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REDUCTIONS IN MESOLIMBIC DOPAMINE SIGNALING AND AVERSION:
IMPLICATIONS FOR RELAPSE AND LEARNED AVOIDANCE

by

Mykel A. Robble

A Dissertation submitted to the Faculty of the Graduate School,
Marquette University,
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy

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ABSTRACT
REDUCTIONS IN MESOLIMBIC DOPAMINE SIGNALING AND AVERSION:
IMPLICATIONS FOR RELAPSE AND LEARNED AVOIDANCE

Mykel A. Robble

Marquette University, 2017

The ability to adjust behavior appropriately following an aversive experience is essential for survival, yet variability in this process contributes to a wide range of disorders, including drug addiction. It is clear that proper approach and avoidance is regulated, in part, by the activity of the mesolimbic dopamine system. While the importance of this system as a critical modulator of reward learning has been extensively characterized, its involvement in directing aversion-related behaviors and learning is still poorly understood.

Recent studies have revealed that aversive stimuli and their predictors cause rapid reductions in nucleus accumbens (NAc) dopamine concentrations. Furthermore, a normally appetitive stimulus that is made aversive through association with cocaine also decreases dopamine, and the magnitude of the expressed aversion predicts drug-taking. However, whether the presentation of a drug cue that reduces dopamine, and evokes a negative affective state, can motivate relapse is unknown.

Here we demonstrate that the presentation of an aversive drug cue both reduces dopamine and causes cocaine-seeking. This finding is provocative because drug seeking in reinstatement designs is typically associated with increased dopamine signaling. Using a combination of fast scan cyclic voltammetry (FSCV) and *in vivo* electrophysiology we subsequently show that the presence of an aversive drug cue abolishes the dopaminergic encoding of other drug cues and alters NAc neuronal activity patterns. Importantly, a subpopulation of neurons that subsequently encode aspects of drug-seeking behavior increase their baseline firing rates during this aversive experience.

We then examine the mechanistic regulation of dopamine signaling by aversive stimuli under more natural conditions. Using FSCV and site-specific behavioral pharmacology we demonstrate that blockade of ventral tegmental area kappa opioid receptors attenuates aversion-induced reductions in dopamine, and prevents proper avoidance learning caused by punishment. By maintaining D2 receptor occupancy within the NAc during punishment, we demonstrate the requirement of aversion-induced reductions in dopamine for aversive learning.

Together, these studies inform an evolving model of striatal physiology. Our findings emphasize a role for both increases and decreases in dopamine signaling that modulate behavior by promoting the stimulus-specific activity of distinct striatal output pathways. The continued interrogation of this model may offer novel targets for therapeutic development aimed at treating neurodegenerative disease and drug addiction.

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CHAPTER I

INTRODUCTION

General Introduction

Learning proper approach and avoidance behavior is a fundamental process that is critical for survival. Dysfunction in these learning processes results in aberrant approach and avoidance behaviors that are the hallmark of many diverse neuropsychiatric disease states. While considerable progress has been made characterizing the neural systems that guide reward and approach, the neural regulation of aversion and avoidance are poorly understood. Understanding these processes requires an understanding of the brain's reward circuitry, including the mesolimbic dopamine system. The following is a detailed account of experimental evidence implicating a role for the mesolimbic dopamine system in aversion-driven behavior.

Early Indications that Dopamine Regulates Reward

The serendipitous observation that a rat will press a lever for electrical brain stimulation (Olds & Milner, 1954) was a breakthrough finding for the study of the neurological systems that govern reward. Initially, the rewarding nature of this stimulation was thought to be controlled by the lateral hypothalamus, an area where electrical stimulation is such a powerful reward that rats will continue to respond even in the face of punishment by painful electric foot shock (Olds, 1958). However, the fact that the lateral hypothalamus is traversed by many

fibers of passage prompted researchers to suggest that electrical brain stimulation may produce its powerful rewarding effects by activating other neurological systems outside of this locus (Nieuwenhuys, Geeraedts, & Veening, 1982; Veening et al, 1982). These effects were subsequently attributed to stimulation of the medial forebrain bundle, as it was revealed that rats respond for electrical stimulation of multiple sites along this fiber tract (Rompre & Miliaressis, 1985; Call, Micco, & Berntson, 1974). The demonstration that pharmacological manipulations of dopamine with systemic antagonists blocked brain stimulation reward (Fouriezos & Wise, 1978), coupled with the knowledge that dopaminergic axons are part of the medial forebrain bundle (Millhouse, 1969), led to the emergence of the dopamine system as a likely candidate for mediating the rewarding effects of electrical stimulation of the medial forebrain bundle. The evolving view that brain dopamine systems mediate the rewarding effects of electrical brain stimulation was further corroborated by the observation that electrical stimulation of the ventral tegmental area (VTA) also supported self-stimulation behavior in the rat, and that the boundaries of effective stimulation clearly mapped onto the anatomical boundaries of the A9 and A10 dopaminergic cells that comprise the mesocorticolimbic and nigrostriatal dopamine system (Corbett & Wise, 1980).

Anatomy of the Mesolimbic Dopamine System

Much of the study of motivated behavior has focused on midbrain dopamine neurons in the VTA, originally called A10 dopamine neurons. These

neurons are tonically active, with firing rates from 1-5Hz, and exhibit stimulus driven phasic burst firing and pauses in activity (Schultz, 1997; Cooper, 2002). VTA dopamine neurons send dopaminergic projections to various brain regions, including the amygdala, prefrontal cortex, septum, hippocampus, and cingulate cortex (Ikemoto, 2007). The most dense dopaminergic project terminates in the nucleus accumbens, an area commonly thought of as a site of limbic motor integration (Mogenson, Jones, & Kim, 1980; Swanson, 1982; Oades & Halliday, 1987; Figure 1.1). Based on histological differences, the nucleus accumbens is divided into two sub regions, the core and the shell (Zaborski et al, 1985). The core is thought to be important for motivated behavior drive by associative processes, while the shell is involved in primary reinforcement (Cardinal, Parkinson, Hall, & Everitt, 2002). The major output neurons of the nucleus accumbens are GABAergic medium spiny neurons (MSNs). These neurons can be classified into two distinct populations based on whether they express D1-like or D2-like dopamine receptors, as estimates indicate only 5% express both receptor types (Bertran-Gonzalez et al, 2008). D1-like receptors have a low affinity for dopamine, in the micromolar range, while D2-like receptors have a high affinity for dopamine, in the nanomolar range (Rice & Cragg, 2008). MSNs also differ in terms of their projection targets. D1-expressing MSNs predominantly project to the ventral pallidum (Kupchik et al, 2015) and VTA (Bocklisch et al, 2013), while D2-expressing MSNs predominantly project to the ventral pallidum (Wall, Parra, Callaway, & Kreitzer, 2013; Kupchik et al, 2015).

These separate populations of output neurons regulate distinct behaviors (Kravitz, Tye, & Kreitzer, 2012).

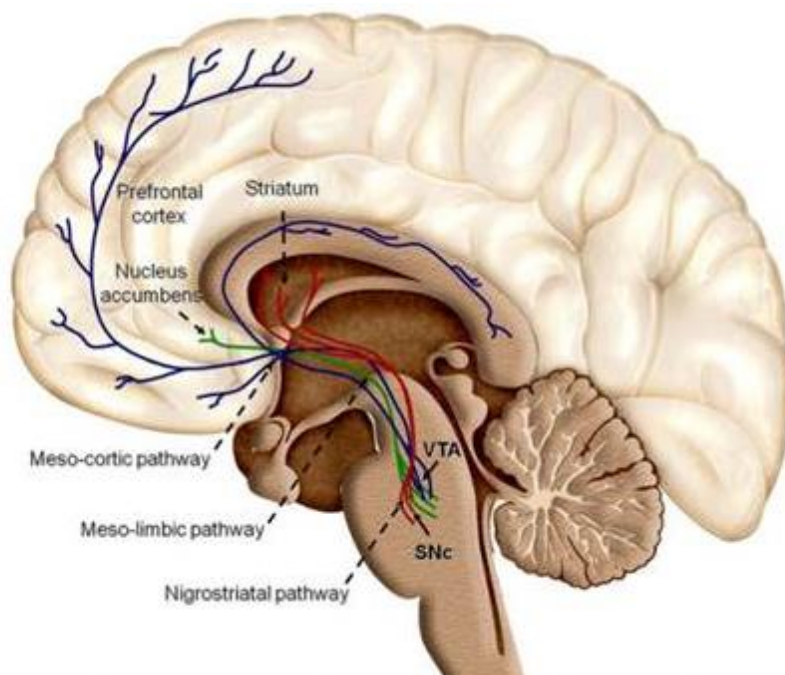


Figure 1.1. Anatomy of the mesolimbic dopamine system. Midbrain dopamine neurons send projections to various forebrain nuclei. The mesocortical pathway is shown in blue, and the nigrostriatal pathway is shown in red. The most dense dopaminergic projection from the ventral tegmental area terminates in the nucleus accumbens and comprises the mesolimbic dopamine system (green). This pathway is a critical regulator of motivated behavior. Adapted from Arias-Carrión et al. 2010.

The nucleus accumbens receives glutamatergic input from several brain areas, including the prefrontal cortex, amygdala, lateral hypothalamus, hippocampus, and VTA (Maldonado-Irizarry, Swanson, & Kelley, 1995; Floresco, Todd, & Grace, 2001; McFarland, Lapish, & Kalivas, 2003; Stuber et al, 2011; Qi et al, 2016). It is thought that dopamine receptor activation on MSNs alters the

sensitivity to glutamatergic drive to influence output signaling (Surmeier, Ding, Day, Wang, & Shen, 2007). Within the nucleus accumbens there are local GABAergic and cholinergic interneuron populations that may regulate MSNs activity (Sadikot, & Sasseville, 1997; Alcantara, Chen, Herring, Mendenhall, & Berlanga, 2003). Furthermore, the distinct MSN subpopulations make collateral inhibitory connections with each other (Dobbs et al, 2016). Understanding the functional connectivity of the nucleus accumbens is vital to the understanding of the role of this structure in the processing of rewarding and aversive stimuli, and associated cues, and how these stimuli guide behavior and learning.

The Emergence of the Anhedonia Hypothesis

Electrical brain stimulation provided a pure framework for the study of reward, and this framework allowed for theoretical integration of other observations of the impact of dopaminergic pharmacological manipulation on natural rewards and drugs of abuse. At the time, evidence was mounting that dopamine antagonists substantially reduce the reinforcing efficacy of intravenous cocaine and amphetamine, as well as that of food rewards (Yokel & Wise, 1976; de Wit & Wise, 1977; Wise & Schwartz, 1981; Ettenberg & Camp, 1986; for review see: Ettenberg, 1989). Taken together, these studies support the interpretation that dopamine neurotransmission is a critical component of reward. Roy Wise classically articulated these ideas by presenting the “anhedonia hypothesis” (Wise, 1982). Wise posited that the dopamine system was a common pathway that all rewarding stimuli act on. Furthermore, he stated that

dopamine was the neurochemical basis for the sensation of pleasure that is evoked by rewarding stimuli, and that the decrease in responding for food, drugs, or electrical brain stimulation caused by dopamine antagonists results from a decrease in the rewarding value of these stimuli. This hypothesis was the first formal explanation of the neurological basis of reward, and became the prevailing view of the time. Importantly, it had clinical relevance for Parkinson's disease (Wise 1982), as pharmacological manipulations that reduce dopamine signaling produce a Parkinson's-like state in the rat, and it would become fundamental to theoretical explanations of drug addiction (Bozarth and Wise, 1987).

Studies measuring terminal dopamine release by *in vivo* microdialysis largely supported the anhedonia hypothesis. Intra-VTA microinjections of opioid receptor agonists increased the rewarding value of electrical brain stimulation (Jenck, Gratton, & Wise, 1987), and elevated ventral striatal dopamine concentrations (Devine et al, 1993). Other drugs of abuse, such as cocaine, and natural rewards, such as food, were also shown to increase ventral striatal dopamine (Hernandez & Hoebel, 1988). Together, these studies were consistent with the hypothesis that the modulation of terminal dopamine release following the experience of either natural or pharmacological rewards creates the subjective experience of pleasure.

Challenges to the Anhedonia Hypothesis

The potential role of dopamine in mediating pleasure that was posited in the anhedonia hypothesis was challenged in several seminal studies. One critical

observation was made using taste reactivity to assess hedonic responses to sucrose. When rodents, or infant primates and humans, receive a sweet taste they make stereotypical orofacial reactions. Conversely, aversive tastants evoke a different set of stereotypical responses. These responses are quantifiable and reflective of the hedonic value of the tastant (Grill and Norgren, 1978; Figure 1.2).

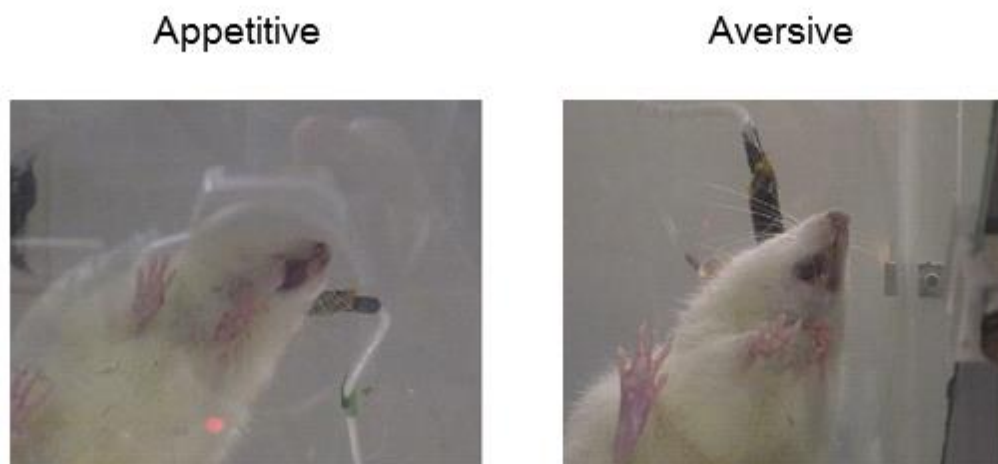


Figure 1.2. Taste reactivity. Characterization of stereotyped orofacial responses to tastants allows for the assessment of the neural encoding of hedonic processing without the potential confound of learning or goal-directed action. Left: Appetitive Taste Reactivity. Lateral tongue protrusions are reliably evoked by palatable taste stimuli. Right: Aversive Taste Reactivity. Rejection responses are reliably elicited by aversive stimuli.

Using this behavioral assessment, Berridge, Venier, and Robinson (1989) examined the impact of dopamine depletion caused by 6-hydroxydopamine (6-OHDA) treatment in the midbrain on the hedonic value of sucrose in rats. Midbrain dopamine depleted rats will not respond for food rewards, nor will they consume food presented to them, despite retaining the motor capacity to perform such responses (Berridge & Robinson, 1998). As was the case with dopamine

antagonists, these deficits were previously explained by the anhedonia hypothesis. However, dopamine depletion was shown to have no effect on taste reactivity responses when the tastant was intraorally infused, removing the requirement for goal-directed action. This observation directly contradicted the hypothesis that dopamine amplifies, or is responsible for, the hedonic aspect of reward. These findings were corroborated by the demonstration that mice that cannot produce dopamine still prefer sucrose to water. Saccharin, a non-caloric sweet taste, was also examined and preferred over water, ruling out the caloric content of sucrose as a potential explanation (Cannon & Palmiter, 2003). Together these studies indicated that dopamine does not mediate the hedonic aspect of rewards. Rather, they suggest that its role in reward processing is more complex and is dissociable from hedonics.

The anhedonia hypothesis was further challenged by Cousins and Salamone (1994). In a concurrent choice design, rats had the option to lever press for food pellets on a low fixed ratio requirement or free access to standard lab chow. On alternating days, the choice was removed and the only option was to lever press for food pellets. Using this elegant paradigm it was demonstrated that nucleus accumbens dopamine depletion resulted in reduced lever pressing and increased standard chow consumption on days when both choices were available. However, on days when lever pressing was the only available option, nucleus accumbens dopamine depletion had no effect on responding. In a subsequent study, this group showed that dopamine depletion did not alter responding for food when the response requirement was low. Rather, dopamine

depletion specifically impaired responding when the required amount of work was high. Furthermore, sated rats, which have reduced motivation to respond for food, were shown to exhibit a reduction in responding regardless of the work requirement (Aberman & Salamone, 1999). Together these studies favor the more nuanced view that, rather than being a pleasure neurochemical, or even being responsible for the motivation to obtain rewards, dopamine is involved in the allocation of effort and effort-based decision making.

These critical experiments represent major challenges to the original view that dopamine is central to hedonic perception. Through the use of elegant experiments designed to dissociate the actions of dopamine under specific circumstances, it became clear that the anhedonia hypothesis was insufficient to explain the role of this neurochemical in reward-seeking behavior. In light of this realization, new theories that offered a more nuanced and precise explanation of the role of dopamine in reward-related behaviors emerged and gained prominence.

Dopamine and Learning

Following the challenges to the anhedonia hypothesis, several studies examined the possibility that dopamine guides motivated behavior by promoting appetitive association. These experiments involved classical conditioning procedures in which a previously neutral stimulus becomes a conditioned stimulus (CS) when it is paired with reward (US) delivery/availability. Through repeated pairings, animals learn that the CS predicts the US, as the CS begins to

elicit a response (Pavlov, 1927). In many cases, it was observed that the CS would elicit anticipatory or preparatory behaviors following learning, as if the CS has become a substitute for the reward (Dickinson, 1980; Schultz, 1997).

Observations that pharmacological dopamine depletions produced deficits in reward seeking or approach behavior (Wise 1982; Beninger 1983), but did not change the hedonic value of the reward itself (Berridge & Robinson, 1998; Berridge et al, 1989) suggested a possible role for DA in learning.

To determine if dopamine is involved in reward learning, researchers began to use *in vivo* electrophysiological recordings during conditioning procedures. One critical finding was reported by Aosaki, Graybiel, and Kimura (1994). This group recorded primate ventral striatal neurons during a classical conditioning design in which a neutral auditory stimulus was paired with a food reward. They found that initially only a small fraction of the neurons recorded responded to the auditory stimulus, but following repeated pairing with the reward the percentage of cells responsive to the CS increased substantially. Furthermore, unilateral dopamine depletion caused a dramatic reduction in responsiveness to the CS only on the side in which the dopamine depletion was performed. This observation had a large impact on the field because it demonstrated that neurons in regions that receive dopaminergic input change their activity coincident with learning, and this activity change depends on a functional dopamine system.

In parallel with these findings, electrophysiological recordings of putative midbrain dopamine neurons revealed a similar phenomenon. Midbrain dopamine

neurons are activated by rewards of various sensory modalities, and by reward-predictive cues (Romo & Schultz, 1990; Schultz & Romo 1990; Ljungberg, Apicella, & Schultz, 1992). Importantly, expectation was determined to be a crucial factor in the responsiveness of these neurons to rewards. In non-human primates, dopamine neurons were found to be activated only by unexpected rewards, and, with conditioning, phasic increases in firing rate shifted from the reward to the CS (Mirenowicz & Schultz, 1994). Notably, when an expected reward was omitted, dopamine neurons ceased firing during the time of expected reward delivery. This indicated that dopamine neurons have a bidirectional response to the unexpected, as increases or decreases in firing rate were coincident with unexpected reward delivery or omission of an expected reward, respectively (Figure 1.3). Perhaps most provocative, with the strongest implications for learning theory, was the finding that when a reward becomes fully predictive, which is only seen with extensive training, responses to the reward disappear (Schultz, Apicella, & Ljungberg, 1993; Figure 1.3).

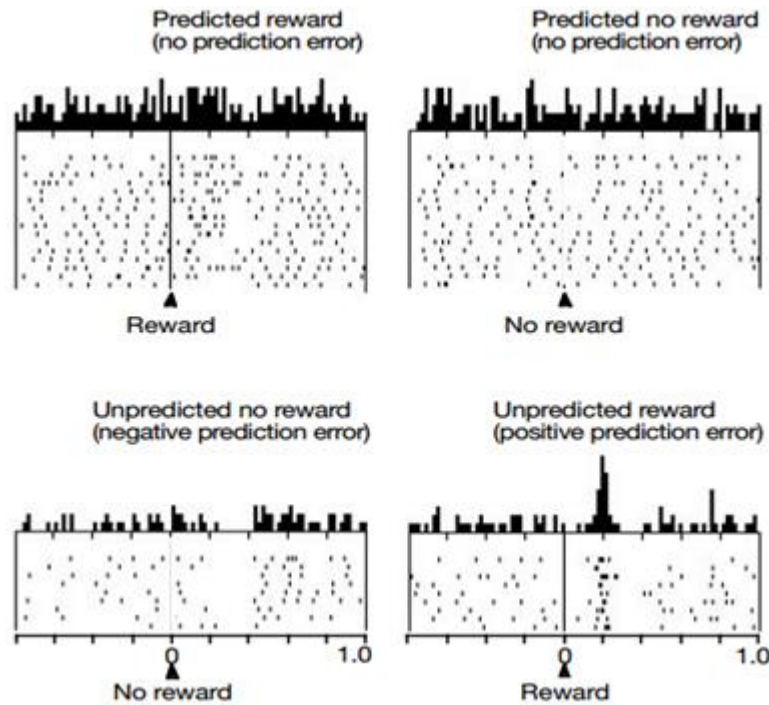


Figure 1.3. Reward-related activity of putative midbrain dopamine neurons is modulated by expectation. Top: Expected rewards, as well as expected reward omission, do not generate prediction errors and do not alter the firing rates of dopamine neurons. Bottom: A situation in which an expected reward is omitted generates a negative prediction error and causes a pause in dopaminergic cell firing. Conversely, a positive prediction error is generated when an unexpected reward is delivered, causing a transient increase in dopaminergic cell firing. Adapted from Waelti et al, 2001.

These observations were of particular significance to the field because they closely aligned with a prominent model of classical conditioning. The Rescorla – Wagner model of conditioning (1972) carefully described the factors that determined the effectiveness of a stimulus to cause learning. One of the most important factors in determining whether a CS could acquire association with a US is predictability, with unexpected reward delivery creating the strongest opportunity for learning. This factor was modeled as the difference between expectation and actual outcome and referred to as “delta”. Learning occurs best

when delta is largest. The observations by Schulz and colleagues indicated that the activity patterns of midbrain dopamine neurons may be the physiological substrate for computing these deviations in expectation, later termed “prediction errors” (Schultz et al, 1995; Schultz 1997), and thus may be the neural system responsible for conveying learning signals.

One important feature of the Rescorla-Wagner model was that it provided an explanation of complex learning phenomena, such as blocking. Blocking refers to the phenomenon by which associations made between a CS (B) and a US can be “blocked” if the CS is presented at the same time as a different, but qualitatively similar, CS (A) that has already been associated with the US. The Rescorla-Wagner model explains this occurrence in terms of expectation and predictability. CS B is blocked from association with the US because it does not offer any additional predictive value to the associative strength of the original CS A for the US (Rescorla & Wagner, 1972; Bakal, Johnson, & Rescorla, 1974). In a seminal study, Schultz and colleagues examined the activity of midbrain dopamine neurons in primates during a blocking design. During initial training, CS “A” was paired with a reward, and CS “B” was explicitly unpaired, such that CS B does not become associated with the reward. Following training a blocking procedure was conducted in which A was now combined with neutral stimulus “X” and presented as a compound CS (AX). Concurrently, neutral stimulus “Y” was combined with “B” and presented as a different compound CS. Each compound CS was paired with reward delivery. Following this procedure, X and Y were presented alone and dopamine neuronal responses were recorded.

