) Absolute magnitude calculated to present changes in both excitatory and inhibitory responses in sign-trackers (blue) and goal-trackers (red). Correlations of PCA Index and magnitude to CS offset (B) and UCS (C) were performed to determine relation of firing rate changes to the attribution of incentive salience to reward-paired cues. An index of -1 to -0.5 indicates GTs, while an index of +0.5 to +1 indicates STs. Data presented as average +/- SEM

Figure 2.19: Excitatory and Inhibitory Responses of Non-Dopamine Neurons to CS Offset and UCS



Firing rates changes were calculated for sign-trackers (blue) and goal-trackers (red) to A and B) CS Offset, the 400ms time period following lever retraction (shaded region) and C and D) receipt of food reward (UCS). Data presented as average +/- SEM

Figure 2.20: Non-Dopamine Response to Cue Interaction



Neurons were analyzed for their response to cue interaction, the last 7 sec of lever presentation during which subjects are engages in a conditioned responses. Responses are either directed toward location of pellet receipt (goal-trackers, red) or towards lever (sign-trackers, blue). A) Magnitude of response (Z-score) was calculated during this time and presented as absolute value to account for both excitatory and inhibitory responses (**$p<0.01$). B) Neurons that were responsive specifically to the cue interaction event were equally excitatory and inhibitory in both sign-trackers and goal-trackers. C) Correlation of magnitude with PCA index, i.e. the propensity to interact with either food cup (towards -1.0) or lever (towards +1.0). Data presented as average +/- SEM

DISCUSSION

Individual differences in approach behaviors to reward-paired cues, and the initial tendency to attribute incentive salience to cues, are reflected in patterns of neural activity in the ventral tegmental area. Differences in coding were manifested in 1) non-dopamine neurons from goal-trackers showed higher firing rates to cue onset than non-dopamine neurons of sign-trackers, 2) sign-tracker, not goal-tracker dopamine neurons showed increased firing rates to cue interaction, 3) magnitude of firing rate was positively correlated with the attribution of incentive salience to the cue, 4) non-dopamine neurons from goal-trackers show significantly more neurons responding to cue offset than STs (population coding), and 5) increased firing rates of dopamine neurons in sign-trackers remain elevated during the entire cue interaction phase, increase further for cue offset and peak during pellet delivery with response magnitudes to UCS significantly higher than goal trackers. Further, not a single dopamine neuron from goal-trackers responded to the cue interaction phase. While the VTA has already been implicated in prediction-error (Schultz et al., 1997) these results also show a role in coding differences in incentive motivation.

The present study used a 2-cue approach to pull apart the coding properties of predictive and incentive cues. This paradigm, in combination with analysis of behavioral differences to approach reward-predictive cues, has allowed us to dissociate the differences of dopamine neurons in prediction-error and incentive motivation. Prior research has shown different population of cells responding to predictive and incentive cues in the ventral pallidum (Ahrens, Meyer, et al., 2016; Tindell et al., 2004, 2005). This study is the first to show coding differences also exist in the ventral tegmental area, and that sign-trackers and goal-trackers employ different coding patterns. The results of this study provide a target for neural manipulation to alter or halt behaviors directed at cues.

Such an intervention may prevent cue-induced reward-seeking behavior, especially those with negative consequences.

The illuminated lever serves as an equal predictor of reward delivery in STs and GTs; only the form of conditioned response differed reflecting the attribution of incentive salience (Meyer, Lovic, et al., 2012; Terry E Robinson, Yager, Cogan, & Saunders, 2014). Our results have shown that both the magnitude of firing (rate code) and the proportions of dopamine neurons (population coding) responsive to CS onset did not differ between STs and GTs. Population coding differences have implications in the release of dopamine into the core and shell of the nucleus accumbens. This indicates that both STs and GTs neurons of the VTA are coding the predictive properties of the cue equally. The responses seen here of dopamine neurons to predictive cues is consistent with others who have looked at neural activity patterns in midbrain dopamine neurons (Jo et al., 2013; Schultz et al., 1997). These studies analyzed neurons in the VTA and substantia nigra in response to cues predictive of hedonic stimuli. Our results differ, however, from our previous study that analyzed neural activity patterns between STs and GTs in the ventral pallidum. Those results showed both significantly greater population coding and rate coding in STs to predictive cues in the VP (Ahrens, Meyer, et al., 2016). The ventral pallidum is a structure downstream from the VTA in the reward circuit. Such differences in neural activity suggest that the results seen here are unique to the VTA. The indirect influence of the VTA over the VP through the nucleus accumbens may also play a role in transcribing the predictive cue into one of incentive value. STs also show an increased magnitude to CS offset (incentive cue) in comparison to CS onset (predictive cue). These results may have implications in dopamine release in the nucleus accumbens and propagation of learning and motivation signals downstream.

While neural activity did not differ between STs and GTs to CS onset, there were important coding differences during the last 7 sec of lever presentation, the cue interaction phase. STs showed significantly higher magnitudes (rate coding) and proportions (population coding) of responsive dopamine neurons. As both sign-trackers and goal-trackers engage in a conditioned response, and they both show similar vigorous motor activation during cue presentation, albeit, to lever vs. food cup, the increased magnitude to the CS interaction period in STs can be interpreted as a neural representation of incentive motivation. Magnitude differences between STs and GTs is not restricted to the VTA. STs also showed a sustained firing to cue interaction in VP neurons (Ahrens, Meyer, et al., 2016). This suggests that these incentive signals from the VTA are transmitted and maintained through the mesolimbic circuit. As sign-trackers attribute incentive salience to the lever and only dopamine cells from sign-trackers show a response to lever presentation, our results support the hypothesis that incentive salience is coded to a greater extent in sign-trackers than goal-trackers in the ventral basal ganglia. These are the first results to show a role for dopamine neurons in incentive motivation.

Dopamine (DA) neurons signal through both phasic and tonic patterns, each providing unique information to downstream sites (Goto et al., 2007; Grace, 1991; Owesson-White et al., 2009). Phasic, in this paper, refers to a short burst of firing lasting 300-400ms (up to 1sec), relating to synaptic levels of dopamine. Tonic refers to periods of baseline activity relating to extracellular dopamine levels. One of the main downstream sites from the VTA is the nucleus accumbens. Studies have indicated that phasic dopamine release may be involved in learning when an environmental stimulus is predictive of an event or reward that leads to a potential change in behaviorally coordinated events (Goto & Grace, 2005; Saddoris et al., 2015). Tonic release, on the other hand, may be involved in the motor responses that should follow a predictive

signal. Phasic dopamine release are regulated by burst firing that occurs at the level of the dopamine cell bodies and is quickly modulated by reuptake of presynaptic transporters. Tonic levels, however, are a result of the number of dopamine neurons firing in concert (i.e. population coding) and are regulated by multiple systems (Floresco, West, Ash, Moore, & Grace, 2003). Efflux of dopamine occurs at a much slower rate and is regulated by multiple systems. The phasic and tonic levels of dopamine are also interconnected, whereby excessive tonic release of dopamine causes the presynaptic upregulation of D2 autoreceptors. As a result, the phasic release of dopamine is extinguished quickly (Goto et al., 2007). Further dopamine release signals unique theories of appetitive behavior. Studies have shown that dopamine release in the nucleus accumbens core is related to learning (i.e. prediction error) while release in shell is consistent with incentive salience (Flagel et al., 2011; Phillips, Stuber, Heien, Wightman, & Carelli, 2003; Saddoris et al., 2015). As population coding is more consistent with extended dopamine release and STs showed higher response proportions to both CS onset, CS offset, and cue interaction, it can be predicted they would also show extended dopamine release in the nucleus accumbens and propagate signals of incentive salience.

One result that was unexpected was the response to pellet delivery. The majority of dopamine neurons from both sign-trackers and goal-trackers responded only to pellet delivery. Magnitude of firing was significantly higher in ST dopamine neurons than GT dopamine neurons as well. Other studies have shown that while dopamine neurons initially fire to reward delivery, once subjects learn the cue-reward association, patterns of firing shift to cue onset and no longer fire to reward delivery (Schultz et al., 1997; Schultz, 1998a). Such patterns are also seen with dopamine release in the nucleus accumbens (Day et al., 2011). Results from our study suggest that both sign-trackers and goal-trackers continue to signal reward receipt long after the cue-reward association is

learned. Recent studies have found that dopamine release codes value of reward, but even more, that there is a ramping up of dopamine release the closer in time a reward becomes available (Hamid et al., 2016). Such a ramping was seen in magnitude in dopamine neurons of both STs and GTs following CS Offset, though it was sustained only in ST dopamine neurons. This suggests that ST place a higher value on the pellet reward. Indeed, the change in firing rates between cue presentation and pellet delivery are actually significantly greater in dopamine neurons of STs than GTs. According to the study by Hamid *et al.* (2016), the change in firing rates, and subsequent release of dopamine, is consistent with their findings that dopamine encodes value of rewards (Hamid et al., 2016).

Non-dopamine neurons, both local and projecting to VTA, serve as a brake on dopamine neuron firing; inhibition of GABA neurons projecting to dopamine neurons causes disinhibition of firing (Creed et al., 2014; Mahler et al., 2014). In the presented study, we found a greater number of inhibitory signaling of non-dopamine neurons in sign-trackers to pellet receipt, which may account for the sustained excitatory response seen in dopamine neurons. We also found a greater number of non-dopamine neurons responding to CS offset in GTs compared to STs. This result may also affect the synaptic changes downstream from the VTA and contribute to the blunted response of GTs to pellet receipt.

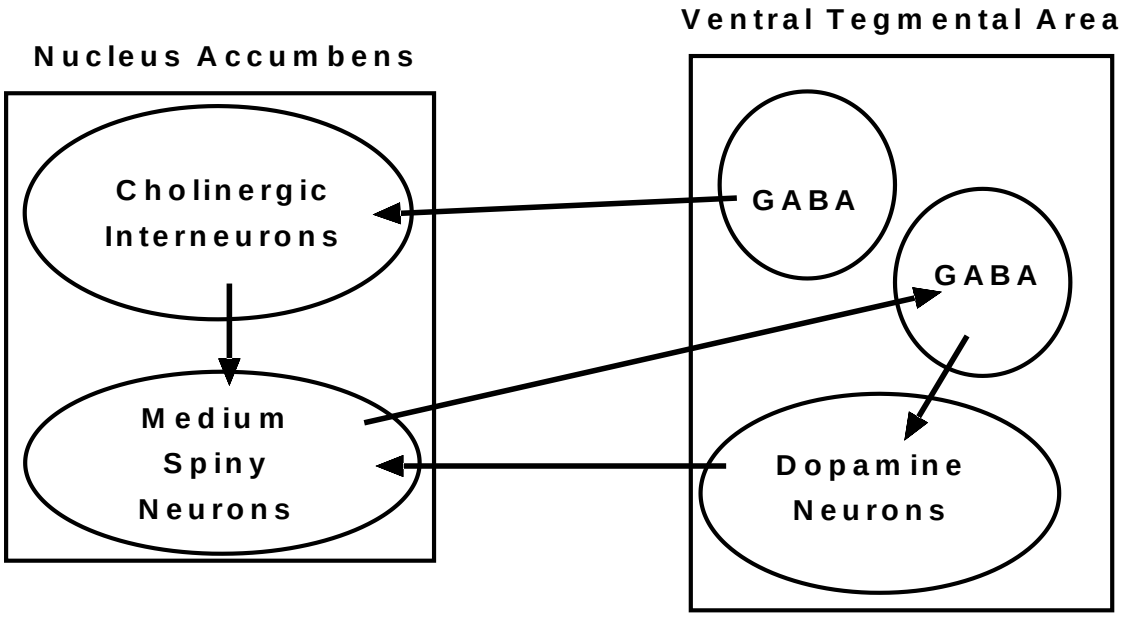
One difficulty in discriminating the role of non-dopamine neurons is the presence of both projection GABA neurons and local GABA interneurons in the VTA (Brown et al., 2012; Creed et al., 2014). These neurons account for roughly 30% of the neural population within the VTA (Dobi, Margolis, Wang, Harvey, & Morales, 2010), with the majority being projection neurons (~25%) (Margolis, Lock, Hjelmstad, & Fields, 2006). Their role in the reward circuit is less well understood. Studies using optogenetic

approaches to target local GABA interneurons have found that stimulation of these neurons result in decreased basal firing of dopamine neurons *in vivo* (Tan et al., 2012), while inhibition of local GABA interneurons results in disinhibition of DA neurons (Bocklisch et al., 2013). Other studies have found that aversive stimuli selectively activate local GABA neurons (Cohen et al., 2012; Tan et al., 2012) indicating a role in learning motivationally relevant properties of associated stimuli. Inhibition of local GABA neurons has resulted from a variety of addictive drugs, like benzodiazepines (Tan, Rudolph, & Lüscher, 2011) and cocaine (Bocklisch et al., 2013), and increased dopamine neuron firing activity as a result. Blockade of local GABA neuron activation eliminates self-administration indicating a role for these neurons in drug addiction treatment. GABA projection neurons target the prefrontal cortex and nucleus accumbens, amongst other areas, similar to dopamine neurons. In the nucleus accumbens, GABA neurons preferentially target cholinergic interneurons, whereby activation of GABA projections results in inhibition of cholinergic interneurons (Brown et al., 2012) (Figure 2.21). Such inhibitions of cholinergic interneurons result in modulation of medium spiny neurons in the nucleus accumbens and increased dopamine neuron firing in the VTA (Alcantara, Chen, Herring, Mendenhall, & Berlanga, 2003; Cachope et al., 2012). Activation of GABA projection neurons during Pavlovian tasks have not altered reward consumption, though activation of GABA interneurons did (van Zessen et al., 2012). Results from these studies indicate a complex interplay between GABA and dopamine neurons and their projection targets in reward-related behavior.

The ability to determine the role of dopamine neurons in reward learning and motivation relies highly on distinguishing dopamine from non-dopamine neurons. The properties of dopamine neurons (firing rate, spike length, etc.) was determined from dopamine recordings in the substantia nigra, which is composed 90% of dopamine

neurons (Aebischer & Schultz, 1984; Hyland et al., 2002). Current characteristics used to distinguish such neural types may overlap with non-dopamine neural properties in the VTA (Margolis et al., 2006), which is only 55% dopamine neurons (Creed et al., 2014; Dobi et al., 2010). With a more diverse area it may be that different characteristics are necessary to delineate neural types. Margolis *et al.* (2006) have found similar action potential duration for neurons staining both positive and negative for tyrosine hydroxylase, a marker of dopamine-releasing neurons. For this reason, results of our study may be confounded and may be one reason that so many inhibitory responses were seen in "dopamine-like" neurons. As no cytochemical analyses were performed to confirm placement of electrode wires over cells staining positive for tyrosine hydroxylase, it may be that those neurons that we classified as dopamine maybe actually be incorrect. Studies that utilize *in vitro* recording within the VTA have found that firing rate, interspike interval standard deviation, and interspike interval skew are more reliable characteristics to distinguish dopamine from non-dopamine neurons (Margolis et al., 2006). Such properties, however, do not always remain consistent during *in vitro* recordings. Care must be taken to interpret the heterogeneous firing properties of dopamine neurons.

Figure 2.21: VTA-NAcc Circuit



The ventral tegmental area (VTA) contains primarily dopaminergic and GABAergic (GABA) neurons. DA neurons project to medium spiny neurons (MSNs) in the nucleus accumbens (NAcc), which project back to the VTA, preferentially inhibiting GABA interneurons. The VTA also contains GABA neurons that project to and inhibit cholinergic interneurons in the NAcc, which in turn modulate MSNs.

Chapter 3: Neural Activity During Cocaine and Food Self-Administration

INTRODUCTION

The ability of the brain to respond to 'natural' rewards like food and sex is important in an evolutionary sense and carries essential benefits for survival and reproduction. It is less important, and sometimes disadvantageous, for 'unnatural' (drugs of abuse) to be coded as rewarding. Yet, many studies have shown that addictive drugs hijack the neural circuitry that encodes the pleasure and motivation for 'natural' rewards (Di Chiara et al., 1998, 1999; Robinson & Berridge, 1993, 2001, 2003). Further, not only do drugs of abuse activate these systems more potently than 'natural' rewards, they also induce cellular, molecular and systemic changes (Everitt & Wolf, 2002; Robinson & Berridge, 1993; White & Kalivas, 1998) including dopamine transmission.

Dopamine circuits have been shown to play a major role in drug dependence. Drugs of abuse have consistently shown to activate brain dopamine systems, and subsequently stimulate motivation to obtain such drugs (for review, see Berridge & Robinson, 2003). Most drugs of abuse exert their effects through the extended release of dopamine in the nucleus accumbens (NAcc). In response to cocaine, dopamine (DA) is released into the NAcc from the ventral tegmental area (VTA). Usually, DA is bound to transporters (DAT) to facilitate reuptake of the neurotransmitter. Cocaine inhibits this dopamine-transport binding to block reuptake allowing for a tonic release of the chemical. Increasing levels of cocaine linearly decreases DA affinity for DAT (Calipari, Ferris, Zimmer, Roberts, & Jones, 2013) resulting in extended DA signaling. The molecular and functional changes that occur following extended cocaine use (such as reduction in D2 receptor expression) has been well studied (Ferrario et al., 2005; Volkow

et al., 2006, 2007). The mechanism under which dopamine serves as a motivational vehicle is still under investigation.

The NAcc core, through its anatomical connections with the prefrontal cortex, somatomotor and autonomic efferents (Haber & McFarland, 1999) has been implicated in goal-directed behavior and assignment of motivational value. Recently, the core has been shown to respond to reward-paired cues as well (Flagel et al., 2011, 2007). The NAcc sends projections to the ventral pallidum (VP) (Heimer et al., 1991; Yang & Mogenson, 1985) and receives reciprocal gamma-aminobutyric acid (GABA)-ergic inputs (Churchill & Kalivas, 1994) in a topographical manner (Figure 3.1). The VP is thought to play an integrative role in reward processing, receiving projections from the amygdala through the NAcc (Yim & Mogenson, 1983) and projecting to the VTA, substantia nigra and thalamus (Heimer et al., 1991; Root et al., 2013) and has implications in drug-seeking behavior (Mahler et al., 2014). The VP as a whole is thought to be important in translating motivational signals into appetitive behaviors (Smith, Tindell, Aldridge, & Berridge, 2009; Tindell et al., 2004). The caudal ventromedial subregion has also been shown to be involved in cocaine-primed reinstatement while the dorsoventral (rostral) region has been implicated in cue-induced cocaine reinstatement (Mahler et al., 2014; Root, Fabbricatore, Ma, Barker, & West, 2010). While the VP has been regarded to be primarily involved in motor outputs, it has also shown to play an important role in reward processing. The VP has also been shown to encode both “liking” (Smith & Berridge, 2005) and “wanting” (Tindell et al., 2009) in response to food cues.

Previous studies have shown electrophysiological changes in the nucleus accumbens and ventral pallidum during drug self-administration (Peoples & West, 1996; Root et al., 2013, 2010). Few have looked at coding differences between individuals who differ in the propensity to attribute incentive salience to cue. Cues linked to rewards, like

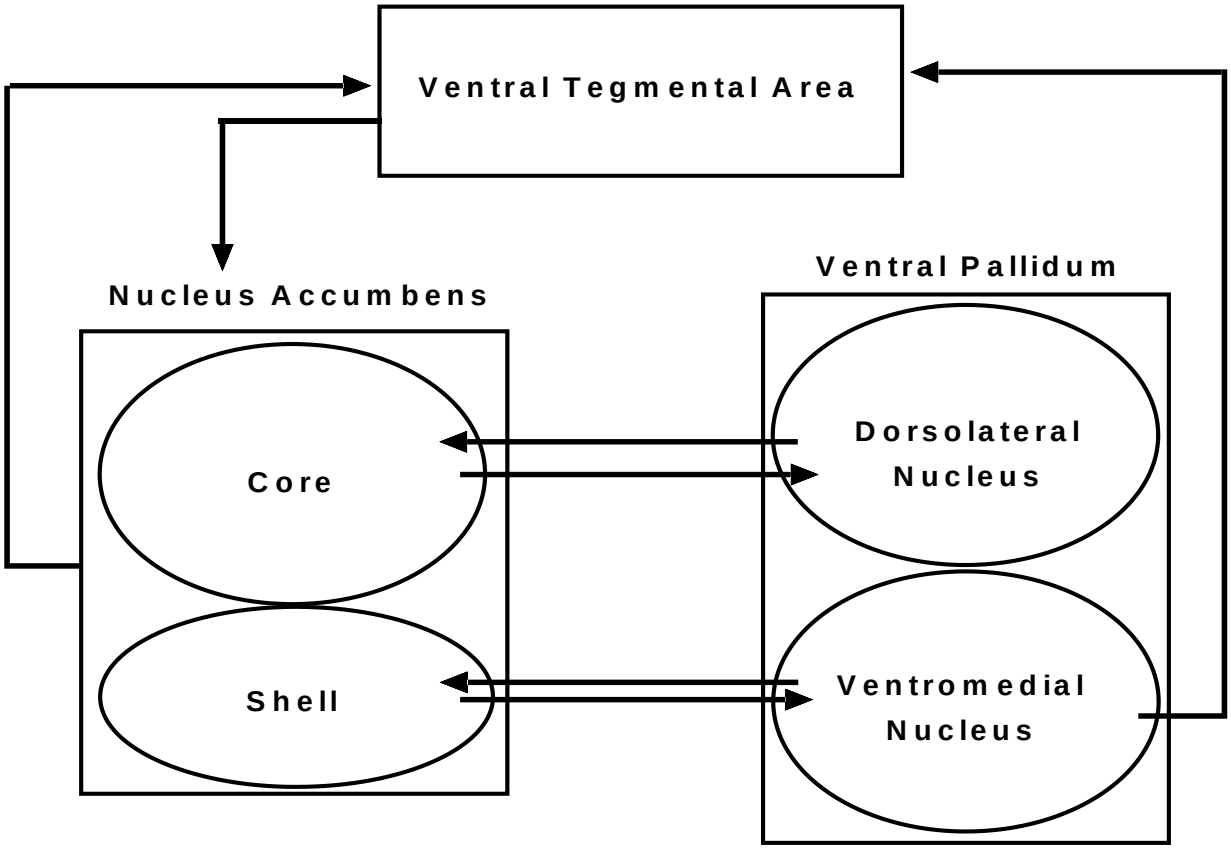
cocaine, exert considerable control over some individuals (i.e. sign-trackers, STs), but not others (i.e. goal-trackers, GTs). Studies have shown that presentation of a drug cue can reinvigorate self-administration in only some individuals following extinction training (Saunders & Robinson, 2011). A reinforcement cue is one that contains incentive, but not predictive properties and can be used to pull apart the neural correlates to these properties. Exploring the neural basis behind the ability of cues to control behavior directed towards them may provide a foundation for therapies directed at drug addiction and relapse. As dopamine release in the NAcc has been implicated in drug seeking and drug taking (Ito et al., 2000; Pettit & Justice, 1991), animals were trained to self-administer different doses of cocaine with the use of a reinforcement cue, and electrophysiological patterns were recorded in the NAcc and VP, structures downstream from the dopamine-rich VTA. How signals of reinforced cues and rewards are processed through these structures for sign-trackers (STs) and goal-trackers (GTs) is not known. Sign-trackers place high motivational value on predictive cues, and may be more responsive to the reinforced cue, thought to be reflected in firing pattern differences from goal-trackers.

The NAcc also contains a hedonic hotspot in the shell region whereby μ-opioid stimulation increases hedonic impact ("liking") and motivation ("wanting") for food rewards (Peciña & Berridge, 2005; Peciña et al., 2006). The ventral pallidum (VP) has also been shown to have a similar hotspot (Smith & Berridge, 2005, 2007). Studies have shown the NAcc and VP hotspots form a microcircuit that work together to elevate "liking" of food rewards (Smith & Berridge, 2007) (Figure 3.2). Little is known about how these hotspots work for drug rewards and reinforcement cues. This study is the first to examine both structures in response to cocaine self-administration.

The purpose of this study is not only to determine the electrophysiological changes that occur with drug exposure, but also the differences in neural firing patterns that change as a result of drug intake, seen through high and low doses. This study utilized a self-administration paradigm for both food and cocaine rewards to compare the neural responses. Typically, with human drug addicts, experimentation with drugs come first and over time environmental stimuli become reinforcement cues for subjects (Cardinal & Everitt, 2004; Everitt & Robbins, 2005). For this reason, a self-administration paradigm was implemented utilizing a reinforced, not predictive cue. In this manner, the subject can determine the rate of responding depending on physiological need, rather than, random presentation of cue.

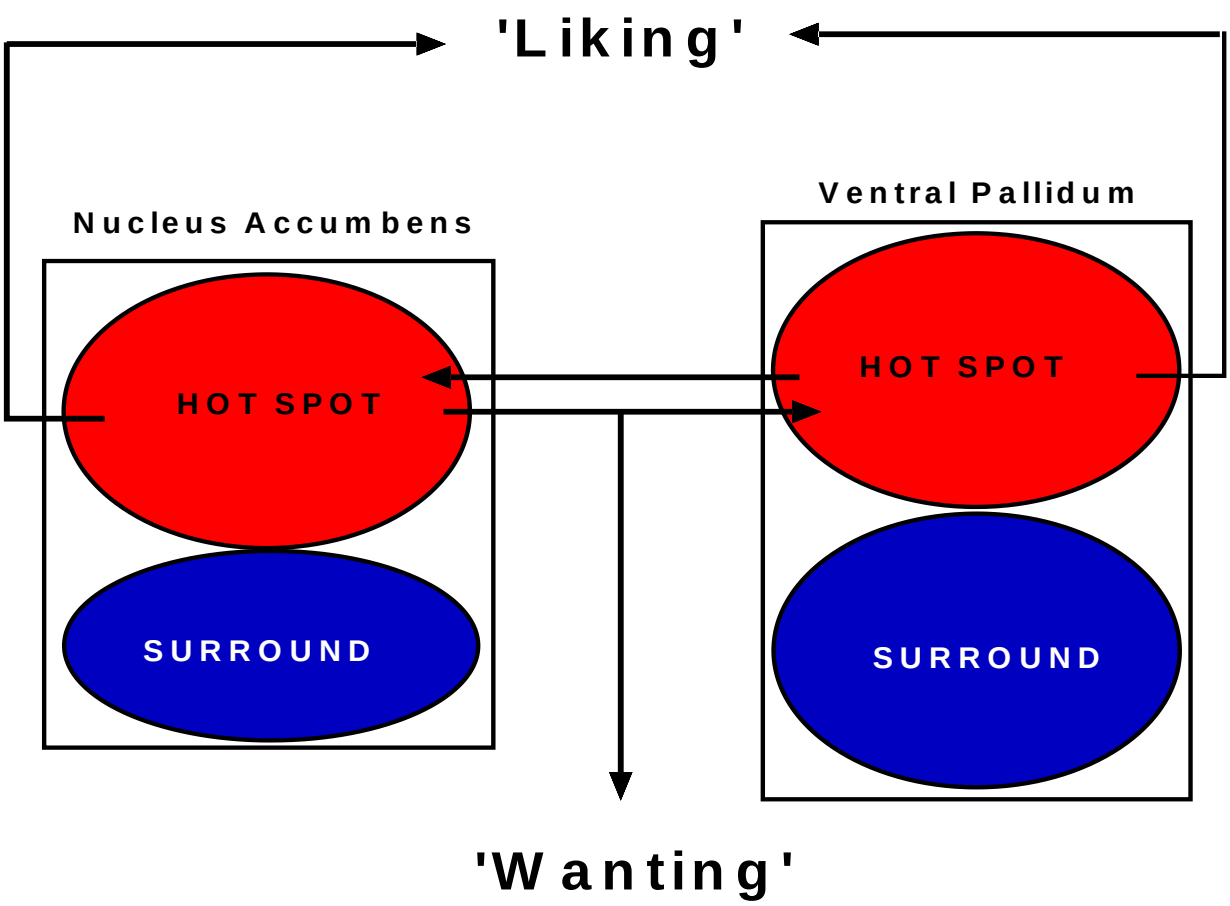
Comparing the neural representations of drug and food self-administrations will give an indication as to how cocaine differentially hijacks mesolimbic circuits. In this study I am purposely recording from NAcc and VP to view how processing reward information in these circuits might differ and/or work together. Also, by exploiting individual differences in the propensity to assign motivational value to reward-paired cues, I will analyze neural activity within the nucleus accumbens and ventral pallidum during self-administration of cocaine and food-reward. Here, I can directly assess how drugs of abuse, i.e. cocaine, specifically alter the communication between neurons of the reward pathway. Previous studies have shown electrophysiological changes in both areas during cocaine self-administration (Peoples & West, 1996; Root et al., 2013; Root, Fabbricatore, Ma, Barker, & West, 2010). My focus, however, is in particular to explore neural representations involved in the associated cues and assignment of incentive value.

Figure 3.1: Simplified Circuit of Mesolimbic Dopamine Circuit



Neurons from the ventral tegmental area (VTA) send projections primarily to the nucleus accumbens (NAcc), which in turn projects to the ventral pallidum (VP), which projects back to the VTA creating a circuit. NAcc and VP regions also contain reciprocal projections, often in a topographical manner. Arrows indicate direction of projection.

Figure 3.2: Mesolimbic Hot Spots



Summary of a "hot spot" microcircuit between the Nucleus accumbens (NAcc) and ventral pallidum (VP) in response to "liking" and "wanting" food rewards. The NAcc and VP both can influence "liking" responses by recruiting participation of the other, which is required to enhance signal. The NAcc, but not VP, can act alone in "wanting" responses to food rewards and can motivate food-seeking behavior.