When presented following blocking, X did not evoke increased dopamine neuronal activity indicating that it was successfully blocked by A. However, Y did evoke increased dopamine neuronal activity as it was not blocked by the previously unpaired CS, B (Waelti, Dickinson, & Schultz, 2001). According to the Rescorla-Wagner model, stimulus X did not become an effective CS because when presented with stimulus A, it did not offer any additional predictive value to the already predictive CS, A. However, Y did become an effective CS when presented with B because B did not have any prior predictive value to it. This study demonstrated that dopamine neuronal activity reflects critical aspects of formal learning theory, and bolstered the view that the dopamine system conveys prediction error signals that guide reward learning.

The findings of Schultz and colleagues were further corroborated by studies using fast scan cyclic voltammetry to measure terminal dopamine release. Like midbrain dopamine neuronal activity, nucleus accumbens dopamine increases following reward receipt and in response to reward-predictive stimuli (Phillips, Stuber, Heien, Wightman, & Carelli, 2003; Day, Roitman, Wightman, & Carelli, 2007; Roitman, Wheeler, Wightman, & Carelli, 2008). Importantly, Day et al (2007) demonstrated that nucleus accumbens dopamine also is modulated by learning. Prior to conditioning, it was demonstrated that a food reward increased extracellular dopamine concentrations. With conditioning, dopamine began to increase in response to a visual cue that was paired with reward delivery, but not with a cue that was explicitly unpaired. Importantly, as dopamine increased to the CS with repeated

trials, the dopamine response to the reward itself began to decrease. This demonstrated that nucleus accumbens dopamine tracks learned associations in a manner that is reflective of the activity of midbrain dopamine neurons. This observation strengthened the work by Schultz and colleagues because it was previously only an assumption that ventral striatal dopamine was involved in learning on the basis of the examination of putative midbrain dopamine neurons. The application of fast scan cyclic voltammetry to scrutinize real time terminal dopamine signaling offered support to the hypothesis that ventral striatal dopamine signaling regulates appetitive association.

Although characterization of dopamine neuronal activity and terminal dopamine release supported the view that dopamine signals prediction errors that guide reward learning, a causal link between dopamine and learning was lacking. This was addressed in a recent study by Steinberg et al (2013) using optogenetics in transgenic rats to selectively and discretely manipulate dopamine neurons. The experimenters employed a blocking design in which they specifically activated VTA dopamine neurons during reward delivery. As a result of this stimulation, rats significantly increased responding to the visual stimulus that was blocked in a separate group of rats that did not receive effective optogenetic stimulation. According to the Rescorla-Wagner model described above, this visual stimulus is normally blocked because it does not offer any additional predictive value not conveyed to the animal by the original CS. However, by selectively activating dopamine neurons during the reward receipt, a prediction error signal was generated. Thus, the additional visual cue added

predictive value to the compound stimulus and consequently became associated with the reward. Furthermore, it was demonstrated that stimulating dopamine neurons during omission of expected reward delivery retarded extinction learning. In this case, the optogenetic activation of midbrain dopamine neurons prevented the negative prediction error normally caused by reward omission (Schultz, Apicella, & Ljungberg, 1993; Waelti et al, 2001) and thus attenuated learning that the reward was no longer available. These findings demonstrate that proper mesolimbic dopamine function is required for prediction error signaling that guides reward learning. Supported by the observation that prediction errors are reflected in human midbrain and ventral striatal BOLD signals (Diederer et al, 2016), this view of the role of dopamine in appetitive behaviors as a learning signal remains prominent to this day.

Dopamine and Motivation

Reward is a complex process with clearly dissociable components. The sensation of pleasure during consumption and the motivational drive to obtain the reward, to which there is a learning component, are both important aspects of reward-seeking behavior. The findings that challenged the anhedonia hypothesis showed that manipulations that reduce dopamine signaling also altered the motivation to obtain a reward, but did not change the hedonic value of the reward itself (Berridge et al, 1989; Cousins & Salamone, 1994, Aberman & Salamone, 1999; Cannon & Palmiter, 2003). These findings suggested that dopamine may, instead, play a role in reward learning (Beninger, 1983). Pharmacological studies

using dopamine antagonists during conditioning revealed that these manipulations did not prevent various stimuli from becoming associated with punishers, as rats tested in the drug-free state showed evidence of associative learning (Beninger, Mac Lennan, & Pinel, 1980; Beninger et al, 1980). Additionally, mice treated with dopamine antagonists exposed to a novel maze showed evidence of learning when re-exposed to the maze in a drug-free state (Ahlenius, Engel, & Zöller, 1977). Furthermore, rats treated with dopamine antagonists during the Pavlovian conditioning phase of a Pavlovian to instrumental transfer design still learned to discriminate between different conditioned stimuli (Beninger & Phillips, 1981). Together, these studies suggested that proper dopamine functioning was not required for various forms of associative learning, regardless of whether the association was to discrete aversive stimuli, contexts, or to rewards. The results of these studies indicate that the role of dopamine in appetitive association is more nuanced than simply strengthening associations.

Reward learning that guides reward-seeking behavior is not limited to the simple association between the cue and the consequence. As an environmental stimulus becomes associated with a reward, it begins to elicit behavioral responses that are reinforced by reward delivery. These responses, driven by conditioned stimuli through reward association, are stimulus-specific preparatory or approach behaviors (Bolles, 1972). The process by which cues associated with rewards gain incentive value, and elicit motivation, is referred to as incentive motivational learning (Beninger, 1983). In light of the findings discussed here that

manipulations that reduce dopamine also reduce responding for rewards (Wise & Schwartz, 1981; Wise, 1982) but do not prevent associative learning, Beninger (1983) argued that dopamine was specifically important for incentive motivational learning. In other words, dopamine signaling regulates the learning process by which reward-associated cues incentivize responding. This idea was supported by the observation that dopamine antagonists prevent incentive learning when administered during the conditioned reinforcement phases of a Pavlovian to instrumental transfer design (Beninger & Phillips, 1980).

Berridge & Robinson made a major theoretical advance to these ideas with the introduction of the “incentive salience” hypothesis of dopamine function (Robinson & Berridge, 1993; Berridge and Robinson, 1998). Originally postulated as a theory of the role of dopamine in drug addiction (Robinson & Berridge, 1993), the incentive salience hypothesis became a more broad explanation of the role of dopamine in motivated behavior (Berridge & Robinson, 1998). At the crux of this hypothesis is the notion that reward can be dissociated into two distinct components: “liking” and “wanting”. Liking refers to the hedonic experience that accompanies reward consumption, while wanting refers to the motivational process “that instigates goal-directed behavior, attraction to an incentive stimulus, and consumption of the goal object” (Berridge and Robinson, 1998: p. 313). They argue that while liking does not involve dopamine (Berridge et al, 1989), wanting is a dopamine-mediated process. Specifically, dopamine signaling is not required for hedonic activation, nor is it required for associative learning. Rather, dopamine signaling is required to imbue a reward-predictive

cue with incentive value so that the cue itself becomes wanted and elicits goal-directed behavior. Several studies of dopamine signaling offer support for the incentive salience hypothesis. DOPAC/dopamine ratios, an indication of dopamine utilization, are elevated in the nucleus accumbens in rats following presentation of a food-predictive cue (Blackburn & Phillips, 1989). This is also reflected in electrochemical monitoring of dopamine release in response to food-predictive cues (Phillips et al, 1993; Roitman et al, 2004). These studies show that dopamine is elevated to cues that initiate preparatory behavior and incentivize responding, a critical facet of the incentive salience hypothesis.

An important implication of the incentive salience hypothesis is that hedonics, associative learning, and incentive motivation are dissociable psychological processes with distinct neural underpinnings. This was elegantly demonstrated by Smith, Berridge, and Aldridge (2011) using a Pavlovian design in which a distal cue preceded a proximal cue for intraoral sucrose delivery. They manipulated opioid and dopamine signaling within the nucleus accumbens and recorded neuronal activity in the ventral pallidum, an area with reciprocal connections to the accumbens that has also been implicated in hedonic and motivational processes (Smith et al, 2009). They found that an intra-accumbens mu-opioid receptor agonist increased ventral pallidum neuronal activity elicited by the incentive cue (proximal), as well as increased reward consumption, (wanting) and hedonic reactions to reward (liking). However, the effect of intra-accumbens amphetamine was specific for motivation, as it increased reward consumption but had no effect on hedonic reactions. Neither of these treatments altered neuronal

activity to the more distal cue, indicating that they did not change the associative strength of that cue with reward. These results indicated, as predicted, that the components of reward are governed by distinct neural signaling systems, and that the dopamine system is selectively important for incentive motivation. This report marked a demonstration of the core tenets of the incentive salience hypothesis.

Finally a recent high-profile study by Flagel and colleagues (2011) provided perhaps the strongest support for the role of dopamine in incentive salience. The study used fast scan cyclic voltammetry to scrutinize real-time dopamine signaling during an autoshaping design. This Pavlovian learning design involves training rats that lever extension is a cue for impending food delivery. The lever extends into the box for a period of time, and food is delivered to the goal area coincident with lever retraction. According to the incentive salience hypothesis, as the lever becomes associated with food delivery it is imbued with the mental representation of the food that incentivizes behavior. Thus, the lever itself elicits a strong conditioned response that involves direct engagement with the lever prior to food delivery. However, there are individual differences in this type of learning. Some rats showed a strong conditioned response toward the lever while others exhibited conditioned approach to the food delivery area. Measuring dopamine responses to the cue and the reward revealed striking differences between rats that reflected differential learning. In rats that learned the strong conditioned response of engaging the cue directly, the cue-evoked dopamine responses were substantially larger than those evoked

by reward. This cue-induced dopamine elevation was absent in rats that did not show the conditioned cue behavioral response. However, this group still approached the food delivery area in anticipation of reward delivery, indicating that the lever served as a predictive cue for food delivery. Furthermore, dopamine antagonist administration selectively disrupted conditioned responses toward the lever. These results support the hypothesis that dopamine is not a critical regulator of basic associative learning, but tracks, and is required for, incentive motivational learning. Importantly, these findings are not at odds with a role for nucleus accumbens dopamine in prediction error signaling, as these signals should be important for learning the conditioned response of cue engagement.

Together, these studies strongly support the role of dopamine in incentivizing reward predictive cues to promote goal directed behavior. Demonstrations like these have helped strengthen the incentive salience hypothesis and have contributed to its continued influence on an evolving understanding of the role of dopamine in motivation.

Dopamine and Effort

Another, perhaps parallel, explanation of the role of dopamine in goal directed behaviors is the hypothesis that dopamine regulates effort-based decision making. This view was inspired by numerous studies that demonstrated that while manipulations that decrease dopamine do not reduce the hedonic value of rewards (Berridge et al, 1989), they do reduce instrumental responding

to obtain rewards (Yokel & Wise, 1976; de Wit & Wise, 1977; Fouriezos & Wise, 1978; Wise & Schwartz, 1981; Ettenberg & Camp, 1986). As described above, seminal work from John Salamone (Cousins & Salamone, 1994; Aberman & Salamone, 1999) showed that dopamine depletions do not impair responding for a food reward. Rather, these manipulations bias rats to choose rewards that require less effort to obtain, and decrease responding for rewards as more effortful responding is required. These studies provided a framework to view dopamine as playing a critical role in the computation of cost benefit analyses that guide reward seeking. Subsequent studies corroborated and further implicated dopamine signaling in this process by showing that blocking D1 or D2 dopamine receptors biased rats to choose smaller, lower cost rewards over larger, higher cost rewards. Furthermore, administration of dopamine receptor agonists were shown to produce the opposite results, biasing behavior towards larger rewards despite increased effort required to obtain them (Bardgett et al, 2009). Together, these findings support a behavioral economics view of the role of dopamine in goal directed behavior, and suggest that dopamine is important for the computation of cost benefit analyses that determine reward seeking.

Critical support for this idea comes from Cargnaird et al (2006) who examined the direct manipulation of dopamine on effort, independent of learning. Using a dopamine transport knockdown mouse, they examined responding for food on a progressive ratio and in the concurrent choice design utilized by Cousins and Salamone (1994). Knockdown of the dopamine transporter results in reduced dopamine clearance in terminal fields. Importantly, this manipulation

also increased tonic firing of midbrain dopamine neurons without affecting phasic burst firing. Mice lacking the dopamine transporter exhibited elevated breakpoints in a progressive ratio task. Furthermore, in the concurrent choice design they showed increased lever pressing for food pellets, despite free access to food presented concurrently. These experiments were performed following training in each task to control for the influence of elevated dopamine during learning. The results represent a significant advancement to field of behavioral neuroeconomics, as they provide a causal role for dopamine in effort-based decision making, and specifically in the sensitivity to the costs associated with effort. These findings comply with previous suggestions that dopamine signaling is related to response vigor and that elevated dopamine levels lead to increased effort to obtain rewards (Robbins & Everitt, 1992; Weiner & Joel, 2002). Indeed, this is also the case in humans; it has been demonstrated that pro-dopaminergic drugs reduce sensitivity to costs in effort-based tasks (Wardle et al, 2011).

Studies that use fast scan cyclic voltammetry to measure dopamine signaling directly have also shown that nucleus accumbens dopamine tracks cost. Using a delay discounting task, Sadoris and colleagues (2015) demonstrated that the dopaminergic response to a reward-predictive cue was largest with no delay between cue presentation and reward delivery. However, the introduction of a delay between cue and reward presentation decreased the dopaminergic response to the cue, and the length of the delay correlated with the attenuation of the cue response. Furthermore, when given free choice trials initiated by a cue, rats exhibited a strong preference for rewards with a

comparatively shorter delay, i.e. a smaller cost. This preference was abolished by optogenetic stimulation of VTA dopamine neuron terminals within the nucleus accumbens during the cue presentation. This result provides a causal role for nucleus accumbens dopamine in cost sensitivity, as preventing the cost-induced dopaminergic scaling of the cue response prevented delay-based costs from influencing response preference. This finding is provocative and supportive of the hypothesis that mesolimbic dopamine is computing cost benefit relationships that determine the allocation of effort.

The basic observation that states of reduced dopamine lead to reduced effort to obtain rewards is reflected in human clinical literature as well. Patients with Parkinson's disease exhibit numerous locomotor deficits, including a reduction in the initiation of voluntary movement (Jankovic, 2008). While this particular deficit has been interpreted as a purely motoric perturbation, recent evidence indicates that it may also involve a motivational component. This reinterpretation arises from evidence that patients with Parkinson's disease reduce their behavioral output as energetic demand is increased, despite demonstrating response capacity that is similar to healthy controls (Mazzoni, Hristova, & Krakauer, 2007). Thus, it may be the case that some of the motor dysfunction reflects a perturbation in cost benefit analysis such that perceived cost of movement is inflated. In other words, these patients perceive rewards that would typically engender responding as not being worth the effort. To examine this, Chong et al (2015) developed a task that not only incorporated effort in responding for a reward, but also gave subjects the choice of whether or not to

initiate a bout of responding. With the amount of effort necessary to obtain a reward in a given trial known ahead of time, subjects could choose if responding was worth it. This allowed for the calculation of “effort indifference points” where subjects chose to respond on 50% of trials. Chong and colleagues found that patients with Parkinson’s disease had a heightened sensitivity to effort requirements (costs) for small rewards, as they chose to respond on a smaller proportion of trials compared to healthy controls. This gap closed as the reward magnitude increased. In these patients, treatment with D2-like receptor agonists, or pro-dopaminergic drugs, increased willingness to work for smaller rewards. The importance of this finding is the demonstration that some of the motor deficits associated with Parkinson’s disease are due to perturbations in motivation, in addition to neurodegenerative disruptions in motor function. Furthermore, it highlights the utility of this disease for the study of the role dopamine in goal directed behavior, as it extends the preclinical literature to the clinical setting to suggest that dopamine is involved in cost benefit analysis to determine motivation to seek rewards.

Attempts to Unify Theories of the Role of Dopamine in Motivation, Learning, and Neuro Economics

The possibility that tonic and phasic dopamine signaling may have distinct functions in goal-directed behavior became the foundation of attempts to unify the seemingly disparate views of dopamine function. Niv et al (2007) proposed a model of dopamine signaling that unites theories of the role of dopamine in

learning (Schultz, 1997), incentive salience (Berridge & Robinson, 1998), and effort-based decision making (Cousins & Salamone, 1994). Rather than focus on performance in discrete trials, their model focuses on situations in which reward is continuously available. This allows for examination of not just the choice to respond, but also latency to respond and response vigor. This model states that reward rate, the amount of available reward over unit time, determines optimal response strategies. In situations in which reward rate is high, the optimal latency to respond is low, and the vigor with which to maintain responding is high. The converse is also true. A lower reward rate results in a higher latency to respond and decreases response vigor. In other words, states of high reward rate increase the cost of inaction and compel effortful responding. Niv and colleagues suggest that reward rate is a state variable, i.e. that it is relatively stable, and this could be signaled by slow neurochemical changes, a role they ascribe to tonic dopamine.

One of the strengths of the model is that it makes testable predictions, among them being that food deprived animals should have elevated tonic nucleus accumbens dopamine when presented with food, as reward rate is higher in a deprived animal. This is corroborated by microdialysis work showing that, indeed, food deprived animals do have an exaggerated dopamine response to food consumption (Wilson et al, 1995). Food deprived rats also have a reduction in dopamine transporter expression and function (Patterson et al, 1998), which elevates tonic dopamine (Cargnaird et al, 2006), and these animals exhibit increased dopamine D2 receptor expression which may decrease

sensitivity to the cost of effortful responding (Thanos et al, 2008). The separation of roles for dopamine based on time scale allows for rapid changes in dopamine to convey learning signals and/or control the attribution of incentive salience to reward predictive cues. Overall, the strength of this model is the ability to synthesize seemingly disparate observations and present a testable framework for researchers going forward.

In a recent high impact study, Hamid et al (2015) furthered the ideas put forward by Niv and colleagues (2007) by using microdialysis and fast scan cyclic voltammetry to examine dopamine signaling on multiple time scales in the same task. In the task, a cue signaled the opportunity to choose one of two response options, and each option resulted in a differential probabilistic reward. In situations in which the reward rate was high, rats exhibited shorter response latencies and received more rewards per minute than when the reward rate was low. Furthermore, using microdialysis to measure slow changes in dopamine signaling, they demonstrated that dopamine concentration was strongly correlated to reward rate. Thus, as predicted by Niv and colleagues, motivational state is positively correlated with reward rate, and this is reflected in slow changes in dopamine.

To examine the role of rapid changes in dopamine in goal-directed behavior they used fast scan cyclic voltammetry to record subsecond dopamine fluctuations during the probabilistic reward task. They found that unexpected rewards evoked larger increases in dopamine than did expected rewards, consistent with prediction error theory (Schultz, 1997). However, reward omission

did not produce a discrete reduction in dopamine, as would be expected if dopamine was conveying a prediction error signal. Rather, reward omission produced more drawn out adjustments to dopamine that the authors suggested reflects changes in reward rate. To clarify this discrepancy, trial-by-trial changes in baseline and peak dopamine concentration following expected and unexpected rewards were analyzed. Prediction error theory would suggest that peak cue-evoked dopamine should decrease across trials as the reward becomes increasingly expected. However, it was observed that as rewards increased in frequency, cue-evoked dopamine remained constant and baseline dopamine concentration was elevated. This pattern of dopamine signaling is, again, more consistent with the interpretation that dopamine is conveying information about state value (reward rate). These findings suggest that dopamine signals reward availability on multiple time scales. This theory reconciles different theories positing a role for dopamine in motivation and learning by characterizing signals that simultaneously convey information about both. Dopamine levels represent a constantly updating signal about the value of a given situation, and this influences the willingness to work to obtain reward. Simultaneously, rapid changes in dopamine are used as learning signals. However, these learning signals are not changes from a steady baseline, as learning theory suggests. Rather, they are fluctuations from a rapidly changing baseline that signals state value.

This latest theory is satisfying because it provides for a role for dopamine in motivated behavior that makes sense considering the anatomical position of

the nucleus accumbens. Given that the nucleus accumbens has the capacity to influence motor output, the notion that rapid dopamine fluctuations purely signal the difference between expectations and outcomes may be too limited. A role for dopamine that facilitates appetitive association, layered on top of a simultaneous signal that conveys the inherent value of the situation allows for dopamine to modulate both learning and motivation to determine effortful responding. This study marks the most recent attempt to unify the existing views of the role of dopamine in goal-directed behavior. Further research will be required to determine the potential generalizability of this model to other experimental situations.

Dopamine Neurons and Aversion

Despite the extensive literature on the characterization of the involvement of nucleus accumbens dopamine in reward related behaviors, the role of this neurotransmitter system in aversion and avoidance behaviors has been the subject of comparatively little examination. Although early reports indicated that midbrain dopamine neurons exhibit a uniform response to rewarding stimuli (Schultz, 1997), subsequent electrophysiological examinations of the changes in firing rates of midbrain dopamine neurons during rewarding and aversive experiences have revealed a more heterogeneous response. As discussed previously, omission of an expected reward inhibits midbrain dopamine neurons (Schultz, 1997; Waelti et al, 2001). Consistent with this, foot pinch also has been shown to inhibit the majority of midbrain dopamine neurons (Maeda & Mogenson,

1982). This was later suggested to be a uniform response (Ungless, Magill, & Bolam, 2004), although more recent evidence suggests a more heterogeneous response profile (Brischoux et al, 2009; Zweifel et al, 2011). An air puff to the face produces inhibitions in four times more dopamine neurons than excitations in monkeys (Matsumoto & Hikosaka, 2009), however this ratio drops to two to one in mice (Cohen, Haesler, Vong, Lowell, & Uchida, 2012). Electric foot shock produces a heterogeneous response that is separable by VTA sub region (Brischoux et al, 2009). To the extent that foot shock evokes an inhibitory response, it appears to do so by exciting midbrain GABAergic neurons that typically act to inhibit dopamine neurons locally (Tan et al, 2012). Despite the lack of a uniform response, it is clear that aversive stimuli of various sensory modalities have some degree of inhibitory effect on midbrain dopamine neurons.

As is the case for reward-associated cues, aversion-associated cues are also encoded by midbrain dopamine neurons, and again, the response is not uniform. Cues for an aversive air puff to the hand have been shown to produce predominantly inhibitory responses in putative dopamine neurons in the monkey (Mirenowicz and Schultz, 1996), while cues for an air puff to the face have also been reported to produce predominantly excitations (Joshua, Adler, Mitelman, Vaadia, & Bergman, 2008). However, this later example focused primarily on substantia nigra neurons, and more recent reports have shown a cue for an aversive air puff produces a mix of excitations and inhibitions in the VTA (Matsumoto & Hikosaka, 2009). Additionally, a shock-predictive cue elicits a

largely inhibitory response by VTA dopamine neurons (Mileykovskiy & Morales, 2011).

It is important to point out that these results may be complicated for a number of reasons. These studies test a variety of aversive stimuli of various intensities and sensory modalities. This may partially explain why differential effects are often observed. Furthermore the state of the animal may explain discrepant results, as some experimental approaches require recordings in anesthetized animals. The presence of anesthesia has been reported to alter dopamine neuron activity (Koulchitsky, De Backer, Quertemont, Charlier, & Seutin, 2012), and furthermore, the magnitude of this effect may be dependent on the specific anesthetic used. Finally, midbrain dopamine neurons project to multiple forebrain regions and responses of these neurons to aversive stimuli may differ by projection targets (Ikemoto, 2007; Lammel, Ion, Roeper, & Malenka, 2011). Thus, in order to assess the extent to which aversive stimuli impact the mesolimbic dopamine system it is necessary to measure dopamine release directly in terminal fields.

Nucleus Accumbens Dopamine and Aversion

Microdialysis is a frequently utilized technique to assess how aversive stimuli modulate nucleus accumbens dopamine concentrations. The majority of these examinations have concluded that a variety of aversive events including foot shock, tail pinch, tail shock, restraint stress, and social defeat all increase dopamine (Abercrombie et al, 1989; Sorg & Kalivas, 1991; Young, Joseph, &

Gray, 1993; Finlay, Zigmond, & Abercrombie, 1995; Kalivas & Duffy, 1995; Tidey & Miczek, 1996; Doherty & Gratton, 1997; Di Chiara, Loddo, & Tanda, 1999; Young, 2004). In addition, several studies have found that cues that predict aversive stimuli also increase dopamine (Young, Joseph, & Gray, 1993; Young, 2004). In a few instances these aversion-induced increases in dopamine do not persist beyond the initial presentation of the aversive stimulus, which has been interpreted as a habituation of the dopaminergic response (Di Chiara, Loddo, & Tanda; Young 2004). However, there are a few notable exceptions. The dopaminergic response to restraint stress has been shown to be biphasic; dopamine increases at the onset of restraint, but decreases below baseline after some time (Puglisi-Allegra et al, 1991). Saccharin normally evokes a dopamine increase, but its presentation results in a decrease when it acquires aversive properties in a conditioned taste aversion design (Mark, Blander, & Hoebel, 1991). There are other examples in which aversive states such as chronic stress, cocaine withdrawal, or food deprivation cause a reduction in basal dopamine concentrations (Weiss et al, 1992; Pothos, Creese, & Hoebel, 1995; Gambarana et al, 1999), although these examples are difficult to interpret due to the potential for long term neuroadaptations that may contribute to this response (Koob & Le Moal, 2000). The discrepancies in these findings, and the lack of coherence with the electrophysiological results, can be explained by methodological differences, as well as differences in the temporal resolution of the assessment techniques. Limitations in temporal resolution may prevent microdialysis from capturing the immediate response to a discrete aversive stimulus, given that the alleviation of

said stimulus is likely rewarding (McCutcheon et al, 2012). Thus, in order to properly characterize the effect of aversive stimuli on dopamine transmission, temporal resolution sufficient to detect sub second changes in dopamine concentration is required.

Few studies have used fast scan cyclic voltammetry to examine the effects of aversive stimuli on dopamine transmission. However this technique has a temporal resolution comparable to *in vivo* electrophysiology which, as mentioned previously, has provided considerable information about dopamine dynamics. Of these, some have focused on dopamine responses to cues that predict the delivery of an aversive stimulus. These studies have reported decreases in dopamine concentration time locked to cue presentation in the nucleus accumbens core (Badrinarayan et al, 2012; Oleson et al, 2012). In regards to primary aversive stimuli, increases in nucleus accumbens dopamine signaling have been reported in response to tail pinch and social defeat stress (Anstrom, Mizcek, & Budygin, 2009; Budygin et al, 2012). As mentioned previously, these stimuli can vary in intensity, the sensory pathways by which they are transduced, and the extent to which they are temporally discrete. Thus, in making these assessments, it is critical to compare responses to rewarding and aversive stimuli that are consist in timing, intensity, and modality. Studies that measure the dopamine responses to intraoral delivery of rewarding and aversive tastants, have found that sucrose, a rewarding tastant, increases dopamine while quinine, an aversive tastant, causes a reduction (Roitman et al, 2008; Wheeler et al, 2011; Figure 1.4).

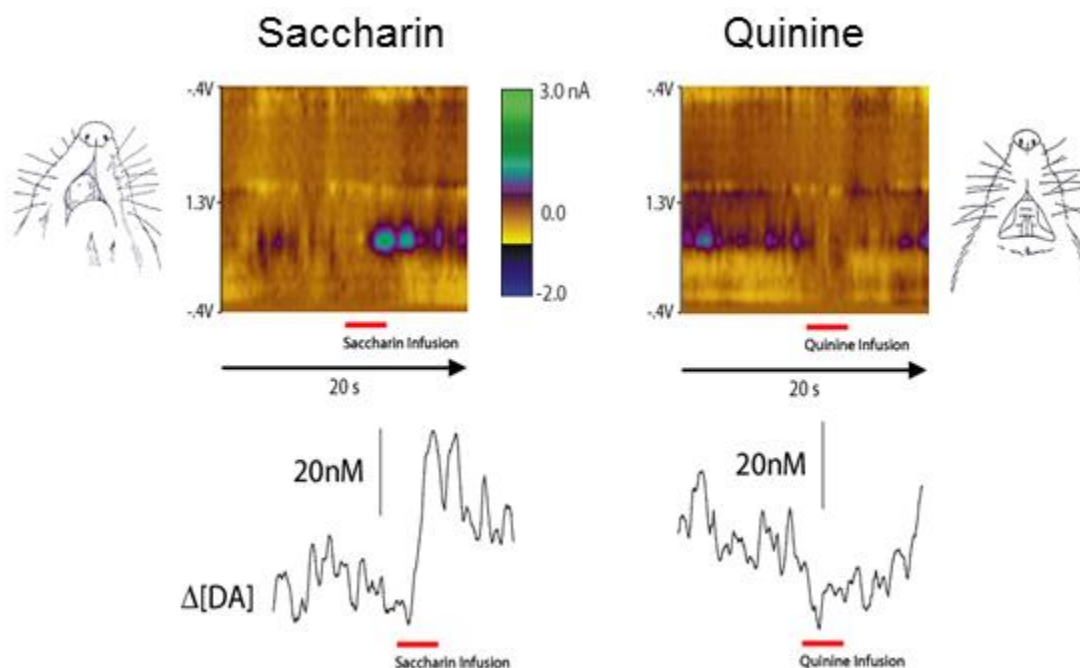


Figure 1.4. Nucleus accumbens dopamine fluctuations in response to rewarding and aversive tastants. Single trial *in vivo* voltammetric recordings in awake and behaving rats receiving an intra-oral infusion of saccharin or quinine. Top: Current changes are shown in color at various potentials (y-axis) across time (x-axis). Taste infusion is indicated by the red bar. Bottom: Change in dopamine concentration across time. Taste infusion is indicated by the red bar. Saccharin infusion elicits a transient increase in dopamine and appetitive taste reactivity while quinine infusion elicits a pause in naturally occurring dopamine release and aversive taste reactivity.

Aside from the benefit of being transduced by the same sensory system, the taste system, the use of these rewarding and aversive stimuli also offers the benefit of direct behavioral assessment of hedonic reactions with taste reactivity (Grill & Norgren, 1978; Berridge et al, 1989). Interestingly, these responses can be altered with conditioning. Tastes that typically increase dopamine concentration (e.g. sucrose or saccharin) come to elicit a negative affective state and decrease dopamine when paired with lithium chloride or cocaine

(McCutcheon et al, 2012; Wheeler et al, 2008; Wheeler et al, 2011). Thus primary or conditioned aversive stimuli cause rapid and pronounced decreases in nucleus accumbens dopamine neurotransmission. The careful characterization of aversion-induced reductions in dopamine is important because these signals have been suggested to play a role in aversive learning and avoidance behavior (Frank, Seeberger, & O'Reilly, 2004; Tan et al, 2012; Danjo, Yoshimi, Funabiki, Yawata, & Nakanishi, 2014). Understanding how aversive stimuli impinge on mesolimbic dopamine signaling may prove invaluable to understanding how these fundamental processes are regulated, and how they may become aberrant in disorders of dysregulated motivation and aversion learning such as drug addiction and Parkinson's disease.

Dopamine and Drug Addiction – Theories of Positive Reinforcement

Drug addiction is a chronic disorder characterized by persistent bouts of relapse following periods of abstinence from drug use. The National Institute of Drug Abuse estimates the economic cost of illicit drug use in the US to be \$193 billion annually, and this does not include the dramatic economic burden related to alcohol and tobacco use (NIDA Trends & Statistics, 2016). Preclinical animal models of addiction have been established to study this disorder and aid in the development of potential treatments, with one of the most useful being the self-administration design, followed by extinction and reinstatement. Briefly, in this paradigm an animal is trained to make an operant response (most commonly a lever press) for an intravenous infusion of a drug. Following several sessions

across days in which subjects are given the opportunity to self-administer drug, the drug is replaced by saline and operant responding is extinguished. Once sufficient extinction is observed, experimenter delivered stimuli, either a priming injection of the previously self-administered drug, drug-associated cues, or aversive stimuli are presented to evoke increased lever pressing, or drug-seeking. This final phase is known as reinstatement and is a model of relapse (Panlilio & Goldberg, 2007).

Many studies of drug addiction have focused on motivational circuitry, as foundational work has shown that many drugs of abuse act on dopamine neurotransmission (Wise, 1982; Bozarth & Wise, 1987). For this reason, influential theories for drug addiction have roots in the theories of dopamine function previously discussed here (Wise, 1982; Berridge & Robinson, 1998). One of the early theoretical explanations involving dopamine that gained widespread appeal was the “psychomotor stimulant” theory of addiction (Bozarth & Wise, 1987). Central to this view was the suggestion that drugs of abuse, across classes, produce their reinforcing effects and locomotor responses by activating the mesolimbic dopamine system. Activation of this common pathway was suggested to be critical for the development and maintenance of addictive behavior. Although tolerance develops following repeated drug use in some situations, this was considered a secondary process and not required for addiction to occur. While influential, this theory has key limitations. It focuses principally on the development of compulsive drug use, and offers little to explain the phenomenon of relapse, perhaps the most insidious aspect of this disease. It

identifies that dopamine is important for the reinforcing efficacy of drugs of abuse, but precisely how it mediates that process is unclear.

Subsequent theories emerged that were more comprehensive and offered a clear role for dopamine in the development of addiction. Perhaps the most currently influential is the “incentive sensitization” theory (Robinson & Berridge, 1993), which was later formally extended to the “incentive salience” hypothesis of dopamine function discussed previously (Berridge & Robinson, 1998; see *Dopamine and Motivation*). This theory posits that the activation of the mesolimbic dopamine system by drugs of abuse results in augmented incentive motivational learning involving drug-associated cues. Repeated drug use produces long lasting neuroadaptations that cement this learning and cause these drug associated cues to remain powerful motivators of behavior in the absence of persistent drug use. In other words, the dopamine response evoked by addictive drugs “sensitizes” the normal attribution of incentive salience to reward associated cues to supra-threshold levels causing these cues to exert unnatural motivational drive over future behavior. This process occurs independently of the hedonic impact of drug consumption, meaning that eventually the drug does not need to be liked to be pursued. This distinction between liking and wanting drugs of abuse provides an explanation for the phenomenon that human drug addicts experience decreased euphoria from drug taking, and exhibit blunted dopaminergic responses to stimulants despite reporting drug craving (Martinez et al, 2007; Volkow et al, 2014; Volkow, Koob, & McLellan, 2016). It also provides an explanation for relapse behavior by

suggesting that sensitized drug cues maintain their motivational strength even following prolonged periods of abstinence. The tenets of this theory helped guide an expansive literature on drug-induced neuroadaptations that occur in the mesolimbic dopamine system, many of which facilitate augmented drive of this circuitry (Nestler, 2001; Wolf, 2016). However, this theory is not comprehensive. A consistent observation in both rodent models and clinical observations of human addiction is that aversive stimuli or stressful events cause relapse (Sinha et al, 2003; Paliwal, Hyman, & Sinha, 2008). This phenomenon cannot be readily explained by incentive sensitization theory.

Dopamine and Drug Addiction – Theories of Negative Reinforcement

An early explanation of how the experience of aversion contributes to drug addiction was put forth by Solomon and Corbit (1974). Termed the, “opponent process” model, this view focuses on homeostatic processes that are engaged by rewarding or aversive events. Solomon and Corbit argue that stimuli that evoke sensations of pleasure and positive affective/emotional states are automatically countered by an opposing response, referred to as the “opponent”, or “B” process. The emergence of the opponent process is a homeostatic response to the engagement of the immediate effect of the stimulus, or “A” process, and these opposing forces govern general motivated behavior. This view of motivation is expanded to drug addiction through the suggestion that drugs of abuse engage these normal processes. Solomon and Corbit propose that the addicted state is a result of a drug-induced dysregulation of these

homeostatic motivational processes. With persistent drug use, the euphoria caused by drug consumption that characterizes the A process dissipates, while the subsequent B process is exaggerated and takes longer to return to baseline. This pronounced B process evokes a negative affective state, a craving state, and the motivation to alleviate this negative state becomes a progressively stronger behavioral regulator. It is suggested that the augmentation of the B process is long lasting, and possibly permanent. Thus, the opponent process model provides a framework for how negative affective states engage negative reinforcement mechanisms to guide motivated drug-seeking behavior (Soloman, 1980).

Theoretical extension and potential neurobiological underpinnings of the opponent process model as an explanation of drug addiction have been put forth in numerous publications by Koob & Le Moal (Koob, 1996; Koob & Le Moal, 2001). Koob has posited that repeated drug use produces a homeostatic dysregulation so severe that the system is unable to return to baseline and thus establishes a new “hedonic set point” outside of the normal range (Figure 1.5). This allostatic process results in the emergence of a negative affective craving state that persists even in the absence of drug taking and is a powerful motivator of relapse. Furthermore, these dysfunctional changes in affective state are associated with a set of neurobiological adaptations that result from repeated drug taking.

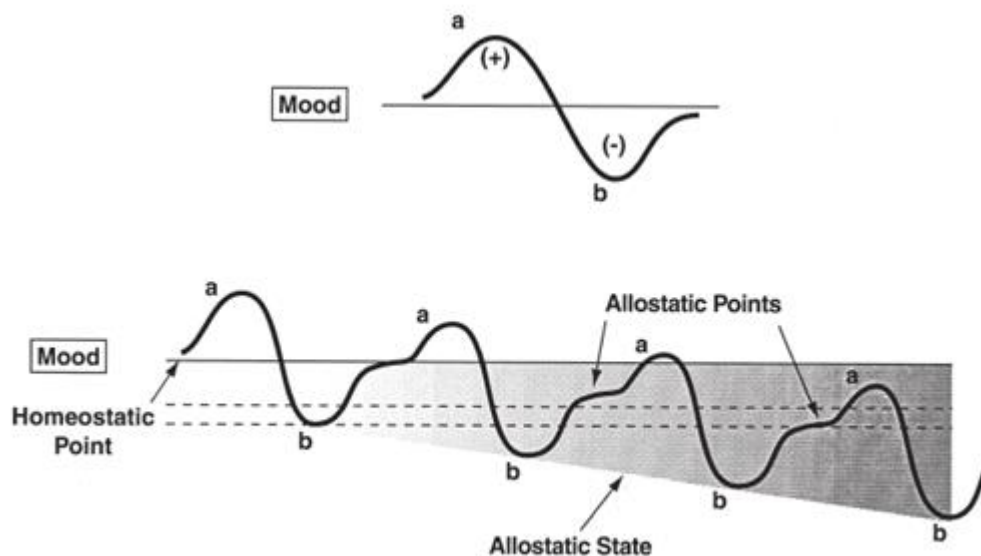


Figure 1.5. Dysregulation of affective state in drug addiction – an extension of the opponent process model. With repeated drug exposure the euphoric aspect of drug experience (the A process) is blunted while the negative affective component (the B process) of drug use is enhanced. Importantly, repeated drug use results in dysregulation of this homeostatic balance such that the baseline resets below the homeostatic point. This allostatic state is characterized by persistent negative affect and drug craving. Adapted from Koob & Le Moal, 2000.

These include increased activity of brain stress and emotional systems that have been conceptually described as the “antireward system” (Koob & Le Moal, 2005; Koob & Le Moal, 2008). Together, these ideas form a model of addiction that is characterized by a persistent, anhedonic negative affective state, driven in part by the activation of the antireward system, which serves as a powerful motivator of recurrent drug use through negative reinforcement.

Numerous observations in preclinical studies have provided support for various aspects of this model. There is considerable evidence that animals in acute drug withdrawal have a reduced sensitivity to reward, demonstrating that

the withdrawal state is characterized by the emergence of negative affect and anhedonia (Markou & Koob, 1991; Schulteis, Markou, Gold, Stinus, & Koob, 1994; Schulteis, Markou, Cole, & Koob, 1995; Epping-Jordan, Watkins, Koob, & Markou, 1998; Kenny, Chen, Kitamura, Markou, & Koob, 2006). A multitude of neuroadaptations have been characterized in drug withdrawal including changes in the activity of endogenous opioid systems and corticotropin-releasing factor signaling (Koob & Le Moal, 2000; Koob & Le Moal, 2005). This negative emotional state is also associated with decreases in nucleus accumbens dopamine signaling (Weiss, Markou, Lorang, & Koob, 1992; Weiss, 1996). The possibility that decreased nucleus accumbens dopamine signaling may be associated with a heightened motivational state is intriguing, as it is not predicted by traditional theories of dopamine and goal-directed behavior. However, this idea and the general notion that withdrawal is a significant driver of drug seeking has been challenged. Naloxone precipitated withdrawal is associated with decreased striatal dopamine, but this treatment failed to promote reinstatement of heroin seeking. In addition, spontaneous withdrawal states have been found to reinstate heroin seeking, but were not found to be associated with reductions in dopamine (Shaham, Rajabi, & Stewart, 1996). Finally, relapse in human addicts remains likely months or even years after the acute effects of withdrawal have dissipated. Still, it remains possible, due to the persistent affective dysfunction characterized by Koob and Le Moal, that drug addicts may have heightened sensitivity to aversive events. These events may cause drug seeking through negative reinforcement by loading on the persistently exaggerated B process.

Aversive Experience and Drug Addiction in Humans

Aversive life events are a significant cause of relapse in human drug addicts. Addicts report that aversive stimuli elicit powerful states of drug craving and precipitate relapse (Wallace et al, 1989), and these reports are consistent with data from the clinical setting demonstrating that drug associated stimuli induce a negative affective state, engage the stress response, and cause cocaine craving in abstinent users (Sinha, Catapano, & O'Malley, 1999; Sinha et al, 2003). Furthermore, the degree of craving predicts the latency to relapse (Paliwal et al, 2008). The relationship between negative emotional states and drug use is further highlighted by the observation that the incidence of PTSD and other mood disorders is higher among cocaine addicts (Cottler et al, 1992), and traumatic episodes, as well as imagery based on previously experienced traumatic events cause drug craving (Brady et al, 1998; Coffey et al, 2002). Specific alterations in the dopamine system have been observed in addicts that appear to be consistent with enhanced sensitivity to aversive stimuli. Alcoholics and cocaine addicts both exhibit a reduction in striatal D2 receptors, and this reduction persists months after cessation of drug use (Volkow & Fowler, 2000). Increases in dopamine transporter function have also been described in the striatum in individuals with cocaine experience (Staley, Hearn, Rutenber, Wetli, & Mash, 1994; Malison et al, 1998; Little et al, 1999), a finding that complies with a similar report in monkeys (Letchworth, Nader, Smith, Friedman, & Porrino,

2001). These changes are indicative of a reduction in striatal dopamine, and may be involved in the dysregulation of motivated behavior.

As progress continues towards a more complete understanding of the causes of drug relapse, the links between negative mood, craving, and drug use and their contribution to relapse will likely be a focal point of treatment strategies. Specifically, given the frequency and unavoidable nature of aversive life events, the effect of aversive stimuli on affective state is of particular concern, making affective regulation an important target of therapeutic interventions aimed at relapse prevention. This highlights the need to better define the neurobiological basis of how aversive events, and the subsequent negative affective state they evoke, engage the brain's motivational circuitry to cause relapse.

Aversive Experience and Reinstatement in Rodents

The self-administration/reinstatement model has been utilized extensively to study the neurobiological regulation of relapse in preclinical animal models. Various aversive events reinstate extinguished drug seeking in rats, including forced swim (Conrad et al, 2010) and food restriction (Shalev et al, 2003). However, much of what is known about the neural regulation of aversion-induced drug seeking comes from studies using intermittent electric foot shock (Ahmed & Koob, 1997). Despite differences in stimulus properties, and the discrepancies in the dopaminergic responses they evoke, all of these aversive events engender motivated behavior, and induce a negative affective state in the animal.

Signaling mechanisms involved in the stress response have been shown to be involved in reinstatement of drug seeking caused by electric foot shock. Central administration of corticotropin-releasing factor (CRF), a peptide classically studied for its function in the HPA axis, causes reinstatement (Mantsch et al, 2008), and systemic delivery of a CRF receptor antagonist blocks reinstatement (Shaham et al, 1997; Graf et al, 2011). The ability of CRF to reinstate extinguished cocaine seeking is not dependent on HPA axis function, as adrenalectomy does not alter this effect (Graf et al, 2011). The involvement of CRF in reinstatement has been localized to the VTA. Electric foot shock increases VTA CRF concentrations (Wang et al, 2005), and blocking VTA CRF receptors also blocks foot shock-induced reinstatement (Blacktop et al, 2011). The observation that shock-induced reinstatement requires activation of VTA CRF receptors indicates a possible interaction with mesolimbic dopamine signaling. Indeed, it has been demonstrated that CRF application inhibits VTA dopamine neurons by activating G-protein coupled inward-rectifying potassium channels (Beckstead et al, 2009). CRF administration has also been shown to decrease nucleus accumbens reward-evoked dopamine release as measured by fast scan cyclic voltammetry (Wanat, Bonci, & Phillips, 2013). These observations are difficult to reconcile with reports that electric foot shock increases nucleus accumbens dopamine measured by microdialysis (Sorg & Kalivas, 1991). Furthermore, reductions in nucleus accumbens dopamine are not typically associated with the performance of a goal-directed behavior such as drug seeking. The apparent contradiction in the dopamine response to foot

shock and the inhibitory actions of CRF on nucleus accumbens dopamine release may be partially explained by the complexity of foot shock as an aversive stimulus and the possible technical limitations of microdialysis to capture this response.

These discrepancies highlight the importance of determining both the physiological response of dopamine signaling in response to an aversive stimulus and the strength of that stimulus to cause reinstatement.

As measured by fast scan cyclic voltammetry, intraoral quinine, a primary aversive tastant, evokes a negative affective state and decreases nucleus accumbens dopamine concentrations (Roitman et al, 2008; Wheeler et al, 2011).

A recent report from our lab examined the capacity of this aversive stimulus to cause reinstatement of extinguished cocaine seeking (Twining et al, 2014). In this study we observed that quinine delivery did reinstate cocaine seeking, and that this effect required VTA CRF receptor activation. Additionally, we showed that quinine decreased nucleus accumbens dopamine on two time scales, and that blockade of VTA CRF receptors prevented the tonic dopamine reduction but did not alter the phasic dopamine response (Figure 1.6).