METHODS

In this experiment, I analyzed how drugs of abuse (cocaine) affect neural coding of sign-trackers and goal-trackers and how the coding to drug rewards differs from food rewards in the same reinforcement task. A total of 30 male Sprague Dawley rats were used with an initial weight of 200-250g (Charles River, Wilmington, MA). Animals were housed in pairs until electrode implant surgery; after which they were singly housed. Here, a unilateral implant targeted both the nucleus accumbens (NAcc) and ventral pallidum (VP) for simultaneous recording. Animals were handled daily and provided enrichment to offset stress responses. All testing was performed during the dark cycle, between 10:00-18:00 with water and food available ad libitum throughout the study (except while in testing chamber). All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA) and Institutional Animal Care and Use Committee (IACUC).

Pavlovian Conditioned Approach:

Animals first underwent PCA training to determine ST/GT phenotype (Flagel et al., 2007). Briefly, animals first learned to retrieve pellets from magazine on day 1 of training, when pellets were delivered on average every 30 sec (no lever presentation). On days 2-6, animals were presented with 25 pairings of a retractable lever with delivery of banana-flavored food pellets (BioServ, Frenchtown, NJ) into a magazine. An illuminated lever (conditioned stimulus, CS) was inserted into the chamber on average every 90 sec. for 8s at which point the lever was retracted and a pellet (unconditioned stimulus, UCS) was delivered into the food magazine. Importantly, pellet delivery was independent of action by subject. At the end of the session, animals were returned to their home cage. Illumination/extinguishing of a house light and white noise signaled start and end of session. Data was collected for number of lever contacts, number of magazine entries,

latency to approach lever, and latency to approach magazine during lever presentation for each trial and session. On the last day of training, data was analyzed to determine scores for latency difference, response bias, and approach probability. Values were averaged to determine PCA index and phenotype (sign-tracker, ST, intermediate, INT, or goal-tracker, GT). Only sign-trackers and goal-trackers underwent cocaine or food self-administration.

Experiment 1: Cocaine Self-Administration:

Catheterization:

After PCA training, ~78 day old STs and GTs were implanted with an intravenous catheter for cocaine self-administration as described previously (Crombag, Mueller, Browman, Badiani, & Robinson, 1999). Catheters were made using shrink tubing (4mm OD, Newark InOne) and silastic tubing (0.94mm ID, 2mm OD, Fisher Scientific) and polypropylene mesh (Amazon). First a 200µl pipette tip (Fisher Scientific) was cut approximately 1cm from the base and a hole was created in one side about 3mm from tip using a soldering iron. A dummy catheter (Plastics One) was bent on the long end of the cannula to a 90^{o} angle. The cannula was then inserted into the pipette tip with the metal part coming out the hole made in the pipette tip. One side of the silicone tubing was cut to 11.5cm long and placed over the metal tubing of the cannula, while the other side was cut at a 45^{o} angle. The shrink tubing was cut to a length of 1.5cm and was placed over the silicone tubing at the base of the cannula as well. A soldering iron was then run over the shrink tubing and silicone tubing to make a tight fight on the metal rod. For the cannula cap (Plastics One), a 2mm was cut from the protruding wire so that it would fit the newly formed cannula. Next, a quarter-size piece of mesh was cut out. The mesh was secured to

the cannula where the pipette tip is open using dental cement (Henry Schein) and left to dry overnight.

Self-Administration Training:

Animals with confirmed catheter patency underwent self-administration training (Carroll & Lac, 1997) with cocaine hydrochloride (25mg/ml, NIDA) dissolved in 0.9% sterile, for infusions with a high dose (50 µl/inf). Animals (~82 days old) were placed in chambers outfitted with 2 nose ports (same size chamber as PCA training). When an animal inserted his nose into an active nose port (randomized for left or right port) the result was the illumination of the nose port (the reinforcement cue) followed by an infusion of drug (lasting 2.6s) on a fixed ratio 1 schedule (one nosepoke into active port stimulated infusion). A 20s timeout period followed, during which the cue light remained illuminated, however nosepokes into either port had no consequences. Nosepoking into the inactive nose port produced similar illumination, but no drug. Animals remained in the training chambers until a specified number of infusions was obtained: 3 days at each 10, 20, and 40 infusions. This was done to keep the total accumulation of drug constant across all subjects. Subjects that did not complete the specified number of infusions after 4hrs or subjects that lost patency (determined when we were unable to flush catheter with gentamycin) were eliminated from the study.

Experiment 2: Food Self-Administration:

A control group was trained to nosepoke for a banana-flavored food reward instead of cocaine (n = 7). A nosepoke into the active port resulted in illumination of light within the port for 20 sec, along with the delivery of a pellet 7s after the nosepoke. The 20 sec illumination period served as a cue for the time-out period, in which no further reward delivery would be given following additional nosepokes. Nosepoke into the

inactive port resulted in illumination of a cue light for same duration but no reward delivery. Subjects underwent training until >80% of nosepokes were in the active port (~3 days). These subjects did not undergo catheterization, but did receive electrode implant and underwent "self-administration" testing, with a total of 25 pellets/session.

Electrodes and Surgery:

Drivable electrodes were manufactured in lab. Two steel cannulae (23G) were soldered 1.4mm apart, into which a silica cannula was threaded. Each silica cannula contained a bundle of 8-16 wires (12.5-50μm in diameter). The silica was adhered to a rotating screw, which was fastened to the board and allowed the silica to advance after being implanted.

Electrodes were surgically implanted into the nucleus accumbens (NAcc) and ventral pallidum (VP). This was the second surgical procedure for rats that previously had catheters implanted. Target for implantation was anteroposterior (AP) = 0.1mm (back bundle) to 1.5mm (front bundle), mediolateral (ML) = -2.1mm, and dorsoventral (DV) = 7.2mm, although histological evidence indicates a range of AP = 0.5 to 1.2mm (averaged between front and back bundle), ML= -2.90 to -1.40mm, and DV = 7.0 to 9.0mm. Electrodes were implanted 1mm dorsal to target and advanced that distance 15min before initial testing session to ensure fresh tissue was being recorded. Electrodes were secured to the skull with bone screws and acrylic cement. Animals were maintained under 2.0-2.5% isoflurane and 1.0μl/min oxygen for the duration of the surgery. Animals were allowed at least 7d to recover before self-administration testing began. At the time of surgery, animals were a maximum of 95 days old.

Self-Administration Testing and Neuronal Recording:

Two different neural recording systems were used. In cocaine-treated animals, neural activity was recorded with a wireless 16-ch system, whereby a headstage and amplifier was connected to electrodes for recording (TBSI), and in control animals, neural activity was recorded with a wired 32-ch system using a tethered system that connected electrode to a headstage and amplifier (Plexon). There were no systematic differences in the quality of recordings obtained from these two systems.

All channels were recorded during the testing period using a laboratory prepared control program, DataTask (J. Wayne Aldridge, University of Michigan, Ann Arbor, MI). A second laboratory program, MTask (University of Michigan) was run in parallel and was used to record the timestamp of when (a) nosepokes are made (into both active and inactive nose ports), (b) the cue light illuminates, and (c) a reward was delivered. Video recordings were taken each test day as well. DataTask, MTask, and video were all synchronized to the same master clock to maintain synchronized timestamps for all measurements.

Animals were tested daily until 40 infusions of cocaine were self-administered or until four hours had passed. This occurred with 4 subjects (6% of all sessions). Subjects of the food group were tested until 40 trials were completed with an average of 25 pellets/session delivered. There was an initial 3 days of reacquisition of self-administration after surgery, during which no electrode advancement took place. After, both bundles were turned 90μm each day for a total advancement of 1mm (9 days of testing).

Twenty-four hours after the final testing day, subjects were lesioned to help detect location of electrode bundle. A small current (0.5mA) was passed for 30 sec through a selected wire in each bundle. This causes a micro-injury at the tip of the electrode bundle

that can be visualized through histology. Subjects were euthanized 48 hrs post-lesioning. In the cocaine group, only animals that completed all days of testing were used in analysis (n=23). All animals in the food group were included in the analysis (n=7).

Neural Analysis:

Neural recordings were discriminated using Offline Sorter (Plexon Inc., Dallas, TX). Neural reactivity was analyzed using a laboratory-prepared custom database application (The Form, University of Michigan) and NeuroExplorer (Plexon, Dallas, TX). Cross correlations were run to ensure no redundancies in the determined neural waveforms. Neural cells were classified on their reactivity to (a) act of making a rewarded nosepoke (bins were 100ms wide from 300ms before and up to 100ms after nosepoke), (b) cue light on (100ms bins from 100-400ms after nosepoke), (c) anticipation for reward delivery (100ms bins beginning 600ms before and up to 400ms after drug infusion or feeder click), and (d) reward delivery (100ms bins beginning 400ms to 6s after infusion began or 400ms - 1s following feeder click). For these neurons a Kruskal-Wallis was performed, comparing the mean firing rates during the periods specified on a trial basis to determine significant rate changes (increase or decrease) during the testing session. A Bonferroni-corrected pairwise Mann U post-hoc was performed on significant units to determine those time intervals that are significantly different ($\alpha<0.05/15$). Further, the Z-score was also calculated to analyze magnitude of firing for responsive neurons. Magnitude of firing during the self-administration period was analyzes using a Friedman's test followed by Dunn's-corrected multiple comparisons to look at effect of drug dose (within cocaine sessions only) and reward type (food vs. cocaine).

For population comparisons, a $\chi 2$ test was used to determine significance between cell counts. The total number of neurons responding and proportions of neurons

responding to specified events (nosepoke, cue, food/drug anticipation, and food/drug) were compared between locations (NAcc core, shell, VP) and session type (food, cocaine). We also compared neurons with "excitatory" and "inhibitory" responses separately for the above events and by location (NAcc Core, NAcc Shell, and VP) and drug dose in cocaine sessions.

A linear regression was also performed on each cell for each epoch to determine a) neural drift (based off background alone), and b) neural response changes over time (based off normalized rates and magnitude indexes). For these, cell responses were normalized to baseline levels (-10 to -5 sec before active nosepoke) on a trial-by-trial basis. Given the typically non-Gaussian distribution of data, linear regressions were analyzed using non-parametric tests, followed by Bonferroni corrected post-hoc tests (Kruskal-Wallis and Wilcoxin-Mann Whitney).

Behavioral Analysis:

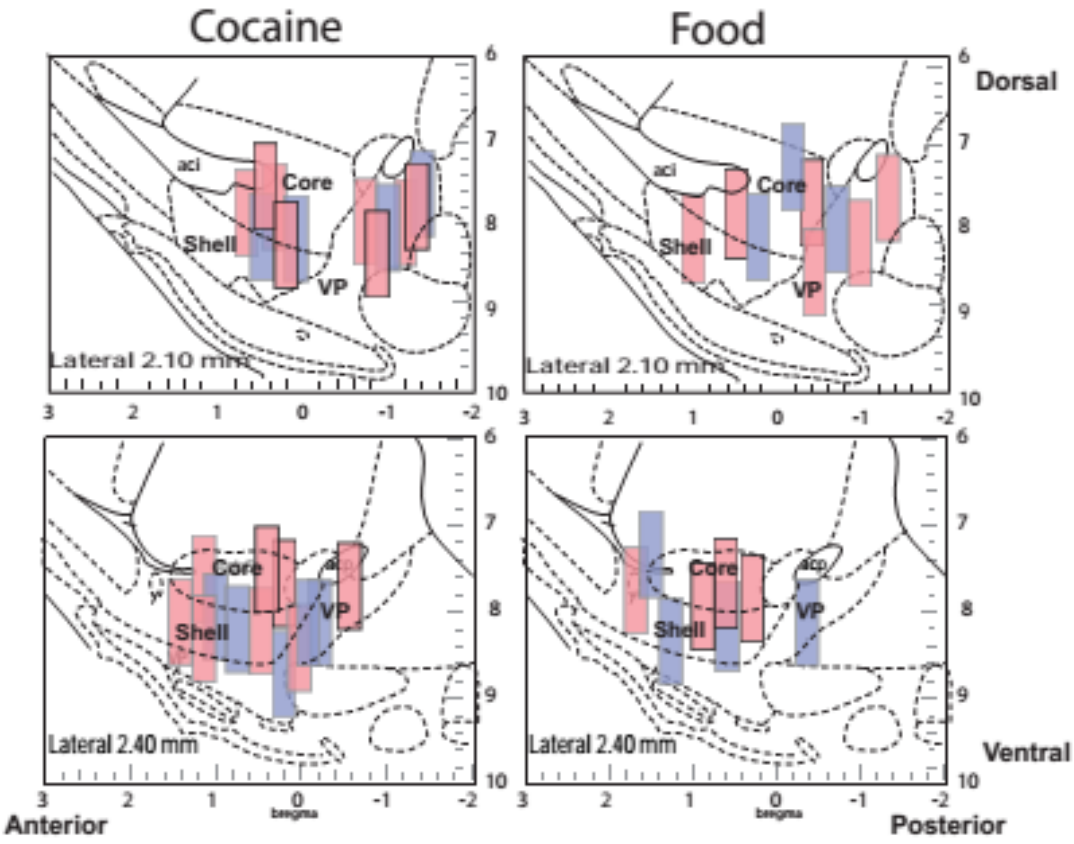
The number of nosepokes (into active and inactive ports) and infusion rates were calculated for each subject on each testing day. Results were averaged across phenotypes (STs, GTs) and dose (high=50µl/ml, low=20µl/ml), resulting in 4 groups (ST high, ST low, GT high, and GT low) in addition to 2 food groups (ST and GT). Data was analyzed using a mixed ANOVA and Bonferroni-corrected post-hoc pairwise t-tests.

Histology:

Fresh brains were frozen rapidly, sliced, and then stained with cresyl violet. Terminal locations of each electrode bundle was then determined (Figure 3.3). Only those units that were found to be entirely within the NAcc (AP: 2.25 to 0.4, ML: 1.5 to 2.5, DV: 7.6 to 9.0) or VP (AP: 0.4 to -1.0, ML: 1.5 to 2.5, DV: 7.6 to 9.0) were used in the analysis (cocaine: n = 211, food: n=29). Of the subject self-administering cocaine, no

cells were in the nucleus accumbens “hot spot” (AP: 1.1 to 3.3mm, ML: 0.8 to 1.2 mm, DV: -6.8 to -8.1 mm). Roughly half of the units from these subjects were within the VP were located in the “hot spot” (AP: -0.5 to – 1.2, ML: 2 to 3.2, DV: -7.6 to -8.5, Smith & Berridge, 2005). Of the 7 food self-administration subjects none of the cells analyzed were in the NAcc hotspot, but ~80% of the VP cells were in the VP hotspot.

Figure 3.3: Electrode Placement



Bars represent recording areas in the nucleus accumbens core, shell, and ventral pallidum (VP). Blue = goal-trackers, red = sign-trackers.

RESULTS

Neural activity was measured from 90 neurons in the nucleus accumbens (NAcc) and 126 from the ventral pallidum (VP), and firing pattern differences were analyzed in these areas as it related to food and drug self-administration. In recordings from 23 animals self-administering cocaine, I observed neurons with activity correlated to behavior in 18 animals (5 STs and 2 GTs at 0.5mg/kg cocaine, 7 STs and 4 GTs at 0.2mg/kg); cells were not seen in the remaining 5 animals. I also recorded from 7 animals self-administering food, and analyzed neurons from 4 - 2 STs and 2GTs. Proportions of responsive neurons did not differ between locations, though neurons from food self-administration sessions showed more responsive neurons than cocaine subjects in the NAcc (χ^2=5.82, $p<0.05$) and VP (χ^2=6.40, $p<0.05$, Figure 3.4). Results show similar patterns of neural firing of food compared to those self-administering cocaine.

Behavior:

All animals demonstrated stable self-administration behavior; however, the animals self-administering a low dose (0.2mg/kg) of cocaine triggered drug infusions at a faster rate than those self-administering a high dose (0.5mg/kg) (significant drug effect, $F_{(3,171)}$=25.02, $p<0.001$) (Figure 3.5A). There were no phenotypic differences; that is, self-administration rates did not differ between STs and GTs, either for the low dose of cocaine (Holm-Sidak corrected multiple comparison t=0.30, ns) or the high dose of cocaine (t=1.72, ns) The number of active nosepokes (which includes nosepokes made during the timeout period that did not lead to drug infusion) did not differ between STs and GTs or between drug dose ($F_{(3,171)}$=1.79, ns) (Figure 3.5B). Nosepokes into the inactive port were counted to measure the general activated behavior, which may result from the stereotypy effects of cocaine. STs self-administering a high dose of cocaine (0.5mg/kg) showed significantly higher inactive nosepokes than all other groups

($F_{(3,171)}$=6.17, Holm-Sidak-corrected t-tests, ts=3.13-3.83, ps<0.05) (Figure 3.5C); however this was due primarily to the contributions of 2 animals.

In the food self-administering group, average number of active nospokes, inactive nosepokes, and magazine entries were averaged across all testing days. I also found no differences in the self-administration of food pellets; there were no differences between STs and GTs in average number of active nosepoke (Holm-Sidak-corrected t=0.93, ns) nor inactive nosepokes (t=0.42, ns) (Figure 3.6). An analysis of magazine entries also showed no differences between STs and GTs (t=0.31, ns). These results indicate that differences in the initial attribution of incentive salience to cues are not depicted in behavioral differences in this self-administration paradigm. As a result, neural data from the food self-administration task from STs and GTs were combined for analysis.

Experiment 1: Neural Coding of Cocaine Self-Administration:

The patterns of neural activation in the nucleus accumbens (NAcc) and ventral pallidum (VP) were compared in both STs and GTs and both high (0.5mg/kg) and low (0.2mg/kg) doses of cocaine. I compared neural firing rates (rate coding) and changes in proportions of responsive neurons (population coding) to the self-administration task. To analyze population coding, I first determined those neurons that were responsive to at least one of the events in the session: nosepoke, cue, drug anticipation, and drug. In the assessment of population responses, no differences between phenotypes in any of the areas recorded were seen (χ^2s=0.52-1.02, ns) (Table 3.1).

Population Coding:

During cocaine self-administration, the majority of neurons in both the NAcc and the VP were unresponsive (Figure 3.7). Of the responsive neurons, there were no differences in proportions of event responses between any of the brain regions for single

or multiple responses (core vs shell, χ^2=4.17, p=0.12; core vs VP, χ^2=2.24, p=0.33; shell vs. VP, χ^2=0.97, p=0.61). When the event(s) to which the neurons were responding to were analyzed, I also found no significant differences between events for the VP (χ^2=0.97, p=0.36), but we did see significant differences in the core (χ^2=15.13, p<0.005), and shell (χ^2=9.587, p<0.05, Figure 3.7). In the core, the majority of neurons responded to the cue and none responded to the drug infusion event. In the shell, the majority of neurons responded to nosepoke. These results suggest that that the core preferentially responds to the cue and the shell preferentially responds to nosepoke events.

Overall, the distribution of excitatory and inhibitory responses differed little or not at all (χ^2=0.35, p=0.84) between regions and drug doses (Figure 3.8). The proportions of excitatory and inhibitory responses were similar in each the nucleus accumbens core (χ^2=1.595, p=0.21), shell (χ^2=0.41, p=0.64), and the ventral pallidum (χ^2=3.742, p=0.05). The proportion of excitations and inhibitions in VP differed only with low dose cocaine with a significantly higher proportion of inhibitions (χ^2=11.39, p<0.01). It should be noted however that overall cocaine self-administration evoked only a small proportion of responsive neurons compared to natural food reward (see below). The cocaine infusions evoked responses equally divided between excitations (n=13, 54%) and inhibitions (n=11, 46%, see examples Figure 3.8). The response differences between core, shell and VP regions to drug infusion indicate a possible location-specific effect for cocaine dose on neural firing patterns.

Rate Coding:

Baseline firing rates in the VP of STs administering the high (0.5mg/kg) dose of cocaine were the highest observed. This high dose effect in STs was higher than low dose cocaine (0.2 mg/kg) and higher than GTs at both low and high doses (t=2.6, p<0.05,

Figure 3.9). In the nucleus accumbens, there were no significant differences with region (core or shell), high or low dose, and STs or GTs (Figure 3.9). For this reason, their neural data was combined.

In order to compare rate changes in populations of neurons and their patterns of firing, a Z-score was calculated for each unit to normalize changes in firing rates. For those neurons that were responsive to any of the events in the session, nosepoke, cue, drug anticipation, and drug, their Z-scores were averaged. Since there were no significant differences between excitatory and inhibitory responses in the nucleus accumbens, I computed the absolute value of inhibition so that all responses would contribute to the population response without canceling out each other (Figure 3.10). This comparison revealed no differences in average rate changes between high and low doses in the nucleus accumbens core ($F_{(1,30)}$=3.09, p=0.09). In the shell there were greater magnitude changes of high dose cocaine to nosepoke, cue, and drug anticipation compared to low dose ($F_{(1,42)}$=4.54, p<0.05).

Neurons in the ventral pallidum (VP) were assessed in the same way as the nucleus accumbens (NAcc) (Figure 3.11) finding changes associated with the nosepoke/cue periods that were significantly higher in magnitude compared to baseline. The high dose of self-administered cocaine evoke significantly greater activation than the low dose ($F_{(119,240)}$=3.556, p<0.001 for dose, $F_{(1,240)}$=120, p<0.001 for change in magnitude, $F_{(119,240)}$=2.46, p<0.001 for interaction). There was also a slightly higher magnitude change in average firing following the drug infusion (4-4.2sec after nosepoke, Holm-Sidak-corrected comparison p<0.05) after high dose self-administration compared to low dose cocaine.

Ventral Pallidum Hot Spots:

In the VP all responsive neurons were compared based on their location in the caudal hedonic "hot spot" (Peciña et al., 2006; Smith & Berridge, 2005) to all responsive neurons surrounding the VP "hot spot" at each dose (Figure 3.12). In this analysis, I compared the direction of changes (excitation vs. inhibition). For animals self-administering a high dose of cocaine, results demonstrated significantly different patterns of responses between neurons in the "hot spot" and those in the surround to nosepoke (Friedman's χ^2=7.6, $p<0.05$), cue (Friedman's χ^2=8.0, $p<0.01$), and drug (Friedman's χ^2=86.39, $p<0.001$). Hot spot VP neurons showed on average an overall increase in the time period around the active nosepoke. In contrast, VP neurons in the surrounding area show overall decreasing changes in firing rate in the period associated with the nosepoke. This indicates that in animals self-administering a high dose of cocaine, neurons in the "hot spot" are responding primarily through excitatory inputs compared to a predominance of inhibitory influences in the surround. Differences were seen between the "hot spot" and surround in animals self-administering a low (0.2mg/kg) dose of cocaine only during drug anticipation (Friedman's χ^2=16, Dunn's corrected multiple comparison, $p<0.05$). These results indicate that drug dose has a stronger impact on neural firing patterns of the VP than the NAcc, both overall, and with neurons in the "hot spot".

Experiment 2: Coding of Food Self-Administration:

In a separate experimental group, animals were trained in an identical paradigm to "self-administer" a tasty banana pellet food reward instead of cocaine infusions. Baseline firing rates were calculated for neurons in the nucleus accumbens (NAcc) and ventral pallidum (VP). There were no differences in the NAcc ($p=0.52$) or VP ($p=0.86$) between food and cocaine self-administration sessions (Figure 3.13).

Population Coding:

Neurons were analyzed in the NAcc and VP in the same way as for cocaine self-administration. A total of 14 neurons from the NAcc and 15 from the VP were assessed determining responsive neurons with a Kruskal-Wallis during the time periods for active nosepoke, cue, pellet anticipation and pellet receipt in comparison to baseline rates (see Methods), averaged over trials/session. In contrast to the cocaine reward, most neurons from in VP and NAcc were responsive to some part of the food self-administration task (65% food vs. 35% cocaine, Figure 3.14). These results were significantly different (χ^2=22.53, p<0.001) and may indicate that there are different populations of neurons that respond to food and drug rewards. Further, there were greater proportions of neurons responding to multiple events in the nucleus accumbens (χ^2=9.48, p<0.01) and VP (χ^2=8.69, p<0.05) during food vs. cocaine self-administration. The analysis of population coding showed that the proportions of neural responses to each event were statistically no different from each other in the NAcc (χ^2=2.50, p=0.47, Figure 3.15). Similarly, in the ventral pallidum the proportions of responsive neurons did not differ (χ^2=2.40, p=0.12). Further, the proportions of neurons responding to each event did not differ from cocaine self-administration in either the NAcc (χ^2=4.38, p=0.22) or the VP (χ^2=1.38, p=0.71).

The nucleus accumbens and ventral pallidum showed similar proportions of excitatory and inhibitory responses in food reward sessions (χ^2=0.06, p=0.80, Figure 3.16). Inhibitory responses were typically associated with nosepoke and/or cue events in the NAcc and VP. In contrast, drug infusion events had mostly excitations. In comparison to how neurons responded in cocaine self-administration, proportions of excitatory and inhibitory were similar in the NAcc (χ^2=1.35, p=0.24). In the VP, however, there were

greater proportions of inhibitory responses with cocaine self-administration compared to food self-administration (χ^2=4.51, p<0.05).

Rate coding:

Firing rates were normalized (Z-score) and averaged across responsive neurons in the NAcc and VP during food self-administration (Figure 3.17). The NAcc showed mostly inhibitory magnitude to nosepoke and excitatory changes to pellet receipt. Firing rate changes in neurons responding to nosepoke and cue events during food self-administration did not differ from those responding during cocaine self-administration ($F_{(2,54)}$=0.68, p=0.51 for control vs. cocaine, Figure 3.17top). The VP showed a combination of excitatory and inhibitory responses throughout all neurons (Figure 3.17 bottom). Firing rate changes were significantly less in the VP during the nosepoke event in food sessions compared to those from cocaine self-administration sessions ($F_{(2,344)}$=15.65, p<0.001 for food vs. cocaine). Differences were only seen between neurons in the VP of food self-administration sessions and those in the VP hot spot of cocaine self-administration (Holm-corrected t-test p<0.01), but not surround (p=0.11). Most of the VP neurons analyzed for food self-administration were in the "hot spot" so differences in firing rate changes seem to be due to reward type (food vs. cocaine).

Background Firing Rates Change Over Time:

During the self-administration of cocaine, the baseline firing rate of many neurons exhibited a gradual change as the animal successfully completed more nosepoke trials (Figure 3.18). To assess these changes, the background firing rates were computed on a trial basis for each unit and session and a linear regression analysis was performed over trials. In cocaine self-administration sessions, results indicated that 61% of the neurons sampled (133 of 216 neurons) showed significant changes in neural firing across trials

with equal numbers in NAcc and VP (Figure 3.19). Linear changes were seen in both responsive (32/133, 25%) and unresponsive (101/133, 75%) units. The majority (75%) showed a decrease in firing over the time of the session (Figure 3.19). A chi-square test indicated significantly more decreasing rates in the NAcc compared to the VP (χ^2=5.44, $p<0.05$) of cocaine self-administration sessions. In the shell region of the nucleus accumbens, there were significantly higher proportions of decreasing rates for high dose vs. low dose of cocaine (χ^2=4.71, $p<0.05$). The core showed a trend for the same results (χ^2=2.87, $p<0.1$). Drug dose did not have significant effects on increasing or decreasing rates in the VP.

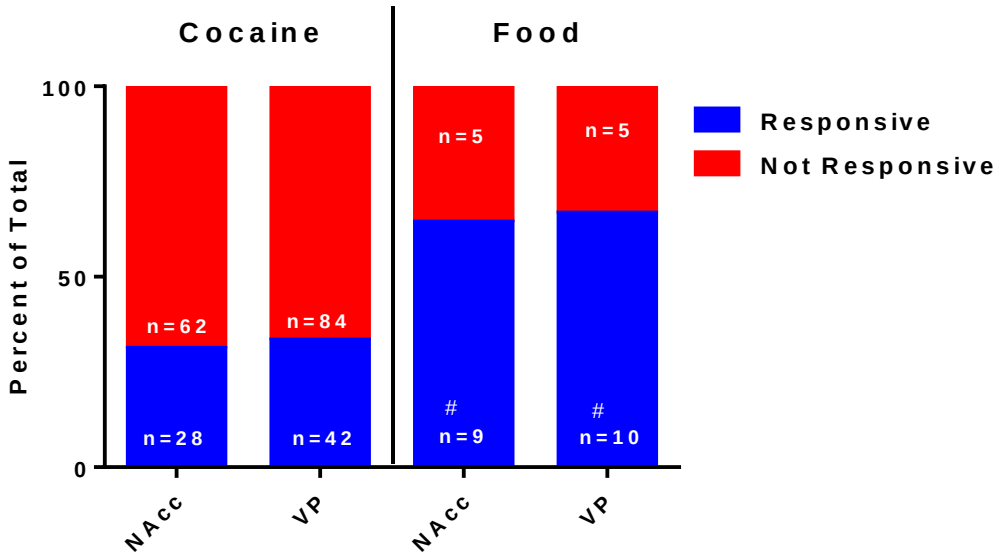
In animals performing the nosepoke task to self-administer food rewards, most neurons (>69%) did not show a change in firing rate across the session (Figure 3.19). Of those that did show a change, only 1 (11%) was responsive to the self-administration task. The proportion of VP neurons showing rate changes with trials was significantly smaller during food vs. cocaine self-administration sessions (χ^2=2.34, $p<0.05$). Like the cocaine session, of the neurons that did show a rate change across trials, most (85%) showed decreases in firing rate over time (Figure 3.19).

I extended these findings to determine if the signal strength of neurons was changing throughout the testing session. A significant change would suggest alteration in salience for cue and/or drug. All of the 216 neurons were analyzed to determine if neural response changed across the trial session. For responsive neurons, mean firing rates were normalized during the responsive period to background (S/B, where S = signal, B = background) on a trial basis and then a linear regression was performed to nosepoke, cue, reward anticipation, and reward events (Figure 3.20). The majority of responses (60%) were stable (linear regression not significant) (Figure 3.21). The remaining 23% showed a stronger response over the self-administration, and 17% showed a weaker response. All

of the weaker responses (decreasing linear regression) were excitatory. This indicates that for 23% of the units, the response to signal got stronger over time. While this is not the majority, the details do extend our knowledge of neural control between the NAcc and VP.

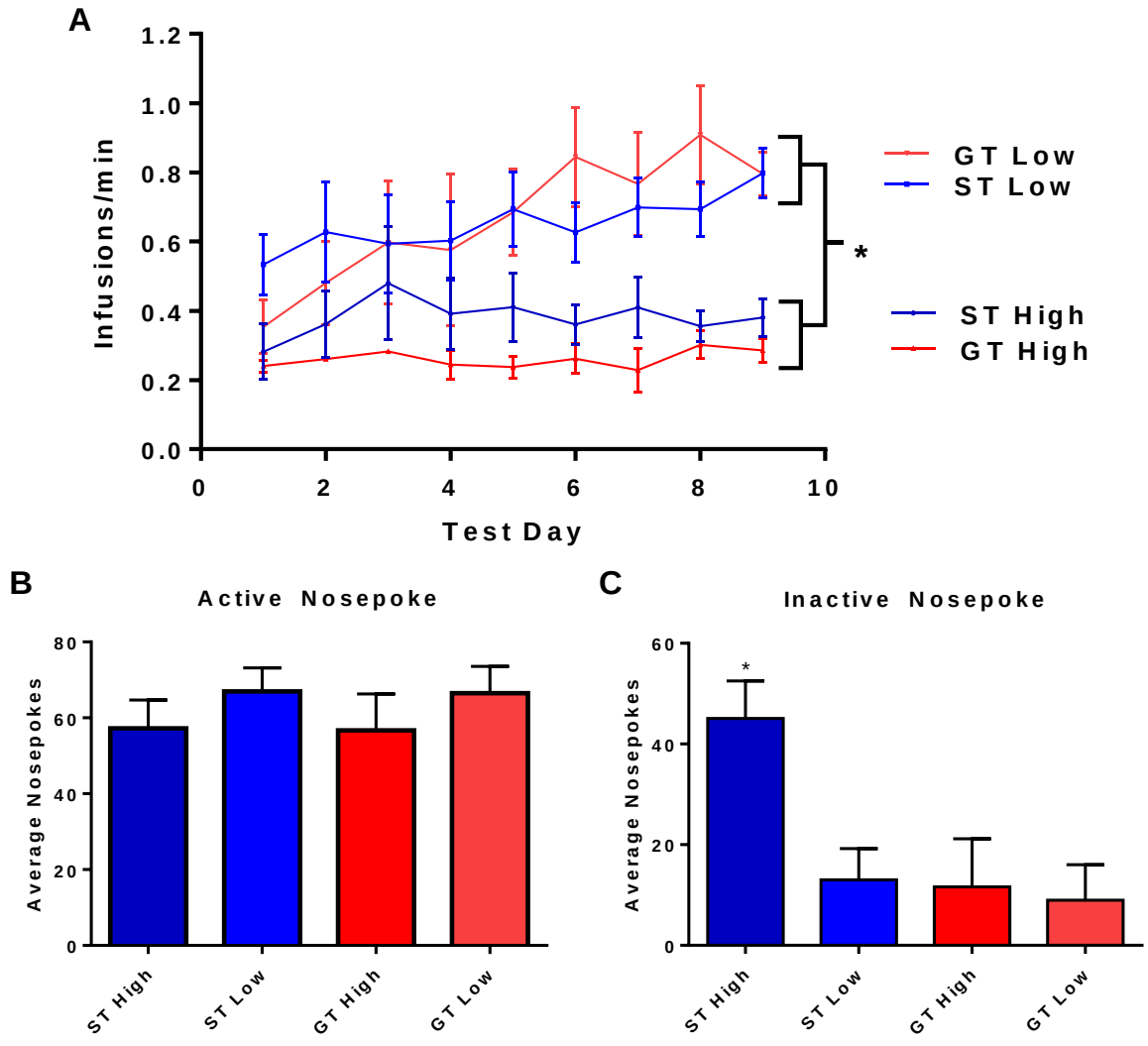
To rule out artifactual changes due to electrode movement or other spike sorting errors, I assessed the spike waveforms carefully across the session by, for example, comparing early and late session trials. Characteristics of the spikes were observed for consistency in amplitude and shape throughout the session. Results provided no evidence to indicate systematic errors such as movement-induced changes in waveform amplitude or shape contributing to spike discrimination errors. In addition, when possible I was able to compare neurons that were recorded from the same wire and/or bundle. If a systemic error were to have occurred, the same changes would be present on all neurons. Indeed, in some instances two units recorded on a single wire showed independent changes (e.g., one unit changed while the other was constant) (Figure 3.22).

Figure 3.4: Proportions of Responsive and Non-Responsive Neurons



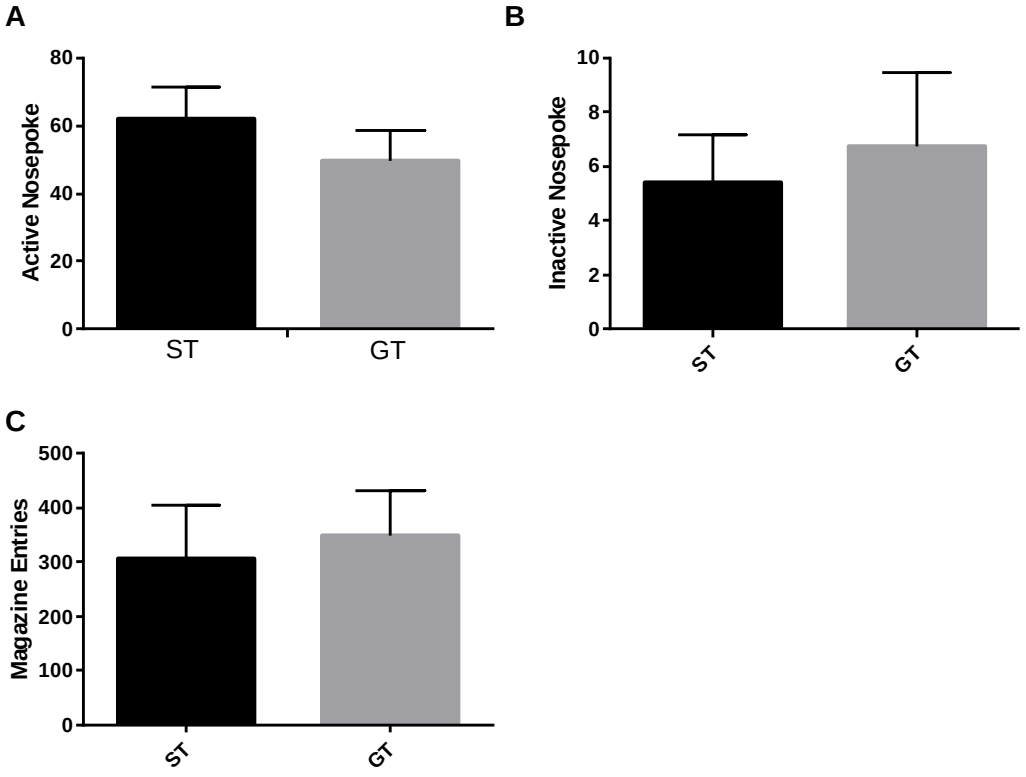
Percentages of neurons responsive to any event in the self-administration task were calculated from the total number of units for each nucleus. Of the 32 responsive units in the VP, 15 are from the VPdl ("hot spot") and 17 are from the VPvm (surround) subregions. #$p < 0.01$, food compared to cocaine proportions.

Figure 3.5: Cocaine Self-Administration Behavior



Animals were trained to nosepoke into an active port to deliver either 0.2mg/kg (low dose) or 0.5mg/kg (high dose) of cocaine. There were significant differences in rates of self-administration (A) for high dose vs. low dose of cocaine, but not for phenotype. There were no differences in the number of active nosepokes made (B) for dose nor for phenotype, however, STs self-administering high dose of cocaine poked in the inactive port significantly more. *$p<0.05$

Figure 3.6: Food Self-Administration Behavior



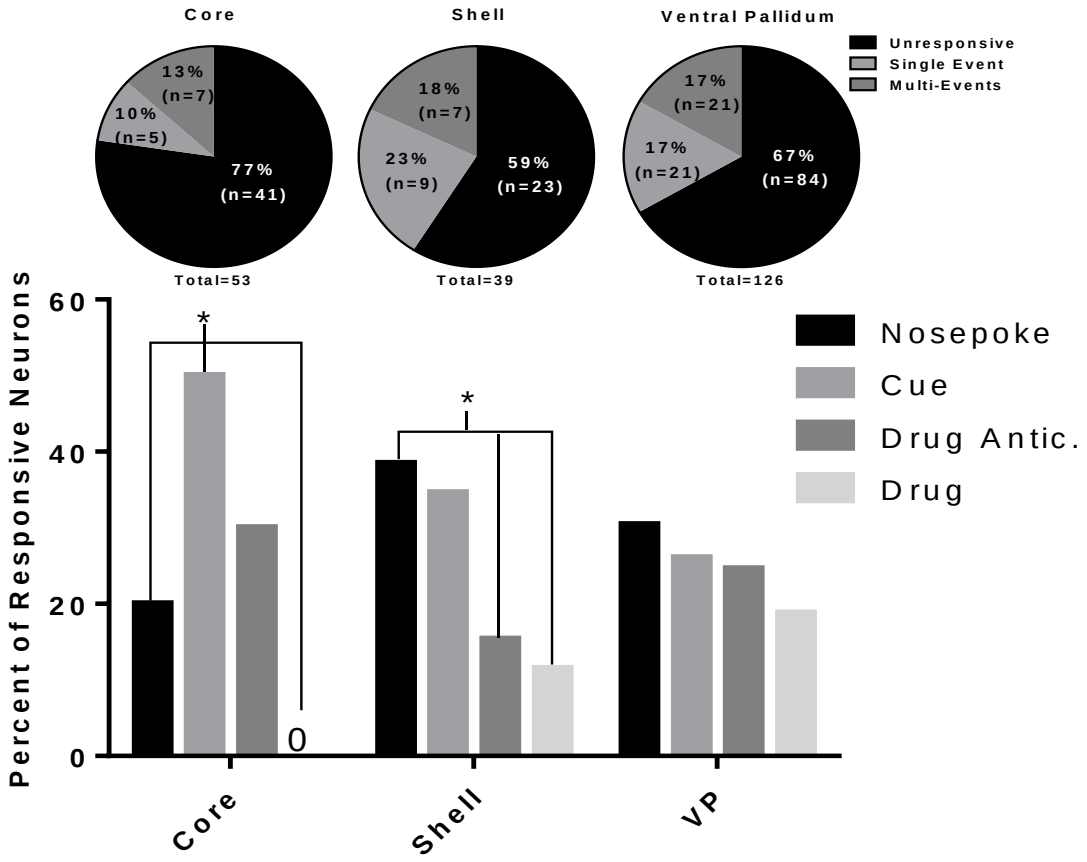
Animals were trained to nosepoke into an active port for a food reward. There was no significant phenotype effect. Subjects did not differ in (A) Active nosepokes, (B) Inactive Nosepokes, or (C) Magazine entries.

Table 3.1: Summary for Sign-Trackers and Goal-Trackers Self-Administering Cocaine

	VP		NAcc Core		NAcc shell		Total	
	ST	GT	ST	GT	ST	GT	ST	GT
Total Units Assessed n	89	37	49	4	30	9	168	50
Responsive n (% of total)	28 (31%)	14 (38%)	11 (22%)	1 (25%)	11 (37%)	5 (56%)	50 (30%)	20 (40%)
Non responsive n (% of total)	61 (69%)	23 (62%)	38 (78%)	3 (75%)	19 (63%)	4 (44%)	118 (70%)	30 (60%)
Cue n (% of responsive)	14 (50%)	4 (29%)	9 (82%)	1 (100%)	8 (61%)	1 (17%)	31 (46%)	6 (21%)
Nosepoke n (% of responsive)	14 (50%)	7 (50%)	3 (27%)	1 (100%)	7 (64%)	3 (80%)	23 (46%)	7 (35%)

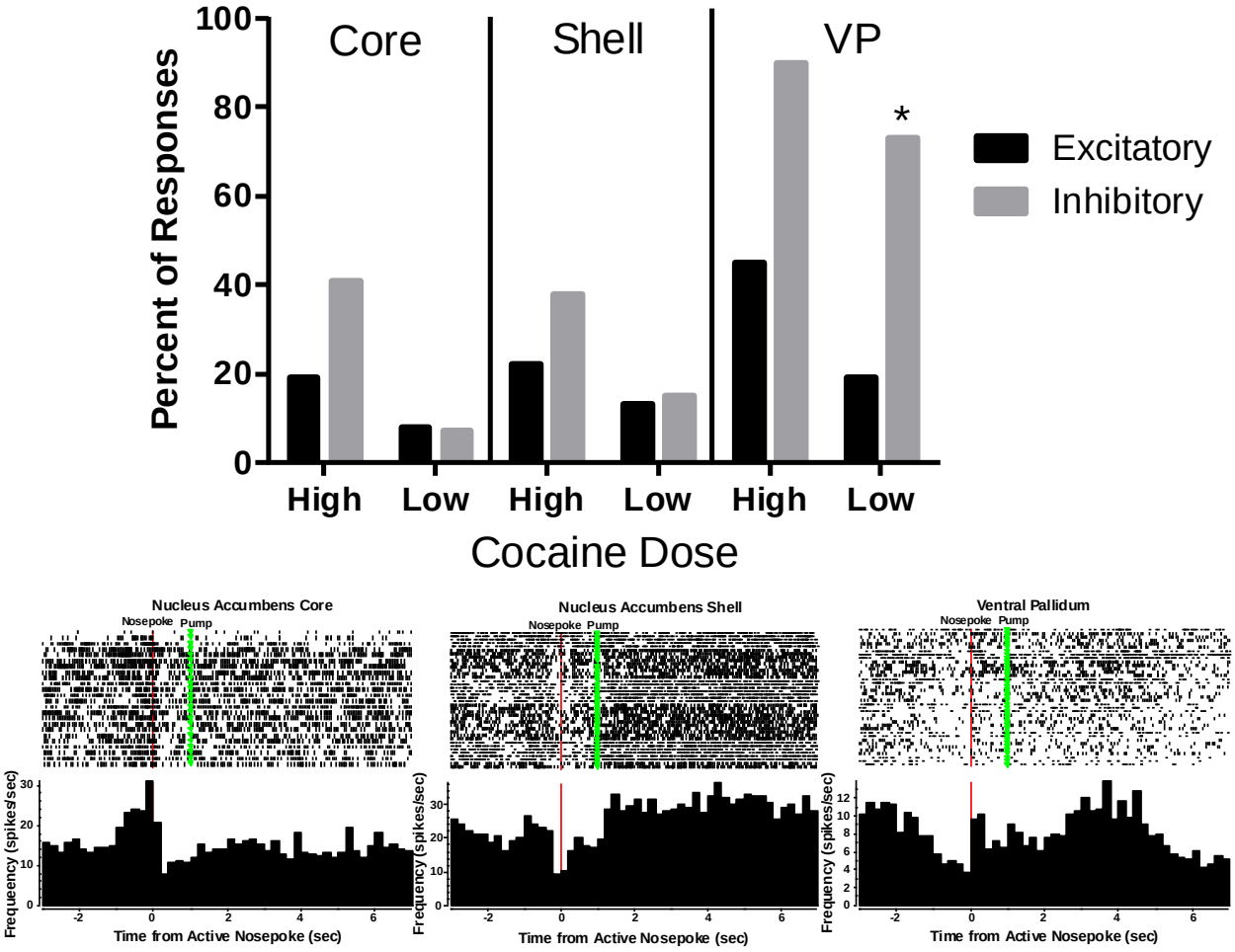
We assessed a total of 15 sign-trackers (ST, 5 high dose, 10 low dose) and 8 goal-trackers (GT, 3 high dose, 5 low dose). We found responsive units in 12 STs (5 high dose, 7 low dose) and 6 GTs (2 high dose, 4 low dose). No differences between phenotypes were seen in proportions of neurons responding to any part of the task.

Figure 3.7: Neural Response Patterns to Cocaine



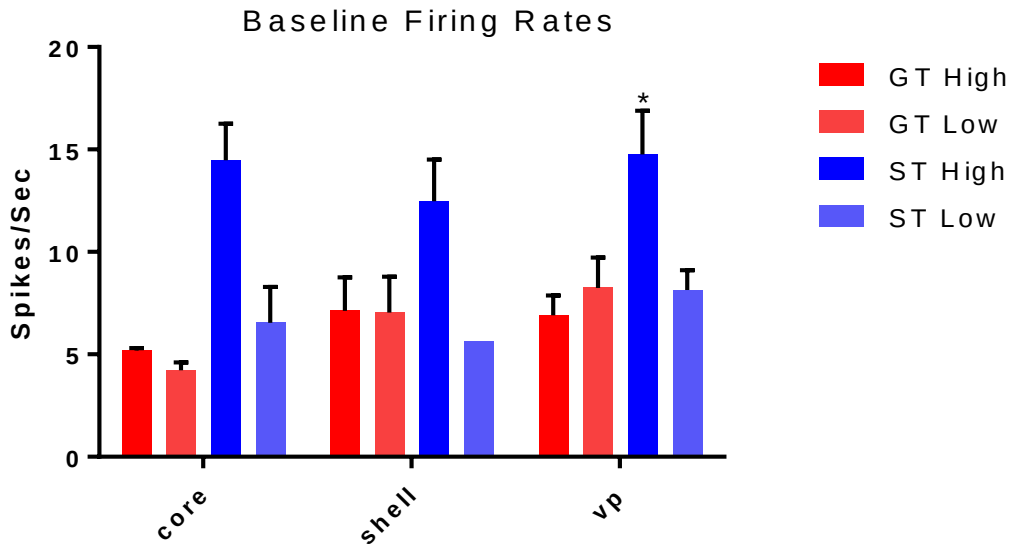
(TOP) Neurons recorded from animals self-administering cocaine were analyzed for responses to a single event (nosepoke, cue, drug anticipation, and drug), or multiple events in the nucleus accumbens core, nucleus accumbens shell, or ventral pallidum. Neurons that did not respond to these events were considered unresponsive. The overall proportions of responsive and unresponsive neurons did not differ across recording locations. (BOTTOM) Among responsive neurons, we observed significantly more responses to cue in the core compared to nosepoke and drug delivery (reward). In the shell we found neurons responding more to nosepoke than drug anticipation or drug. *$p<0.05$

Figure 3.8: Excitatory and Inhibitory Activation



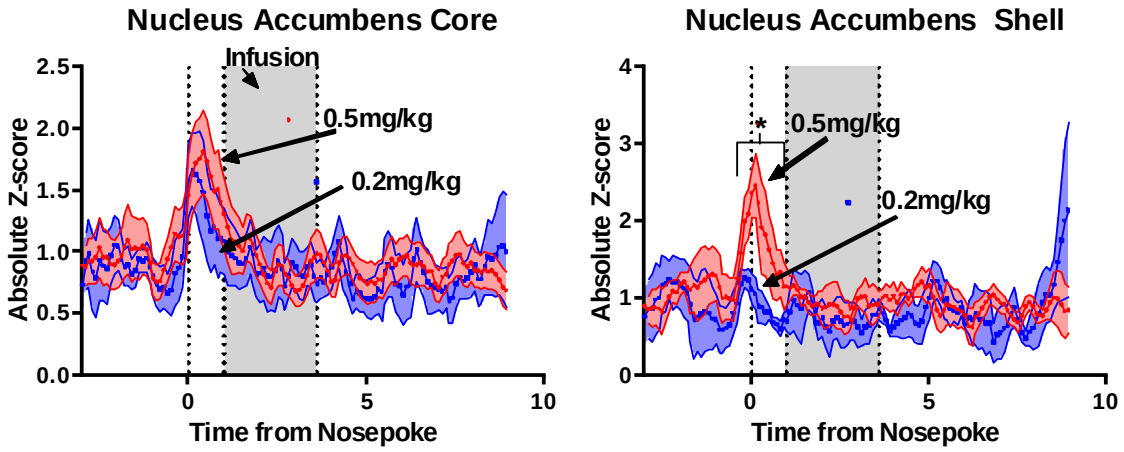
(TOP) The bar graphs indicated the proportions of inhibitory and excitatory responses. Note that the totals in ventral pallidum (VP) exceed 100% as many neurons exhibited both excitation and inhibition (see bottom example). The perievent time histograms (BOTTOM) are aligned to nosepoke (time = 0, red line) in nucleus accumbens core (Left) shell (middle) and VP (Right). Drug infusion began at 1se following nosepoke (green marker). The core neuron (Left) exhibits preparatory activation for the nosepoke. The shell neuron shows an inhibition at moment of nosepoke and slight excitation following initiation of drug delivery (at 1 sec). The VP neuron (Right) has a preparatory inhibition to nosepoke, which triggers a brief excitation at cue onset and a latter vigorous activation during the drug incorporation period (2.6 to 4.5 sec). The latter is followed by a slow decline in firing rate.

Figure 3.9: Baseline (Intertrial Interval) Firing Rates for Cocaine Self-Administration



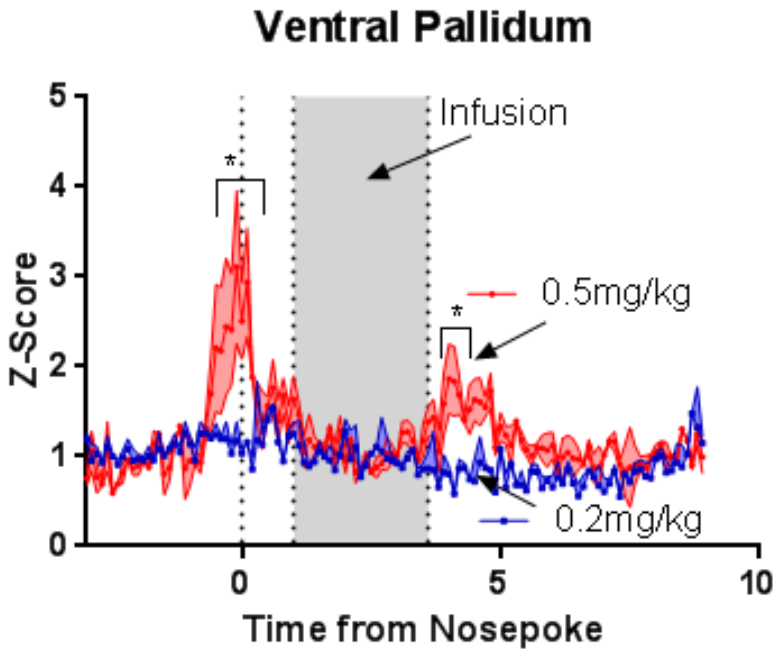
Neural firing rates during the intertrial interval (baseline) were determined from all neurons in the nucleus accumbens core, shell, and ventral pallidum (vp). Baseline firing rates were significantly higher in all regions in sign-trackers self-administering high doses of cocaine and was significantly higher than the other groups only in the VP (*$p<0.05$).

Figure 3.10: Population Neural Responses to Cocaine Self-Administration Task



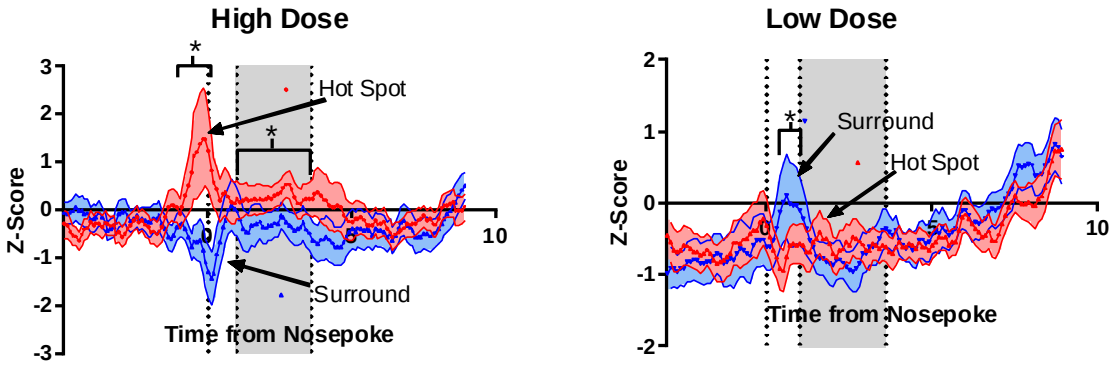
Neurons that were found to be responsive in the (LEFT) nucleus accumbens core, and (RIGHT) nucleus accumbens shell were averaged for low dose (blue) and high dose (red) cocaine self-administered. Firing rates were normalized (Z-score) and rectified (absolute value) with respect to background to allow changes in firing rate to events to be weighted equally across neurons. Dotted line represents moment of nosepoke into active port. Grey region represents time for cocaine infusion. The predominant response was an increase in activation in relation to the nosepoke. There were no significant differences between high (0.5mg/kg) and low (0.2mg/kg) dose of cocaine in core. In the shell high dose neurons fired more than low dose neurons beginning at nosepoke until infusion of drug (+1 sec). *$p<0.01$

Figure 3.11: Comparison of Cocaine Dose on Average Firing Rate Changes in the Ventral Pallidum



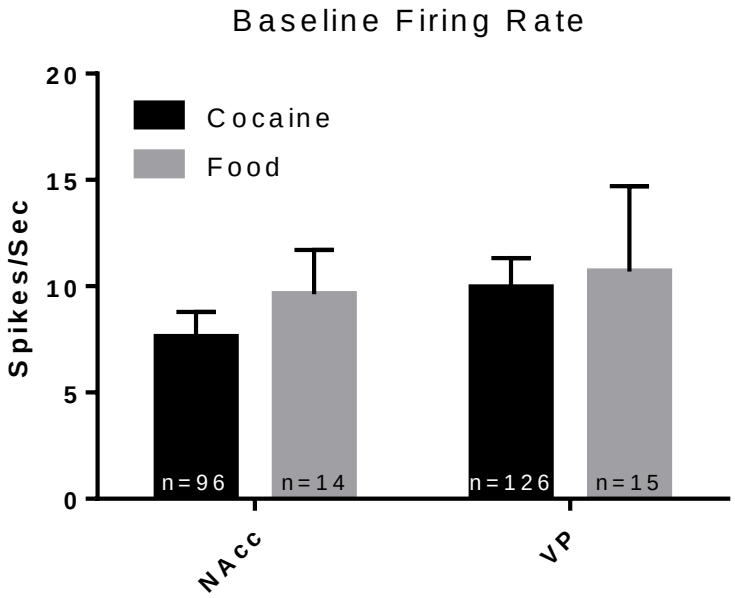
The Z-score of each responsive neuron was calculated and then average for high (red) or low (blue) cocaine dose being self-administered. The magnitude of firing was significantly higher in neurons from animals self-administering a high dose of cocaine to the nosepoke, cue, and drug infusion event. *$p<0.05$ compared to low dose