The finding that an aversive stimulus that decreases nucleus accumbens dopamine causes drug seeking is provocative. Early theories of addiction suggested that negative reinforcement could guide drug seeking (Soloman & Corbit, 1974), however little experimental evidence has supported this idea.

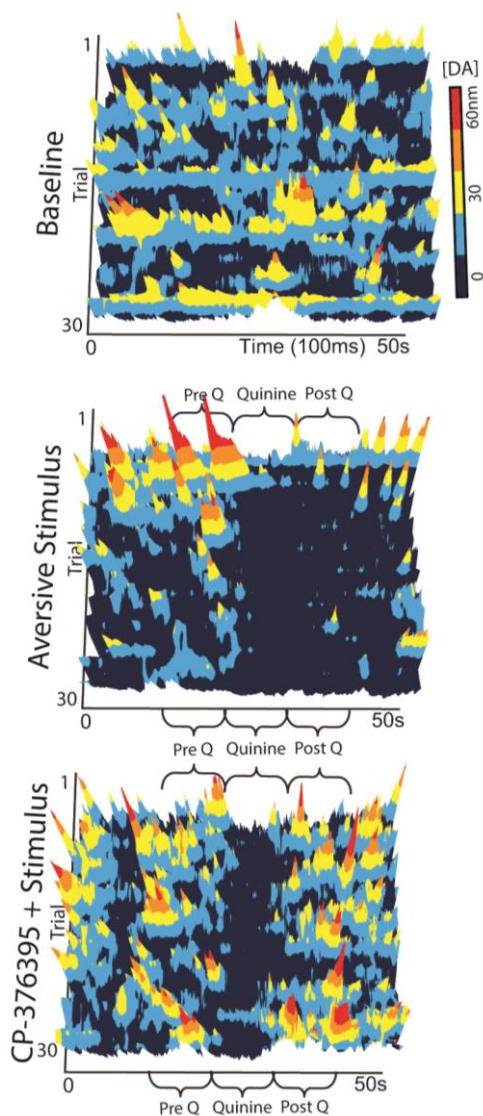


Figure 1.6. Intra-VTA CRF receptor blockade prevents aversion-induced reductions in dopamine. Dopamine signaling is shown in color within (x axis) and across (y-axis) trials. Top: Baseline collection period showing naturally occurring dopamine signaling. Middle: Dopamine signaling across trials of repeated quinine delivery. Importantly, quinine decreases dopamine acutely, coincident with experience of the taste, but also decreases dopamine broadly across trials (y-axis). Bottom: Intra-VTA administration of the CRF receptor antagonist, CP-376395, prevents the broad, or “tonic”, decrease in dopamine while leaving the acute, or “phasic” decrease intact. Adapted from Twining et al, 2014.

A potential interpretation of our data is that the aversion-induced reduction in dopamine evokes a negative affective state, and motivates drug seeking behavior in an effort to alleviate this state. While seemingly at odds with evidence that other aversive stimuli increase dopamine to cause drug seeking, these observations can be reconciled when considering a model of striatal signaling (Figure 1.7).

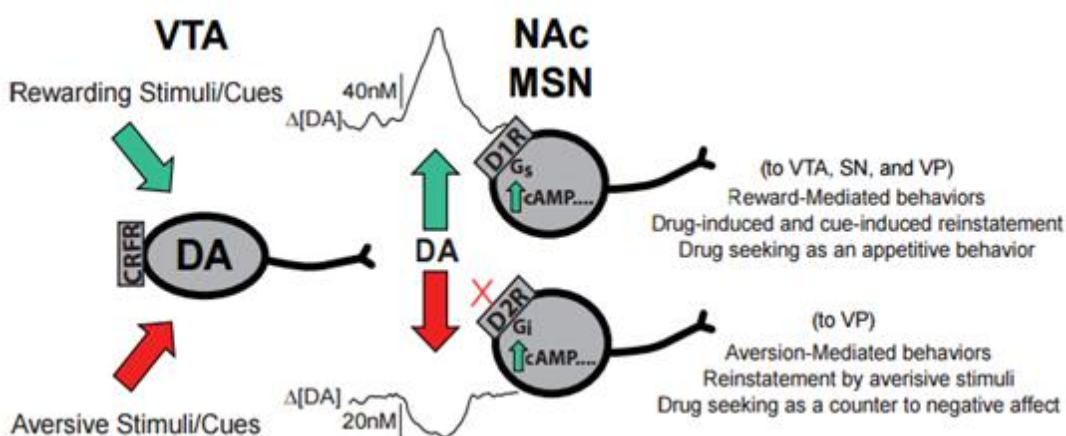


Figure 1.7. Model of dopaminergic modulation of striatal circuitry and drug-seeking. Rewarding stimuli and their predictors activate VTA dopamine neurons, causing an increase in nucleus accumbens dopamine concentration. This promotes excitability of D1-expressing medium spiny neurons that have been implicated in drug-primed and cue-induced reinstatement of drug seeking. Conversely, aversive stimuli activate CRF receptors in the VTA to inhibit the activity of dopamine neurons, causing a reduction in dopamine tone in the nucleus accumbens and the induction of a negative affective state. This promotes the excitability of D2-expressing medium spiny neurons to drive drug-seeking via negative reinforcement.

Stimuli that increase dopamine and cause reinstatement may activate low affinity D1 receptors to increase the sensitivity of D1-expressing MSNs to glutamatergic

drive to promote behavior. In contrast, stimuli that decrease dopamine may reduce occupancy of high affinity D2 receptors, increasing the sensitivity of D2-expressing neurons to glutamatergic drive to promote avoidance. Indeed, optogenetic inhibition of dopamine neurons has recently been shown to be aversive (Danjo et al, 2014), and activation of D2-expressing MSNs has been implicated in avoidance behavior (Kravitz, Tye, & Kreitzer, 2012; Hikida et al, 2013; Francis et al, 2015).

One potential caveat with this interpretation is that reinstatement caused by electric foot shock is not thought to involve nucleus accumbens dopamine, as blockade of D1 or D2 receptors in this area does not prevent reinstatement (Shaham & Stewart, 1996). However, it has been shown that local delivery of a D3 receptor antagonist does block stress-induced reinstatement of cocaine seeking (Xi et al, 2004). There is ample evidence for a role of prefrontal cortical dopamine in stress-induced reinstatement (Capriles, Rodaros, Sorge, & Stewart, 2003; McFarland, Davidge, Lapish, & Kalivas, 2004; Mantsch, Baker, Funk, Lê, & Shaham, 2015), and it is possible that aversion-induced reductions in nucleus accumbens dopamine are not critical for reinstatement caused by aversive stimuli. Alternatively, it is possible that quinine is qualitatively different than foot shock, and can engender motivated behavior via distinct neural circuitry. Further experiments are necessary to determine the involvement of aversion-induced reductions in nucleus accumbens dopamine to stress-induced reinstatement.

Conditioned Cues and Relapse

In preclinical models, the presentation of drug-associated cues has been shown to be sufficient to reinstate extinguished drug seeking for a variety of different drugs (Di Chiara et al, 1999; Crombag, Bossert, Koya, & Shaham, 2008). These cues associated with immediate drug delivery increase nucleus accumbens dopamine signaling (Phillips et al, 2003) and activate nucleus accumbens neurons (Hollander & Carelli, 2007). It is commonly thought that environmental stimuli become incentivized through association with the drug, and the ability of a stimulus to evoke the neural representation of the drug is the reason these cues elicit drug seeking (Robinson & Berridge, 1993).

However, under certain conditions drug-associated cues may not elicit a mimetic physiological response. An extension of the opponent process model (Solomon and Corbit, 1974) is that a drug-associated cue may be able to evoke a response opposing the response evoked by the drug itself, known as the B-process. There is ample evidence to suggest that some drug-associated cues do evoke such a conditioned compensatory response. When rodents are presented with morphine-associated cues followed by a placebo they exhibit hyperalgesia, which opposes the normal analgesic properties of morphine (Siegel, 1975). The notion that a compensatory response may be conditioned is also used to explain accidental heroin overdoses that occur following drug-taking in a novel context. The drug-associated context elicits a conditioned compensatory response, and as a result the user will increase drug intake to experience the desired drug effects. When taken in a novel context, this compensatory response is absent,

and thus the same dose becomes more powerful (Siegel, Hinson, Krank, & McCully, 1982). Furthermore, conditioned hyperthermic responses have also been observed following presentation of cues associated with both opiates and ethanol (Siegel, 1978; Drummond, Cooper, & Glautier, 1990). Importantly, it has been posited that the temporal relationship between cue exposure and drug delivery is a crucial component of these responses. In order for a cue to elicit a conditioned compensatory response it must be conditioned in such a way that it precedes drug delivery (Tzschentke, 1998; Siegel & Ramos, 2002). If a drug-predictive cue were to induce a conditioned compensatory response, any resulting drug-seeking motivation would almost certainly not be the result of the evoked mental representation of the drug, but instead would be the result of negative reinforcement.

There is ample experimental evidence that cocaine has aversive properties, and that it can support aversive conditioning. Approach-avoidance conflict has been described in rodents in the runway self-administration model, and conditioned place aversion to cocaine is observed when conditioning is done 15 minutes following cocaine injection (Tzschentke, 1998; Ettenberg, 2004). These cocaine effects have been suggested to be driven by reductions in dopamine signaling. It has been shown that cocaine has a biphasic response in the VTA; an initial excitation, followed by an inhibition mediated by activation of the lateral habenula. The subsequent inhibitory response is required for aversive conditioning to cocaine effects (Jhou et al, 2013). Normally appetitive tastants elicit a negative affective state through association with future cocaine availability

(Grigson & Twining, 2002). This negative affective state is associated with reduced dopamine and altered activity of nucleus accumbens neurons (Wheeler et al, 2008; Wheeler et al, 2011). Furthermore, the magnitude of the conditioned aversion predicts cocaine taking (Wheeler et al, 2008). These findings appear to parallel observations in human drug users that drug-associated cues can elicit a negative affective state, characterized by feelings of anxiety and craving, and that craving predicts relapse (Sinha, Fuse, Aubin, & O'Malley, 2000; Sinha et al, 2003; Robbins, Ehrman, Childress, Cornish, & O'Brien, 2000; Figure 1.8)

Together, these findings suggest that cue-induced negative affect is an important factor in drug taking and relapse. It is possible that this cue-induced negative affective state is an important motivator of relapse through negative reinforcement. However, whether an aversive cocaine cue that decreases dopamine and causes negative affect can reinstate extinguished cocaine seeking remains unknown. This remains to be experimentally verified, and must be before conditioned changes in affective state can be considered as reasonable targets for therapeutics aimed at relapse prevention.

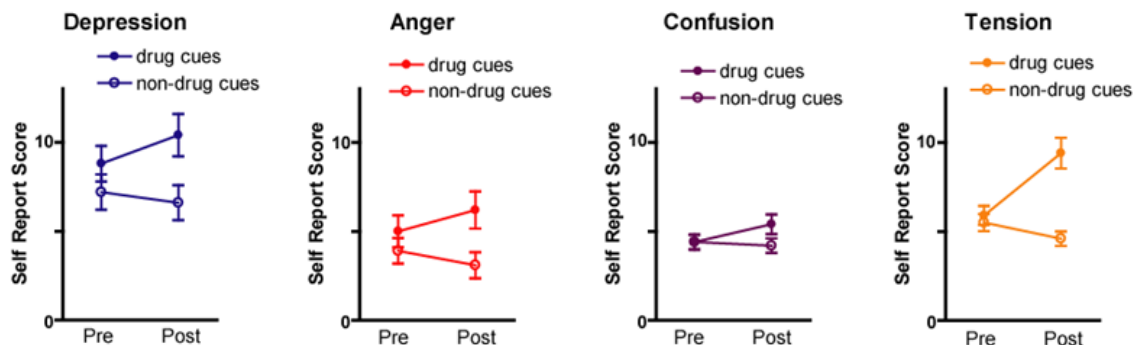


Figure 1.8. Drug-associated cues induce a negative affective state in human drug users. Cocaine-dependent human subjects were presented cocaine-associated stimuli and non-drug associated cues. The presentation of cocaine-associated stimuli increased self-reported scores on indices of depression, anger, confusion, and tension. Subjects also reported feelings of craving induced by drug associated cues. Adapted from Robbins et al, 2000.

Current Studies

The studies included in this dissertation center around the role of aversion-induced reductions in nucleus accumbens dopamine signaling in motivation and learning under normal conditions and in the dysregulated state of drug addiction. We first set out to determine whether an aversive drug cue that decreases dopamine and evokes a negative affective state could cause drug seeking, and how the aversive drug cue altered dopaminergic and nucleus accumbens neuronal responses to other drug associated stimuli to drive behavior. We then examined the mechanistic regulation of aversion-induced reductions in dopamine in the VTA, and the importance of this signal to aversive learning in the non-addicted state. Furthermore, we examined the involvement of nucleus accumbens dopamine receptors in the signaling of aversion-induced

reductions in dopamine and requirement of reduced dopamine receptor activation to promote aversive learning. Using fast scan cyclic voltammetry coupled with site specific *in vivo* behavioral pharmacology, we present evidence in support of a model of striatal signaling by which increases and decreases in mesolimbic dopamine influence motivation and learning via distinct pathways. The importance of reduced dopamine signaling in this model is discussed in its relevance for our understanding of the general principles that guide how aversive stimuli influence behavior. Furthermore, these findings may have relevance for the understanding and development of treatments for disease states that involve dysregulated dopamine signaling, motivation, and learning.

CHAPTER II

DRUG PREDICTIVE CUES ACTIVATE AVERSION-SENSITIVE STRIATAL NEURONS THAT ENCODE DRUG SEEKING

Introduction

Informed by drug addicts' emotional and physiological reactions to drug-associated cues (Childress et al, 1999; Garavan et al, 2000), theories of drug addiction and relapse often posit an influence of cues on drug-seeking behavior (Di Chiara, 1999; Robinson & Berridge, 2003; Everitt et al, 2008). These theories often note the desirability of these incentivized cues, which have a well-known neural representation in both mesolimbic dopamine signaling and cell firing in the nucleus accumbens. Such signals of immediate drug reward reliably increase striatal DA (Phillips et al, 2003), activate nucleus accumbens neurons (Hollander & Carelli, 2007), and promote drug seeking (Shaham et al, 2003).

However, human addicts report that drug-associated cues induce negative feelings related to drug craving and anxiety that predict relapse (Sinha et al, 2000; Sinha et al, 2003). The ability of drug cues to cause negative affect can be studied in animal models, and such studies have demonstrated that predictors of delayed drug access can cause avoidance (Tzschentke, 1998; Grigson and Twining, 2002), negative affect (Wheeler et al, 2008), a reduction in dopamine concentration (Wheeler et al, 2011), and enhanced excitatory activity in reward circuitry (Wheeler et al, 2008). Additionally, the degree of aversion correlates with enhanced drug-seeking motivation (Wheeler et al, 2011; Nyland & Grigson, 2013; Colechio et al, 2014). The fact that appetitive and aversive stimuli can

differentially affect striatal circuitry has led us to hypothesize that aversive stimuli disinhibit a distinct aversion-sensitive striatal circuit that also drives drug seeking (Twining et al, 2014), possibly as an avoidance behavior (Baker et al, 2004; Koob & Le Moal, 2008). While recent studies have shown that correcting a reduction in DA signaling can attenuate drug taking and seeking (Twining et al, 2014; Willuhn et al, 2014), it is not clear how an aversive stimulus that decreases dopamine signaling simultaneously impacts striatal cell firing to promote drug seeking.

Here we directly tested if an aversive cue can cause drug seeking, if it does so in a state of low dopamine tone, and if it activates neurons that encode the drug-seeking act. Additionally, we investigated the impact of the aversive drug cue on the processing of another drug-associated stimulus and drug-seeking behavior. The potential change in the physiological response to a proximate cocaine cue (i.e., a cue that signals immediate drug infusion) was of particular interest because aversive stimuli can interact synergistically with proximate drug cues to drive drug seeking (Liu & Weiss, 2002; Shelton & Beardsley, 2005; Buffalari & See, 2009). The pattern of observed results supports the hypothesis that an aversive drug cue causes a sustained reduction in dopamine signaling, induces negative affect, and activates a subpopulation of striatal neurons that encode drug seeking and other drug-associated cues.

Materials and Methods

General Experimental Design

The novel procedure presented here was inspired by traditional studies of context-induced renewal of drug seeking in which the self-administration context is shifted during extinction, and returned during test (i.e., ABA renewal; Bouton, 2004; Crombag & Shaham, 2002). This treatment is typically compared to a control group that receives a context shift between self-administration and extinction, but not between extinction and test (the ABB control). In the present design, the Paired group of rats received passive exposure to a normally appetitive saccharin solution prior to the opportunity to self-administer cocaine (Figure 2.1) and the Unpaired control group received cocaine access following a waiting period. During extinction, Paired subjects experienced a waiting period followed by an extinction session, whereas Unpaired subjects received saccharin infusions before extinction. Finally, Paired rats experienced a reinstatement test session in which saccharin preceded extinction, and Unpaired rats received another normal extinction session that was used as a test session for behavioral and physiological comparisons. One potential pitfall in this novel design concerns the fact that saccharin is not a motivationally neutral stimulus, even in the Unpaired group. However, by comparing the final extinction session in the Paired and Unpaired groups with the test session in the Paired group, we can determine the potential effect of the aversive cocaine cue, an inherently-appetitive unconditioned stimulus, and no stimulus, on affect and drug-seeking behavior.

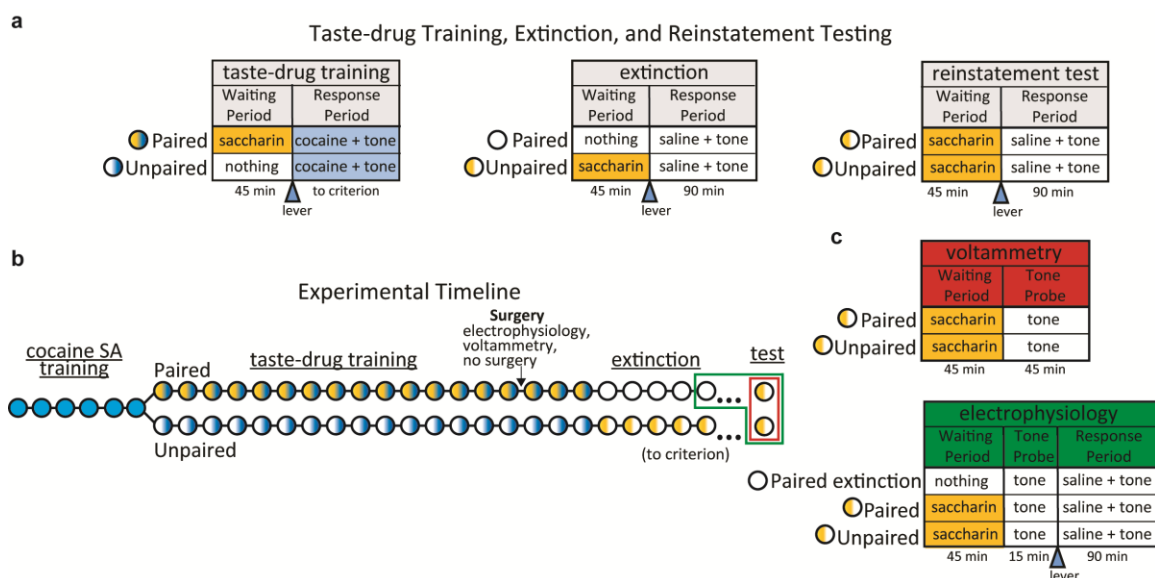


Figure 2.1. Overview of experimental design. (a) Details of the three experimental phases. During taste-drug training, rats experienced a waiting period during which they received 45 saccharin infusions (yellow) or nothing (white), followed by lever extension (triangle), and a response period during which cocaine and a co-occurring tone stimulus could be self-administered (blue). During the response period of the extinction and reinstatement phases, cocaine was replaced by saline (white). Paired rats received saccharin exposure during the taste-drug training and reinstatement phases, and unpaired rats received saccharin during the extinction and reinstatement phases. (b) Timeline of training and testing. Before being divided into groups, all rats self-administered cocaine with no waiting period for six daily sessions (circles). During the three experimental phases, rats received saccharin (yellow) or nothing (white) during the waiting period, and cocaine (blue) or saline (white) during the response period. Green and red boxes denote electrophysiology and voltammetry recording sessions, respectively. (c) Details of voltammetry and electrophysiology testing. Following extinction, both groups received saccharin infusions followed by noncontingent presentations of the tone while rapid dopamine release (voltammetry) or neuronal firing rate (electrophysiology) was monitored. In the electrophysiology study, this was followed by a response period during which rats could self-administer saline.

Subjects

Male Sprague-Dawley rats (Harlan Laboratories) weighing 275-300 g were individually housed in a temperature and humidity controlled AAALAC-accredited vivarium. Rats were maintained on a 12/12 reversed dark/light cycle and all experimental procedures were conducted during the dark phase (starting at 0700 hours). Animals all had ad libitum access to food and water except where otherwise noted. All experimental protocols were approved by the Institutional Animal Care and Use Committee at Marquette University in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. A total of 48 rats were used in these studies, trained as described above. Subsets of this group were assayed for electrochemical and electrophysiological encoding of various aspects of the task. Fifteen (8 Paired and 7 Unpaired) were used for only for behavior. Twenty-five (16 Paired and 9 Unpaired) and 8 (4 Paired and 4 Unpaired) were used for electrophysiology and voltammetry assays.

Surgical procedures

All surgical procedures were conducted under ketamine/xylazine (100 mg/kg; 20 mg/kg, i.p.) anesthesia. Intraoral and intrajugular catheter implantations were conducted as previously described (Wheeler et al, 2008). To prepare for voltammetric recordings, electrode guide cannula (BASi) were implanted above the NAcC unilaterally (AP: +1.3; ML: \pm 1.3), a bipolar stimulating electrode (Plastics One) was placed above the ipsilateral VTA (AP: -5.2; ML: \pm 1.0; DV: -8.0), and an Ag/AgCl reference electrode was placed contralateral to the guide cannula. For electrophysiological recordings, eight-wire microelectrode

arrays (N-B Labs) were implanted bilaterally in the nucleus accumbens at AP: +1.7 mm, ML: ± 0.8 (NAcS) to ± 1.3 mm (NAcC), DV: -6.3 mm. For each array, another wire was wrapped around a skull screw to serve as a ground. For all surgical procedures, rats were treated with the anti-inflammatory meloxicam (1% oral suspension) the day of, and for two days following surgery to reduce inflammation and postoperative pain. To maintain patency, the catheters were flushed daily with dH₂O (i.o.) or heparinized saline and the antibiotic cephalosporin (i.v.).

Apparatus

Subjects were tested in standard operant chambers (Med Associates), interfaced with a computer, and housed in sound attenuating Faraday cages. Each operant chamber had two dedicated syringe pumps for intravenous and intraoral infusions that were delivered to each rat via a dual-channel fluid swivel (Instech Laboratories). Two retractable levers entered the chamber on the right side wall with a cue light directly above each. A food-pellet dispenser delivered 45-mg grain pellets (BioServe) to a recessed foodcup positioned between the two levers. A speaker capable of producing a 65-dB 1-KHz tone was located on the left sidewall. Under each chamber, a camera was positioned to allow for recording behavioral responses (taste reactivity). The chamber floor was clear acrylic glass and a house light was positioned on the door of the sound attenuating chamber, outside of the operant chamber, to ensure recording quality.