Figure 3.12: Ventral Pallidum Neurons in the Hot Spot



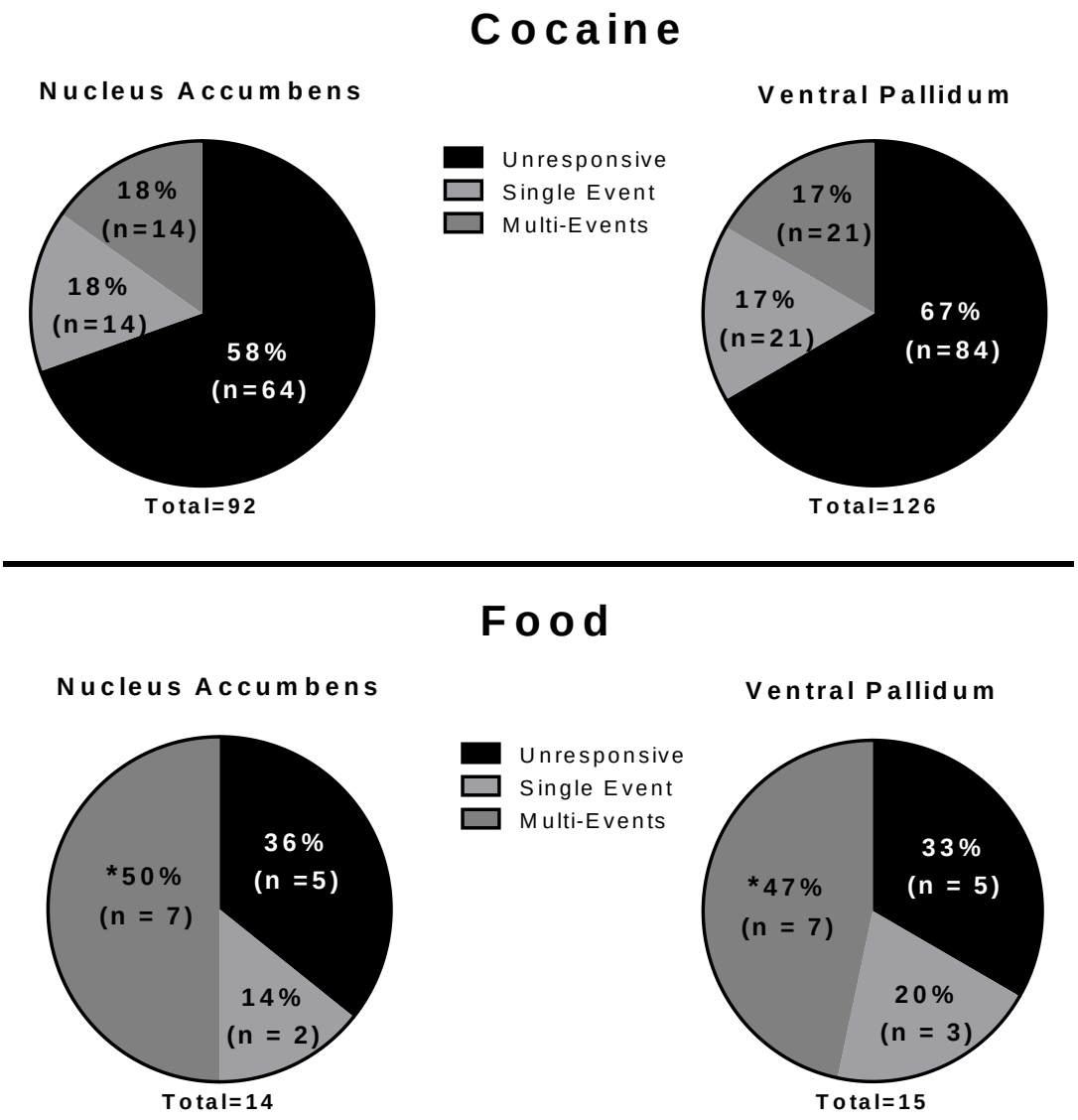
Responsive neurons of the ventral pallidum were analyzed depending on if they were in the "hot spot" (red) or not (i.e. the surround, blue), both for high (0.5mg/kg) dose of cocaine (LEFT), and low (0.2mg/kg) dose cocaine (RIGHT). All of the responsive neurons in the hot spot of subjects self-administering a high dose of cocaine were excitatory to the task, and were significantly different from the surround which were primarily inhibitory in nature. *$p<0.05$

Figure 3.13: Baseline Firing Rates for Food and Cocaine Self-Administration



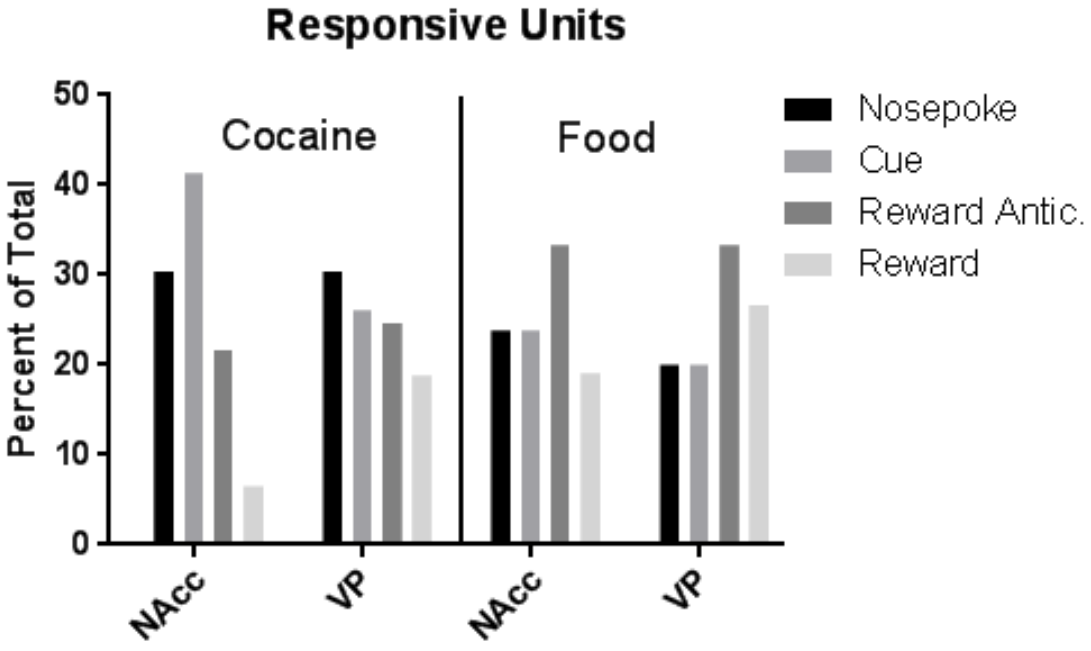
Neurons were recorded in the nucleus accumbens (NAcc) and ventral pallidum (VP). Baseline firing rate was calculated during a 5sec period prior to nosepoke for cocaine infusion/pellet delivery. There were no differences between cocaine and food self-administrations in baseline firing in the NAcc or VP. Data is presented as average +/- SEM.

Figure 3.14: Proportions of Food and Cocaine Neurons in Relation to Self-Administration Task



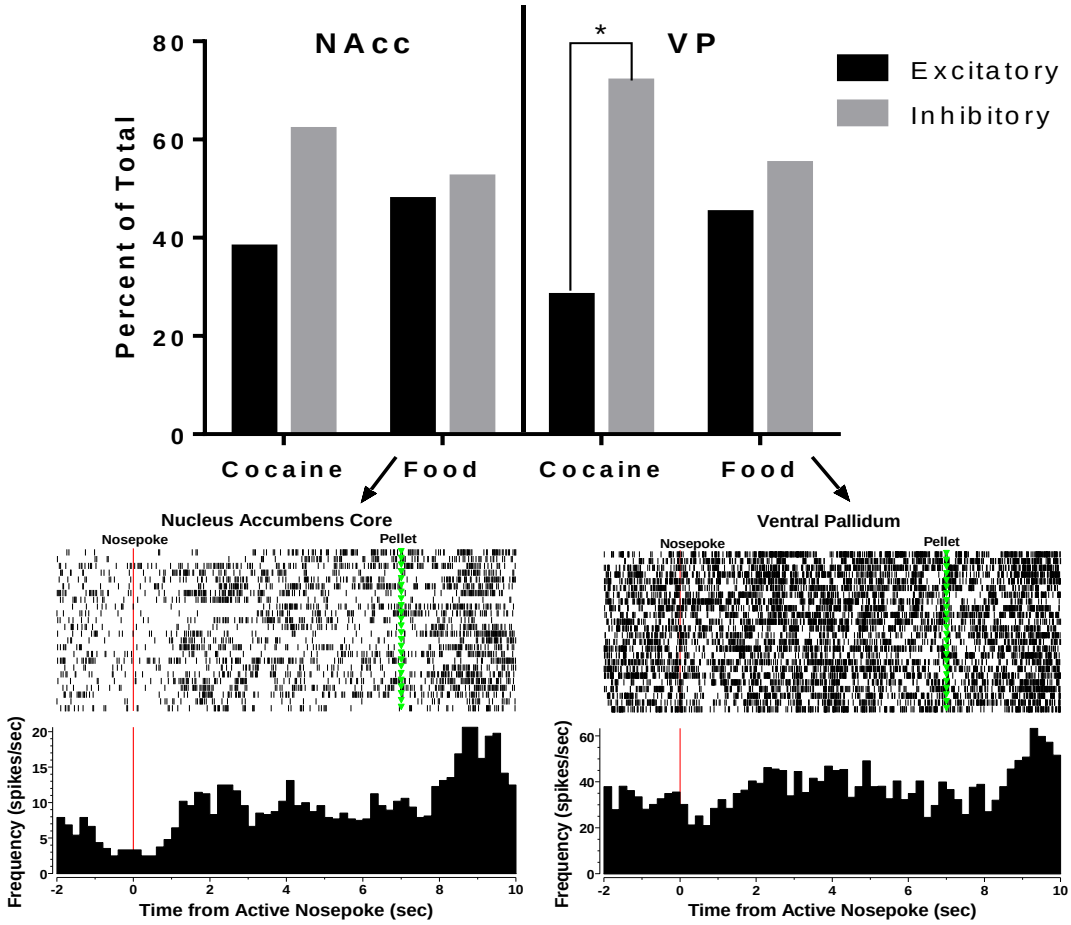
Cocaine (TOP) and Food (BOTTOM) neurons were analyzed for responses one or more events: nosepoke, cue, reward anticipation, and reward receipt. There were significantly more units responding to multiple events in food vs. cocaine self-administration. *$p<0.05$

Figure 3.15: Events of Responsive Neurons to Food and Cocaine Self-Administration



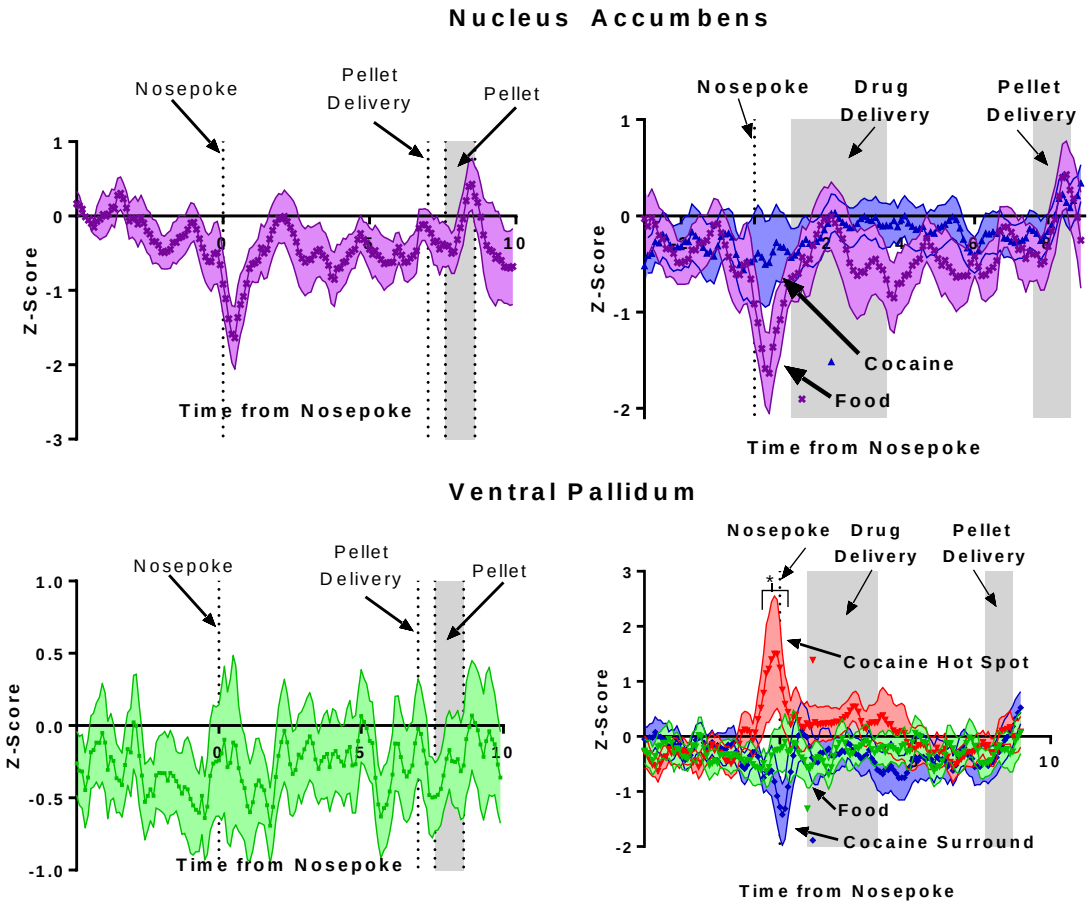
Neurons were analyzed to be responsive to active nosepoke, cue, reward (pellet or cocaine) anticipation, and reward delivery in the nucleus accumbens (NAcc) and ventral pallidum (VP). There were no differences in the number of neurons responding to each event between food and cocaine self-administration.

Figure 3.16: Response Types for Food and Cocaine Self-Administration



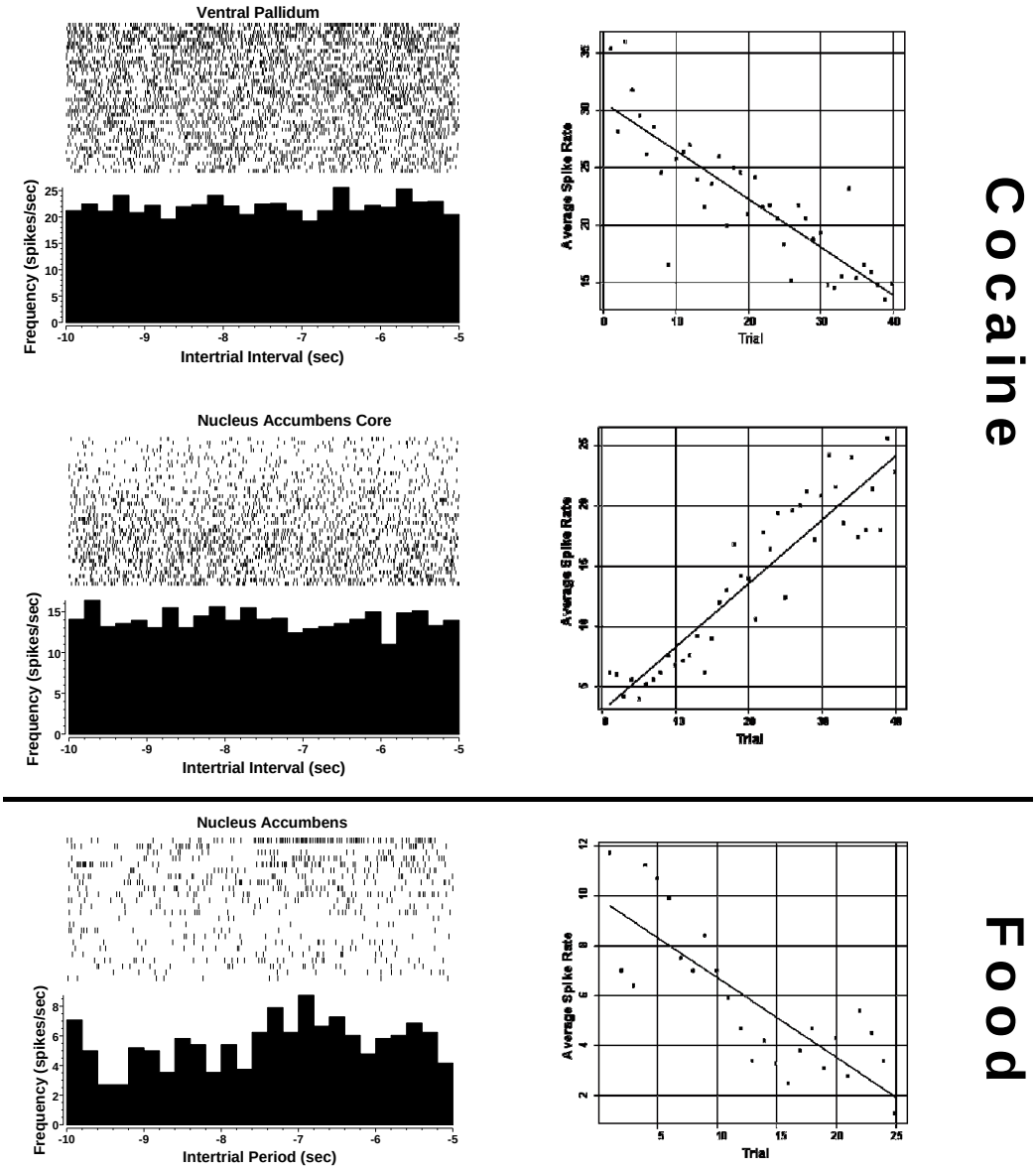
Neurons were analyzed for increase (excitatory) and decrease (inhibitory) responses compared to baseline in the nucleus accumbens (NAcc) and ventral pallidum (VP). (TOP) The proportions of excitatory and inhibitory neurons did not differ between food and cocaine sessions in the NAcc. In the VP there were significantly more inhibitory responses in cocaine self-administration sessions compared to food (*$p<0.05$). (BOTTOM) Neuron from the NAcc (LEFT) was aligned to nosepoke and shows an inhibitory response to nosepoke that returns to baseline 1sec after and shows excitatory response to food pellet. Neuron from VP (RIGHT) shows inhibition to cue event only.

Figure 3.17: Magnitude Changes in Food Self-Administration Task



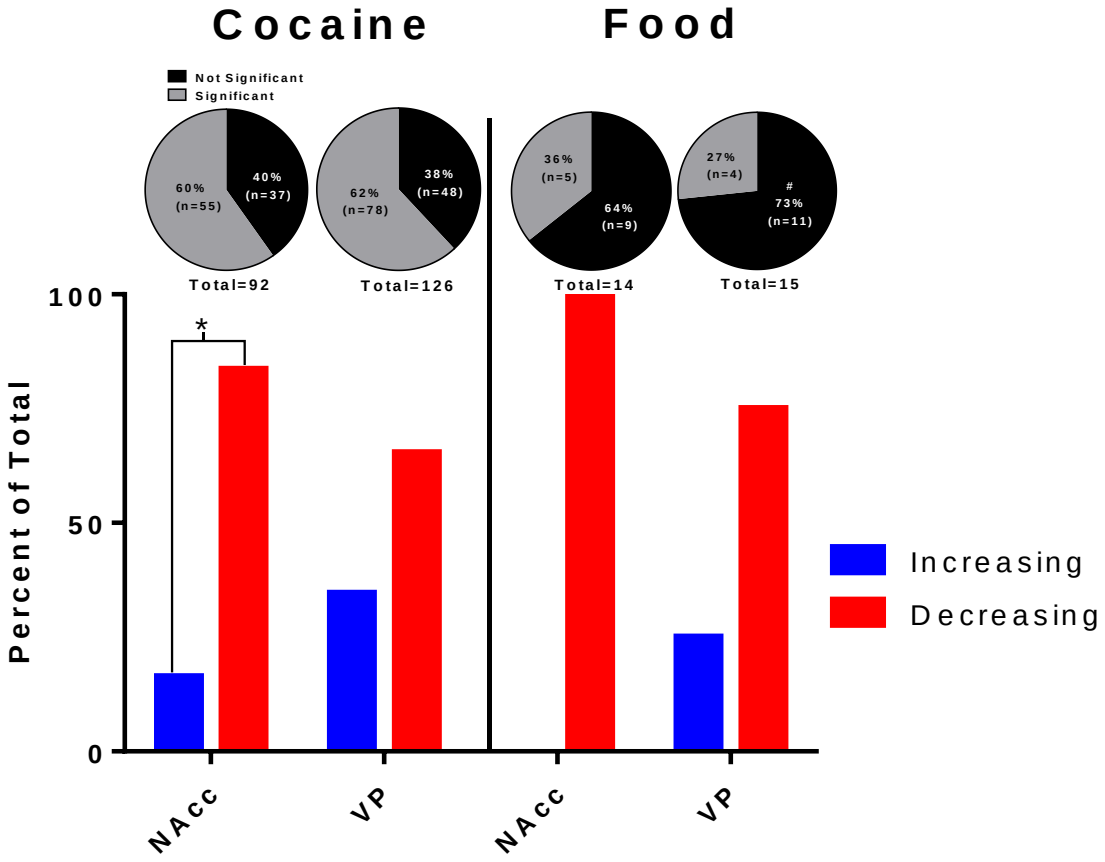
Neurons that were responsive to the food self-administration task were normalized (Z-score) and averaged in the nucleus accumbens (TOP) and ventral pallidum (BOTTOM). Neurons in the nucleus accumbens showed a strong inhibition to nosepoke and excitation to pellet (LEFT). Changes in firing did not differ from neurons responding to cocaine self-administration (RIGHT). Neurons of the ventral pallidum showed excitatory and inhibitory responses throughout food self-administration task. These changes were significantly different from those seen in neurons in the VP hot spot during cocaine self-administration sessions (*$p<0.05$).

Figure 3.18: Baseline Firing Rates Change Over Session



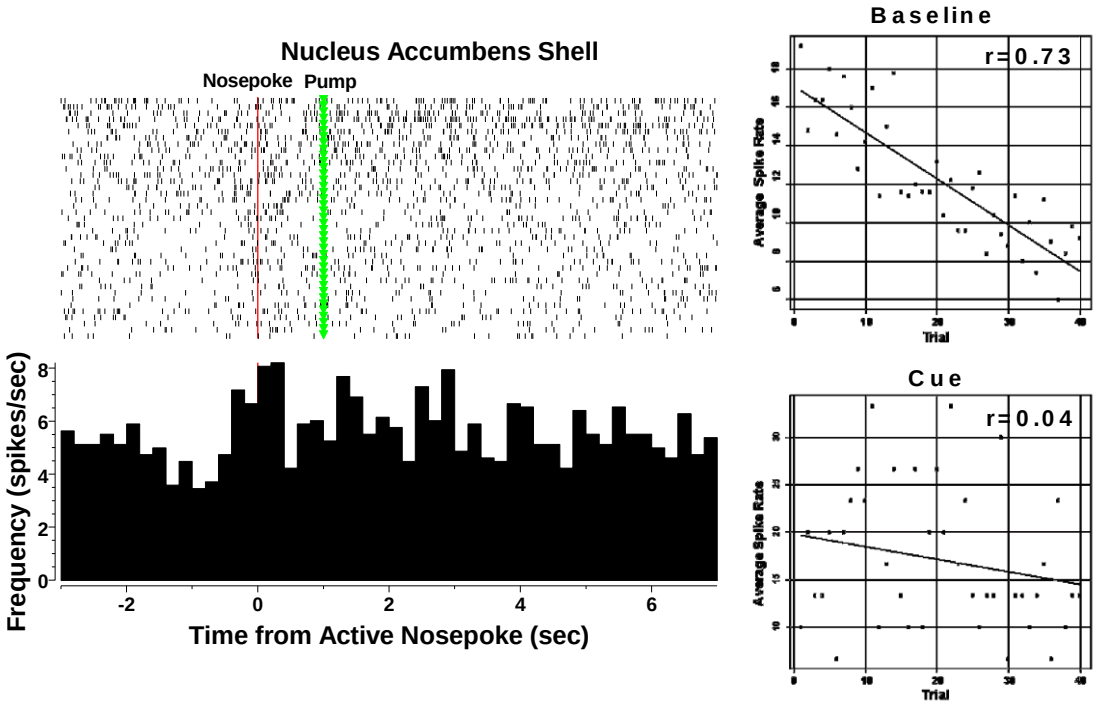
Neurons from cocaine and food self-administration sessions were analyzed for firing rate changes. Neurons showed both increasing and decreasing baseline rates during cocaine sessions (TOP), while they were primarily decreasing in food session (BOTTOM). Neurons were aligned and analyzed during a 5sec period of time prior to active nosepoke.

Figure 3.19: Changes in Baseline Rate During Self-Administration Session



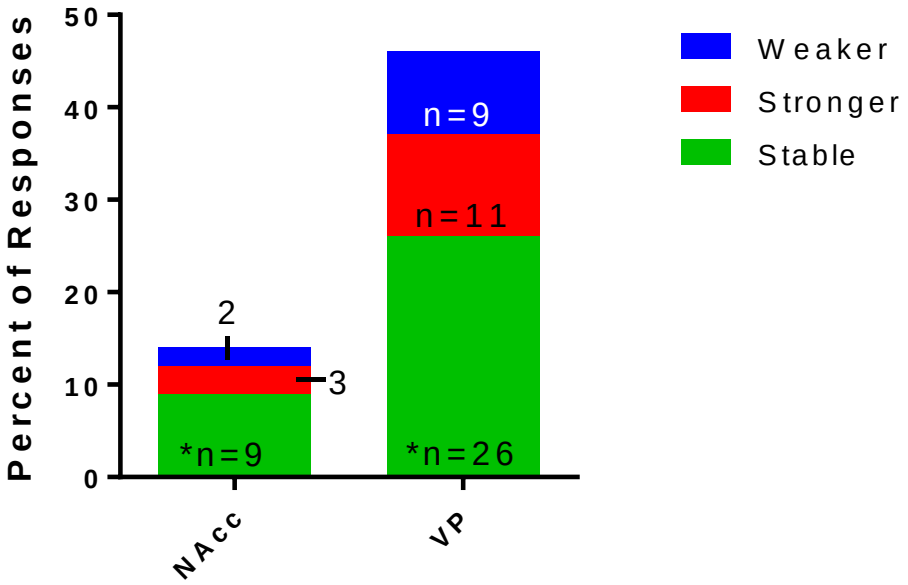
Neurons from both cocaine and food self-administration sessions showed a change in baseline firing rates over time of session in the nucleus accumbens (NAcc) and ventral pallidum (VP). More neurons from cocaine sessions showed this change than from food sessions. χ^2=2.34, #p<0.05 VP food compared to VP cocaine sessions. Further, the majority of the changes seen were a decrease in firing rate over time. There were significantly more decreasing changes in the NAcc compared to increasing during cocaine self-administration (*p<0.05).

Figure 3.20: Neurons with Baseline Rate Changes Show Stable Responding



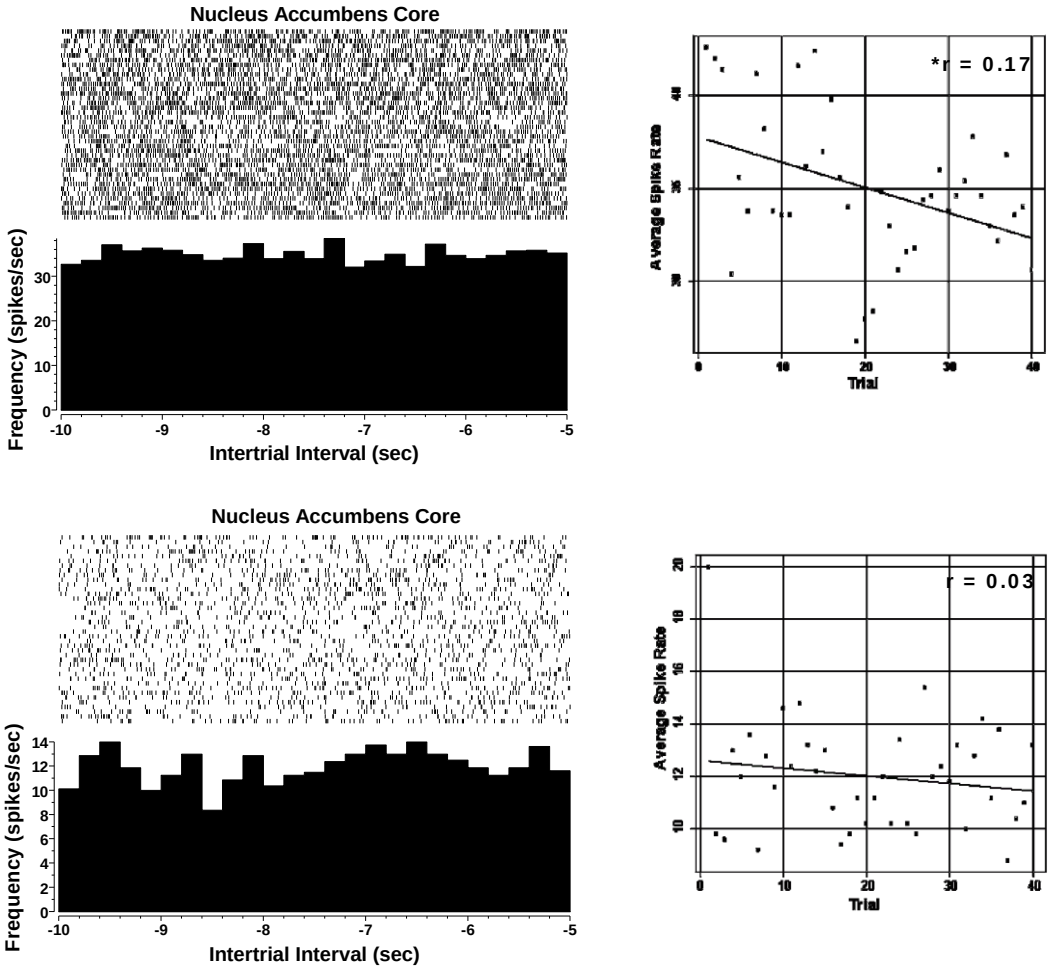
Neurons from cocaine self-administration sessions showed changes in baseline firing rate over time. Some neurons were responsive to the self-administration task. The magnitude of response of a majority of these neurons did not change. (LEFT) Responsive neuron showing excitatory response to active nosepoke. Spikes are aligned to active nosepoke; cocaine infusion (pump, green) began 1sec later. (TOP RIGHT) Linear regression of baseline firing, showing a decreasing number of spikes per trial of cocaine self-administration (*$p<0.05$). (BOTTOM RIGHT) Linear regression (ns) during time period for cue event (+100ms to +400ms from active nosepoke) showing no change in firing. Spikes were normalized (S/B, S = signal, B = background) on a trial basis and plotted over time of self-administration session.

Figure 3.21: Responses to Self-Administration are Stable



Baseline firing rates changed over cocaine self-administration sessions. Some changes were seen in neurons responding to the self-administration task. These were analyzed to determine whether signal strength changed over the session as well. A significant proportion of neural responses remained unchanged (*$p<0.05$, compared to stronger and weaker responses) both in the nucleus accumbens (NAcc) and ventral pallidum (VP).

Figure 3.22: Baseline Changes Not Due to Electrode Movement



Baseline firing rate changes were seen in the majority of neurons recorded during cocaine self-administration. The neurons were assessed for indications of artefactual causes, like electrode movement. Here we present 2 neurons recorded from the same wire in the nucleus accumbens core. (TOP) Neuron showing a significant linear regression (decreasing baseline, *$p<0.05$). (BOTTOM) Neuron showing no change in baseline firing. Time period is during the intertrial interval, prior to a nosepoke for cocaine.

DISCUSSION

The response patterns of the nucleus accumbens (NAcc) and ventral pallidum (VP) differed in response to food and drug cues, particularly in the hot spot of the VP. In particular results established 1) compared to food responses, significantly smaller populations of neurons were responsive to drug rewards; this was true in both the NAcc and VP, 2) high dose cocaine self-administration evoked firing rate magnitudes that were higher to the incentive cue, particularly in the VP hotspot, 3) baseline firing rates drifted during cocaine self-administration, and 4) even with this changing baseline, the relative signal strength to background in responsive events remained constant.

Behaviors of sign-trackers (STs) and goal-trackers (GT) also remained consistent between subjects in either food or drug administration sessions. The cue light illuminates upon nosepoke and serves as a reinforcement cue facilitating self-administration behavior (Deroche-Gamonet, Piat, Le Moal, & Piazza, 2002; Schenk & Partridge, 2001). It does not have predictive properties, but it may have incentive value (Deroche-Gamonet et al., 2002). As sign-trackers have been shown to place greater incentive value on reward-associated cues, we anticipated a greater number of active nosepokes in STs than GTs, but this was not the case. In a previous study analyzing effects of a reinforcement cue on self-administration, number of nosepokes for cocaine and infusion rates were similar between STs and GTs during the acquisition and maintenance stages and only differed upon cue removal (Saunders & Robinson, 2010). Studies have also shown that the cue light plays an important role in the acquisition of self-administration behavior, but not maintenance (Deroche-Gamonet et al., 2002). At the time of testing, self-administration behavior had been well established and is likely to contribute to the behavioral similarities seen in STs and GTs.

One surprising finding was the dramatic difference in number of responsive neurons in food and cocaine self-administration sessions. Carelli and colleagues have found that the majority of neurons in the NAcc (93%) respond to either 'natural' (food or water) or cocaine reinforcement but not both (Carelli, Ijames, & Crumling, 2000; Carelli & Ijames, 2001; Carelli & Wondolowski, 2003; Carelli, 2002). They argue that drugs of addiction tap into the reward circuit of 'natural' rewards, eliciting the same responses, only from a unique set of neurons. Results from this study seem to support such a statement, though I did not directly test this idea as our food and drug recordings were done in separate animals. Overall, cocaine rewards activated smaller proportions of neurons compared to food (34% vs. 63%). Despite the difference in overall responses, the percentage of neurons responding to the different events of self-administration was similar in food and drug sessions. This suggests that cocaine is not changing *how* the neurons of the NAcc and VP respond, rather, cocaine is altering the population coding of this neural representation, or perhaps more appropriately, activating different neural populations. These results seem to support that cocaine takes over the excitability of a subset of neurons in both the NAcc and VP. It may be that in doing so, dopamine is filtering the neural responses to strengthen those that modulate initiation and maintenance of drug-related behavior, as proposed by others (Peoples, Lynch, Lesnock, & Gangadhar, 2004). Additionally, the self-administration paradigm used here requires the subject to nosepoke to receive a drug infusion. This type of low motor paradigm may explain the lower number of responsive neurons and may add to the discrepancies of our results.

Time periods that subjects are allowed to self-administer cocaine also plays a large role in dopaminergic changes seen in the NAcc and VP (Calipari et al., 2013) as does dose of self-administered cocaine (Pettit & Justice, 1991) and may play a role in decreased responsiveness seen in these neurons. Long access, as was allotted here, has

been shown to facilitate tolerance, rather than sensitization (Calipari et al., 2013). As the total accumulation of cocaine increases, the ability of cocaine to inhibit dopamine clearance decreases, resulting in an overall reduction in extracellular dopamine (Calipari et al., 2013) and ability of dopamine to activate neurons of the NAcc. Others have seen that such an extended access paradigm makes cocaine less efficacious at activating the NAcc (Macey, Rice, Freedland, Whitlow, & Porrino, 2004). This could be another reason for the low percentage of responsive units.

One goal of this research was to elucidate representation of incentive salience in neural mechanisms. I found the proportions of neurons in the core were more responsive to cue while the shell was more responsive to the nosepoke event during self-administration of cocaine, results that have been seen in other studies as well (Ito et al., 2000; Owesson-White et al., 2009; Phillips et al., 2003; Saunders, Yager, & Robinson, 2013). In one study, a cue light previously associated with self-administered cocaine was presented non-contingently, which led to increase extracellular dopamine only in the core, not shell of the NAcc (Ito et al., 2000). Further, when a dopamine antagonist was injected into the NAcc core, cue-induced reinstatement was attenuated in rats self-administering cocaine (Saunders et al., 2013). The nosepoke and cue events are tightly timelocked. The nosepoke event may represent neuronal signaling to response initiation, while the cue may be one that supports maintenance of self-administration behavior. Studies have supported a role for the NAcc in initiating motor movements for cocaine self-administration, though they did not distinguish core from shell neurons (Chang, Paris, Sawyer, Kirillov, & Woodward, 1996). Others argue that the Nacc shell is important for learning behavioral sequences that result in reward delivery (Ghitza et al., 2004; Root et al., 2013). Our results do support this claim as the nosepoke event is the behavioral requirement that delivers reward, in our case an infusion of cocaine. The cue

itself, because it comes after the nosepoke event and thus does not elicit an approach, but rather reinforces the approach, could be one reason why the shell does not respond highly to cue. In this particular paradigm, the nosepoke is more predictive of reward, and supports the role of the NAcc shell found by others (Fabbricatore, Ghitza, Prokopenko, & West, 2010; Ghitza, Fabbricatore, Prokopenko, Pawlak, & West, 2003; Root et al., 2010). These support an important role for both the core and shell in self-administration behavior.

Results also demonstrated a dose effect of cocaine in that a high dose of cocaine produced greater magnitude of firing to nosepoke and cue events in the NAcc shell and VP but not core. This drug effect may be a result of the way cocaine activates specific receptors in the NAcc. Other studies have shown that activation of the NAcc core through intracranial injection of dopamine resulted in increased firing in VP neurons, specifically in the region now classified as a hot spot in the VP (Smith & Berridge, 2005; Yang & Mogenson, 1989). Further, when D1 receptors were activated prior to D2 receptors (using agonists), the magnitude of firing was greater in VP neurons than when activating D1 receptors alone (Yang & Mogenson, 1989). Results shown here with cocaine suggest that perhaps low doses of cocaine activate only D1 receptors in the NAcc, while higher doses activate both receptor types. There is evidence that increasing self-administered dose of cocaine results in corresponding increases in extracellular dopamine (Pettit & Justice, 1991) which impacts the binding of dopamine receptor types. This is consistent with the reports of the differences in affinity for dopamine in D1 and D2 receptors. D1 receptors have been shown to have a low affinity for dopamine, while D2 receptors have a high affinity (Di Chiara, Morelli, & Consolo, 1994; Jarvie & Caron, 1993). In order for D2 receptors to be activated, D1 receptors must first be saturated (Yang & Mogenson, 1989). It may be that extracellular concentrations for dopamine resulting from low dose of

cocaine self-administered may not be high enough to activate D2 receptors to the same extent as a high dose of cocaine.

The opposite excitatory and inhibitory effects of high dose of cocaine on VP neurons in the hot spot and surround may be due to differences in D1 and D2 receptor ratios in the NAcc core and shell. The magnitude of firing within the VP was similar, only the "hot spot" produced primarily excitatory responses and the surround produced primarily inhibitory responses. The core and shell has been shown to project to distinct subregions in the VP (Root et al., 2013) with the core projecting to what we call the "hot spot" and the shell projecting to what we call "surround". Others have also shown that dopamine and cocaine cause a hyperpolarization of D1 receptors and depolarization of D2 receptors (Nicola et al., 2000; Uchimura, Higashi, & Nishi, 1986; Uchimura & North, 1990). Activation of receptors causes opposing effects on acetylcholine (Ach) release with D1 receptors increasing Ach release while D2 receptors inhibit it (Bertorelli & Consolo, 1990; Consolo, Girotti, Russi, & Di Chiara, 1992), which will have opposite impact on firing patterns of projection neurons. As we did not analyze receptor densities, we are unable to conclude such a statement, but it warrants further analysis.

The high proportion of significant linear regression results show evidence of a negative feedback system within the mesolimbic circuit. Prior research has postulated that neurons of the nucleus accumbens (NAcc) feed back to the ventral tegmental area (VTA) and synapse with both dopamine (DA) and γ-aminobuteric acid (GABA) neurons to regulate neural firing (Einhorn et al., 1988). This can result in an increase in firing rate, when synapsing with DA neurons, or decrease, when synapsing with GABA neurons. Such results were seen in the current research, and we further show that the feedback is transmitted to the downstream ventral pallidum (VP). Prior research has shown a recovery from such feedback mechanisms after roughly 8 minutes (Einhorn et al., 1988).

The rate changes in this study were observed continuously over hours of self-administration. I refer to this extended change as "neural drift", the change in baseline firing rates of neurons. The change was not observed in all neurons, nor was it restricted to a single region or population of neurons. In fact, 60% of all neurons analyzed were shown to have a linear change in firing rate. Other studies have found similar percentage in neurons exhibiting a change in firing, though these studies compare the entire self-administration session (of nicotine) to a single baseline level (Guillem & Peoples, 2011). In both the VP and NAcc core, the majority of units showed a decreasing regression, whereas in the NAcc shell, increasing and decreasing patterns were equal. The results seen here seem to be specific to cocaine as very few responses were seen in neurons from food self-administering sessions. However, one study looking at the changes to sucrose also found changing baseline rates, with equal proportions of increasing and decreasing linear regressions of units (26% vs 20% respectively, Peoples et al., 2011).

One explanation for this "neural drift" could be due to DA release from the VTA. Voltammetry studies have shown that extracellular DA release from the VTA into the NAcc shell remains elevated through reward-seeking paradigms for as long as 9sec after reward has been obtained (Ito et al., 2000). This may allow longer excitations of post-synaptic DA receptors and account for the higher number of neurons showing an increase in neural drift. Cocaine blocks reuptake of dopamine, allowing extracellular dopamine to send signals longer. Studies have indicated that as a result, neurons begin to express dopamine autoreceptors to help regulate levels of neurotransmitter release. The NAcc core expresses such receptors more than the shell, allowing dopamine to diffuse longer distances in the shell region (Phillips et al., 2003).

Chapter 4: Modulation of Dopamine Neurons in the VTA Affects Cue-Driven Behaviors in a Pavlovian Task.

INTRODUCTION

Rats express differences in their motivation to approach and interact with learned reward-paired cues. Pavlovian conditioning in which an approachable cue (a lever) precedes a reward by a few seconds will expose these individual differences. As rats learn the association of a cue and a paired reward, they diverge into different groups. Some rats approach and interact with the Pavlovian cue when its presented (sign-trackers) while other individuals respond to the cue by moving to and engaging the reward delivery apparatus (goal-trackers). The natural expression of divergent cue-motivated behavior suggests there must be a neural circuitry difference between the groups of animals that underlies their differences in behavioral tendencies. To investigate these circuit differences, the impact of the ventral pallidal influence on the ventral tegmental area (VTA) was examined. Specifically, how does altering information flow in this neural pathway affect behaviors regulating reward acquisition?

Studies have shown that STs and GTs differ in dopamine release in the nucleus accumbens core, with STs releasing more dopamine following presentation of Pavlovian cues than GTs (Flagel et al., 2007). In Chapter 2, I showed that firing patterns of ventral tegmental dopamine neurons are more active in STs than GTs to presentation of incentive cues. These studies demonstrate an important role for dopamine in cue-induced reward-seeking behavior. One of the main afferent inputs to the ventral tegmental area is the ventral pallidum, specifically from the ventromedial subregion (VPvm) (Zahm & Heimer, 1988; Zahm, 1989). A large portion of the VPvm lies in the rostral pole of the VP. The VPvm region has shown a role in motivated behaviors. In particular, studies utilizing a

Pavlovian instrumental transfer paradigm have shown activation of rostral VP neurons projecting to the VTA are important in motivated behavior related to Pavlovian cues (Leung & Balleine, 2013, 2015). The rostral VP has also been implicated in cue-induced reinstatement of drug-seeking behavior (Mahler & Aston-Jones, 2012; Mahler et al., 2014). Due to the important role the rostral VP plays in modulating reward-seeking behaviors related to Pavlovian cues, it was the target for this study.

The VP is made up primarily (~80%) of GABAergic neurons (Gritti, Mainville, & Jones, 1993) that can be either projection neurons or interneurons (Pang, Tepper, & Zaborszky, 1998). The remaining neurons are cholinergic (Kupchik & Kalivas, 2013) and very few (~2.5%) are glutamatergic (Geisler et al., 2007). Modulation of VP GABAergic neurons influences firing of ventral tegmental dopamine neurons, which in turn affect downstream activity in the nucleus accumbens. Neurons of the ventromedial VP provide tonic inhibitory signals to the VTA through GABAergic innervations (Floresco et al., 2003; Root et al., 2012). The VP acts to regulate populations of active VTA dopaminergic neurons and as a result may alter tonic dopamine release in the nucleus accumbens (Floresco et al., 2003). Inhibition of the ventral pallidum has been shown to cause an increase in the number of dopamine neurons firing (population) in the VTA, which was followed by increase in the release of dopamine in the nucleus accumbens (Floresco et al., 2003).

Most studies that look at how firing patterns of dopamine neurons alter dopamine release use electrical or chemical stimulation, which would be expected to activate the entire population of dopamine neurons. It would be difficult to determine which brain structures or sets of neurons were contributing to the behavioral effects under this non-specific paradigm. An advantageous way to analyze how modulation of a specific subset of dopamine neurons will alter downstream dopamine release is through manipulation of

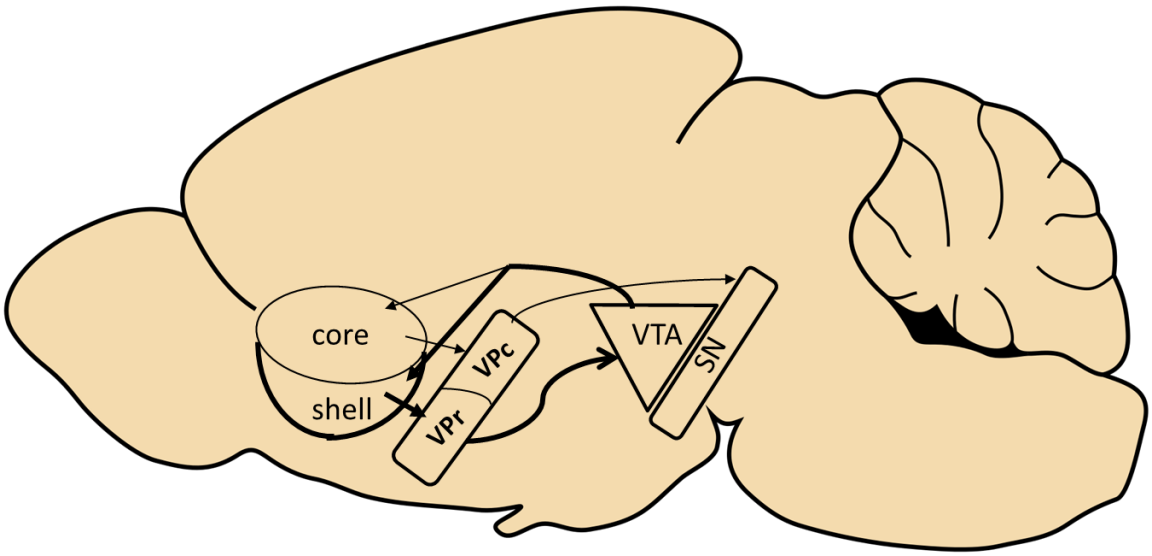
endogenous neural pathways. This study utilizes such an approach using designer receptors exclusively activated by designer drugs (DREADDs) and analyzes the effects DREADD-activated circuits have on behaviors directed towards Pavlovian cues. The DREADDs used in this experiment are G_i-protein coupled proteins that mimic the signaling cascade of GABAergic and glutamatergic neurons (Armbruster, Li, Pausch, Herlitze, & Roth, 2007; Pin, Galvez, & Prézeau, 2003). Specifically, these inhibitory DREADDs activate potassium channels causing a hyperpolarization and neuronal silencing (Armbruster et al., 2007; Pin et al., 2003).

The genetic material encoding DREADDs are delivered to neurons through viral vectors. With these viral vectors, specific cell types were targeted via their efferent and afferent connections. Then, by injecting an otherwise innocuous drug, clozapine-n-oxide (CNO), DREADDs located in target neural groups can be activated. DREADD activation can cause excitation or inhibition of neurons, depending on the type of agent used. CNO does not show any appreciable affinity for any other receptors and remains highly unchanged upon injection in rodents and humans (Armbruster et al., 2007). This study used a dual vector approach to specifically target DREADD expression in neurons projecting from the rostral VP to the VTA. The neural manipulation in this study, in combination with behavioral scoring, will give an indication as to how the circuitry is controlled and how the key areas of reward are related.

The current study aims to observe how changes in VP to VTA firing patterns affect cue-related rat behavior to determine the specific role of this distinct pathway (Figure 4.1). By inhibiting the rostral VP during Pavlovian conditioning, I expect to increase the incentive salience of reward-paired cues by the mechanism of an increase in population coding of ventral tegmental dopamine neurons and tonic dopamine release in the NAcc. Therefore, I hypothesize that DREADDs will cause goal-trackers to attribute

greater incentive salience to the cue and initiate sign-tracking behaviors. I expect to see the same trend in sign-trackers with more intense sign-tracking behavior, but the change will be less than in goal-trackers, as STs already attribute incentive salience to reward-related cues.

Figure 4.1: Simplified Mesolimbic Reward Circuit



Dopamine neurons from the ventral tegmental area (VTA) project to the nucleus accumbens core and shell. Neurons of the core and shell project to, respectively, the caudal dorsolateral region of the ventral pallidum (VPc) and the rostral ventromedial ventral pallidum (VPr). The VPc then sends neural efferents to the substantia nigra (SN), while neurons of the VPr project to the VTA. Work from our lab and other studies have shown a distinct role for the VTA-shell-VPr (thick black arrows) in coding motivation towards reward-paired cues. Viral vectors of the current study target this microcircuit, specifically neurons that project from the VPr to VTA.

METHODS

Animals and Care:

A total of 26 male Sprague Dawley rats were used with an initial weight of 200-250g (Charles River, Wilmington, MA). Males were housed in a reverse light:dark (14:10) cycle with lights off at 10:00. Upon arrival, they received 2 days to habituate to their new surroundings. They remained in pairs for duration of study. All testing was performed during the dark cycle, between 10:00-18:00 with water and food available *ad libitum* throughout the study (except while in testing chamber). All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA) and Institutional Animal Care and Use Committee (IACUC).

Pavlovian Conditioned Approach (PCA):

This paradigm has been shown to effectively identify the behavior of many types of mammals (rats, mice, voles) in terms of their level of attributing incentive salience to cues (see Anselme, 2015). In this procedure, animals are placed in a metal and Plexiglass chamber situated with a house light and white noise speaker on one wall. Opposite that in the center, approximated 1 cm from the floor is a magazine for food delivery. To the left or right (placed randomly for each animal) roughly 6cm from the floor is an illuminated retractable lever. Session began with illumination of house light and white noise.

Training on Day 1 began with 25 trials to familiarize the animals to delivery of banana-flavored food pellets (BioServ, Frenchtown, NJ) into the magazine. During magazine trials, pellets (unconditioned stimulus, UCS) were delivered into the magazine on a variable time 30 schedule (average 30 sec, range 15-45 sec). PCA training followed magazine training for 5 days. The Pavlovian trial had a predictive cue, consisting of an illuminated lever (conditioned stimulus, CS) inserted through the wall into the cage for 8

seconds. The reward pellet was released at the moment the lever was retracted and delivered into the magazine 600msec later. Note that pellet delivery required no response by subject. Trials were presented on a variable time 90 schedule (average 90 sec, range of 30-150 sec).

At the end of each training session, animals were returned to their home cage. All subjects learn the predictive nature of the cue (Flagel et al., 2007). Some direct their attention towards the lever during presentation (sign-trackers, STs, n=10), while other direct their attention towards location of pellet receipt (goal-trackers, GTs, n=10). Still others oscillate between both (intermediates, n=6).

PCA indexing:

Sign-tracking and goal-tracking phenotypes are apparent and stable by 4-5 days of training (Flagel et al., 2007), and can be quantified by calculating PCA index (Meyer, Lovic, et al., 2012). The PCA index is determined by (a) latency difference [(time to approach magazine during CS – time to approach lever)/8], (b) response bias [(# lever deflections - # magazine entries)/(# lever deflections + # magazine entries)], and (c) approach probability difference [(probability of contacting lever – probability of contacting magazine)]. A score of <-0.5 indicates a GT phenotype, >+0.5 indicates a ST phenotype and -0.5 to +0.5 indicates an intermediate phenotype (INT) (Figure 4.2).

Viral Vector Infusion:

We used a dual vector approach to target specifically the neurons that project from the VP to the VTA. An adenovirus-free adeno associated virus containing inhibitory DREADDs with a double floxed inverted open reading frame (rAAV8/hSyn-DIO-hm4D (Gi) mCherry) was injected into each hemisphere of the rostral VP (University of North Carolina Vector Core, Chapel Hill). These DREADDs express the fluorescent marker

mCherry and requires an enzyme Cre-recombinase in order to be expressed in cells. A second canine adenovirus virus containing Cre-recombinase (CAV-Cre) was injected into the VTA (Montpellier Vector Platform, Montpellier, France). This type of virus is retrogradely transported (Soudais, Laplace-Builhe, Kissa, & Kremer, 2001). Using a dual vector approach, only those cells that take up both DREADDs and CAV-Cre viruses express inhibitory receptors (Boender et al., 2014). These neurons were targeted by a systemic injection of an otherwise inert drug clozapine-N-oxide (CNO) (NIDA).

Animals were anesthetized using 2% isoflurane and secured to an apparatus. Hair from the top of the skull was removed and a local analgesic was injected subcutaneously. A 2 inch incision was made in a rostral-caudal direction started from between the eyes and the skull was exposed. Bregma and lambda were identified and coordinates were calculated so that the dorsal ventral measurements were within 10μm to ensure head was level. A 1mm craniotomy was created over the rostral VP at AP: +0.42, ML: +/- 1.7, DV: 8.0, and VTA at AP: -5.1, ML: +/- 1.0, DV: 7.0 for a total of 4 craniotomies. A volume of 0.5μl of virus was injected using a Hamilton microinjection syringe, with DREADD virus selectively injected into the VP and CAV-Cre virus selectively into the VTA. Viruses were injected slowly at a rate of 0.2ul/min using an electronic pump. The syringes were left in place for an additional 15min following injection. Subjects were sutured with silk sutures using a continuous stitch method or using staples. For two days following surgery, rats were given intra-peritoneal (ip) injections of penicillin (0.1 mL) and flunixin (2.5 mg/kg) to prevent infection and provide pain relief, respectively. Following surgery they returned to their homecage and rested for 2-3 weeks.

Experiment 1:

Animals (n=12) were first trained on a Pavlovian conditioning task and analyzed for their propensity to approach cue or location of reward delivery. Following training, they underwent surgery for implant of designer receptors exclusively activated by designer drugs (DREADDs). Following a 3-week incubation, subjects underwent testing with DREADD activation of using clozapine-n-oxide (CNO) (Figure 4.3).

Behavioral Testing and Analysis:

In a previous experiment, a subset of animals were injected with DREADDs and euthanized at 2 weeks (n=2) or 3 weeks (n=2) to determine incubation time necessary to observe DREADD expression. We found good expression (both at cell body and projections) at 3 weeks. Thus, following viral implant, subjects rested undisturbed for 3 weeks in their home cage. One week of testing followed. During testing procedures, subjects were first given 25 lever/pellet presentations as in PCA training. Immediately after they were given an injection of either CNO (3.0mg/kg, ip) or saline (volume yoked to CNO treatment), and another 25 lever/pellet pairings followed. Therefore, each session consisted of 25 trials (referred to as "pre-injection" or "baseline" trials), followed by another 25 trials (referred to as "post-injection" or "treatment" trials) that occurred when CNO or saline were on board.

Effects of CNO-activated DREADDs on changes in neural firing were previously observed 20 min after a systemic injection and effects lasted 25-60 min (Mahler et al., 2014; Scofield et al., 2015). To determine effects of DREADDs on behavior, within-subject comparisons were made between the first 10 post-injection trials and the last 10 post-injection, when CNO is expected to be modulating neural firing. Comparisons were made in CNO and saline sessions using Holm-corrected paired t-tests.

Pre-injection and post-injection behavior was also compared on testing days 1 and 7 in 5-trial blocks for: 1) Probability to approach lever or magazine, 2) latency to lever press or enter magazine, and 3) average lever contacts and magazine entries per trial. A two-way (treatment x time) Repeated Measures ANOVA and Holm-corrected post-hoc tests were performed for each variable. PCA Index scores were compared across all days of testing following injection of either saline or CNO. Significant changes in scores were compared across days using a linear regression analysis. Slopes were also compared between phenotypes (ST, INT and GT).

Based on the expectation that all animals were hypothesized to show more sign-tracking characteristics with DREADD activation, we grouped intermediates with goal trackers for analysis and contrasted those findings to sign trackers. This is a conservative combination as intermediates have characteristics closer to sign trackers and combining them with goal trackers only diminishes the group average impact of changes toward sign tracking.

Experiment 2:

Animals (n=14) first received implant of inhibitory DREADDs targeting VP neurons projecting to the VTA. Following a 2 week incubation period, subjects underwent Pavlovian conditioned approach (PCA) training determine initial phenotype. Immediately following, subjects were tested in a Pavlovian task for an additional 2 weeks of PCA testing combined with injection of either CNO or saline. Over one week animals received injections of CNO, and one week they received injections of saline. Animals were randomized as to whether they received CNO in the first week or second (Figure 4.4).

Behavioral Testing and Analysis:

During testing procedures, subjects were first given 25 lever/pellet presentations, then they were given an injection of either CNO (3.0mg/kg, ip) or saline (yoked to CNO treatment). Another 25 lever/pellet pairings followed. After 1 week, treatments switched for CNO and saline injections. Latency to lever press, latency to magazine entry, total number of lever presses, and total number of magazine entries were recorded for every trial.

Statistical analyses were performed with GraphPad Prism (Version 6.06) to explore 1) probability to approach lever or magazine, 2) latency to lever press or enter magazine, and 3) average lever contacts and magazine entries per trial, and 4) PCA Index.

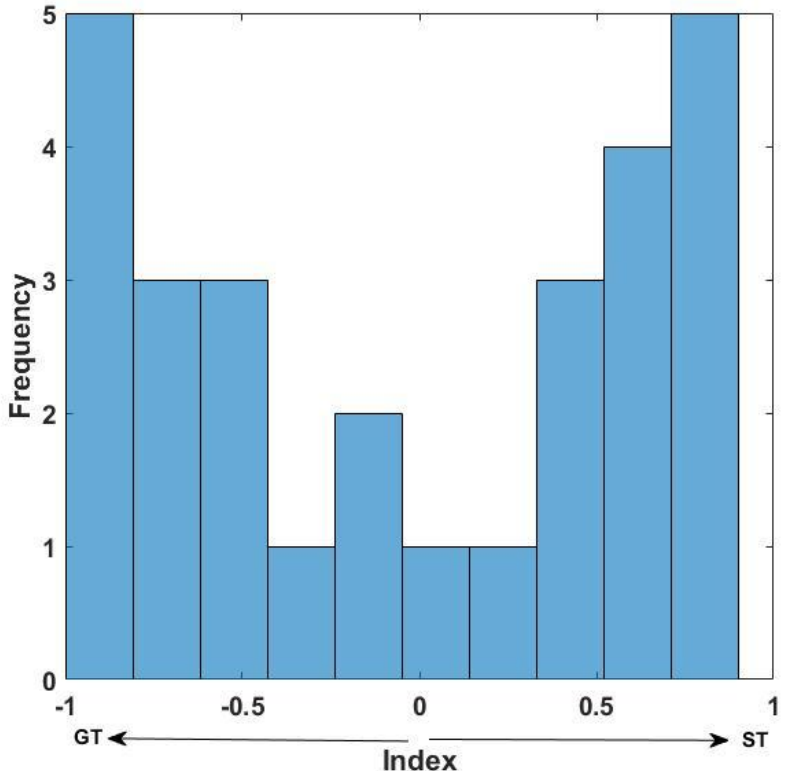
Behavioral changes during the PCA sessions were analyzed in 5-trial blocks for: 1) Probability to approach lever or magazine, 2) latency to lever press or enter magazine, and 3) average lever contacts and magazine entries per trial. We compared pre to post injection values to determine when CNO exerts greatest effects. A two-way (treatment x time) Repeated Measures ANOVA and Holm-corrected post-hoc tests were performed for each variable. PCA Index scores were compared across all days of testing following injection of either saline or CNO. Significant changes in scores were compared across days using a linear regression analysis. Slopes were also compared between phenotypes (ST, INT and GT).