Self-Administration training

Following catheter implantation, subjects were allowed to recover until they reached pre-surgery bodyweight. Subjects were then food restricted to 90% bodyweight, and trained to press one of the two levers for food pellets. Upon acquisition of lever-press behavior, subjects were trained to self-administer cocaine on a fixed-ratio 1 schedule of reinforcement. The beginning of each cocaine self-administration session was signaled by the entry of both levers into the box and the illumination of two cue lights. Responses on the active lever resulted in a 3-s cocaine infusion (0.25 mg/0.1 ml) accompanied by cue-light offset, onset of a 5-s tone, and a 20-s timeout period. Responses on the active lever during the timeout resulted in no programmed consequences and are not included in the data analyses. The conclusion of the time out period was signaled by cue light onset. Each session was completed only when each subject reached a maximum infusion cap (detailed below).

Self-administration sessions occurred in a series of 6 experimenter-controlled 6-day cycles consisting of 3 days of cocaine self-administration and 3 days without cocaine access in the home cage. The maximum number of infusions taken per session increased each cycle from a 20-infusion cap in the first cycle, to a 50-infusion cap in the final cycle. Upon completion of cycles 1 and 2, subjects were separated into groups, counterbalanced for weight and average latency to reach to the infusion cap in the second cycle. Rats were initially trained for 6 sessions without a waiting period in order to balance the groups and to ensure the acquisition of a tone-cocaine association independent from saccharin

exposure. For Paired rats, all subsequent cocaine self-administration sessions were preceded by a 45-min waiting period during which each subject received 45 intraoral saccharin infusions (0.15%; 0.2 ml/6 s; 1 inf/min). Unpaired subjects received an equivalent waiting period with no taste prior to cocaine access.

Extinction and reinstatement

Upon completion of 6 cycles of cocaine self-administration all subjects were transitioned into the extinction phase. Before every extinction session, Unpaired rats received 45 intraoral infusions of saccharin, while Paired rats experienced a waiting period. Following the saccharin period, a 90-min extinction session began in which presses on the formerly active lever resulted in cue light offset, tone onset, and an infusion of saline. After a minimum of 5 extinction sessions, subjects were tested for reinstatement when they registered an average of 10 presses or less on the formerly active lever (excluding presses made during the 20 s time out period) over two consecutive sessions. Extinction responding was analyzed with a mixed ANOVA comparing the Paired and Unpaired groups across matched extinction sessions.

Once each subject reached extinction criteria they were tested for saccharin-induced reinstatement of cocaine seeking. This session was procedurally identical to an extinction session with the exception that all subjects received saccharin infusions. Reinstatement was assessed by comparing responding in the Paired group at test with the final extinction session in both the Paired and Unpaired groups using t-tests. In order to assess the relationship between reinstatement behavior and aversion, a regression analysis was

performed (Pearson's R). Because of variability in baseline rates of lever pressing at the end of extinction, a reinstatement ratio was computed for the regression analysis by dividing the total number of lever presses during test by the sum of number of lever presses during test and extinction.

Taste reactivity scoring/analysis

Taste reactivity was analyzed in a frame-by-frame analysis using digital video recorded on the test session in Paired and Unpaired rats. Appetitive and aversive responses were counted in the saccharin period using the technique of Grill and Norgren (1978). Bouts of 'wet dog shaking', paw flailing, and mouth movements that matched the 'triangle' shape for a duration exceeding 90 ms were counted as aversive. Instances in which the tongue protruded and crossed the midline were counted as appetitive. The rates of aversive and appetitive events (per trial) were computed for each animal. Taste reactivity data were analyzed with a mixed ANOVA and subsequent planned comparisons of appetitive and aversive taste reactivity (events/trial) in the Paired and Unpaired groups.

Voltammetry testing and data collection

Subjects underwent fast-scan cyclic voltammetry surgery after their 15th day of cocaine self-administration training. Following recovery, subjects received their final 3 days of self-administration and extinction as previously described. To familiarize the rats with the recording situation, the VTA stimulating electrode was harnessed to a rotating commutator (Crist Instruments) during at least one of the final extinction sessions. Once subjects reached extinction criteria, DA responses

to saccharin and tone were measured. A carbon fiber electrode was lowered into the nucleus accumbens core. The fiber was held at -0.4 V against Ag/AgCl between scans and then driven to +1.3 V and back in a triangular fashion at 400 V/s for each voltammetric measurement. The application of this triangle waveform causes oxidation and reduction of chemical species that are electroactive within this potential range, producing a change in current at the carbon-fiber. Specific analytes (including dopamine and pH) are identified by plotting these changes in current against the applied potential to produce a cyclic voltammogram (Heien, Johnson, & Wightman, 2004; Heien et al, 2005). The current arising from electrode processes was removed by using background subtraction. For data collected during testing, the background period was taken as the minima during the 10-s before saccharin or cue presentation. This practice does not subtract the presence of phasic dopamine release events because the background was explicitly selected for the absence of dopamine signals. Measurements were made every 100 ms and, after driving the electrode into the nucleus accumbens core, the electrode equilibrated for 40 min before any data were collected. The position of the microelectrode was then optimized by monitoring the presence of spontaneously occurring dopamine release events (Roitman et al, 2008; Wheeler et al, 2011). Once this was observed, the electrode was locked in place and data collection proceeded. The experiment consisted of a 20-min baseline dopamine monitoring period, then a 45-min saccharin delivery period, followed by a 45-min tone test period during which the proximate cocaine cues (tone onset and cue light offset) were presented every

90 s. Orofacial expressions during saccharin administration were recorded and scored. Following the conclusion of testing, several electrical stimulation trains that varied in number of pulses (1, 2, 5, 10 and 20) were administered for the generation of a training set for principal component analysis for the detection of dopamine and pH changes during the behavioral session. Due to technical limitations of our voltammetry testing apparatus, dopamine was not measured during lever-press behavior.

Analyte identification and quantification were achieved using principal component regression analysis described in detail elsewhere (Heien et al, 2004). All data presented here fit the resulting model at the 95% confidence level. Briefly, training sets were generated from background-subtracted cyclic voltammograms collected during and after electrical stimulations. At least ten voltammograms were obtained for dopamine and pH. The resulting current amplitude was converted to DA concentration based on calibration of the electrode in a flow injection analysis system after the *in vivo* experiment. To convert current due to oxidation of dopamine, 500 nM and 1 μ M dopamine in a buffer (pH 7.4) were used in the flow injection analysis system. Data from each saccharin or cue probe presentation were background subtracted using a 1-s block at the local minima in the 10 s prior to presentation. The resultant current changes over time were converted into dopamine concentrations over time using a principle component regression.

Voltammetry data analysis

For each rat, trials were aligned to saccharin or tone presentation and averaged together. To analyze the acute effects of saccharin on dopamine, average concentration during a baseline epoch (10 s before infusion onset) was compared to average concentration during an effect epoch (10 s after infusion onset). Additionally, the diffuse effect of saccharin on non time-locked dopamine was examined by blocking baseline epoch measurements into thirds (early, middle, and late). Unlike the acute dopaminergic response to saccharin, which lasts several seconds, the observed effect of the tone was rapid (~1 s). Thus, to analyze the effect of the tone on dopamine concentration, the peak dopamine concentration observed during the 5-s tone was compared to the peak dopamine concentration observed in the final 5 s of the baseline epoch. Changes in dopamine were analyzed using repeated measures ANOVAs and planned contrasts were used for direct comparisons.

Electrophysiology recording procedures

Subjects underwent electrode implantation surgery after their 15th day of self-administration training. Recordings were conducted with microelectrode arrays featuring eight stainless steel wires (50 μm diameter) arranged in a 2 x 4 configuration (N-B Labs). Following recovery, subjects received their final 3 days of self-administration and extinction. To familiarize the rats with the recording situation, they were connected to a flexible recording cable (Plexon Inc.) attached to a commutator (Crist Instruments) during these sessions. In order to assess encoding of the tone independent of lever pressing, 8 Paired and 8 Unpaired

subjects also experienced noncontingent tone presentations during the last extinction session, and during the test session. Fifteen tone trials were delivered at 60-s intervals after the saccharin-infusion period. After the tone testing, the levers were extended, and extinction/reinstatement testing commenced. The headstage contained 16 miniature unity-gain field effect transistors. Nucleus accumbens activity was recorded differentially between each active wire and an inactive wire chosen for an absence of neuronal activity. Online isolation and discrimination were accomplished using a commercially available neurophysiological system (OmniPlex system; Plexon Inc.). Multiple window discrimination modules and high-speed analog-to-digital signal processing in conjunction with computer software enabled isolation of neuronal signals on the basis of waveform analysis. The neurophysiological system incorporated an array of digital signal processors (DSPs) for continuous spike recognition. The DSPs provided a continuous parallel digital output of neuronal events to a computer. Another computer controlled behavioral events of the experiment (Med Associates) and sent digital outputs corresponding to each event to the OmniPlex to be time-stamped along with the neural data. Criteria for identifying different neurons on a single wire have been described in detail elsewhere (Roitman, Wheeler, & Carelli, 2005). Briefly, discrimination of individual waveforms corresponding to a single neuron was accomplished using template and principle component analysis procedures provided by the PlexControl software system. The template analysis procedure involves taking a sample of the waveform and building a template of that extracellular waveform. Subsequent

neurons that match this waveform are included as the same neuron. Cell sorting was further accomplished after the experiment was over using additional principle components analysis in Offline Sorter V3.3.2 (Plexon Inc.).

Phasic response categorization

Phasic encoding of the saccharin, tone, and lever-press response was characterized by generating peri-event histograms (500-ms bins) surrounding each event in Neuroexplorer 4.126 (Nex Technologies). To ensure a reliable histogram, lever-press responses were only considered if the subject registered 10 or more responses spaced at least 20 s apart. Each histogram was divided into a baseline epoch and an effect epoch based on the type of event: 1) 10 s immediately following the onset of the saccharin infusion, 2) 5 s immediately following the onset of the tone, 3) 5 s immediately preceding a lever press, 4) 5 s immediately following the lever press. Individual bins within the effect epochs were then standardized based on the mean and standard deviation of the appropriate baseline epoch. Neurons that exhibited two or more bins with z-scores that exceeded or fell below three standard deviations were classified as excitatory or inhibitory. Because some inhibitions reached 0 Hz, but still did not fall below three z-scores, inhibitory responses were also included if two or more bins had the minimum possible z-score, provided that the effect epoch showed at least twice as many minimum bins as the baseline epoch. Neurons were also categorized based on a t-test comparison of the frequency bins of neurons that exhibited >15% shifts in frequency from baseline to the effect epoch ($\alpha = .05$). After neurons were characterized, Fisher's exact tests were used to detect any

differences in phasic responses and the distribution of excitatory and inhibitory responses in the core and shell during Paired extinction, Unpaired extinction, and Paired testing.

Baseline analysis

An average firing rate (Hz) for each trial was drawn from the baseline epoch for the saccharin infusion and transformed ($\log_{10}(x+.001)$) in order to normalize the distributions (Guillem, Ahmed, & Peoples, 2014). Trials were grouped in thirds to capture any changes in baseline firing that might occur during the saccharin period. Previously categorized tone and lever-press encoding neurons were selectively analyzed with repeated-measures ANOVAs to determine whether their baseline firing rates are altered during saccharin exposure. One inhibitory tone-encoding neuron in the Unpaired group did not have sufficient data for a complete time-course analysis.

Histology

After voltammetry and electrophysiology testing, subjects were euthanized with CO₂. To verify placements of voltammetry recordings, small electrolytic lesions were created by running a current (250 μ A) through a stainless steel electrode placed at the depth at which the recording took place. Brains were then removed and submerged in 10% formaldehyde for 14 days. For electrophysiology recordings, a current (20 μ A) was run through the implanted microwires, and brains incubated in a 10% formaldehyde/4% potassium ferrocyanide solution. All brains were then sliced into 40- μ m sections, mounted,

stained with 0.25% thionin, and coverslipped. Figure 2.2 shows the electrode placements from the voltammetry and electrophysiology experiments.

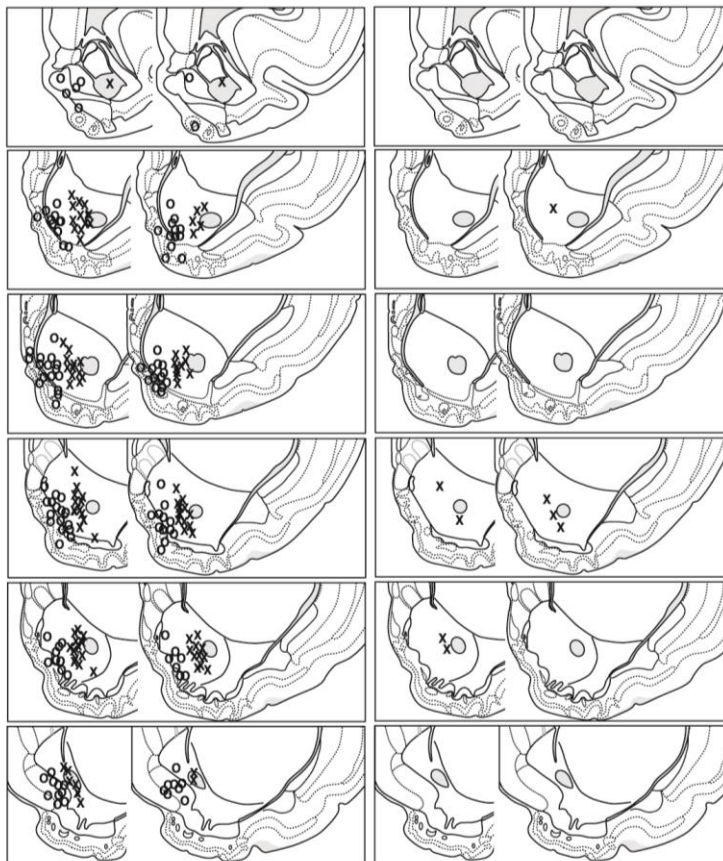


Figure 2.2. Electrode placements. (a) Electrophysiology electrode placements in the NAC (Xs) and NAcS (Os) in paired (left; $n=16$) and unpaired (right; $n=9$) rats. (b) Voltammetry electrode placements in paired (left; $n=4$) and unpaired (right; $n=4$) rats.

Results

The aversive drug cue reinstates extinguished drug seeking

While prior studies have demonstrated a predictive relationship between cue-induced negative affect and drug taking, here we tested the ability of an aversive drug cue to cause reinstatement. Because self-administration sessions had a quota/cap, all subjects took the same amount of cocaine. To examine the extinction data (Figure 2.3a), responses on the formerly active lever (excluding those made during the time out period) were analyzed. Both groups decreased responding similarly on the active lever across sessions (session: $F_{(4, 52)} = 22.41$, $p < .01$; all other $ps > .19$). Taste reactivity to saccharin at test resembled previous reports (Figure 2.3b), with Paired rats showing more aversive ($p < .02$) and fewer appetitive ($p < .01$) responses relative to Unpaired rats (group x taste reactivity: $F_{(1, 13)} = 25.75$, $p < .01$; all other $ps > .45$). Paired rats exhibited enhanced drug seeking relative to their final extinction session (Figure 2.3c; $t_{(7)} = 3.85$, $p < .01$), and Unpaired rats at test ($t_{(13)} = 2.90$, $p < .02$). In contrast, responses on the inactive lever did not differ between Paired (mean = 2.88; SE = 1.08) and Unpaired rats (mean = 2.88; SE = 1.08; $t_{(13)} = 1.68$, $p = .12$). Furthermore, the aversive reaction to cocaine-associated saccharin was correlated with reinstatement in Paired rats (Figure 2.3d; $r_{(6)} = 0.74$, $p < .04$).

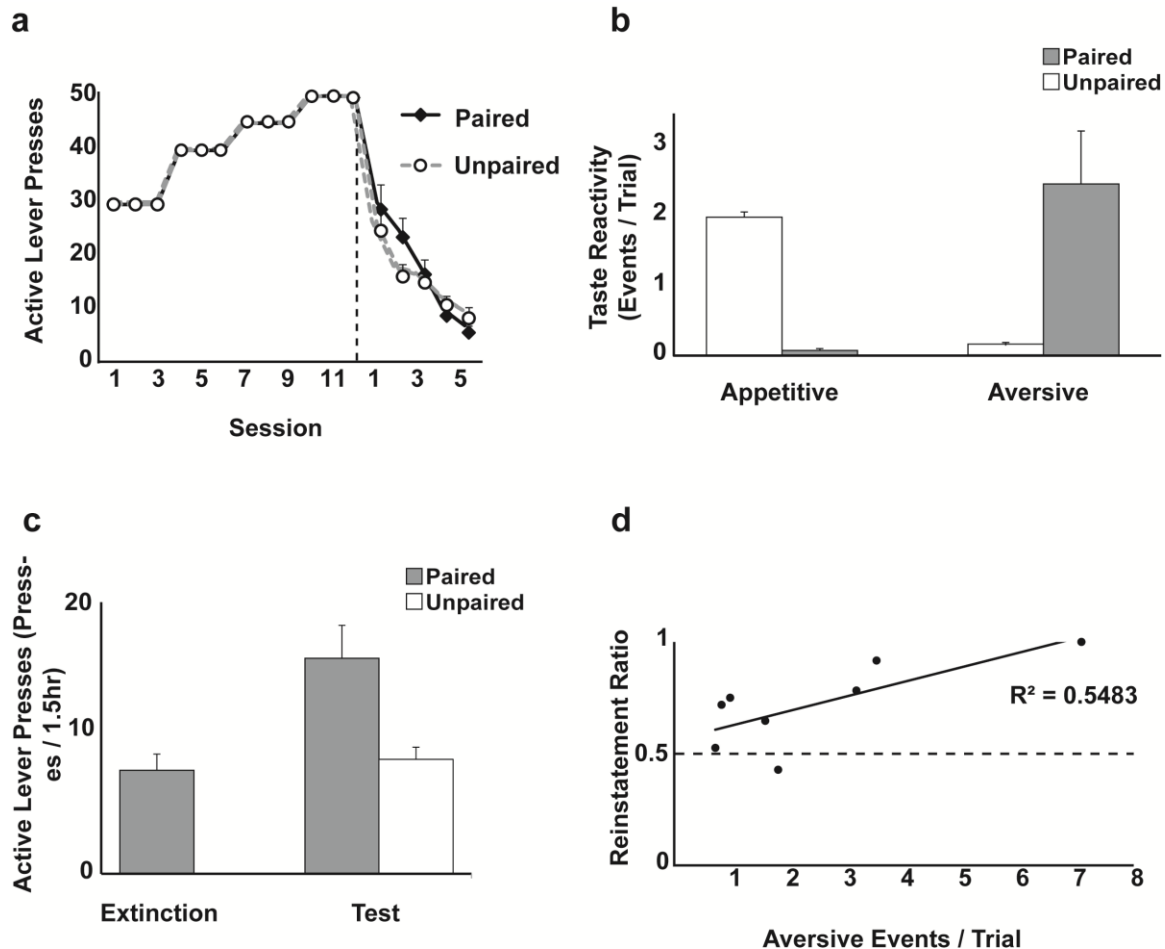


Figure 2.3. The aversive drug cue causes drug seeking. (a) Lever pressing during self-administration and extinction. Session duration varied during self-administration because all rats responded until they reached a progressive cap. During extinction, session duration was 1.5 h. The vertical dashed line depicts day of switch to nonreinforced responding. Responding during extinction was similar in paired ($n=8$) and unpaired ($n=7$) rats, mean+SE. (b) Paired rats showed more aversive taste responses to intraoral saccharin infusions per trial than unpaired rats ($p<0.02$), whereas unpaired rats showed more appetitive responses than paired rats ($p<0.01$). (c) Paired rats increased responding on the previously active lever from extinction to test ($p<0.01$) and responded more than unpaired rats ($p<0.02$). (d) The magnitude of the aversion caused by the drug cue predicted reinstatement ($p<0.04$). A ratio >0.5 indicates an increase in responses on the previously active lever during test compared with extinction.

The aversive drug cue reduces dopamine and enhances neural excitation acutely

Consistent with observations in the nucleus accumbens shell (Wheeler et al, 2011), the aversive and appetitive stimuli had opposite effects on nucleus accumbens core dopamine levels (Figure 2.4a and b). Cocaine-predictive saccharin caused an acute decrease in nucleus accumbens dopamine concentration in the Paired group, whereas appetitive Unpaired saccharin caused an acute increase in DA concentration (group X epoch: $F_{(1, 6)} = 10.45$, $p < .02$; group: $F_{(1, 6)} = 6.42$, $p < .01$; epoch: $p > .50$).

Also consistent with prior reports, aversive and appetitive stimuli had differential effects on nucleus accumbens neuronal firing rates. Figure 2.4c shows examples of inhibitory and excitatory neural encoding of the saccharin stimulus at test. Saccharin infusions were encoded by 48.39% ($n = 60/124$) of the Paired neurons and 38.84% of the Unpaired neurons ($n = 40/103$). Encoding of the aversive drug cue was predominantly excitatory in nature ($n = 35/60$) rather than inhibitory ($n = 25/60$). This ratio was reversed ($p < .01$) in the Unpaired group, in which encoding was predominantly inhibitory ($n = 28/40$) rather than excitatory ($n = 12/40$). Figure 2.4d depicts the proportion of responsive neurons in each subregion.