End Point and Histology:

After the final testing day, subjects were euthanized using an overdose of sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals). They were perfused transcardially using PBS followed by 4% paraformaldehyde. Brains were stored in paraformaldehyde for 24 hours then transferred to 30% sucrose for at least 3 days. Brains were sliced

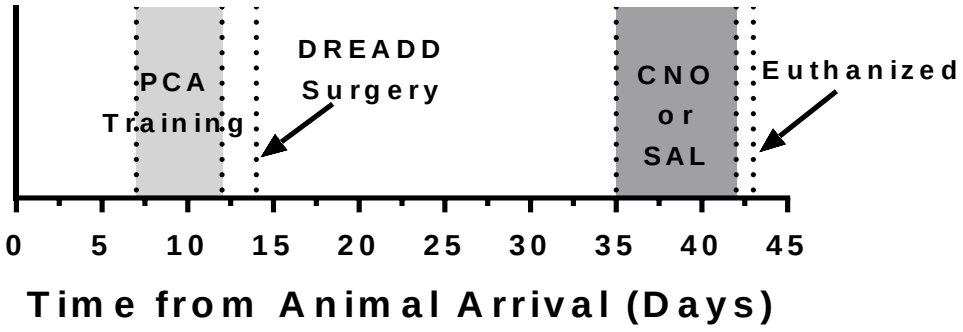
coronally and stored in cryoprotectant until analysis. Coronal sessions containing rostral and caudal VP were mounted and left to dry overnight. DREADD expression in the ventral pallidum was confirmed through fluorescent examination with help of the Paxinos and Watson brain atlas (1997) (Figure 4.5). DREADD receptors contain a mCherry tag that naturally fluoresces between 587-610nm. This allowed for the accurate assessment of CNO activation of VTA-projecting VP neurons. Behavioral analysis was only performed in animals confirmed for DREADD expression.

Figure 4.2: Distribution of Phenotypic Index



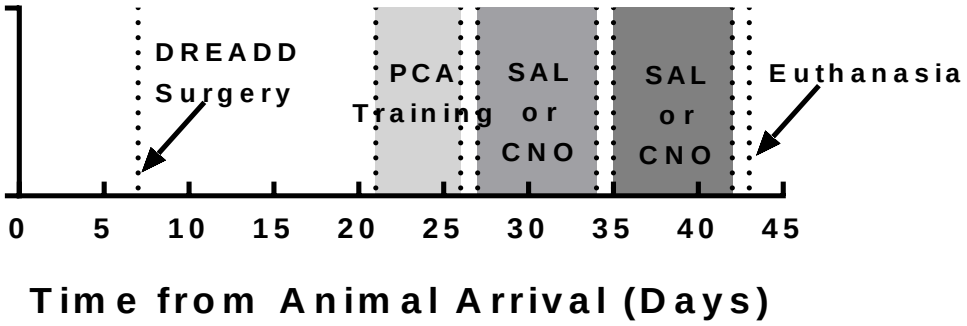
This represents all animals used in this study. Animals were trained in a Pavlovian conditioning task and behaviors to approach lever or magazine were scored to determine goal-tracking (GT), intermediate, or sign-tracking (ST) phenotypes. Scores less than -0.5 indicate a GT and scores greater than +0.5 indicate a ST phenotype.

Figure 4.3: Experimental Timeline for Experiment 1



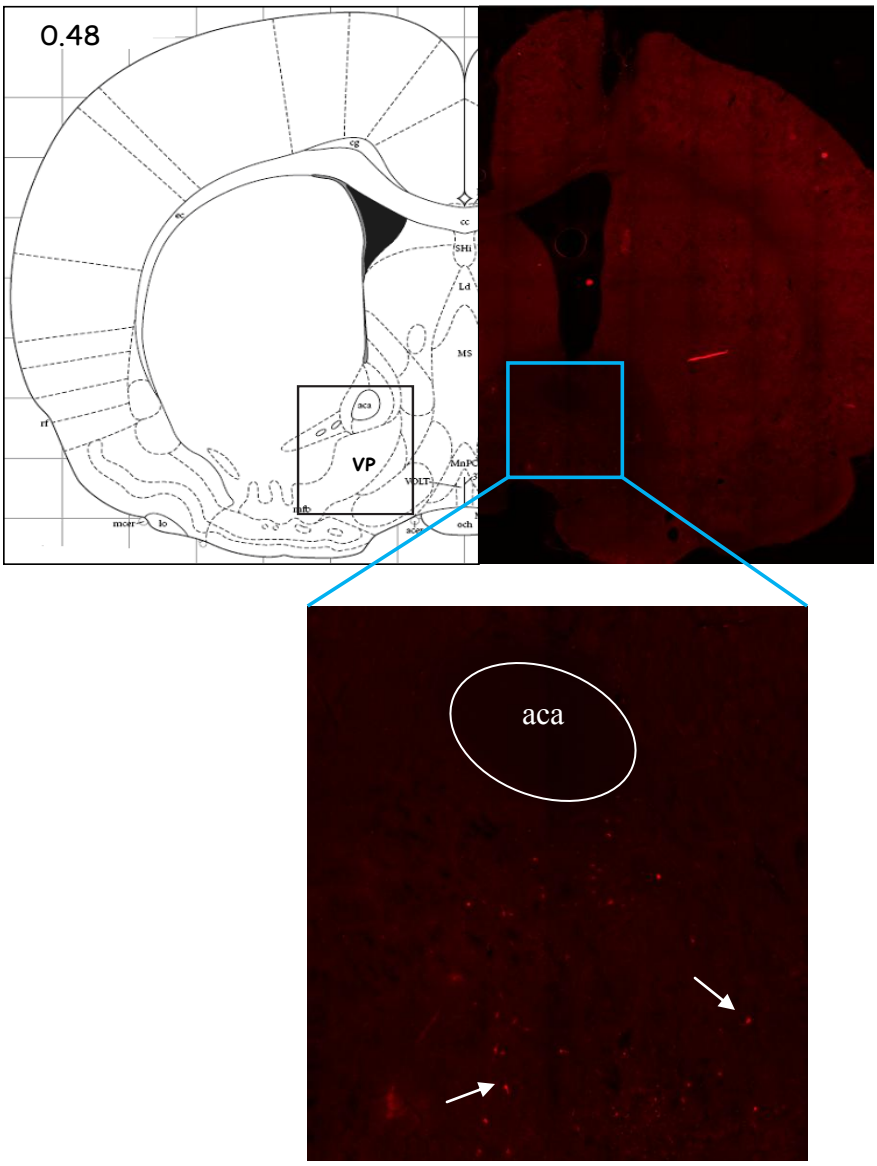
Animals arrived at our facility on Day 0 and were given 3 days to adjust to their new environment before being handled daily for 4 days. On Day 7, we began training (5 days). Implant of viral vectors (DREADDs) took place on day 14 and were given 3 weeks to incubate. Testing with either clozapine-n-oxide (CNO) or saline (SAL) began on Day 35. Subjects were euthanized on Day 43.

Figure 4.4: Experimental Timeline for Experiment 2



Animals arrived on Day 0 and were given 3 days to habituate to their environment before handling. Implant of viral vectors occurred on Day 7 and were given 2 weeks to incubate before Pavlovian training (5 days). Testing with clozapine-n-oxide (CNO) or saline (SAL) began on day 27 for 7 days and then treatment switched for an additional 7 days. Subjects were randomized as to whether they received CNO or saline first.

Figure 4.5: Visualization of DREADD virus with mCherry expression



Subjects were injected with inhibitory DREADDs and CAV-Cre viruses. Expression of viral receptors on cell bodies was visualized using antibodies targeting mCherry (1:500, Abcam, UK). Arrows point to examples of 2 cells expressing DREADDs.

RESULTS

Phenotype expression remained stable throughout Pavlovian conditioning sessions with both saline (SAL) controls and receptor activation testing with clozapine-n-oxide (CNO) injections. We compared the first 10 trials to the last 10 trials. There were no significant lever- or magazine-directed behavioral changes (Holm-corrected paired t-tests, ts=0.0-4.43, ns).

We compared within subject differences resulting from DREADD activation by comparing the last 10 trials of Pavlovian sessions prior to CNO or SAL injection to the last 10 trials of Pavlovian sessions after CNO or SAL injection. This period represents the time of peak CNO activation of DREADDs. We found no significant differences to any of the behaviors described (see Methods) in GTs (Holm-corrected paired t-test, ts=0-2.89, ns) or STs (ts=0-2.65, ns). This indicates that DREADD activation does not have an immediate significant behavioral impact on sign-tracking or goal-tracking.

Experiment 1:

Behavioral Changes Over Time:

With daily training sessions over 7 days, all animals showed a tendency to exhibit more sign-tracking-like behavior following DREADD activation. This was true for both goal-trackers and sign-trackers. The fact that sign-trackers already exhibit sign-tracking meant that the intensity change of sign-tracking behavior was more difficult to detect in face of the natural ceiling effect. Goal-trackers, on the other hand, clearly demonstrated the shift to more sign-tracking-like behavior. CNO-driven activation of DREADDs exaggerated this shift to a sign-tracking phenotype over and above the gradual shift to more sign-tracking-like behavior (Figure 4.6). A linear regression of the average change in phenotypic index scores showed significant change towards 1.0 (a perfect sign-tracker)

in STs (R^2=0.68, p<0.05) and GTs (R^2=0.66, p<0.05). The slopes of the lines were overall identical ($F_{(1,10)}$=0.03, ns) indicating rate of change was similar between STs and GTs. All subjects showed an increase in phenotypic index scores with DREADD activation. Surprisingly, GTs receiving saline injections also showed a significant change in phenotypic index scores towards 1.0 (R^2=0.76, p<0.05) (Figure 4.7). Scores of STs receiving saline injections remained constant (R^2=0.49, ns). The differences between the slopes of GTs and STs receiving saline injections were significant ($F_{(1,10)}$=14.46, p<0.01).

Behavioral Changes Toward Lever and Magazine:

Potentially due to a ceiling effect, the analysis of behavior directed towards lever or magazine did not show any significant in between STs receiving CNO or SAL treatment on days 1 or 7 (ts=0-2.09, ns). However, DREADD activation did alter the relationship between STs and GTs on these days of testing. In particular, results demonstrated significant group differences in the probability of approaching the lever both during baseline (pre-injection, Two-Way ANOVA $Fs_{(3,40)}$=6.42-21.31, ps<0.001) and treatment (post-injection, $Fs_{(3,40)}$=11.04-24.35, ps<0.001) sessions, but not to time ($Fs_{(4,40)}$=0.13-0.40, ns) nor interaction ($Fs_{(12,40)}$=0.05-0.25, ns). On Day 1, GTs showed a greater probability of contacting the lever both before (Holm-corrected t-tests, t=4.41, p<0.01) and after (t=5.85, p<0.001) CNO-driven DREDD activation compared to GTs receiving SAL (Figure 4.8). The difference was to a greater magnitude following DREADD activation. During the baseline session, both groups of GTs were significantly different from STs (ts=3.2-6.4, ps<0.01). With the shift toward sign-tracking evoked by DREADD receptor activation, the difference in GTs from STs in the saline group (t=2.14, ns) dissipated. By Day 7 of testing, while the probability to approach lever in all GTs increased, only those in the saline group showed significant differences from all STs both

pre-injection (ts=3.14-3.70, ns) and post-injection (ts=4.45-4.59, $p<0.001$). Following treatment on Day 7, probability of GTs with DREADD activation showed greater probability of lever contact than GT SAL ($t=3.14$, $p<0.01$). The probability of approaching magazine also showed significant group differences in baseline ($F_{(3,40)}=3.98$, $p<0.05$) and treatment ($F_{(3,40)}=4.63$, $p<0.01$) sessions, but not to time ($Fs_{(4,40)}=0.13$-0.49, ns) or interaction ($Fs_{(12,40)}=0.14$-0.42, ns) on day 1. In both sessions GTs were significantly different from the ST saline group (ts-2.65-3.45, $ps<0.05$) only on day 1. By day 7, there were no significant differences between groups or across time in the probability to approach magazine during either baseline or treatment sessions (Fs=0.05-2.58, ns).

The latency to approach lever did not change as a result of DREADD activation on day 1 nor day 7 (Figure 4.9). The latency to approach lever decreased in GT following CNO-driven DREADD activation, but it was not significant (Holm-corrected paired t-test, $t=2.2$, ns on day 1, $t=2.09$, ns on day 7). There were group differences both on day 1 and day 7 to baseline (pre-injection, $Fs_{(3,40)}=12.12$-20.71, $p<0.001$) and treatment (post-injection, $Fs_{(3,40)}=11.68$-22.03, $p<0.001$) sessions, but not time ($Fs_{(4,40)}=0.58$-1.0, ns) or interactions ($Fs_{(12,40)}=0.34$-1.0, ns). On day 1 GTs in the DREADD activation and SAL control group showed significantly higher latencies to approach lever than both ST groups in baseline ($ts=3.76$-6.35, $p<0.01$) and treatment ($ts=2.41$-6.93, $p<0.05$) sessions. GTs in the DREADD activation group also approached the lever at shorter latencies that GTs in the saline group in both baseline ($t=3.24$, $p<0.01$) and treatment ($t=4.81$, $p<0.001$) sessions. By day 7, GTs showed differences to GTs in the SAL group during baseline ($t=3.59$, $p<0.01$) and treatment ($t=4.31$, $p<0.001$) following DREADD activation, but not to either STs with DREADD activation (ts=1.67-2.15, ns) nor ST SAL controls (ts=1.08-2.32). There were also no immediate effects of DREADD activation on the latency to

enter magazine. The latency to enter magazine was only significant between STs and GTs during the baseline session on day 1 ($F_{(3,40)}$=3.45, p<0.05), where GTs with DREADD activation showed significantly shorter latencies than STs in the SAL group (t=3.09, p<0.05). No other groups showed significant differences on day 1 (ts=0.02-2.70, ns) nor day 7 (ts=0.01-2.58).

Activation of DREADDs also did not effect the number of lever contacts or magazine entries. Both showed significant changes across sessions and days of testing, however. On day 1 of testing, there were significant group differences in baseline ($F_{(3,40)}$=19.1, p<0.001) and treatment ($F_{(3,40)}$=22.21, p<0.001) sessions in average lever contacts (Figure 4.10). Both GT groups had significantly less lever contacts per trial than both ST groups in baseline and treatment sessions (ts=3.23-6.78, p<0.01). On day 1 GTs with DREADD activation also showed significantly more lever contacts than GTs in the SAL group before (t=2.77, p<0.05) and after (t=3.94, p<0.01) injections. By day 7 there were no longer significant differences between GTs in either group (baseline t=1.84, ns, treatment t=1.51, ns). All GTs showed significantly less lever contacts on day 7 compared to STs during baseline sessions (ts=3.31-5.20, p<0.01). However, with DREADD activation, GTs showed significant differences from STs with the same treatment (t=4.85, p<0.001) not STs in the SAL group (t=2.28, ns). In regards to magazine entries, there were no differences between groups on either day 1 (ts=0-1.25, ns) or day 7 (ts=0.05-2.33, ns) in baseline sessions. In treatment sessions on day 1 only, however, GTs with DREADD activation showed more magazine entries than STs in both groups (ts=2.99-3.24, ps<0.05). Magazine entries decreased slightly in all groups over days 1 and 7 of testing. There were no significant differences during treatment sessions on day 7 (ts=0.03-2.32, ns).

Experiment 2:

Overall Change in Behavior:

In this experiment, subjects received one week of saline injections and one week of CNO-driven DREADD activation (order randomized), and were analyzed for effects of DREADD activation on behaviors expressed during lever presentation: probability of approaching lever or magazine, latency to approach lever of magazine, and number of lever contacts or magazine entries. The behavioral changes across all 5 days of testing with DREADD activation and all 5 days of saline-controlled activation can be expressed in phenotypic index score changes. Both sign-trackers and goal-trackers showed a shift in phenotypic index towards a greater sign-tracking phenotype (R^2=0.39 and 0.54 respectively) (Figure 4.11). Though these were not significant, goal-trackers showed a trend towards significance (p=0.15). The rate of change varied between individuals, but the average was similar between STs and GTs ($F_{(1,6)}$=0.42, ns). Over the 5 days that STs were receiving saline injection, their sign-tracking phenotype became stronger (R^2=0.93, p<0.01), while GTs showed stable phenotypic indexes during this time (R^2=0.27, ns) (Figure 4.12). There were also no differences in the slopes of STs and GTs to phenotypic index ($F_{(1,6)}$=2.08, ns).

Behavioral Changes Toward Lever and Magazine:

There were also no differences on day 1 between goal-trackers with DREADD activation or saline injections for any of the behaviors analyzed (ts=0.01-0.94, ns). Sign-trackers receiving saline or DREADD activation only showed differences to lever contact on day 1 of testing following injection ($F_{(3,120)}$=13.76, p<0.001) (Figure 4.13). Results demonstrated that STs made more lever contacts following DREADD activation compared to controls (t=3.39, p<0.01). All other variables were similar between ST

groups both pre- and post-injection for day 1 (ts=0.04-1.23, ns) and day 5 (ts=0.03-1.05) of analysis. Number of magazine entries did not differ between groups of STs on either day 1 or 5 of testing (ts=0.02-0.16, ns). GTs did not differ in the number of magazine entries on day 1 (ts=0.3-1.29, ns), but by day 5 GTs receiving DREADD activation made significantly less magazine entries than those receiving saline before ($t=2.96$, $p<0.01$) and after ($t=2.93$, $p<0.01$) injections. Both groups of GTs made more magazine entries than both groups of STs in all sessions on day 1 (ts=5.90-8.14, ps<0.001) and day 5 (ts=4.53-7.88, $p<0.001$).

GTs demonstrated a tendency for shifting phenotype toward sign-tracking. With DREADD receptor activation by CNO, the probability of approaching the lever was higher compared to those receiving saline on day 5 prior to injection ($F_{(3,110)}=19.90$, $p<0.0001$, Holm-corrected t-test $t=2.51$, $p<0.05$) but not after injection ($t=1.204$, ns) (Figure 4.14). Probability scores of GTs in both treatment groups were significantly less than both ST groups on days 1 and 5 of testing both before (ts=3.47-6.49, ps<0.001) and after injections (ts=3.47-5.26, ps<0.01). GTs also showed *lower* probabilities to approach magazine on day 5 compared to goal-trackers receiving saline both before ($t=2.78$, $p<0.05$) and after ($t=2.72$, $p<0.05$) CNO-driven DREADD activation. Probability to approach magazine was still higher in both groups of goal-trackers compared to both groups of sign-trackers in both sessions on days 1 (ts=7.13-12.41, ps<0.001) and 5 (ts=6.14-9.91, ps<0.001).

The latency to approach lever or magazine was also different between GTs on day 5 of testing. DREADD activation induced shorter latency to approach lever in GTs compared to saline controls only before injection ($F_{(3,120)}=12.21$, $p<0.001$, $t=2.64$, $p<0.05$), but not after ($t=1.51$, ns) (Figure 4.15). STs and GTs also differed in latency scores related to lever and magazine. On day 1 of testing, GTs showed significantly

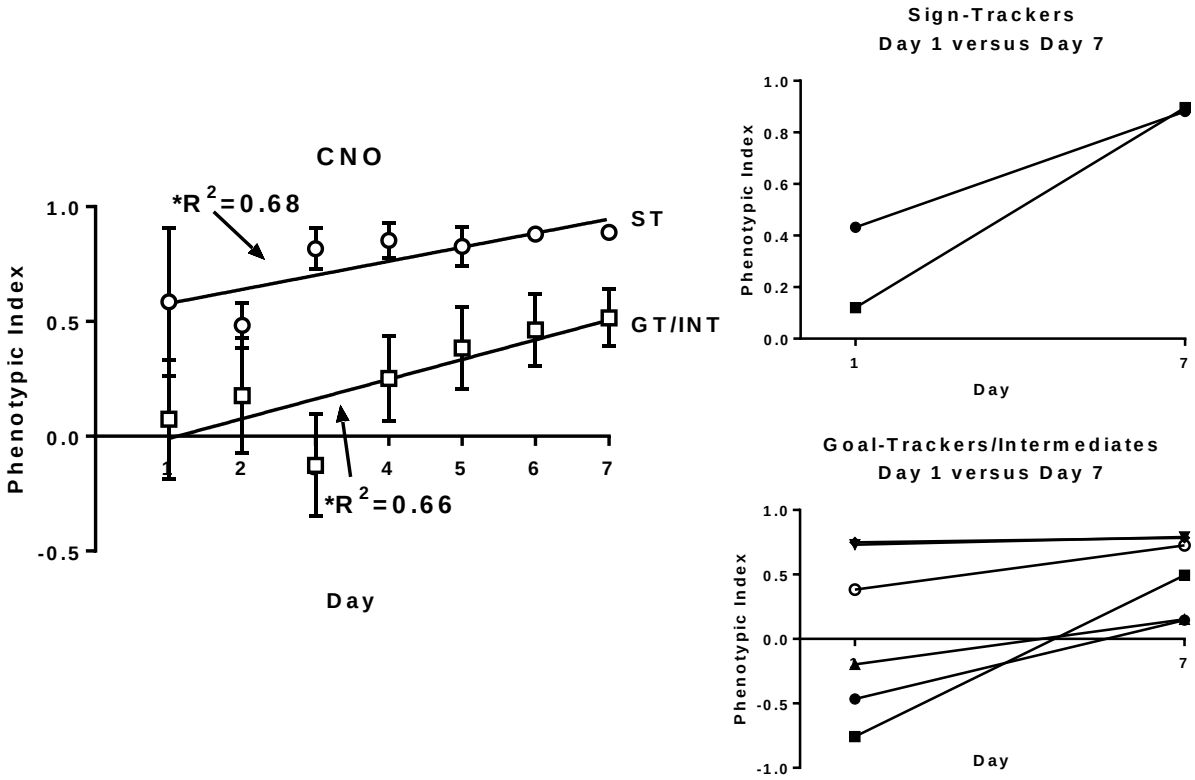
higher latency to approach lever compared to STs with DREADD activation (ts=2.79-3.59, $p<0.05$) not SAL controls (ts=1.48-2.28, ns) following CNO-driven inhibition of rostral VP neurons. On day 5, all STs showed significantly shorter lever latency compared to all GTs before (ts=2.40-5.35, $ps<0.05$) and after (ts=2.61-4.09, $ps<0.05$) injections. Similarly all STs showed significantly longer magazine latency compared to all GTs before (ts=4.91-9.31, $ps<0.001$) and after (ts=4.45-7.24, $ps<0.001$) injections on days 1 and 5. GTs also did not differ in latency to approach magazine on day 1 (ts=1.34-1.99, ns) or day 5 (ts=1.30-1.67, ns).

Effects of Experimental Paradigm:

Differences in effects of DREADD activation on cue-directed behaviors were seen as a result of experimental set up, particularly in goal-trackers. In experiment 1, animals were trained in the Pavlovian task prior to DREADD implant and expression. When given a 3-week break between training and testing, goal-trackers showed a shift in phenotypic expression and became more similar to sign-trackers (Figure 4.16A,B). In comparing phenotypic scores of subjects following inhibition of rostral VP, results demonstrated significant differences over time ($F_{(2,10)}=8.98$, $p<0.01$). By the end of training all goal-trackers had phenotypic scores significantly different from sign-trackers ($t=3.6$, $p<0.01$) but not intermediates ($t=2.0$, ns). On day 1 and day 7 of testing, however, there were no differences between phenotypes (ts=0.50-1.72, ns). GTs in the saline control group also showed changes in phenotype over time ($F_{(2,4)}=46.67$, $p<0.01$). In this group, GTs showed significant difference from STs after 5 days of training ($t=12.39$, $p<0.001$) and on day 1 of testing ($t=13.87$, $p<0.001$), but not on day 7 of testing ($t=2.44$, ns).

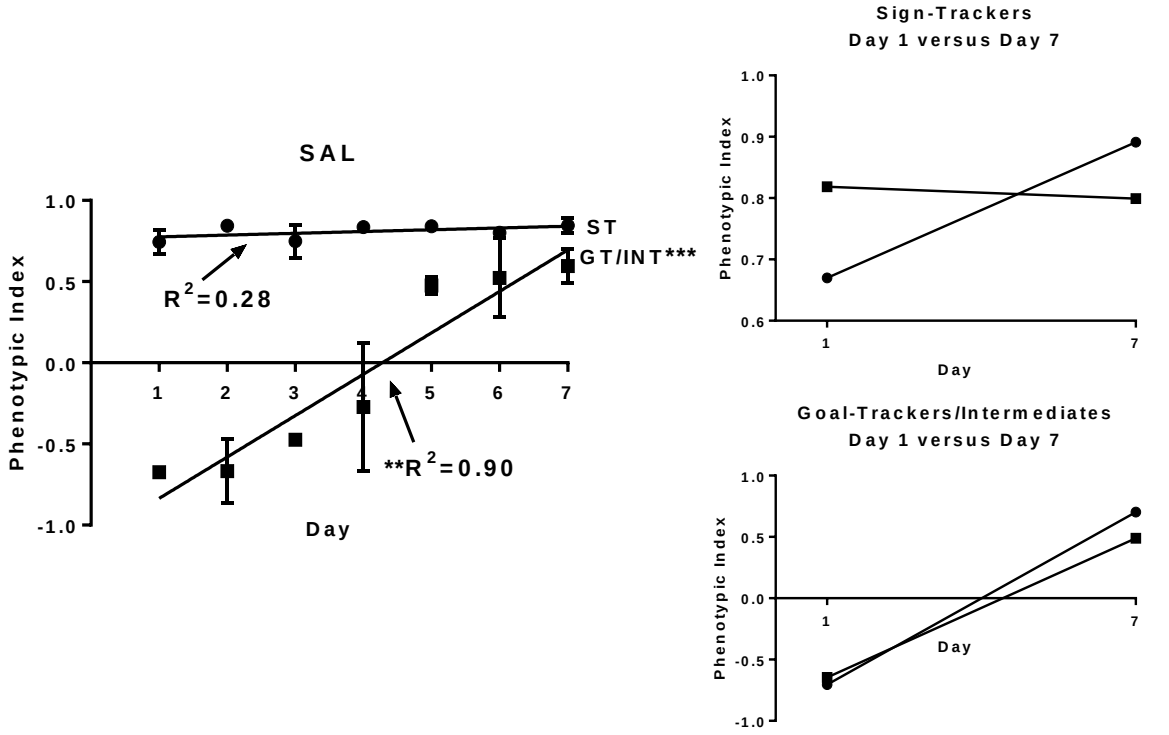
In experiment 2, DREADDs were given time to express prior to training and testing. As a result, cue-directed changes over this time were slight and did not significantly affect phenotype of subjects (Figure 4.16C,D). Comparisons between training and testing days with DREADD activation, there were significant differences between phenotypes ($F_{(2,11)}$=34.29, $p<0.001$) and over time ($F_{(2,22)}$=3.57, $p<0.05$). Specifically, phenotypic index scores were significantly different between GTs and both STs and INTs after training (ts=3.51-7.45, $p<0.01$) and on days 1 (ts=4.4-6.28, $p<0.001$) and day 7 (t=3.08-5.32, $p<0.01$) of testing. STs only showed significant differences between INT after training (t=2.76, $p<0.01$), but not on day 1 or 7 of testing (ts=0.83-1.38, ns).

Figure 4.6: Change in Phenotypic Index with DREADD Activation Following a 3-week Suspension Period



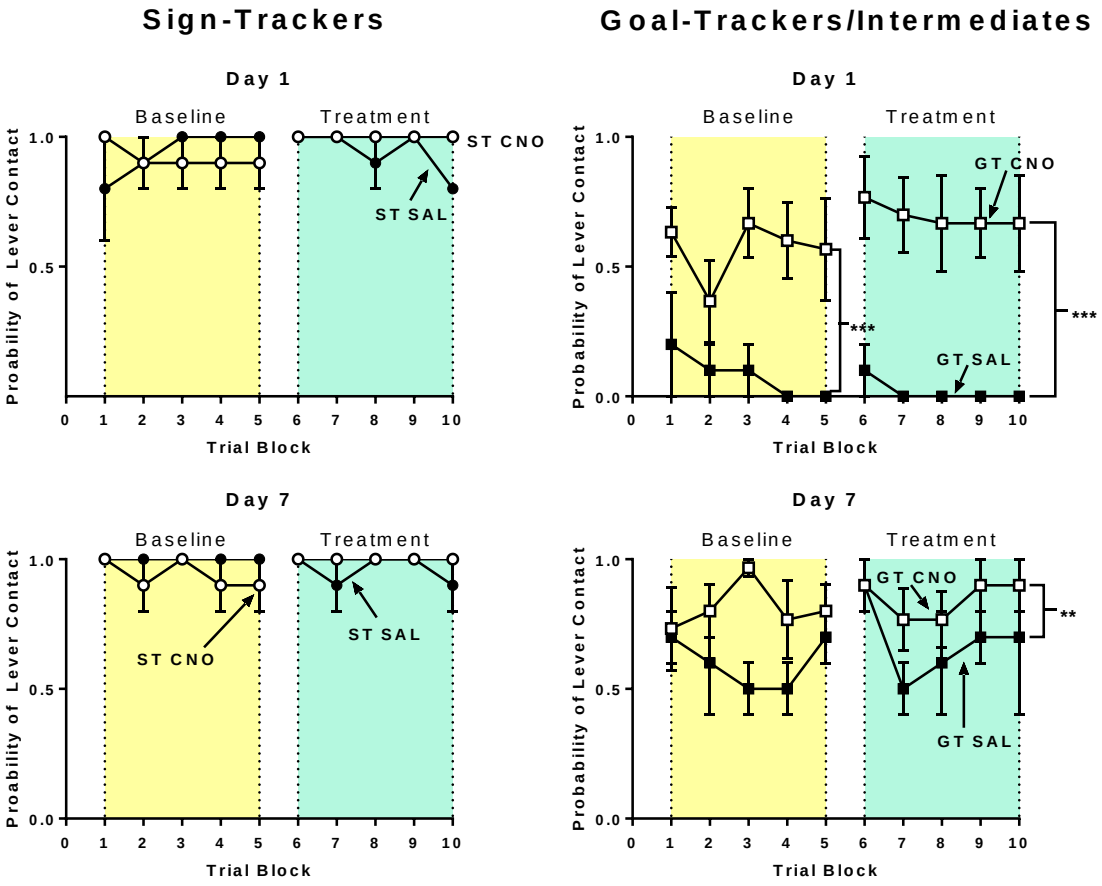
Phenotypic index scores were calculated from averaging latency to approach lever or magazine, probability of approaching lever or magazine, and number of lever or magazine interactions. (Left) Average +/- SEM of phenotypic index scores from sign-trackers (ST) and goal-trackers/intermediates (GT/INT) receiving an injection of clozapine-n-oxide (CNO). (TOP Right) Change in phenotypic index of individual sign-trackers (n=2) receiving CNO injections. (Bottom Right) Change in phenotypic index of individual goal-trackers (n=6) receiving CNO injections. A linear regression analysis showed a significant change in PCA index for STs and GT/INTs following CNO injection, but no difference between the groups. *$p<0.05$

Figure 4.7: Change in Phenotypic Index with SAL Control Injections Following a 3-week Suspension Period



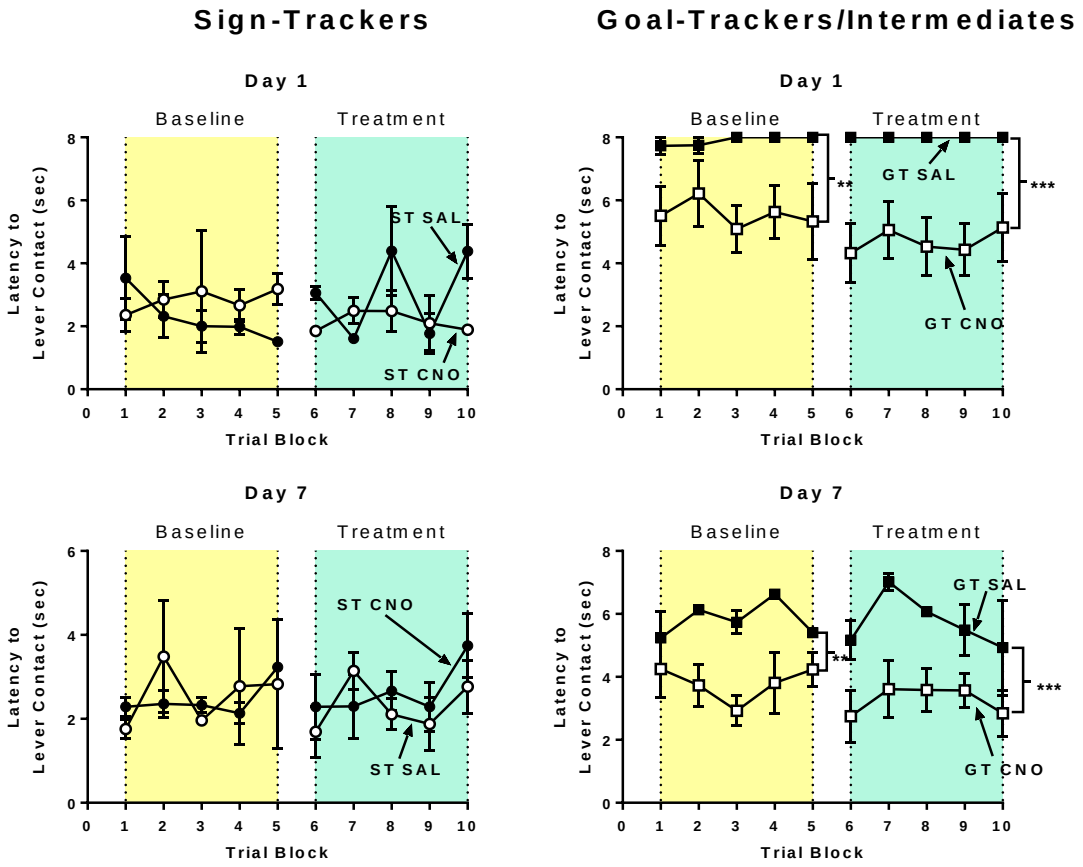
Phenotypic index scores were calculated from averaging latency to approach lever or magazine, probability of approaching lever or magazine, and number of lever or magazine interactions. (Left) Average +/- SEM of phenotypic index scores from all animals receiving an injection of saline (SAL). (TOP Right) Change in phenotypic index of individual sign-trackers (n=2) receiving SAL injections. (Bottom Right) Change in phenotypic index of individual goal-trackers (n=2) receiving SAL injections. A linear regression analysis showed a significant change in phenotype for GTs and a significant difference in slopes between STs and GT/INTs, **p<0.01, ***p<0.001.

Figure 4.8: Probability of Contacting Lever



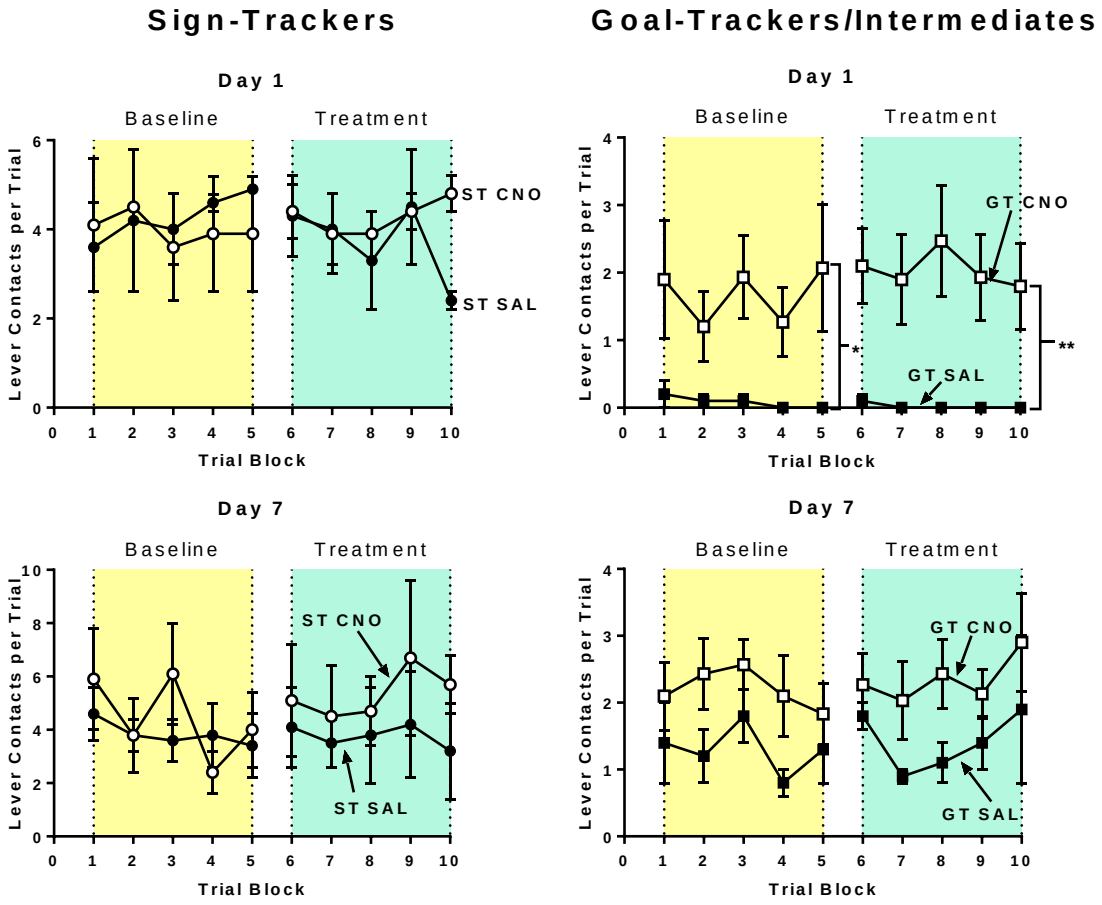
During testing sessions subjects underwent a typical Pavlovian conditioning paradigm (25 lever/pellet pairings). The first session served as a baseline of behavior (gold). Following, subjects received and injection of CNO (3mg/kg) or saline (SAL, yoked volume) and underwent an additional 25 lever/pellet pairing (blue). The probability of a subject approaching the lever and magazine was recorded for each trial on days 1 and 7. Scores were organized into trial blocks, the averaged probability over 5 trials each during baseline and treatment. A two-way repeated measures ANOVA and Holm-corrected pairwise t-tests were performed. Sign-tracker (ST), goal-tracker (GT), clozapine-n-oxide (CNO), saline (SAL), **p<0.01, ***p<0.001 GT CNO vs. GT SAL

Figure 4.9: Latency to Contact Lever



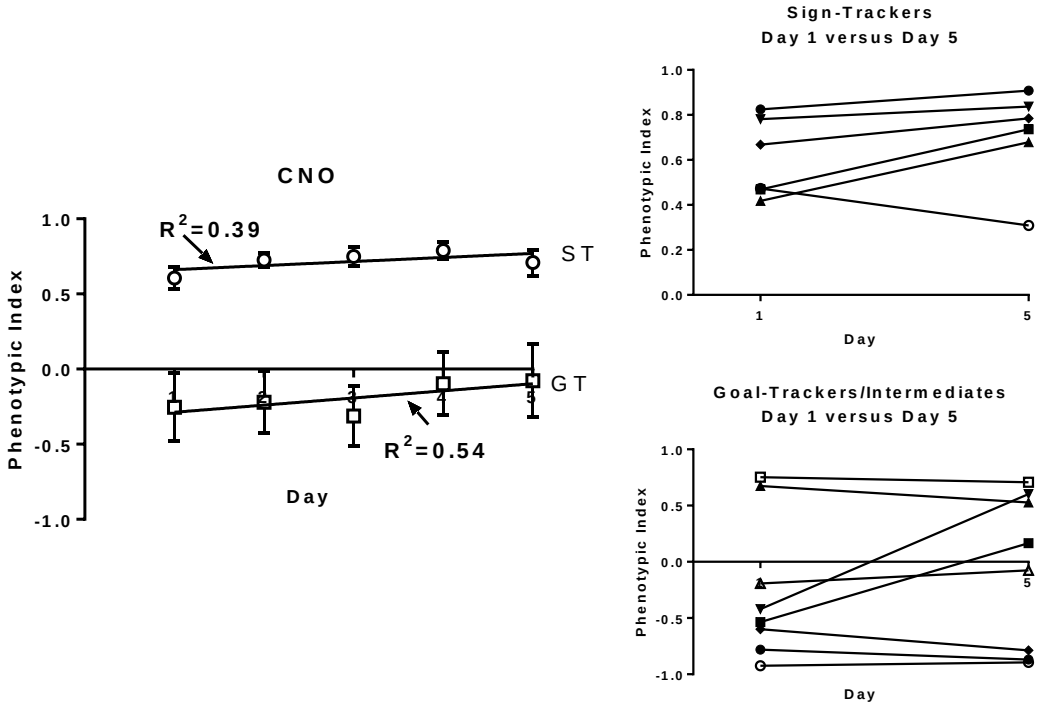
During testing sessions subjects underwent a typical Pavlovian conditioning paradigm (25 lever/pellet pairings). The first session served as a baseline of behavior (gold). Following, subjects received and injection of CNO (3mg/kg) or saline (SAL, yoked volume) and underwent an additional 25 lever/pellet pairing (blue). The probability of a subject approaching the lever and magazine was recorded for each trial on testing days 1 and 7. Scores were organized into trial blocks, the averaged probability over 5 trials each during baseline and treatment for sign-tracker (ST) and goal-trackers (GT). A two-way repeated measures ANOVA and Holm-corrected pairwise t-tests were performed. Significant differences were seen between GTs receiving clozapine-n-oxide (CNO) and saline (SAL). ***p<0.001

Figure 4.10: Average Lever Contacts per Trial



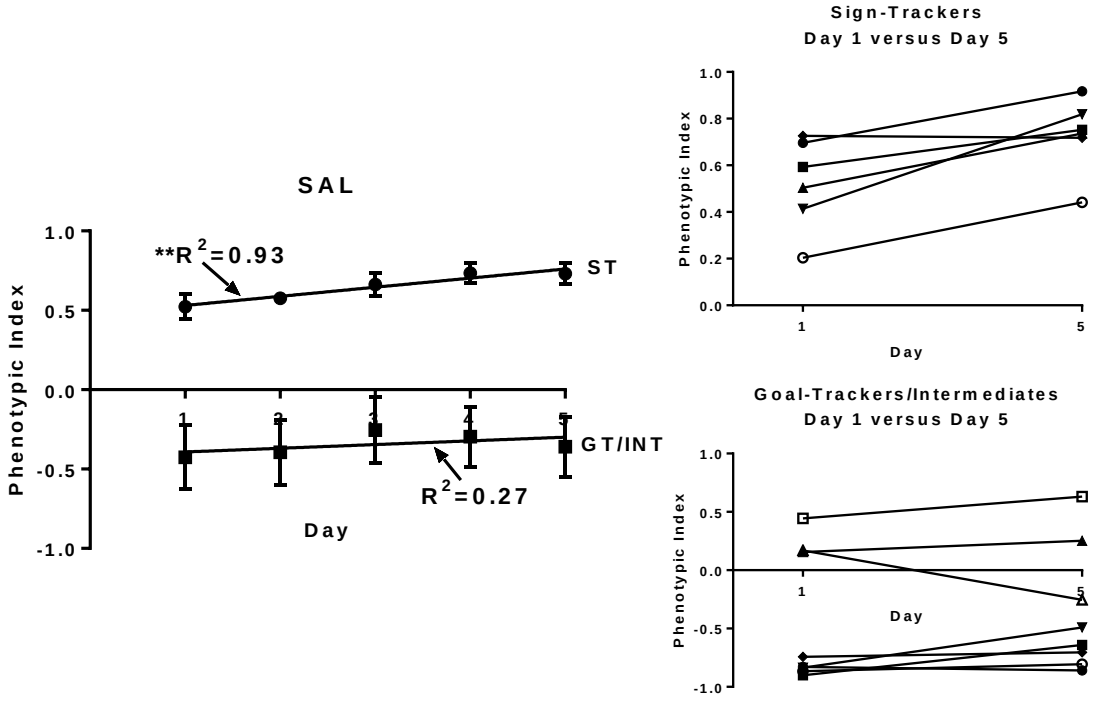
During testing sessions subjects underwent a typical Pavlovian conditioning paradigm (25 lever/pellet pairings). The first session served as a baseline of behavior (gold). Following, subjects received and injection of CNO (3mg/kg) or saline (SAL, yoked volume) and underwent an additional 25 lever/pellet pairing (blue). The probability of sign-trackers (ST) and goal-trackers (GT) approaching the lever and magazine was recorded for each trial on days 1 and 7 of testing. Scores were organized into trial blocks, the averaged probability over 5 trials each during baseline and treatment sessions. A two-way repeated measures ANOVA and Holm-corrected pairwise t-tests were performed. **p<0.01 GT SAL compared to GT CNO

Figure 4.11: Change in Phenotypic Index Following DREADD Activation Performed Immediately Following Training



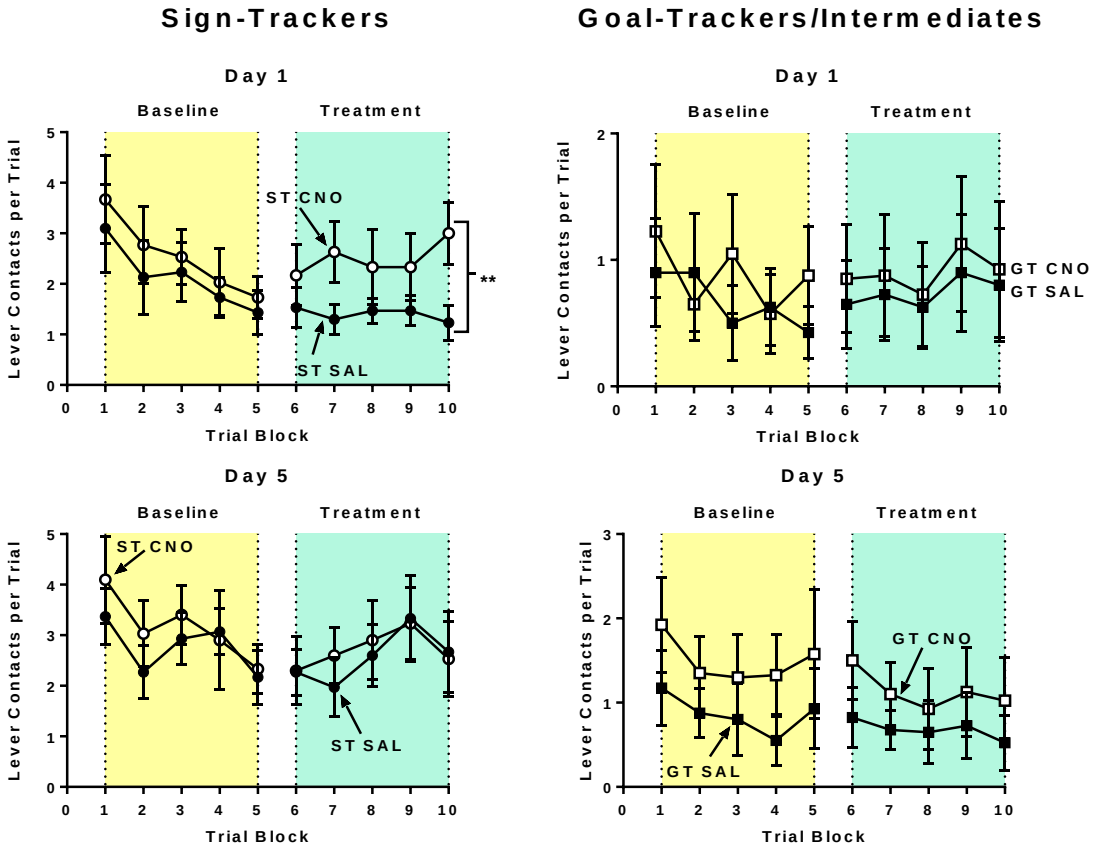
Phenotypic index scores were calculated from averaging latency to approach lever or magazine, probability of approaching lever or magazine, and number of lever or magazine interactions. (Left) Average +/- SEM of phenotypic index scores from all animals receiving an injection of clozapine-n-oxide (CNO). (TOP Right) Change in phenotypic index of individual sign-trackers (n=6) receiving CNO injections. (Bottom Right) Change in phenotypic index of individual goal-trackers and intermediates (n=8) receiving CNO injections. A linear regression analysis showed no significant changes in PCA index for either group following CNO injection, though there was a trend in GT (p=0.15).

Figure 4.12: Change in Phenotypic Index with SAL Control Injections Performed Immediately Following Training



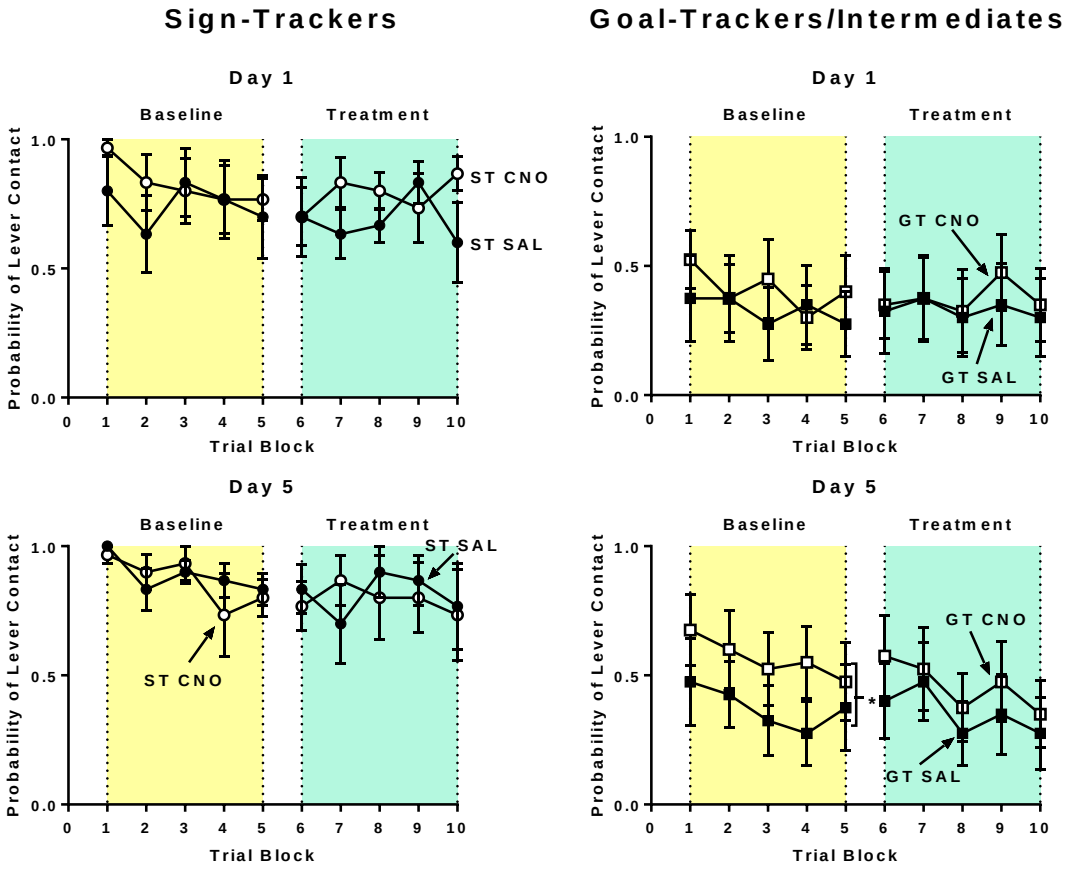
Phenotypic index scores were calculated from averaging latency to approach lever or magazine, probability of approaching lever or magazine, and number of lever or magazine interactions. (Left) Average +/- SEM of phenotypic index scores from all animals receiving an injection of saline (SAL). (TOP Right) Change in phenotypic index of individual sign-trackers (n=6) receiving SAL injections. (Bottom Right) Change in phenotypic index of individual goal-trackers/intermediates (n=8) receiving SAL injections. A linear regression analysis showed a significant change in phenotype for STs **$p<0.01$.

Figure 4.13: Probability of Contacting Lever



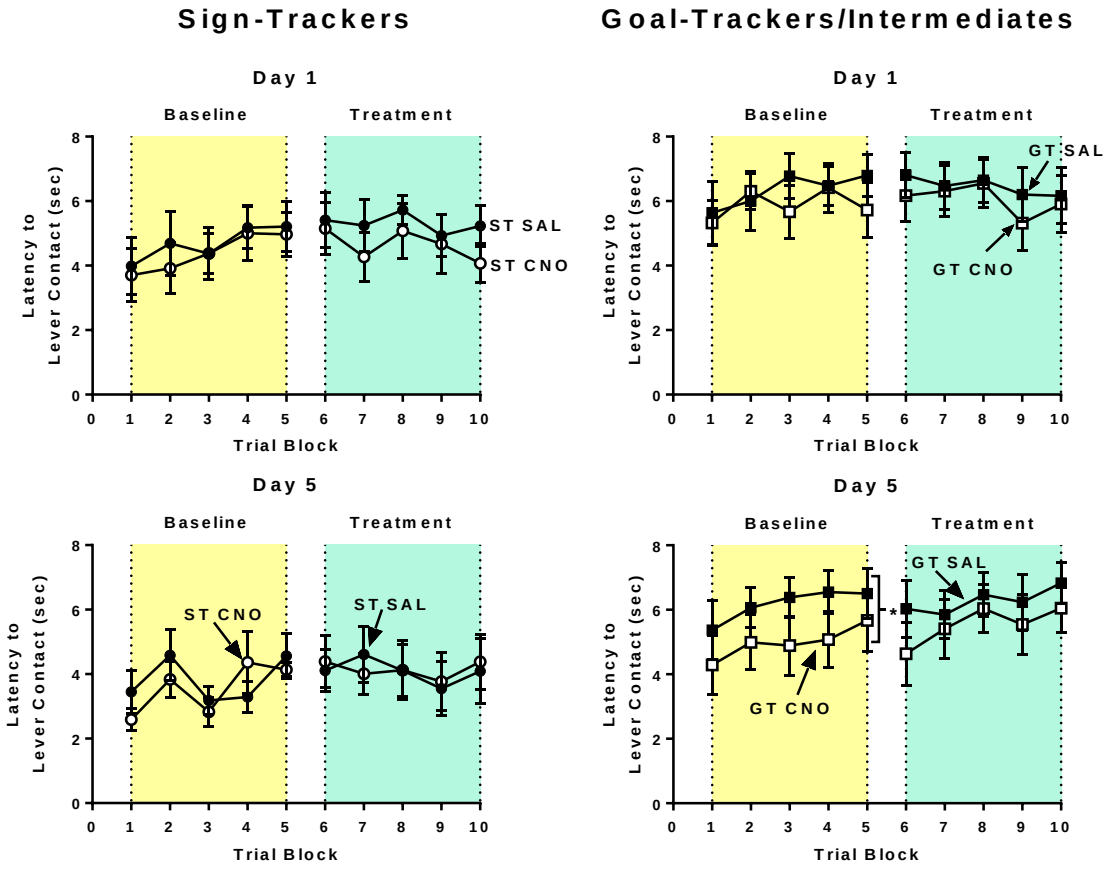
During testing sessions subjects underwent a typical Pavlovian conditioning paradigm (25 lever/pellet pairings). The first session served as a baseline of behavior (gold). Following, subjects received and injection of CNO (3mg/kg) or saline (SAL, yoked volume) and underwent an additional 25 lever/pellet pairing (blue). The probability sign-trackers (ST) and goal-trackers (GT) approaching the lever and magazine was recorded for each trial on days 1 and 5. Scores were organized into trial blocks, the averaged probability over 5 trials each during baseline and treatment. A two-way repeated measures ANOVA and Holm-corrected pairwise t-tests were performed. **p<0.01 ST SAL vs ST CNO

Figure 4.14: Latency to Contact Lever



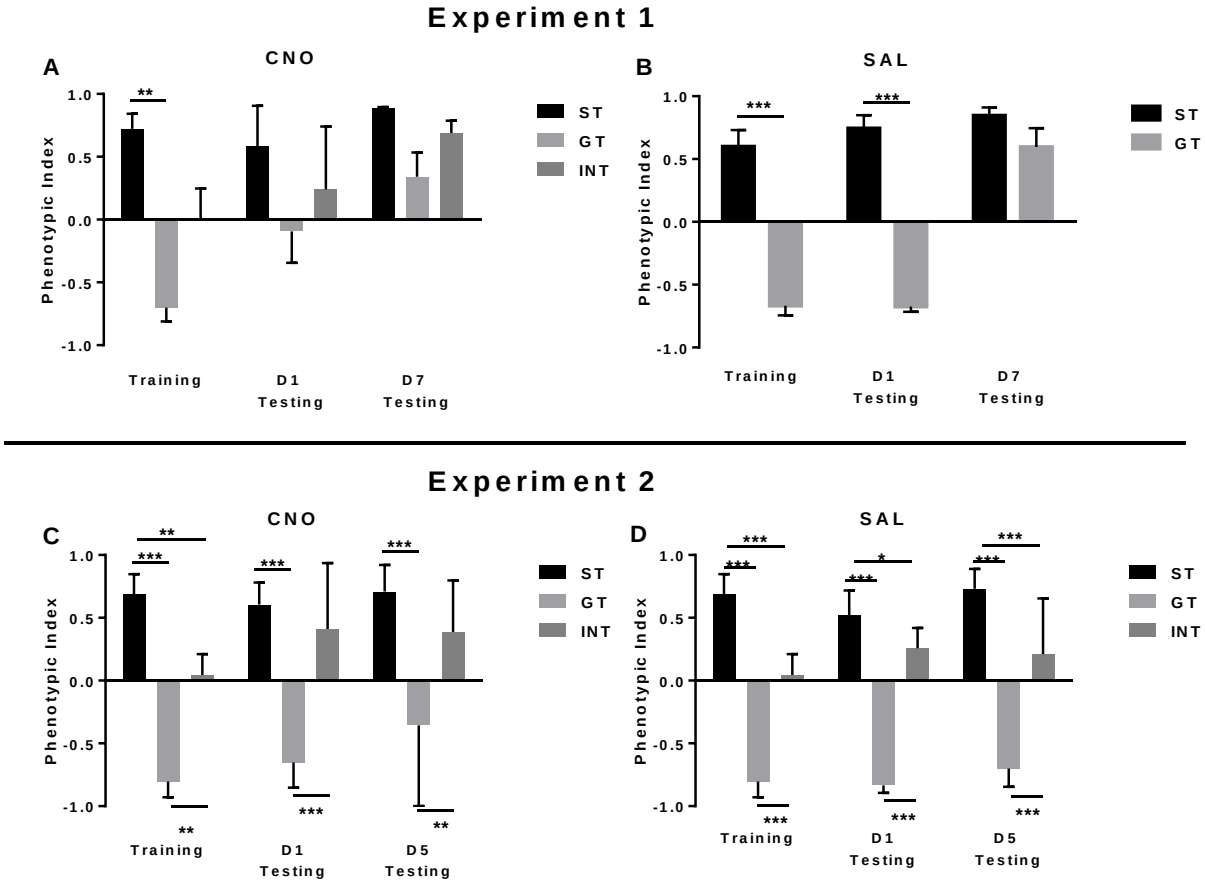
During testing sessions subjects underwent a typical Pavlovian conditioning paradigm (25 lever/pellet pairings). The first session served as a baseline of behavior (gold). Following, subjects received and injection of CNO (3mg/kg) or saline (SAL, yoked volume) and underwent an additional 25 lever/pellet pairing (blue). The probability of a subject approaching the lever and magazine was recorded for each trial on testing day 1 and 5. Scores were organized into trial blocks, the averaged probability over 5 trials each during baseline and treatment. A two-way repeated measures ANOVA and Holm-corrected pairwise t-tests were performed. Sign-trackers (ST), goal-trackers (GT)

Figure 4.15: Average Lever Contacts per Trial



During testing sessions subjects underwent a typical Pavlovian conditioning paradigm (25 lever/pellet pairings). The first session served as a baseline of behavior (gold). Following, subjects received and injection of CNO (3mg/kg) or saline (SAL, yoked volume) and underwent an additional 25 lever/pellet pairing (blue). The probability of a subject approaching the lever and magazine was recorded for each trial on testing days 1 and 5. Scores were organized into trial blocks, the averaged probability over 5 trials each during baseline and treatment. A two-way repeated measures ANOVA and Holm-corrected pairwise t-tests were performed. Sign-trackers (ST), goal-trackers (GT)

Figure 4.16: Summary of Change



Phenotypic index scores were calculated from averaging latency to approach lever or magazine, probability of approaching lever or magazine, and number of lever or magazine interactions on the 5th day of training as well as on Day 1 and Day 7 of testing. In A and B, subjects were given a 3-week break between training and testing. A) Phenotypic Index was calculated using trials following systemic injection of clozapine-n-oxide (CNO, 3mg/kg). B) Phenotypic index was calculated using trials following systemic saline injection (SAL). A two-way repeated measures ANOVA showed significant differences between sign-trackers (ST) and goal-trackers (GT), but not intermediates (INT). In C and D, testing took place immediately following training. C) Following injection with CNO, phenotypic index scores were significantly different between STs, GTs, INT. D) Following saline injections, all groups showed significant differences on all days analyzed. (*p<0.05, **p<0.01, **p<0.001)

DISCUSSION

In Experiment 1, subjects were first trained on a Pavlovian conditioning task, then were injected with viral vector targeting DREADD expression in VP neurons projecting to the VTA and were left undisturbed for 3 weeks to allow for expression of receptors. Animals were tested in the Pavlovian task for 25 trials then immediately given an injection of clozapine-n-oxide or saline before testing with 25 additional trials. Our results demonstrated both an effect of DREADD activation on behaviors towards lever and magazine as well as behavioral changes over time. Specifically, goal-trackers with DREADD expression showed increased probability to approach the lever following CNO injection. They also show increased lever contacts and magazine entries following CNO injections by day 7 of testing. Results also showed behavioral changes over the 7 days of testing. GTs given CNO showed 1) increased probability to approach lever and decreased probability to approach magazine, 2) decreased latency to approach lever and increased latency to enter magazine, and 3) increased lever contacts and decreased magazine entries.