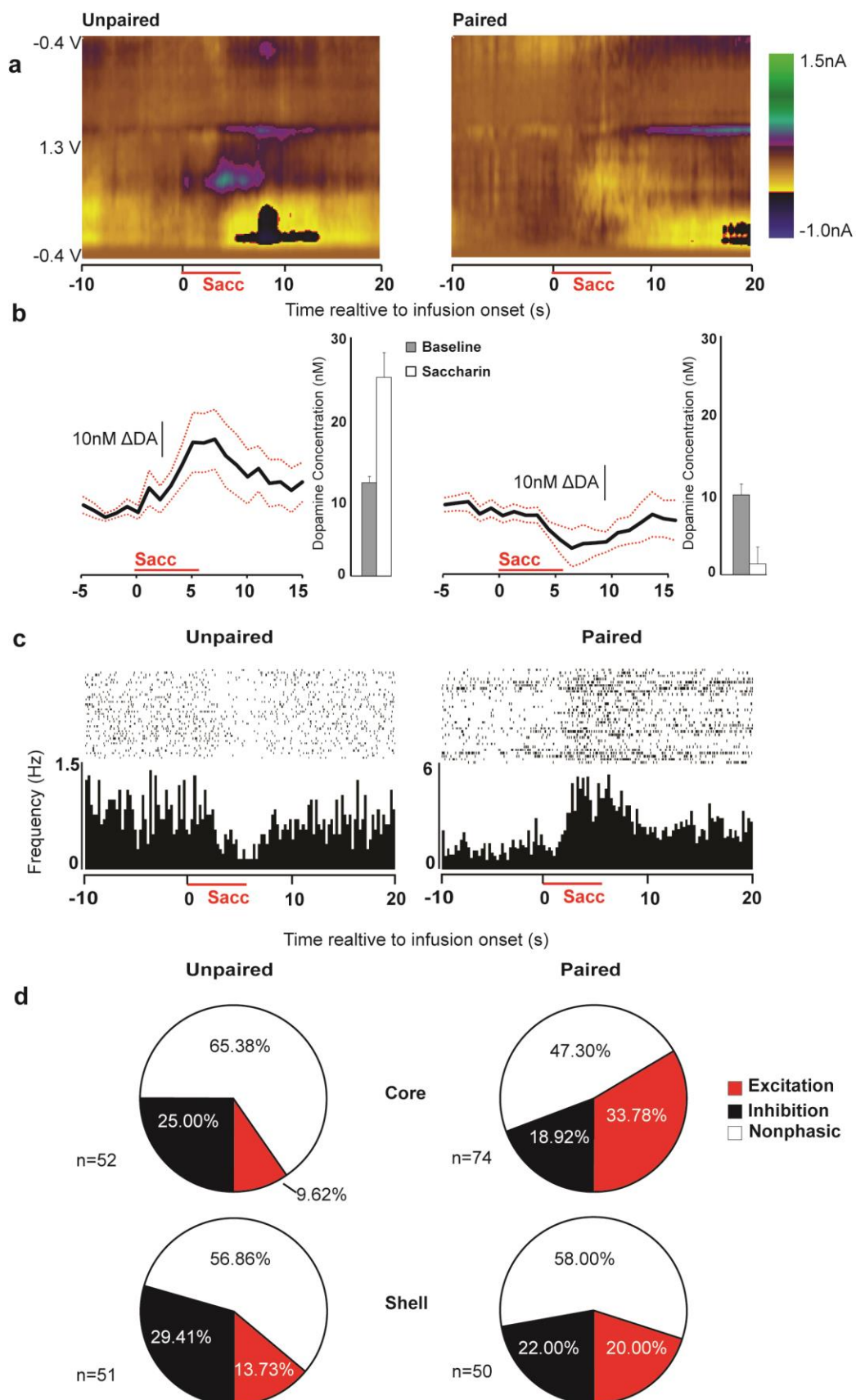


Figure 2.4. The aversive drug cue reduces DA and enhances neural excitation acutely. (a) Average color plots from representative unpaired (left) and paired (right) rats showing dopaminergic responses to saccharin (horizontal red line). The figures show current changes (nA; z-axis) plotted against voltage (V; y-axis) and time (s; x-axis). (b) Relative to the 10 s baseline epoch, saccharin acutely increased DA concentration (nM) in unpaired rats (left) and decreased DA concentration in paired rats (right; $p < 0.02$). Black lines show average concentration over time and red lines indicate SE for each group. Bars depict overall averages from the baseline and saccharin epochs. (c) Rasters and histograms depict examples of inhibitory (left) and excitatory (right) changes in firing rate time-locked to the presentation of saccharin. Individual action potentials during each saccharin presentation are shown in the raster, and firing rate (Hz) is shown in the histogram below. (d) Categorization of responses to saccharin in all recorded neurons. Unpaired neurons (left) show more excitatory than inhibitory responses to saccharin, whereas paired neurons (right) show more inhibitory than excitatory responses ($p < 0.01$).

The aversive drug cue eliminates dopaminergic encoding of the extinguished proximate drug cue

The tone was presented after saccharin exposure to determine whether reinstatement behavior co-occurs with renewed dopamine encoding of the extinguished proximate drug cue. The results revealed the opposite (Figure 2.5a and b; group X epoch: $F_{(1, 6)} = 6.30$, $p < .05$). The proximate drug cue caused a rapid, transient increase in DA in the Unpaired group ($p < .01$), but this dopamine response was not observed in the Paired group ($p > .70$). Surprisingly, these data suggest that dampened dopamine signaling caused by aversive cocaine-predictive saccharin persists during the time in which an animal transitions to drug seeking and diminishes the dopaminergic encoding of other drug predictive cues.

The aversive drug cue augments excitatory neural encoding of the extinguished proximate drug cue

Considering the suppression of dopamine encoding of the tone in the Paired group, the neural encoding of this stimulus was of particular interest. The tone was encoded by 27.78% ($n = 20/72$) of Paired neurons on the last day of extinction and 25.37% ($n = 17/67$) of Paired neurons at test. In the Unpaired group, 22.0% ($n = 16/73$) of Unpaired neurons encoded the tone at test, indicating no effect of group or treatment on the overall proportion of tone-encoding neurons in the nucleus accumbens ($p > .40$). However, the nature of these phasically-active neurons (excitations or inhibitions) varied based on the treatment and brain region (Figure 2.5c and d). Tone-encoding neuronal responses were roughly evenly distributed between excitations and inhibitions in the Unpaired condition (excitation/inhibition ratio: core ns = 4/3, shell ns = 3/6; $p = .62$), as well as the Paired condition during extinction (core ns = 3/4, shell ns = 6/7; $p = .63$). However, the presence of the aversive, drug-predictive cue induced a significant shift toward excitatory responses in the nucleus accumbens core in the Paired group at test (ns = 7/0) relative to the NAcS (ns = 4/6, $p < .04$). Thus, a shift toward excitatory encoding co-occurred with the suppression of dopamine encoding.

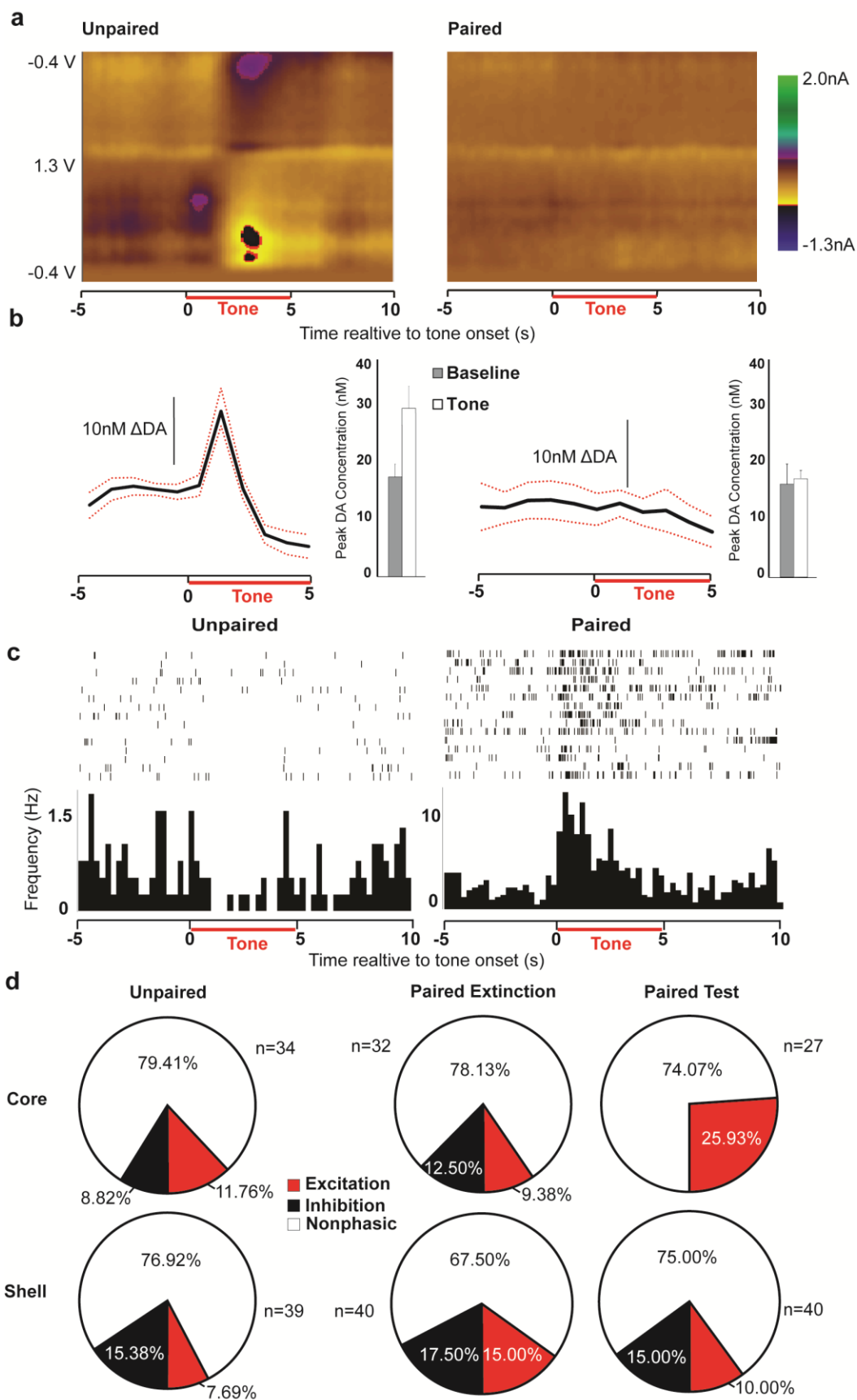


Figure 2.5. The aversive drug cue eliminates DA encoding and augments excitatory neural encoding of the proximate drug cue. (a) Average color plots from a representative unpaired (left) and paired (right) rat showing the dopaminergic response to the proximate drug cue (tone; horizontal red line). The figures show current changes (nA; z-axis) plotted against voltage (V; y-axis) and time (s; x-axis). (b) Relative to the 5 s baseline epoch, the tone caused a brief increase in DA concentration (nM) in unpaired rats ($p < 0.01$), and this response was abolished by the preceding experience of the aversive drug cue in paired rats ($p = 0.70$). Black lines show average concentration over time and red lines indicate SE for each group. Bars depict average peak DA from the baseline and tone epochs. (c) Rasters and histograms depict examples of inhibitory (left) and excitatory (right) neural encoding of the tone. Individual action potentials during each tone presentation are shown in the raster, and firing rate (Hz) is shown in the histogram below. (d) Categorization of responses to the tone in all recorded neurons. Paired neurons (right) show more excitatory encoding in the core relative to the shell at test ($p < 0.04$), but this difference in excitatory encoding was not apparent in paired neurons during extinction (center; $p < 0.63$) or in unpaired neurons (left; $p = 0.62$).

The examination of neural encoding of drug-seeking was restricted to rats that registered enough lever presses to produce a reliable histogram, which limited the analysis to rats in the Paired group at test ($n = 5$). Overall, 34.38% ($n = 11/32$) of nucleus accumbens core and 37.5% ($n = 6/16$) of nucleus accumbens shell neurons encoded one or more aspects (pre- and/or post-response) of the lever-press behavior. Of the phasically-active nucleus accumbens core neurons, 54.55% ($n = 6/11$) exhibited some form of excitatory encoding, and 54.55% ($n = 6/11$) exhibited inhibitory encoding (1 neuron exhibited a phasic decrease before and a phasic increase after the response), whereas nucleus accumbens shell neuronal responses were 33.33% ($n = 2/6$) excitatory and 66.67% ($n = 4/6$) inhibitory (Figure 2.6a and b).

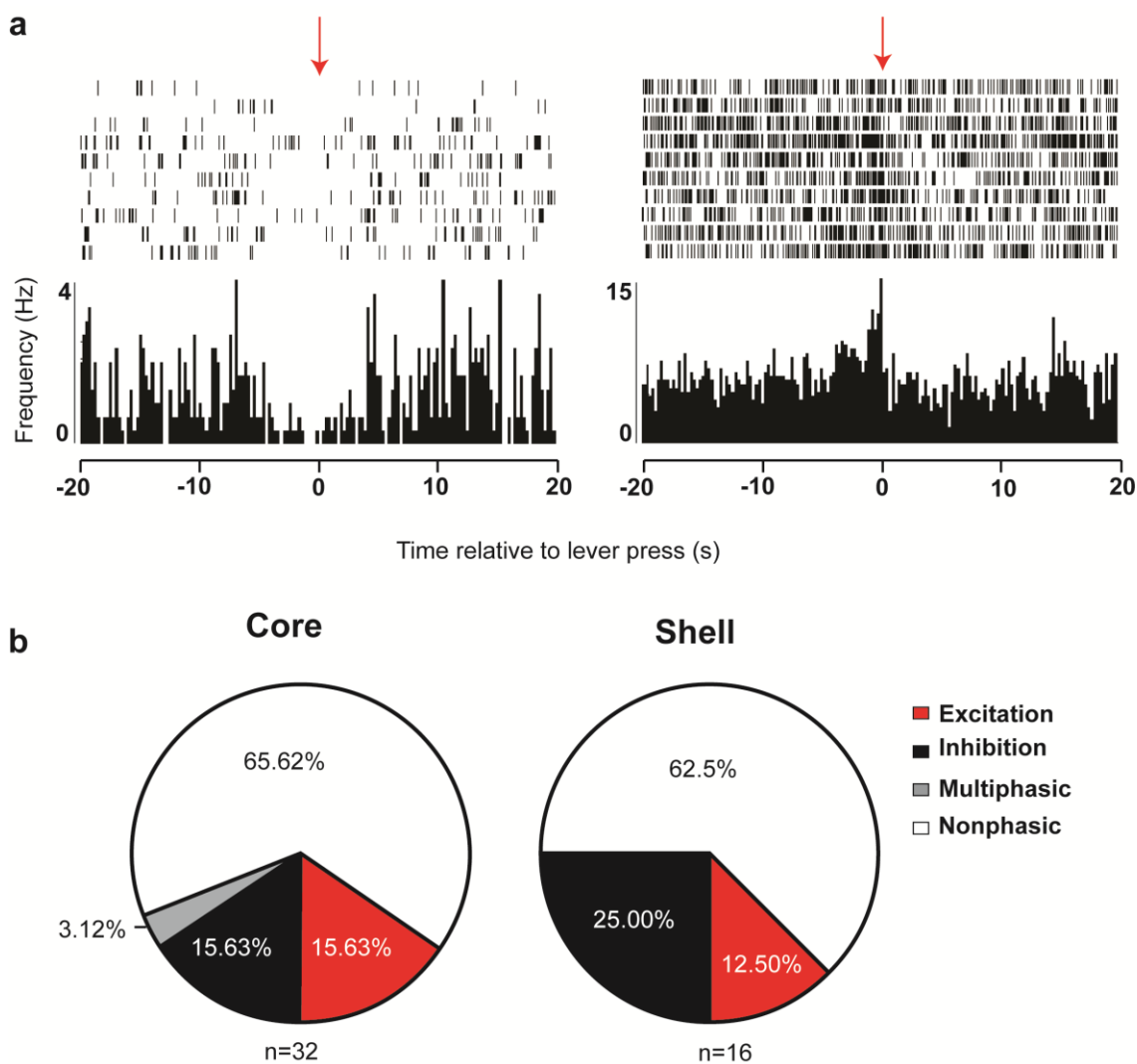


Figure 2.6. NAc neurons encode responses during reinstatement of cocaine seeking. (a) Representative inhibitory (left) and excitatory (right) encoding of lever press at test. Individual action potentials aligned to each lever press (red arrows) are shown in the raster, and firing rate (Hz) is shown in the histogram below. (b) Categorization of lever-press encoding in paired neurons at test.

The aversive drug cue reduces baseline dopamine and enhances the baseline excitability of neurons that will encode future drug-seeking behavior

In the nucleus accumbens shell, an aversive drug cue causes a long term, diffuse depression of dopamine concentration as well as an acute time-locked

dopamine decrease (Wheeler et al, 2011). The presence of the diffuse depression might be especially important for reinstatement of drug-seeking (Twining et al, 2014). We tested for the presence of this phenomenon here by performing an analysis of baseline (10 s pre infusion) dopamine concentration in the nucleus accumbens core across trials during the saccharin exposure period (Figure 2.7a). The baseline period was blocked into early and late session dopamine concentration. One subject in the Paired group lacked sufficient data to construct a complete time-course and could not be included in this analysis, but it was clear that baseline dopamine concentration changed in a non time-locked manner across trials differentially depending on group (Figure 2.7c; group X trial block: $F_{(1, 5)} = 22.39$, $p < .01$; all other p s $> .20$). Planned comparisons revealed that appetitive, unpaired saccharin increased basal dopamine concentration across trials ($p < .02$), and aversive, cocaine-predictive saccharin decreased basal dopamine concentration across trials ($p < .03$).

Because fluctuations in dopamine can modulate neural excitability (Surmeier, Ding, Day, Wang, & Shen, 2007), we were interested in how diffuse changes in DA might relate to the baseline excitability of nucleus accumbens neurons. Furthermore, we isolated neurons that would later go on to encode the proximate drug cue or drug-seeking behavior in the next phase of testing to determine whether paired saccharin differentially impacted the behavior of these neurons (Figure 2.7d). Because of the limited number of previously categorized neurons, both categories of responses (inhibitory and excitatory) were combined for this analysis. Changes in baseline firing rates were assessed by blocking the

saccharin period into thirds and generating 3 average baseline firing rate measurements for each neuron. An ANOVA revealed a significant interaction between all factors (trial block X group X subregion: $F_{(2, 56)} = 4.20, p = .02$) and a marginal interaction between group and trial block ($F_{(1, 25)} = 3.87, p = .05$) but no other effects ($ps > .17$). A post-hoc analysis showed that the aversive cocaine cue caused an increase in the baseline firing rates from the beginning to the end of the saccharin period specifically in nucleus accumbens core neurons that would later encode the tone cue (Figure 2.7d; $p < .05$; all other $ps > .80$). Notably, all of the Paired core neurons that increased their baseline firing rates were recorded in subjects that exhibited reinstatement (reinstatement ratios $> .50$), whereas the single neuron that did not increase firing rate across the saccharin session was recorded in a subject that did not show appreciable aversive taste reactivity (< 0.50 aversive events/trial) or reinstatement.

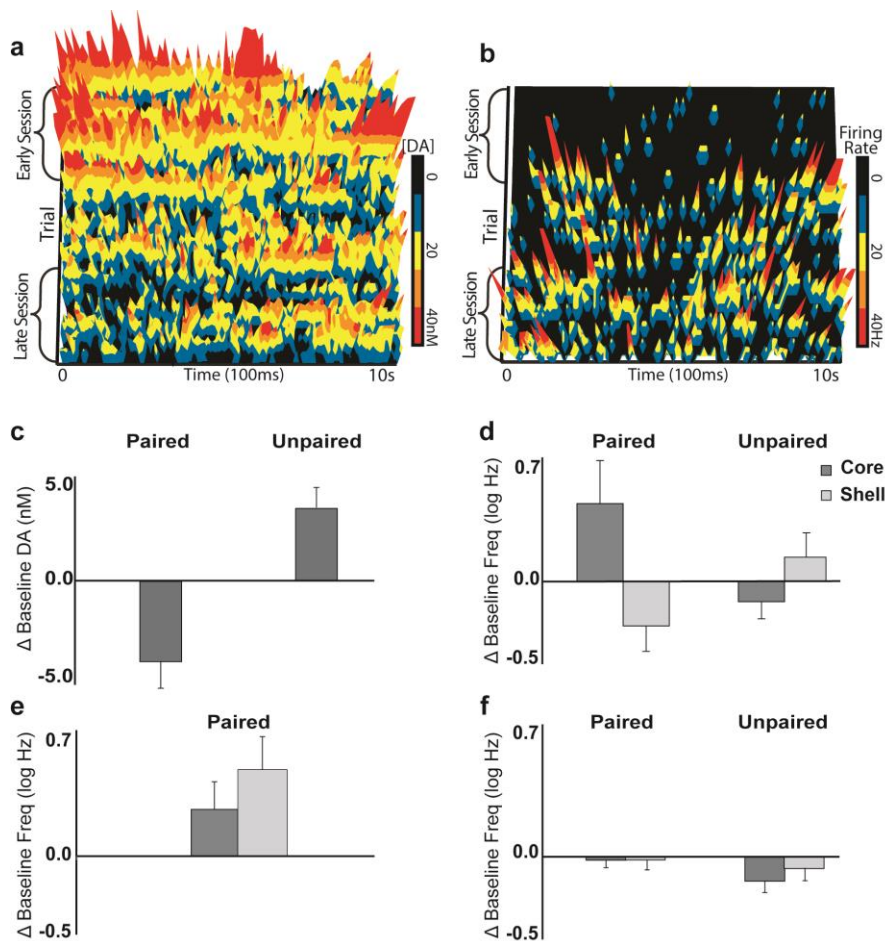


Figure 2.7. The aversive drug cue reduces DA and enhances excitability of neurons that will encode future drug-seeking behavior. (a) Baseline DA (nM; z-axis) across trials (y-axis) throughout the saccharin period in a representative paired rat. (b), Baseline firing rate (Hz; z-axis) across trials (y-axis) throughout the saccharin period in a representative tone-encoding neuron from a paired rat. (c) Difference scores (late–early) of average baseline DA concentrations (nM) in paired and unpaired rats. Saccharin reduced baseline [DA] over time in paired rats ($p < 0.03$), and increased baseline [DA] in unpaired rats ($p < 0.02$). (d) The change in baseline firing rates (log Hz) in units that subsequently encoded the tone. In the paired group, saccharin increased firing rates in tone responsive units in the NAcC ($p < 0.05$), but had no effect in the NAcS or in unpaired rats (p values > 0.80). (e) The change in baseline firing rates in lever press response units in paired rats. Saccharin increased baseline firing rates in both the NAcC and NAcS ($p < 0.03$). (f) The change in baseline firing rates in all recorded units before saccharin infusion in paired and unpaired rats. Saccharin reduced baseline firing rates in the full population of recorded neurons ($p < 0.01$).

Similar to tone-encoding neurons in the core, neurons that encoded drug seeking exhibited a change in baseline activity during exposure to the aversive saccharin stimulus (Figure 2.7e). In this case, baseline firing rates in both the core and shell neurons increased during the saccharin period ($F_{(2, 18)} = 4.51$, $p < .03$; all other p s $> .09$). Importantly, an analysis of all neurons (including non-encoding neurons) showed that most baseline firing rates decreased during the saccharin period regardless of group (Figure 2.7f; $F_{(2, 420)} = 4.88$ $p < .01$), and baseline activity in the Paired group was generally higher than the Unpaired group ($F_{(1, 210)} = 7.37$, $p < .01$; all other p s $> .07$). Considering the general downward trend in baseline activity, the increase in firing rate in neurons that encode drug seeking or the proximate drug cue is exceptional.

Discussion

The present findings show that a natural reward that signals delayed access to cocaine becomes aversive and reinstates drug seeking after extinction. The presence of this aversive drug cue decreases acute and diffuse dopamine release in the nucleus accumbens core and increases the baseline firing rates of neurons that will subsequently encode the proximate cocaine cue and cocaine-seeking behavior. Furthermore, the aversive drug cue also eliminates the dopaminergic encoding of the proximate drug cue. This demonstrates a pronounced depression of dopamine signaling that persists long after the aversive drug cue is removed. At the same time, excitatory encoding of the proximate cue is enhanced specifically in the nucleus accumbens core. This

relationship between dampened dopamine signaling and enhanced neural excitability provides insight into how negative affect can drive compensatory drug-seeking behavior.

Behavioral design

The present design differs from studies of noncontingent drug cue- and context-induced reinstatement (Crombag & Shaham, 2002; Saunders, Yager, & Robinson, 2013) because it allows for direct assessment of the affective state of the animal during cue exposure. It differs from cue-induced reinstatement designs in which subjects receive response-contingent presentations of an unextinguished drug cue (Davis & Smith, 1976) because it is not directly driven by the conditioned incentive value of the saccharin cue (which is presumably punishing). As such, it is capable of providing unique insight to the physiological mechanisms underlying the induction of negative affect by drug cues, and is useful for modeling cue-induced changes in affective state that may promote relapse in human addicts.