In experiment 2, DREADDs were given time for expression before training began and testing immediately followed training. Significant changes in phenotypic index were only seen in STs when given saline, though all scores showed a trend towards greater sign-tracking behavior over the 5 days. Immediate behavioral effects due to DREADD activation with CNO was only seen in STs on day 1 of testing, expressed as increasing lever contacts. DREADD activation showed changes in goal-trackers over the 5 days of testing. Specifically, results demonstrated increased probability to contact lever, decreased latency to approach lever, increased latency to enter magazine, decreased probability of a magazine entry, and overall less magazine entries.

The increased approach and interaction with Pavlovian cues following systemic CNO administration seen in both experiments (both STs and GTs in experiment 1, mainly STs in experiment 2) was expected. By inhibiting the rostral VP using DREADDs, we were inducing a tonic inhibition of VP neurons. This has been seen by others studying the role of VP in cocaine self-administration behavior (Mahler et al., 2014; Root et al., 2012) and leads to a tonic disinhibition of dopamine neurons (Hjelmstad, Xia, Margolis, & Fields, 2013; Mahler et al., 2014). Inhibition of VP neurons have also shown to increase the population of responsive dopamine neurons and results in tonic, extrasynaptic release of dopamine (Floresco et al., 2003). In chapter 1, tonic firing of dopamine neurons was associated with the attribution of incentive salience in sign-trackers and may facilitate the adopted sign-tracking behavior seen in goal-trackers as well.

The change in phenotype from goal-trackers to sign-trackers seen in the first experiment indicates a malleable neural circuit change had occurred over the 3-week incubation period between Pavlovian training and testing. Changes in phenotypic score were observed on day 1 of testing indicating that the change was not solely due to a relearning of the cue-reward association. The ventral pallidum (VP) has previously shown to code predictive and incentive cues differently. One study found that sign-trackers showed greater population coding and firing rate changes to predictive vs. incentive cues compared to GTs and INTs (Ahrens, Meyer, et al., 2016). In a study of amphetamine sensitization effects on firing patterns of posterior VP neurons, a shift in the coding of VP neurons from predictive cues to incentive cues was identified (Tindell et al., 2005). The behavioral changes seen in this study suggest a similar shift in neural coding of VP cells. Another study found enhanced behavioral responding to a Pavlovian cue associated with a food reward following amphetamine injection into the NAcc shell, indicating that stimulation of the mesolimbic circuit with a dopamine agonist is able to

induce “wanting” of reward cues regardless of their drug or food association (Wyvell & Berridge, 2000).

In this study, we did not expose subjects to any drug that would have altered dopamine transmission between training and testing. Still, the effects seen on goal-trackers indicate a type of behavioral sensitivity. Sign-trackers and goal-trackers differ in their sensitivity to cues (Meyer et al., 2014). STs have shown sensitivity to discrete cues and cue-induced reinstatement following extinction. Goal-trackers on the other hand show sensitivity to contextual cues (Robinson et al., 2014). Other studies have also demonstrated the power of context in cocaine sensitization (Mattson et al., 2008) and drug seeking (Crombag, Bossert, Koya, & Shaham, 2008). These studies present data that show following extinction, the magnitude of responding towards a discrete drug-paired cue increased during reinstatement only in the context previously associated with reward delivery. Results from the present study indicate that goal-trackers were more sensitive to the 3-week incubation (i.e. withdrawal) period than sign-trackers. Thus, when placed back in the same chambers for testing, it induced a behavioral sensitivity that was directed at the lever in the Pavlovian task.

Behavioral sensitization has been shown following a withdrawal period from drugs such as cocaine (De Vries, Schoffelmeer, Binnekade, Raasø, & Vanderschuren, 2002) and amphetamine (Paulson, Camp, & Robinson, 1991; Paulson & Robinson, 1991). Few have documented such changes following training with Pavlovian cues. This study warrants further investigation into such a phenomenon, as evidence of behavioral sensitization was only seen in experiment 1, not experiment 2.

Another surprising result was the lack of significant behavioral changes following DREADD receptor activation. The medial rostral VP, where viral expression was targeted, has been shown to contain a majority of GABA neurons thought to be

extensions of the medium spiny neurons from the nucleus accumbens shell (Kupchik & Kalivas, 2013). The rostral VP contains more glutamatergic neurons than the caudal VP as shown by densely packed VGLUT2 mRNA (Geisler et al., 2007). It is unknown as to whether these glutamatergic neurons target dopamine, GABA, or glutamate neurons of the VTA, but they are sure to attenuate the inhibitory component of VP GABA neurons projecting there. This may be one reason behind the blunted behavioral effects following receptor activation seen in both experiments. Alternatively, histological analysis of viral expression demonstrated few cells expressing DREADDs. As we did not combine electrophysiological or voltammetry procedures, we were unable to confirm effectiveness of DREADD activation following CNO injection. The blunted behavioral differences following inhibition of VP neurons may have been too limited (or not at all) to notice significant changes in sign-tracking and goal-tracking behavior.

The behavioral analyses performed may not have been appropriate to detect subtle changes in behavior. In experiment 2, we did not see significant changes in goal-tracker behavior in regards to lever contact. It may be, however, that the behavioral changes expressed were not detected by the computer. In one study analyzing the ability of cocaine cues (a lever) to be attributed with incentive salience, it was found that approach behavior, in the form of orienting and sniffing, increased with stimulus-reward learning (Uslaner, Acerbo, Jones, & Robinson, 2006). Other studies have found similar approach behavior with reward-stimulus learning that was not always followed by contact (Peterson et al., 1972; Woodruff & Williams, 1976). However, in the previous studies, the reward was not "consumable" by the subjects and may reflect the lack of cue contact, as they suggest. As video recordings were not performed in this study, approach behavior was unable to be scored. Future studies may want to incorporate such analyses.

Few studies have analyzed effects of neural firing patterns following DREADD activation with CNO in behaving subjects. One study has indicated that CNO takes 20 min following systemic injection to effect neural firing (Mahler et al., 2014). Another has found neural changes within 10 minutes, with activation peaking at 20 min (Scofield et al., 2015). However, immediate behavioral changes following CNO injections were seen in this study. Future work will alleviate such discrepancies by performing electrophysiological recordings from target sites of viral vector implants (i.e. VP and VTA) in animals as they exhibit their cue-directed behavior in PCA sessions. This will provide direct correlations between changing firing patterns and changes in observed behaviors.

Chapter 5: General Discussion

The purpose of this dissertation was to determine neural representations of the ventral basal ganglia in reward and motivation, specifically in the attribution of incentive salience to reward-paired cues. That is, the ability of cues themselves to become attractive to individuals eliciting approach and serving as potent conditioned reinforcers. The studies presented here utilized a Pavlovian Conditioned Approach (PCA) paradigm well known to elicit approach behavior (Brown & Jenkins, 1968) and differentiate individual differences in the location of their directed attention. Sign-tracking was a term originally coined to describe individuals directing attention towards or away from a cue (Hearst & Jenkins, 1974). Currently sign-tracker specifically describes individuals directing their attention towards a discrete cue, while goal-tracker describes those who direct their attention towards reward delivery. In the last decade there has been a reinvigoration of studies regarding the individual differences expressed towards reward-paired cues. Many have examined the individual behavioral effects of reward cues on motivation; few have focused on the neural firing patterns correlated with such divergent characteristics. My studies focused on neural coding within the ventral tegmental area (VTA), nucleus accumbens (NAcc) and ventral pallidum (VP), areas known to be involved in reward. They addressed my central hypothesis that neural coding to cue presentation would be greater in STs than GTs due to the differences in attribution of incentive salience.

Taken together, results from these studies indicate that STs and GTs employ different coding patterns in the mesolimbic circuit. In chapter 2, I found that the initial tendency to attribute incentive salience to reward-paired cues is coded in neurons of the VTA. Specifically, dopamine neurons respond to both predictive and incentive properties

of food-paired cues. In STs the magnitude of neural response during the last 7sec of lever presentation (cue interaction) and during lever retraction (CS Offset) was significantly greater than GTs. Further, neural population coding was greater in STs than GTs to cue interaction. As only sign-trackers place incentive salience on reward cues, these results demonstrate a role for dopamine neurons in coding incentive motivation. We also found greater magnitude of firing to CS Onset and a lesser magnitude to Cue Interaction of non-dopamine neurons in GTs compared to STs. As non-dopamine neurons have been shown to regulate dopamine firing, these results indicate that GTs employ a different regulatory mechanism in the mesolimbic circuit.

In chapter 3, I found differences in neural coding to food and drug administration in both STs and GTs. Specifically, there was a smaller population of neurons in the NAcc and VP responsive in the cocaine vs. food self-administration task. Further, cocaine dose size seemed to be encoded in the NAcc shell and VP hot spot. This was evidenced by greater firing magnitudes to nosepokes and cue periods associated with high doses compared to low doses of cocaine and food reward. I also found similar behaviors in STs and GTs to infusion rate and number of nosepokes in both food and drug self-administration tasks. This indicates that individual differences in the initial tendency to attribute incentive salience to reward-paired cues are not evident in behaviors in a well-learned self-administration task following a short period (7 days) of withdrawal.

In chapter 4, I found that inhibition of VP neurons projecting to the VTA modulate the mesolimbic circuit of GTs and STs to a different extent and is expressed in behaviors directed towards Pavlovian cues. Specifically, DREADD activation enhanced cue-directed behavior in STs in the form of increased lever contacts, increased probability of approaching lever, and decreased latency to approach magazine. Following a 3 week “withdrawal” period, GTs also showed enhanced cue-directed behavior both

with and without DREADD activation. These results indicate the neurons of the VP act to regulate the coding of incentive salience to reward cues.

The results of the studies presented in this thesis provide a thorough understanding of how the mesolimbic circuit works to encode and propagate signals of motivation. While others have indicated the role of dopamine release in the attribution of incentive salience to reward cues (Flagel et al., 2011), we have shown that differences lie in the firing patterns of neurons in the VTA, which in turn effect synaptic and extrasynaptic dopamine concentration. Further, the behavioral changes that were seen in both STs and GTs demonstrate neural plasticity in the circuit, showing the possibility of altering the value of environmental stimuli through cue exposure (across testing days) and withdrawal (i.e. sensitization). Following a short withdrawal period with food (chapter 3 and 4) or drug (chapter 3), GT behavior shifted towards sign-tracking, to the point ST and GT behavior no longer differed in response to cues. The short period of withdrawal that occurred between training and testing (due to recovery following electrode implant) may have induced an increased motivational state for the reward, a result seen by others following 1 month of drug abstinence (Hollander & Carelli, 2005). This suggests incentive value of cues can change. As DREADD activation of neurons projecting from VP to VTA also changed motivation for reward cues, we have shown that the value of cues can be manipulated pharmacologically and indicates a neural target for treating cue related disorders to potentially inhibit cue-induced reward-seeking behavior.

A current map of the mesolimbic circuit specifies unique roles for the NAcc core and shell in coding reward-paired stimuli (Figure 5.1). We present results showing responses of both core and shell to drug and food reinforcement. The responses from the NAcc were differentially transmitted to the VP subregions, with core neurons that project

to the dorsolateral ventral pallidum (VPdl, hot spot) induced excitatory responses, while the shell projections to the ventromedial ventral pallidum (VPvm, surround) induced inhibitory responses to drug self-administration. The core, with its connection through the VPdl and to the substantia nigra may be involved in the enhanced locomotor behavior seen in STs and GTs following a period of sensitization. The NAcc core showed equal magnitude to both drug doses, but the downstream VPdl showed greater firing to higher dose of drug. The magnitudes of these responses in both VP subregions were correlated with dose, showing greater magnitude of firing to a higher dose of cocaine. The neural responses to high dose of cocaine were greater than the neural responses to food in the VPdl, not VPvm, perhaps associated with heightened motor output. Studies have shown an increase in head bobbing, sniffing and general locomotor activity following different doses of experimenter-injected cocaine and this was associated with dose-dependent activation of striatal neurons (Rebec, 2006; White, Doubles, & Rebec, 1998). This also appears to depend on dopamine release in the nucleus accumbens (Kalivas & Duffy, 1990; Kalivas & Stewart, 1991; White et al., 1998) and strengthens our argument that the core is motor related. The NAcc shell through connections with the ventromedial VP (VPvm) and ventral tegmental area (VTA) may be involved in coding of incentive value. The NAcc shell showed greater magnitude of firing to a high dose of cocaine compared to low dose, suggesting the shell may be more sensitive to drug dose (value). We also found greater magnitude of firing in the NAcc to food cues vs. drug. Studies have shown that when given the choice, the majority of rats prefer food to drug (Perry, Westenbroek, & Becker, 2013). This suggests that food and associated cues have greater motivational value over drug cues, correlating with greater neural activation. The projection from the VPvm also functions to regulate the motivational properties of cues. Following inhibition of neurons projecting to the VTA, there was an enhanced approach behavior to food-

paired cues. Results presented here show that enhanced responding to Pavlovian cues is associated with increased firing of dopamine neurons in response to cue presentation. This corroborates studies showing that inhibition of the VPvm results in disinhibition of VTA dopamine neurons (Hjelmstad et al., 2013; Liu, Pu, & Poo, 2005; Mahler et al., 2014). The greater coding of cues seen in the VTA, NAcc, and VP regions indicates that motivation is propagated through the entire mesolimbic circuit.

The ventral pallidum, with its diverging projections to the substantia nigra and ventral tegmental area, has been implicated in both motor and motivation (Mogenson et al., 1980; Yang & Mogenson, 1989). Its role in motivation has been the focus of these studies. Recent studies of GABAergic neurons in the VP have found 2 populations of neurons with distinct morphological, electrochemical, and synaptic inputs (Kupchik & Kalivas, 2013). Specifically, GABAergic neurons of the rostral VP are rich in a "type 2" neuron that is typically hyperpolarized, has a low spontaneous firing rate, densely packed channels (suggesting high density of dendritic spines) and are largely similar to and seem to be extensions of medium spiny neurons of the NAcc shell and/or extended amygdala (Kupchik & Kalivas, 2013). In contrast, "type 1" GABAergic neurons that have been more extensively studied are generally depolarized, have higher firing rates and spontaneous activity and dominate most of the lateral and caudal regions of the VP (Kupchik & Kalivas, 2013). Further type 1 GABA neurons are mainly innervated by GABAergic input, while type 2 neurons are primarily regulated by glutamatergic afferents. The differences in electrophysiological, physical, and connective properties of the neural types may contribute to the functional differences seen in the subregions of the VP and through the mesolimbic circuit.

Studies have suggested that drugs of abuse hijack the area of the mesolimbic circuit used to encode 'natural' rewards (Carelli et al., 2000; Carelli & Wondolowski,

2003). This is seen as neurons that fire to food and water are not the same as those that respond to cocaine (Carelli et al., 2000; Carelli & Wondolowski, 2003), which leaves neurons responding to sexual behavior rewards. Activation of D2 receptors in the shell, not core, has shown to be critical in the formation of pair bonding in prairie voles (Aragona et al., 2006). Studies have also demonstrated that activation of D2 receptors is involved in behavioral sensitization and reinstatement of drug-seeking to cocaine, heroin, and amphetamine (Clark & Bernstein, 2006; De Vries et al., 1999; De Vries et al., 2002). Pair bonding upregulates D1R mRNA and DA release in the NAcc to maintain these social bonds (Resendez et al., 2016). An upregulation of D1R is also seen following exposure to cocaine and morphine (Terwilliger, Beitner-Johnson, Sevarino, Crain, & Nestler, 1991). Further, following pair bonding in prairie voles, these individuals show neural protection against drugs like amphetamine (Liu, Young, Curtis, Aragona, & Wang, 2011; Resendez et al., 2016). The neuroprotection seems to be mediated through D1, not D2, receptors in the NAcc shell (Liu et al., 2011). The parallels of drugs and pair bonding (an example of sexual behaviors) support the idea that drugs tap into the neural circuit of a selective 'natural' reward. Further, it appears from the results of the studies presented here, and others that activation of neurons in the mesolimbic circuit through specific dopamine receptors is important in coding of incentive salience of all reward-paired cues.

OTHER AREAS MODULATING MESOLIMBIC ACTIVATION

The mesolimbic circuit does not act in isolation. The VTA receives innervations and interconnections between the prefrontal cortex (PFC), amygdala, and medial preoptic area (Carr & Sesack, 1999, 2000; Simerly & Swanson, 1988; Yim & Mogenson, 1983). These areas also affect dopaminergic firing and behavioral output. Their role was not explored in this thesis, nor is it known how they contribute to the sign-tracker and goal-

tracker differences in the attribution of incentive salience. They are worth mentioning as they do influence the mesolimbic circuit and may also reflect modulatory differences in STs and GTs.

The VTA, NAcc and VP receive glutamatergic input from the prefrontal cortex and basolateral amygdala (Brog, Salyapongse, Deutch, & Zahm, 1993; Carr & Sesack, 1999; Geisler et al., 2007; Maslowski-Cobuzzi & Napier, 1994). These connections have been implicated in coding prediction error and are involved in drug-seeking behavior (Jo et al., 2013; Lu, Xue, Steketee, Rebec, & Sun, 2012; Scofield et al., 2015). Glutamatergic innervations of the NAcc has been shown to modulate the rewarding aspects of cocaine and other drugs of abuse (Hoffman & Lupica, 2001; Schramm-Sapyta, Olsen, & Winder, 2006; White & Kalivas, 1998). GABAergic neurons from the mPOA project to the VTA and have been shown to enhance neural firing of the NAcc (Tobiansky et al., 2013) and increase locomotor activity following cocaine administration (Tobiansky et al., 2013; Will, Martz, & Dominguez, 2016). These GABA neurons express D2 receptors indicating the ability of dopamine to regulate firing as well (Tobiansky et al., 2013). Further, dopamine release into the mPOA facilitates male sexual behavior (Dominguez & Hull, 2005; Stolzenberg & Numan, 2011).

LIMITATIONS

In chapter 3, the number of responsive neurons in the nucleus accumbens core and shell was less than the desired amount. However, the low proportions of responsive neurons in the NAcc to cocaine self-administration have also been seen by others who report 43-47% of cells responsive to both food and cocaine (Carelli et al., 2000). The "withdrawal" period present in our study may also account for the low number of responsive neurons in the NAcc core and shell. Studies have shown that a 3- or 21-day

abstinence following chronic intraperitoneal injections of cocaine (30mg/kg), resulted in a reduction of D2R function and increase in D1R function in the nucleus accumbens core *in vitro* (Perez, Ford, Goussakov, Stutzmann, & Hu, 2011; Terwilliger et al., 1991). In addition to altering D1R and D2R function, following a 7-day withdrawal period from ip injections of cocaine (30mg/kg), studies have shown decreased synaptic dopamine in the NAcc (Robertson, Leslie, & Bennett, 1991). One consequence of this is lowered excitability of neurons. Future studies would benefit by combining electrode implant surgery with catheterization. This would allow neural recordings during testing period to occur immediately following training.

The behavioral similarities of STs and GTs seen in cocaine self-administration indicate that attribution of incentive salience to cues is not predictive of drug-taking behavior. In self-administration, each initial nosepoke into the active port resulted in stimulation of drug infusion, thus is specific to "consummatory" (drug-taking) not appetitive (drug-seeking) behaviors (Roberts, Gabriele, & Zimmer, 2013). While STs and GTs do not appear to differ in drug-taking, perhaps they differ in drug-seeking behavior. This could be corrected by having a noseport on a wall away from where animals nosepoke for drug delivery. The function of the new port would be to activate the ports for drug delivery would allow for analysis of both appetitive and consummatory behavior. Alternatively, using an extinction paradigm may help to differentiate motivational differences in drug-taking and drug-seeking behaviors in sign-trackers and goal-trackers. STs have shown to be more resistant to extinction of Pavlovian cues than GTs (Ahrens, Singer, Fitzpatrick, Morrow, & Robinson, 2016) but less resistant to cue-removal paradigms when reward is still delivered (Saunders & Robinson, 2010). Whether behavioral differences of STs and GTs in such extinction paradigms are reflected in neural firing patterns remains to be determined. Differences in neural encoding of such

behavior would point to target areas for therapeutic interventions and predict their ability to impact drug-seeking behavior in individuals.

SIGNIFICANCE

These studies are the first to analyze neural coding pattern differences in individuals varying in the attribution of incentive salience of Pavlovian cues. Specifically, the results presented are the first to show coding of dopamine neurons to motivation through tonic activation. Further, results from the presented studies indicate that behaviors directed towards reward-paired cues, a measure of incentive salience, can be modulated through the rostral ventral pallidum.

Results from chapter 3 were the first, to my knowledge, to report electrophysiological changes in the NAcc and VP simultaneously to food and drug self-administration. Other studies have stimulated the NAcc with pharmacological manipulations and analyzed firing patterns in the VP. They have found that that activation of both D1 and D2 receptors in the NAcc was required for significant increases in firing in the VP (Yang & Mogenson, 1989). It was previously discussed in this thesis that D2 receptors are more abundant in the NAcc shell than core and respond more to tonic levels of dopamine (DA). This, in combination with results that demonstrate the role of tonic dopamine release in encoding incentive salience (chapter 2), suggests that increased firing in the VP as a result of NAcc stimulation is propagating the incentive signal.

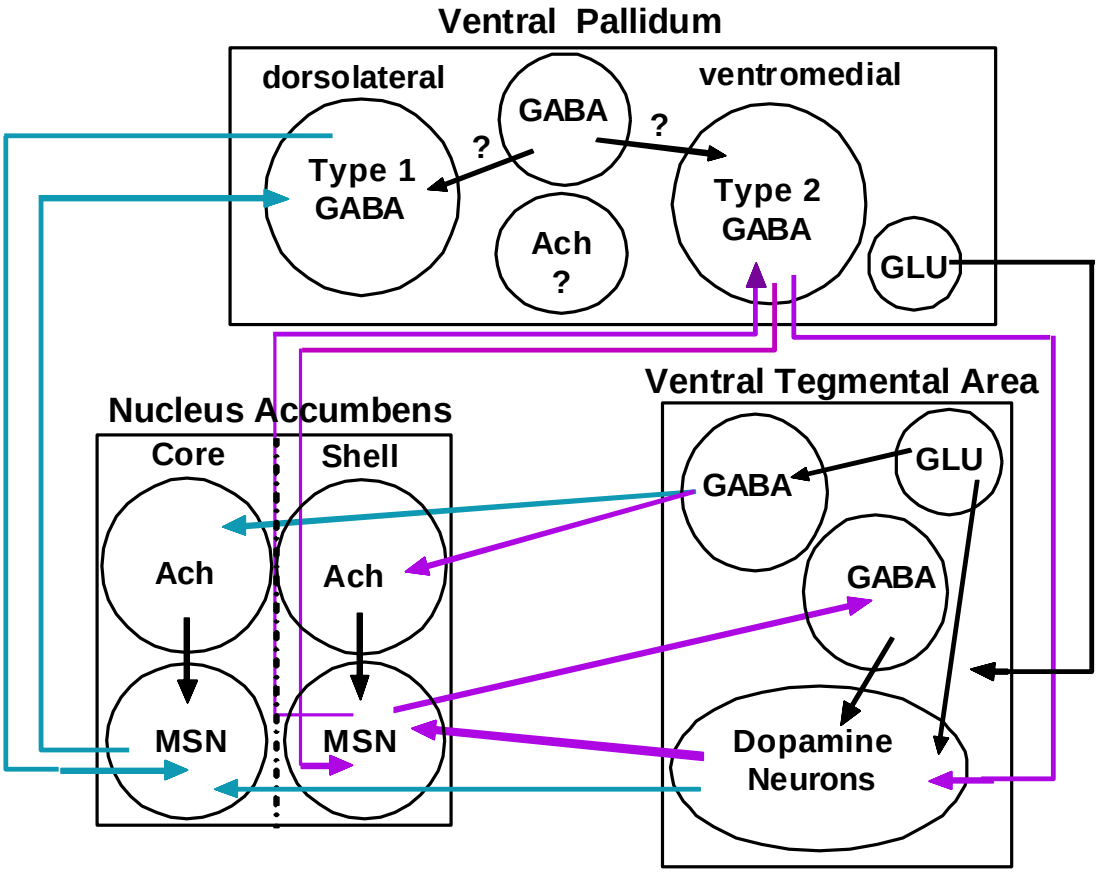
FUTURE DIRECTIONS

With the current use of viral vectors to target specific neurons and microcircuits, the understanding of the relationship between neurons in a circuit has become better known. Future studies would benefit by combining viral vectors (DREADDs or

optogenetics) with pharmacological manipulations (D1/D2 receptor agonist/antagonist) to describe how particular neurons activate or inhibit their target sites. Further, combining electrophysiological recordings with DREADD activation *in vivo* would allow for the direct correlation of behavioral changes with changes in firing patterns. This is an avenue we are currently pursuing. The benefit of DREADDs is the translational potential to treat some cue-related disorders.

Further, results presented in these studies indicate the malleable potential of the mesolimbic circuit in some individuals (i.e. goal-trackers). The behavioral changes seen were specifically quantified as approach behavior. Perhaps other behavioral expression changed as well, such as towards more impulsive actions (like those seen in sign-trackers). Also, analyzing the neural firing patterns and/or dopamine release as a result of increasing propensity to approach reward-paired cues (and perhaps changes in impulsivity) in goal-trackers would provide greater evidence to the occurrence of circuit alterations.

Figure 5.1: Detailed Schematic of the Mesolimbic Dopamine Circuit



Studies have identified two microcircuits of the mesolimbic circuit – one from the Ventral Tegmental Area to Nucleus accumbens shell, to the ventromedial ventral pallidum and back to the VTA. The other is from the VTA to the nucleus accumbens core to the dorsolateral ventral pallidum (and to the substantia nigra). The circuit involving the NAcc core (blue) may be more involved with motor behavior, while the shell (purple) may be involved in encoded motivational value of rewards and associated cues. Types of neurons: γ-aminobutyric acic (GABA), glutamate (GLU), cholinergic (Ach)

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