The present design resembles other context-induced reinstatement models in that the extinction context (Unpaired saccharin in our design) could be an omission cue. Thus, the critical behavioral and physiological differences between the groups could be due to the presence of an omission signal suppressing behavior in the Unpaired group, rather than the presence of an aversive drug cue in the Paired group inducing behavior. Several observations make this unlikely. First, both groups extinguished at the same rate, suggesting that the Unpaired cue was not being used as an omission signal to guide

behavior. Also, electrophysiological responses to the tone did not differ during extinction in the Paired and Unpaired groups, indicating that the presence of a potential omission signal did not influence encoding of the proximate cue. Finally, recent findings indicate that the presence of an innately aversive taste stimulus is sufficient to cause drug seeking. We observed a similar level of reinstatement following exposure to quinine, an inherently-aversive stimulus that had no association with drug access (Twining et al, 2014). Similarly, in the present study the magnitude of the reinstatement effect was positively correlated with the expression of aversion. Together these observations indicate that it is the aversive status of the cocaine cue, and the negative affect and neurochemical environment that it engenders, that induce drug seeking.

The impact of an aversive drug cue on dopaminergic encoding of a proximate drug cue

The observation of persistent dopaminergic encoding of an extinguished drug cue in the Unpaired group may be surprising, but it is consistent with contemporary theory regarding the product of extinction. Extinction produces suppression of behavior, but without direct physiological intervention (Lee, Milton, & Everitt, 2006; Otis, Dashew, & Mueller, 2013) or perhaps precise timing (Monfils, Cowansage, Klann, & LeDoux, 2009), it does not result in a loss of learning (Bouton, 2004; Miller & Matzel, 2006). Neural processing of an extinguished drug cue is an interaction between memories of reinforcement and nonreinforcement, and the dopamine response likely reflects this ambiguity. As such, it is not surprising that brain chemistry is more reactive to an extinguished

drug cue than a non-associated behaviorally inert stimulus, which does not typically elevate nucleus accumbens dopamine (Phillips et al, 2003).

The fact that reinstatement behavior was accompanied by a suppression of the dopaminergic encoding of a proximate drug-cue was surprising. Even so, the present result is more intuitive when informed by observations of dopamine signaling during cocaine self-administration. For example, rats that exhibit escalated drug use actually show attenuated dopamine encoding of drug-taking behavior (Willuhn et al, 2014). Furthermore, drug taking is reduced when positive dopamine encoding is restored through pharmacological intervention. That is, rats seek more cocaine because dopamine encoding is depressed, and reduce responding when it is restored (Willuhn et al, 2014). In this light, it is provocative to consider the possibility that if the acute dopamine elevation mediates the suppression of drug taking; it is possible that positive dopamine signaling also works to suppress drug seeking after extinction. The aversive drug cue may spur behavior, in part by eliminating this neurochemical event. This explanation is consistent with the observation that vigorous cocaine seeking/taking behavior tends to be preceded by reductions in nucleus accumbens dopamine (Wise et al, 1995; Tsibulsky & Norman, 1999; Stuber, Wightman, & Carelli, 2005).

In contrast to the present results, it is important to recognize that some aversive stimuli can increase dopamine and reinstate drug seeking. For example, electric foot shock increases nucleus accumbens dopamine as measured by microdialysis, and this increase in dopamine is sustained as drug seeking is reinstated (McFarland, Davidge, Lapish, & Kalivas, 2004). There are many

factors that may contribute to this discrepancy (Wenzel, Rauscher, Cheer, & Oleson, 2014). The nociceptive stimulation produced by electrical foot shock is typically very brief, discrete, and naturally engages robust locomotor behaviors that facilitate escape from the experimental context. In contrast, the taste infusions used here are relatively long, occur over an extended period of time, and induce behaviors of gustatory rejection that involve little locomotion (culminating with passive rejection). It is noteworthy that aversive gustatory stimuli can elevate dopamine in the nucleus accumbens (Bassareo, De Luca, & Di Chiara, 2002), indicating that modality is not the only critical factor. Regardless of the root cause(s) of this discrepancy, it is important to examine these different situations because both can lead to enhanced drug seeking, and may do so through dissociable mechanisms.

The role of NAc neural and neurochemical signaling in drug seeking

Broadly, activity in the nucleus accumbens mediates reinstatement phenomena (Bossert, Marchant, Calu, & Shaham, 2013). Disrupting activity in the nucleus accumbens attenuates stress-, cue-, cocaine-, and context- induced reinstatement (Cornish & Kalivas, 2000; Fuchs, Evans, Parker, & See, 2004; McFarland et al, 2004; Fuchs, Ramirez, & Bell, 2008; Xie et al, 2012). Specifically, corticostriatal glutamatergic projections drive relapse phenomena (McFarland et al, 2003; McFarland et al, 2004; Stefanik et al, 2013). In the present experiments, reinstatement was associated with enhanced excitability in two ways. First, exposure to the aversive drug cue elevated the baseline excitatory activity of neurons that would eventually encode the proximate drug

cue and/or drug-seeking behavior. This observation reveals a physiological mechanism that directly connects cue-induced negative affect to the subsequent drug-seeking act. Second, the aversive drug cue caused drug seeking and enhanced excitatory encoding of the proximate drug cue. This change in cue reactivity could contribute to the reinstated drug seeking observed here and in studies that show behavioral synergy between aversive stimuli and proximate drug cues (Liu & Weiss, 2002; Buffalari & See, 2009), even if the proximate drug cue has been extinguished (Shelton & Beardsley, 2005).

The relationship between decreased dopamine signaling and a shift toward excitatory activity

The effect of dopamine on striatal neural activity is complex and depends on a number of factors, such as D1-like and D2-like receptor expression, as well as the collateral influence of dopamine-sensitive interneurons (Surmeier et al, 2007). In general, D1-like receptor activation enhances excitability (Surmeier, Bargas, Hemmings, Nairn, & Greengard, 1995); appetitive stimuli preferentially activate D1-expressing neurons (Xiu et al, 2014); and excitatory encoding of motivated behavior coincides with and is sometimes contingent upon elevated dopamine signaling in the nucleus accumbens (Owesson-White et al, 2009; Cacciapaglia, Wightman, & Carelli, 2011). In contrast, D2-like receptor activation inhibits neuron excitability (Hernandez-Lopez et al, 2000); D2-expressing neurons are preferentially activated by aversive stimuli (Xiu et al, 2014); and acute inhibition of dopamine signaling induces aversion by activating D2-expressing neurons in the nucleus accumbens (Danjo et al, 2014). Given our

coincident observations of decreased dopamine signaling, increased nucleus accumbens activity, and behavioral signs of negative affect, we hypothesize that the current results reflect a disinhibited D2-mediated aversion-sensitive striatal circuit (Figure 2.8).

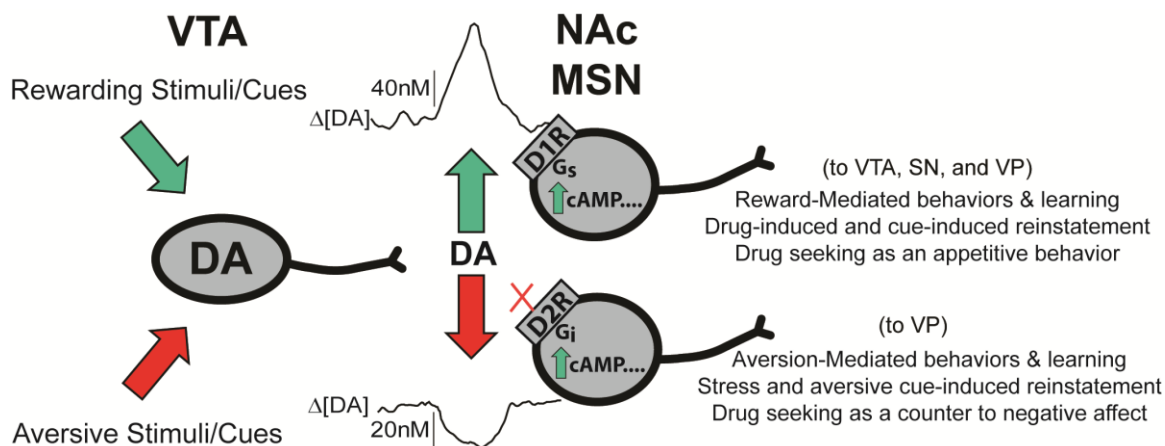


Figure 2.8. Model of striatal circuitry involved in cue-induced relapse. Rewarding stimuli and their predictors activate VTA dopamine neurons, causing an increase in nucleus accumbens dopamine concentration. This promotes excitability of D1-expressing medium spiny neurons that have been implicated in drug-primed and cue-induced reinstatement of drug seeking. Conversely, aversive drug cues impinge on the VTA which causes a reduction in dopamine tone in the nucleus accumbens and the induction of a negative affective state. This promotes the excitability of D2-expressing medium spiny neurons and avoidance behavior. In this model, drug seeking can also be viewed as an avoidance behavior. In this case, drug seeking results from the avoidance of an induced negative affective state.

The product of aversion-induced neural activation has been linked to behavioral inhibition (i.e., no-go; Nakanishi, Hikida, & Yawata, 2014), but activation of D2-like expressing neurons is also important for directing new behaviors that avoid aversive outcomes (Mehta, Swainson, Ogilvie, Sahakian, &

Robbins, 2001; Yawata, Yamaguchi, Danjo, Hikida, & Nakanishi, 2012; Porter-Stransky, Seiler, Day, & Aragona, 2013). This convergent evidence strongly suggests that cue- and aversion-induced reductions in dopamine signaling play a critical role in enhancing the excitability of neurons that encode proximate drug cues and drug-seeking behavior. The interaction between negative affect, dampened dopamine signaling, and enhanced neural excitability highlights a physiological substrate by which negative reinforcement mechanisms can augment cue reactivity and drive behavior. This aversion-driven behavioral modulation could be directed toward escape in adaptive behavioral contexts, but in a drug seeking context, it may promote drug seeking in an effort to correct the negative affective state. Future experiments will be needed to investigate the precise causal role of physiologically-relevant dopamine reductions in initiating this maladaptive cascade of events.

CHAPTER III

LEARNED AVOIDANCE REQUIRES VTA KOR-MEDIATED REDUCTIONS IN DOPAMINE

Introduction

The ability to adjust behavior appropriately following the experience of an aversive outcome is an essential and evolutionarily conserved brain process, as failure to learn from adverse experiences predisposes animals to future danger. Interestingly, variability in this complex trait is noticeable in humans and thought to give rise to specific personality characteristics (Carver & White, 1994). Clinical research suggests that more extreme variance in punishment sensitivity contributes to a diverse array of mental disorders that are characterized by lack of behavioral restraint, including conduct disorder, attention deficit hyperactivity disorder, and compulsive gambling (Luman, Oosterlaan, Knol, & Sergeant, 2008; Fairchild et al, 2009; de Ruiter et al, 2009) and is present in dopaminergic neurodegenerative disease (Frank, Seeberger, & O'reilly, 2004). Yet despite the obvious clinical importance, the neural circuits that regulate punishment learning remain largely uncharacterized.

Recent data indicate that both rewarding and aversive experiences shape future behaviors by acting on the mesolimbic dopamine system. Considerable convergent evidence indicates that rewarding stimuli promote appetitive association, in part, by increasing mesolimbic dopamine signaling. Far less is known about how avoidance-inducing environmental situations impact this system. However, there is mounting evidence from electrophysiological studies

of midbrain dopamine neurons and electrochemical monitoring of terminal dopamine release that aversive stimuli and their predictors can induce rapid reductions in nucleus accumbens dopamine signaling (Ungless et al, 2004; Roitman et al, 2008; Badrinarayan et al, 2012; Tan et al, 2012; Twining et al, 2014). These aversion-induced reductions in dopamine commonly occur with the induction of a negative affective state (Wheeler et al, 2008; Wheeler et al, 2011), and the activation of aversion-sensitive striatal neurons (Wheeler, Robble et al, 2015). The coincidence of aversion-induced reductions in dopamine signaling and striatal activation is consistent with the proposal that reductions in dopamine signaling disinhibit dopamine D2 receptor-expressing MSNs (Frank et al, 2004; Dreyer, Herrik, Berg, & Housgaard, 2010). Activation of this specific NAc output pathway has been linked to behavioral suppression (Lobo et al, 2010; Kravitz et al, 2012) and the ability to properly learn about aversive outcomes (Yawata et al, 2012; Porter-Stransky et al, 2013; Danjo et al, 2014). However, the mechanisms by which aversive stimuli impact NAc dopamine to influence aversion learning remain largely uncharacterized.

The kappa opioid receptor (KOR) system is a likely mechanism by which aversive stimuli impinge on mesolimbic dopamine signaling. Activation of KORs is aversive, and induces dysphoria and depressive-like behaviors in both humans and rodents (Pfeiffer, Brantl, Herz, & Emrich, 1986; Shippenberg & Herz, 1986). KORs and the dynorphins, their endogenous ligands, are highly enriched in limbic and midbrain areas involved in motivation and learning, specifically the NAc and the VTA (Pickel, Chan, & Sesack, 1993; Mansour et al, 1994;

Muschamp et al, 2014). Stress-induced behavioral responses are blocked by systemic KOR antagonist treatment and are absent in dynorphin knockout mice (Land et al, 2008). Furthermore, activation of KORs decreases NAc dopamine which likely contributes to their aversive properties (Britt & McGehee, 2008; Ebner, Roitman, Potter, Rachlin, & Chartoff, 2010). While the regulation of aversion-related behaviors by KORs has been studied extensively by systemic and local manipulation in limbic regions (Van't Veer & Carlezon, 2013), considerably less is known about the potential role for VTA KOR regulation of dopamine release and aversion learning.

Recent studies have begun to characterize the functional role of VTA KORs in aversion learning (Chefer, Backman, Gigante, & Shippenberg, 2013; Enrich et al, 2015). However, it is still unclear under what circumstances activation of these receptors regulates mesolimbic dopamine signaling. Electrophysiological studies of midbrain dopamine neurons have yielded conflicting results concerning whether or not KORs on nucleus accumbens-projecting dopamine neurons are functional (Ford, Mark, & Williams, 2006; Margolis et al, 2006). Notably, evidence of functional KORs expressed presynaptically on VTA glutamatergic and GABAergic inputs reveals the potential for these receptors to modulate nucleus accumbens-projecting dopamine neuronal activity (Margolis, Hjelmstad, Bonci, & Fields, 2005; Polter et al, 2014), but whether KOR activation is necessary for aversion-induced reductions in dopamine remains unknown. This question is critical to understanding how aversive stimuli engage brain circuitry that guides future behavior through

learning. In these studies, we scrutinized how blockade of VTA KORs impacted aversion-induced reductions in nucleus accumbens dopamine, and punishment learning. Our findings demonstrate the necessity of both VTA KOR activation and reductions in nucleus accumbens dopamine for aversive learning.

Methods and Materials

Subjects

42 male Sprague-Dawley rats (275–300 g; Harlan Laboratories, St. Louis, Missouri) were individually housed in a temperature- and humidity-controlled, Association for Assessment and Accreditation of Laboratory Animal Care accredited vivarium. Rats were maintained on a 12/12-hour reversed cycle (lights off at 7 AM) and had *ad libitum* access (unless otherwise noted) to water and food (Teklad; Harlan Laboratories). All experimental protocols were approved by the Institutional Animal Care and Use Committee at Marquette University in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgery

All surgical procedures were conducted under ketamine/xylazine (100 mg/kg / 20 mg/kg, intraperitoneal) anesthesia. Intraoral catheter implantations were conducted as previously described (Twining et al, 2014). Guide cannulas for microinjections (26-gauge; Plastics One, Roanoke, Virginia) were implanted bilaterally immediately above the VTA (AP: -5.6; ML: +/-2.2 at 11° angle; DV: -7.0) or above the nucleus accumbens shell (AP: -1.7; ML: +/-1.9 at 9° angle; DV:

-6.7). To prepare for voltammetric recordings, electrode guide cannula were implanted above the nucleus accumbens shell unilaterally (AP: +1.7; ML: +/-0.8), and a silver/silver chloride reference electrode was placed contralateral to the guide cannula. For all surgical procedures, rats were treated with meloxicam (1% oral suspension) during recovery.

Microinjections

Microinjectors extended 0.5 mm from the end of the guide cannula. Sterile saline or the KOR antagonist nor-Binaltorphimine (Nor-BNI, 2.5 µg/120s) (Graziane, Polter, Briand, Pierce, & Kauer, 2013) was bilaterally injected into the VTA. Sterile saline or the dopamine D2-like receptor agonist quinpirole (1.0 µg/60s) (Porter-Stransky et al, 2013) was bilaterally injected into the nucleus accumbens shell. Microinjectors were left in place for 2 minutes after each injection to allow for diffusion.

Voltammetric recordings

Twenty-four hours prior to recordings, rats (n=10) received bilateral microinjections of either vehicle or Nor-BNI into the VTA. Following injection, rats were habituated for 2 hours in the voltammetric recording environment, consisting of a clear Plexiglas chamber (Med Associates, St. Albans, Vermont) housed in a custom-designed Faraday cage. The headpiece was harnessed to a rotating commutator (Crist Instrument Co., Hagerstown, Maryland), and one intraoral cannula was harnessed to a fluid swivel (Instech Laboratory, Plymouth Meeting, Pennsylvania) that could receive fluid from a syringe pump (Razel, St. Albans, Vermont). On the following day, voltammetric recordings were conducted

as previously described (Wheeler et al, 2011). Briefly, a carbon fiber electrode was lowered into the nucleus accumbens shell, a fluid line was attached to the intraoral cannula, and the behavioral session was initiated. The experiment consisted of a 30-minute baseline dopamine monitoring period followed by a 50-minute quinine delivery period. Throughout the quinine delivery period, a 6-second infusion of 0.2mL quinine (0.001M) was delivered approximately every minute in the absence of any audio/visual cues. Orofacial reactions to quinine delivery were recorded and scored to determine whether Nor-BNI altered the palatability of quinine.

Voltammetric data analysis

Specific analyte identification and quantification were achieved using a principal component regression analysis as previously described (Heien et al, 2004). Dopamine was detected with a chemometric analysis using previously recorded training sets matched for dopamine oxidation potential and average amplitude of spontaneous dopamine release events (Twining et al, 2014). The resulting current amplitude was converted to dopamine concentration based on average post experiment electrode calibration (Wheeler et al, 2011). Individual trials were aligned to the initiation of a quinine infusion. Data from each trial (10s pre, and 30s post quinine infusion) were background subtracted at the local minima in the 10 seconds prior to quinine infusion. For each rat, data were averaged across the quinine infusion trials in the 10 seconds following the initiation of the quinine infusion period (Quinine) compared with the previous 10-second period (Pre Quinine) and the next 10-second period (Post Quinine). The

resultant current changes over time were analyzed for dopamine changes using principle component regression. Changes in dopamine were analyzed using a 2X3 mixed ANOVA and planned contrasts were used for direct comparisons. Statistical analyses of all behavioral and electrochemical data were performed using commercially available software (Statistica).

Punishment behavioral design

Rats were placed under mild food deprivation and maintained at 90% body weight for the entirety of the experiment. Once body weight reached criteria, subjects began training to lever press for an intraoral infusion of sucrose (20% sucrose, 0.1ml/inf). Subjects were trained on each lever in separate sessions across days and the training order was randomized (n=12). Training sessions had a maximum duration of 1 hour, but terminated earlier if 50 rewards were obtained. The schedule of reinforcement was then increased from a fixed ratio (FR) 1, to FR3, and finally to FR5 as subjects met criteria. Following training, subjects were given 5 daily 1 hour sessions in which they received access to both levers simultaneously (Maintenance, Figure 2A). Responses on either lever resulted in sucrose delivery on an independent variable interval (VI) 45s schedule of reinforcement. Following the 5th session, subjects received bilateral intra VTA microinjections of either Nor-BNI (2.5 μ g) or vehicle. Twenty-four hours following microinjection, subjects received a punishment session in which responses on one lever resulted in intraoral infusion of quinine (0.001M, 0.1ml/inf) on a VI45s schedule of punishment and responses on the other lever had no programmed consequences (Punishment, Figure 2B). The punished lever

was counterbalanced. A pilot study showed that within session punishment learning is not apparent in this design (data not shown). As a result rats were tested in extinction on the following day to assess punishment learning (Test, Figure 2C). In this session, responses on either lever resulted in no programmed consequences. Following testing, subjects were euthanized and brains were collected for histological verification of cannula placement. In a subsequent experiment, the D2-receptor agonist, quinpirole, or vehicle was microinjected into the nucleus accumbens shell 15 minutes prior to the punishment session (n=14). All other aspects of the experiment were identical.

Punishment data analysis

Responses made during the final day of Maintenance, Punishment, and the subsequent Test phase were analyzed. To account for generalized punishment effects, a criterion of 10 lever presses during the final test session was imposed and rats that did not meet this response requirement were excluded from data analysis (n=6). To account for variability lever-press behavior, a preference ratio was calculated and analyzed. Preference was calculated as the number of responses on the previously punished lever / total number of responses made on both levers. The same ratio was calculated for the 5th day of training. A ratio of .5 indicates no preference, whereas any value below .5 indicates avoidance of the punished lever. Behavioral results were analyzed using a mixed ANOVA and subsequent direct comparisons were made using planned contrasts.

Taste reactivity scoring

Taste reactivity was analyzed in a frame-by-frame analysis using digital video recorded on the voltammetry test day in Nor-BNI and vehicle injected rats (n=5 in each group). Appetitive and aversive orofacial movements were counted during and for 4 seconds immediately following quinine infusion using the technique of Grill & Norgren (1978). Mouth movements that matched the triangle shape for a duration exceeding 90 msec, as well as “paw flails” and “wet dog shakes” were counted as aversive. These criteria excluded all neutral and ingestive mouth movements.

Histology

Upon completion of each experiment, rats were euthanized with carbon dioxide. To verify recording electrode placement in rats used for voltammetry testing, small electrolytic lesions were created by running a current (250mA) through a stainless steel electrode placed at the depth at which the recording took place. Brains were removed and fixed in 10% formaldehyde for at least 14 days. Brains were then sliced into 40µm sections, mounted, stained with 0.25% thionin, and cover slipped. Representations of electrode and cannula placement from voltammetry and behavioral studies are shown in Figure 3.1.

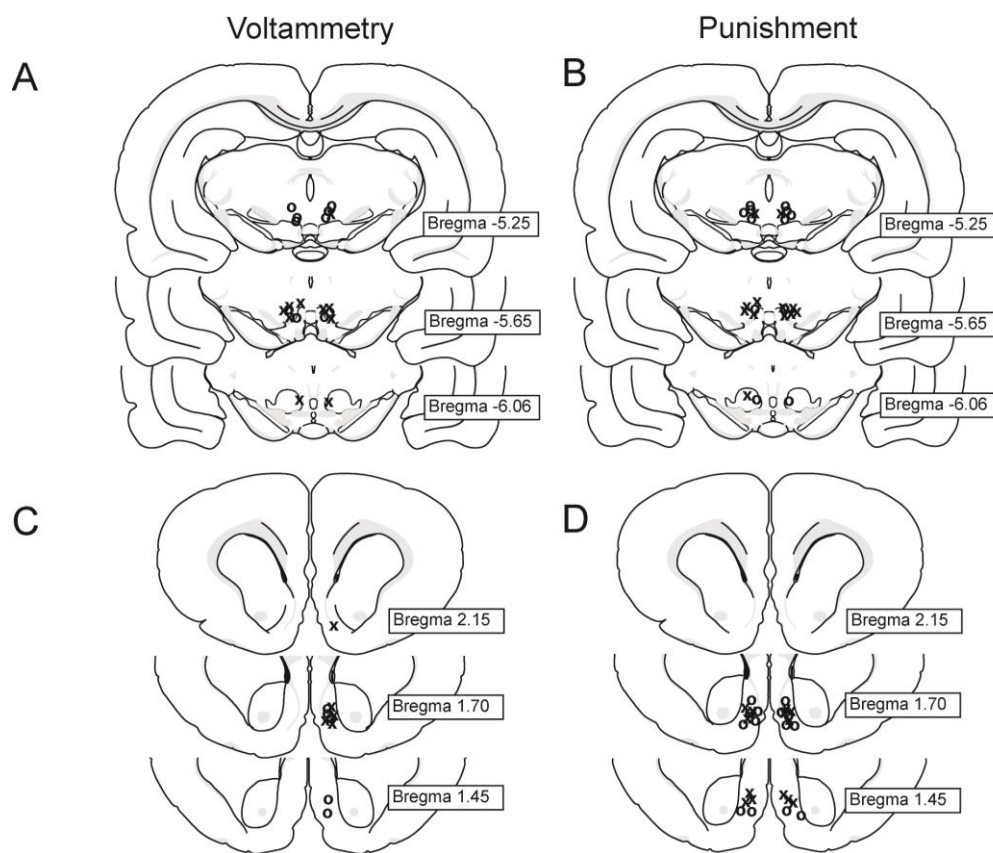


Figure 3.1. Histological reconstruction of recording electrode and cannula placements from voltammetry and punishment experiments. Infusions of Nor-BNI / quinpirole are represented with “X”s and vehicle infusions are represented with “O”s. (A) Placements of bilateral Nor-BNI injections from voltammetry recordings (n=10). (B) Placements of bilateral Nor-BNI injections in punishment behavioral task (n=12). (C) Placements of voltammetry recording electrodes in the NAc (n=10). (D) Placements of bilateral quinpirole infusions in punishment behavioral task (n=14).

Results

Blockade of VTA kappa opioid receptors attenuates aversion-induced reductions in nucleus accumbens shell dopamine.

To test whether KOR activation contributes to aversion-induced reductions in dopamine signaling, we monitored subsecond changes in nucleus accumbens shell dopamine concentration in response to an aversive taste stimulus using fast scan cyclic voltammetry. Prior to the recording session, rats received bilateral VTA microinfusions of either the KOR antagonist, nor-Binaltorphimine (Nor-BNI) or vehicle. Following a dopamine monitoring period, rats were given 50, brief intra-oral infusions of quinine. Individual trials were aligned to quinine infusion onset, and changes in dopamine concentration were then averaged. These averages were blocked into three periods, each 10s in duration (pre-quinine, quinine, post-quinine) for statistical analysis. A 2X3 mixed ANOVA (Group X Period) revealed significant main effects of group ($F(1,8) = 12.00, p < 0.01$) and of period ($F(1,8) = 9.89, p < 0.01$). No significant interaction was observed ($p > 0.1$). Planned contrasts revealed that groups did not differ during the Pre Quinine period ($F(1,8) = 1.11, p > 0.3$). However, quinine caused a greater reduction in dopamine concentration in vehicle treated rats compared to Nor-BNI treated rats ($F(1,8) = 12.48, p < 0.01$, Figure 3.2b and c). One reliable characteristic of a quinine-induced reduction in dopamine concentration is that it persists beyond the termination of quinine delivery. A comparison of vehicle and Nor-BNI groups in the post quinine period revealed that vehicle treated rats also exhibited significantly lower dopamine concentration in this period ($F(1,8) = 6.58, p < 0.05$,

Figure 3.2c). Orofacial reactions to quinine delivery were recorded and scored to determine the influence of VTA KOR blockade on the hedonic perception of quinine. Groups did not significantly differ in their disgust reactions to quinine ($F(1,8) = 0.17, p = 0.69$; Figure 3.2d). Together, these results indicate that VTA KOR activation is an important modulator of aversion-induced reductions in nucleus accumbens dopamine signaling, but does not impact palatability.

Blockade of VTA kappa opioid receptors prevents punishment learning.

KOR signaling has been implicated in modulating aversion, and while we found a pronounced regulation of dopamine signaling, we found no effect on hedonic processing. Based on this, we examined a role for VTA KOR signaling in punishment learning (Figure 3.3). In this design, all rats acquired operant responding for sucrose similarly. Following training rats were given the opportunity to respond for intraoral infusions of sucrose on each of two levers in daily 1 hour sessions from 5 days (Maintenance, Figure 3.3a). Following pretreatment with intra-VTA Nor-BNI or vehicle, sucrose seeking was punished by responses on one lever resulting in an intraoral quinine infusion while the other lever was inactive (Punishment, Figure 3.3b). On the following day rats were tested in extinction to assess avoidance learning following punishment (Test, Figure 3.3c).

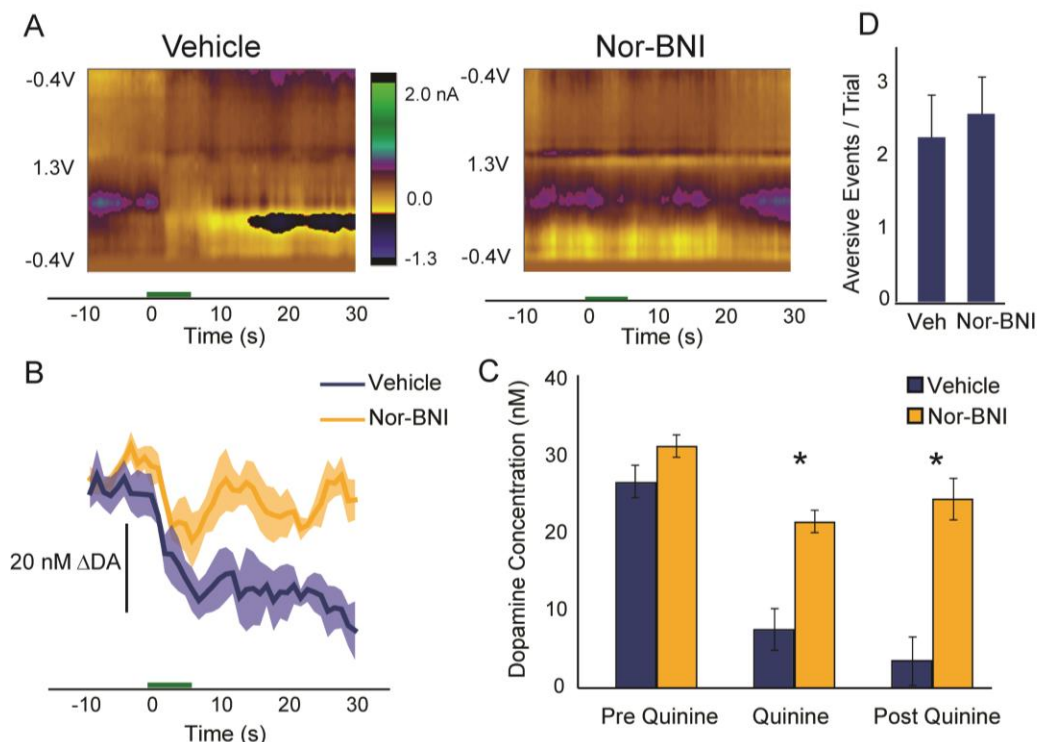


Figure 3.2. VTA kappa opioid receptor blockade attenuates aversion-induced reductions in nucleus accumbens shell dopamine. (A) Average color plots from individual recordings showing time averaged changes in dopamine concentration in response to quinine. Voltage is shown on the y-axis, time is shown on the x-axis, and current changes are shown in color. Intra oral quinine infusion (6s) occurred at time 0 and is designated by the green bar. Each animal was pretreated with either vehicle (left) or Nor-BNI (right). (B) Aversive taste reactivity responses (mean \pm SEM) in response to quinine. Vehicle and Nor-BNI pretreated animals did not differ in aversive responses to quinine ($p > 0.6$). Changes in dopamine concentration determined via principal component analysis are plotted in (C) and quantified in (D) ($n=10$). (C) Time-averaged dopamine concentration changes during intraoral quinine delivery. (D) Average dopamine concentration at 3 time points: Pre Quinine (-10-0s), Quinine (0-10s), and Post Quinine (10-20s). The quinine-induced dopamine reduction was significantly greater during the Quinine and Post Quinine periods ($p < 0.05$) in vehicle treated subjects compared to Nor-BNI treated subjects. Data are presented as mean \pm SEM.

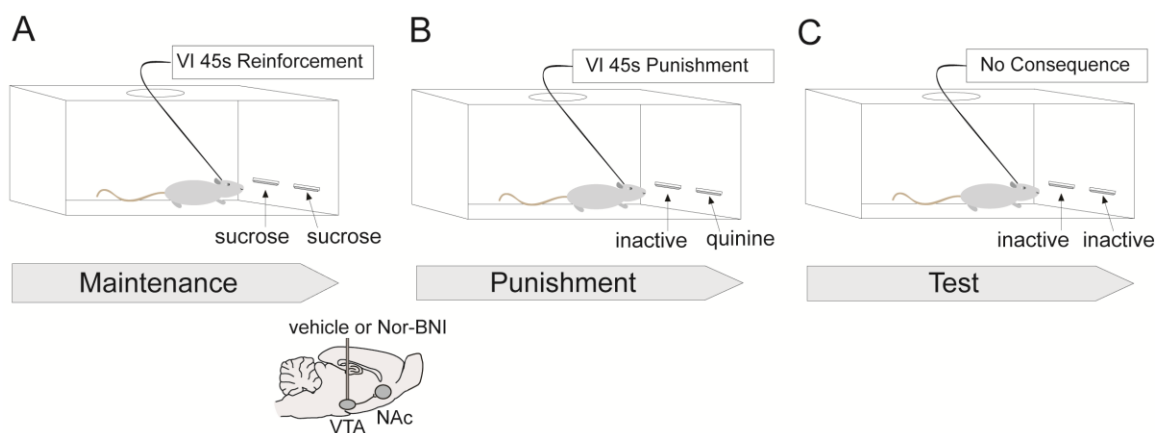


Figure 3.3. Overview of behavioral design. Subjects were trained to lever press for intraoral delivery of sucrose (20%, 0.1ml/inf). Experimental phases are shown in (A), (B), and (C). All sessions were 1 hour in duration. (A) Maintenance. Subjects received 1 session each day for 5 days during which responses on either lever yielded equivalent delivery of sucrose (20%, 0.1ml/inf). (B) Punishment. Delivery of pharmacological agents took place either 24 hours (Nor-BNI) or 15 minutes (quinpirole) prior to Punishment. All subjects received a punishment session during which responses on one lever resulted in delivery of intraoral quinine (0.001M, 0.1ml/inf), and the other lever was inactive. (C) Test. On the following day all subjects received a test session to assess punishment learning. During the Test session both levers were inactive. Intraoral infusions were always delivered in the absence of discrete cues. During Maintenance and Punishment, stimuli were delivered on independent VI45s schedules.

There were no between groups differences in sucrose consumption during Maintenance ($p > 0.3$). Following vehicle or Nor-BNI treatment, groups did not differ in quinine consumption during the Punishment phase (Figure 3.4a, $p > 0.4$). However, a significant interaction indicated that the ability to learn from the aversive experience depended on drug condition (group X session: $F(2,20) = 4.62$, $p < 0.05$). The vehicle group had a significantly lower preference for the punished lever at Test compared to Maintenance ($F(1,10) = 5.72$, $p < 0.05$),

indicating effective punishment learning. However, preference for the punished lever did not differ between Test and Maintenance in Nor-BNI treated animals ($F(1,10) = 1.56, p > 0.2$) (Figure 3.4b). Together, these results show that quinine is an effective punisher of operant sucrose seeking, and that VTA KOR activation is required for punishment learning.

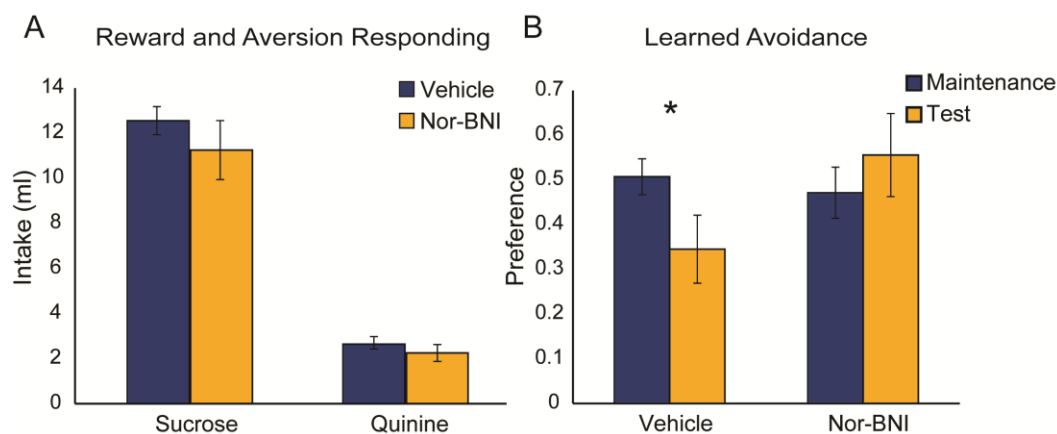


Figure 3.4. VTA kappa opioid receptor blockade prevents punishment learning. (A) Sucrose consumption during Maintenance ($p > 0.3$) and quinine intake during Punishment did not differ between the groups ($p > 0.4$), indicating an equal opportunity to learn. (B) Subjects receiving intra-VTA vehicle microinfusions ($n=6$) significantly reduced their preference for the punished lever ($p < 0.05$) indicating effective punishment learning. Learning was prevented in subjects pretreated with intra-VTA Nor-BNI ($n=6$) ($p > 0.2$). Preference = responses on the punished lever / total responses. Data are presented as mean \pm SEM.

Nucleus accumbens shell D2 receptors regulate punishment learning.

Our results suggest that KOR-mediated reductions in nucleus accumbens dopamine are important for aversive learning. If this reduction in dopamine signaling is an essential component of aversive learning, then it is likely acting through reduced occupancy of high affinity D2-like dopamine receptors in the

NAC (Dreyer et al, 2010; Porter-Stransky et al, 2013; Danjo et al, 2014). To test this, we microinfused the D2 receptor agonist, quinpirole, into the nucleus accumbens shell prior to Punishment in the same learning design. We hypothesized that if reduced D2 receptor occupancy is involved in aversive learning, then quinpirole would prevent the downstream effect of aversion-induced dopamine reductions and interfere with punishment learning. Rats were again trained to respond for 20% sucrose, and following Maintenance were given a Punishment session. Fifteen minutes prior to the Punishment session rats received intra-nucleus accumbens shell microinjections of either vehicle or quinpirole. On the following day, rats received a Test session to assess learning. Groups did not differ in sucrose consumption during Maintenance ($p > 0.5$) or quinine consumption during Punishment ($p > 0.4$), suggesting that each group had the same opportunity to learn (Figure 3.5a). A repeated measures ANOVA revealed a significant interaction (Group x Session: $F(2,24) = 3.71, p = 0.04$) indicating that the effectiveness of the punishment training depended on drug treatment condition. Rats treated with vehicle showed punishment learning, as their preference for the punished lever was significantly lower at Test than during Maintenance ($F(1,12) = 7.08, p = 0.02$). However, rats that had received quinpirole did not show evidence of learning, as preference was unchanged from Maintenance to Test (Figure 3.5b, $F(1,12) = 0.001, p > 0.9$). These results indicate that preventing aversion-induced reductions in D2 dopamine receptor activation during the experience of an aversive stimulus impairs the ability to learn to avoid that aversive stimulus.

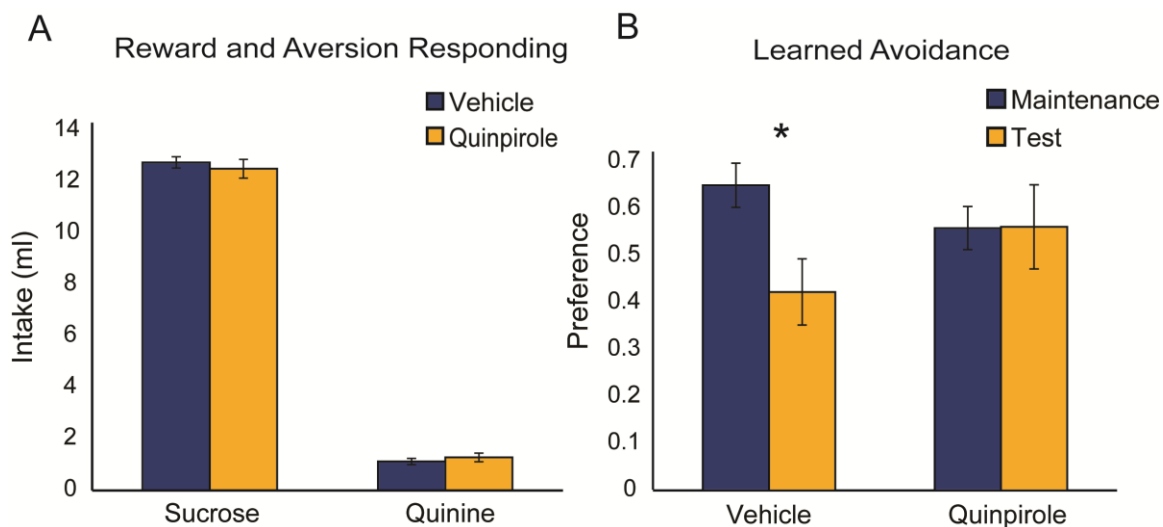


Figure 3.5. Activation of nucleus accumbens D2 receptors prevents punishment learning. (A) Sucrose consumption during Maintenance ($p > 0.5$) and quinine intake during Punishment did not differ between the groups ($p > 0.4$). (B) Subjects receiving intra-NAc vehicle microinfusions ($n=7$) significantly reduced their preference for the punished lever ($p < 0.05$) indicating effective punishment learning. Learning was prevented in subjects pretreated with intra-NAc quinpirole ($p > 0.9$) ($n=7$). Preference = responses on the punished lever / total responses. Data are presented as mean \pm SEM.

DISCUSSION

The present study assessed the contribution of VTA KORs to aversion-induced reductions in nucleus accumbens dopamine signaling and aversion learning. Electrochemical monitoring has previously revealed that exposure to the aversive tastant, quinine, simultaneously induces negative affect and reduces NAc dopamine (Roitman et al, 2008; Wheeler et al, 2011; Twining et al, 2014). These experiments replicated the prior results and demonstrated that the aversion-induced reduction in dopamine signaling, but not the aversion-induced

negative affect, was attenuated by blockade of VTA KORs. To further test the behavioral relevance of this attenuated aversion-related signal, we adapted a punishment design (Marchant, Khuc, Pickens, Bonci, & Shaham, 2013) in which quinine was used to punish sucrose seeking in rats. We found that aversive conditioning with quinine caused rats to avoid the punished outcome during a subsequent test, and that this learning was dependent upon activation of VTA KORs. Finally, we tested the necessity of aversion-induced reductions in dopamine signaling in avoidance behavior by maintaining nucleus accumbens D2 receptor tone during punishment. We found that activation of D2 receptors during conditioning prevented avoidance learning caused by punishment. Together, these experiments demonstrate the necessity of activation of VTA KORs for aversion-induced reductions in dopamine that facilitate avoidance, and highlight the importance of nucleus accumbens D2 receptor signaling for aversive learning.

Since the observation that KOR activation is aversive in rodents and humans, the dynorphin/kappa opioid system has been extensively studied for its role in stress-induced and aversion-related behaviors (Pfeiffer et al, 1986; Shippenberg & Herz, 1986). Systemic administration of a kappa receptor agonist increases immobility in a forced swim test, as well as increases reward thresholds for intra-cranial self-stimulation, indicating that these agents produce depressive-like behavioral phenotypes and anhedonia (Carlezon et al, 2006). Systemic as well as local injections of KOR agonists in various brain areas, including the VTA and nucleus accumbens cause a conditioned place aversion

(Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993; Zhang, Butelman, Schlussman, Ho, & Kreek, 2005). The depressive effects of KOR activation are blocked by treatment with a kappa receptor antagonist, and are lacking in mice possessing a nonfunctional prodynorphin gene or genetic KOR knockout (McLaughlin, Marton-Popovici, & Chavkin, 2003; Land et al, 2008). Furthermore, numerous studies have reported that, coincident with these depressive-like behaviors, KOR agonists also produce a reduction in nucleus accumbens dopamine signaling as measured by microdialysis and fast scan cyclic voltammetry (Carlezon et al, 2006; Britt, & McGehee, 2008; Ebner et al, 2010). Together, these studies indicate that activation of KOR receptors induces a negative affective state coincident with a decrease in nucleus accumbens dopamine. The link between KOR-mediated depressive-like behaviors and inhibition of NAc dopamine release has led to a recent focus on the role of VTA KORs in aversion.

KORs are well positioned to modulate motivated behavior, being expressed not only in the nucleus accumbens, but also both postsynaptically on dopaminergic neurons, as well as presynaptically on glutamatergic and GABAergic afferents in the VTA (Pickel et al, 1993; Margolis et al, 2005; Polter et al, 2014; Chefer et al, 2013). Activation of VTA KORs reduces the firing rates of VTA dopamine neurons (Muschamp et al, 2014), although this effect may not be uniform on mesolimbic and mesocortical projections (Ford et al, 2006; Margolis et al, 2006). While the behavioral significance of VTA KOR signaling has not received much attention, several recent studies have highlighted a key role for

this system in the regulation of aversion related behaviors. Recently it was shown that knockdown of KORs on dopamine transporter-expressing neurons reduced anxiety in an open field and light/dark box (Van't Veer et al, 2013). Subsequently, Chefer et al (2013) demonstrated that mice lacking KORs on DAT-expressing neurons were unable to learn kappa agonist-induced conditioned place aversion. Furthermore, these mice also lacked the characteristic kappa agonist-induced decrease in nucleus accumbens dopamine as measured by microdialysis. In an elegant rescue experiment, intra-VTA viral-mediated expression of KORs rescued both the dopamine response to a kappa agonist as well as the aversive learning. Together, these reports highlight the importance of VTA KOR signaling in the regulation of aversion-related behaviors and nucleus accumbens dopamine. However, the interpretation of these studies is limited by the usage of pharmacological KOR agonists to produce aversion. While it is clear that KOR activation is sufficient to decrease nucleus accumbens dopamine and induce negative affect, it is unclear from these findings under what physiologically relevant conditions this occurs. The present study extends these findings by demonstrating that VTA KORs are activated by acute environmental aversive stimuli, and that this activation is required for aversion-induced reductions in dopamine and for proper avoidance learning caused by punishment.

Interestingly, Enrich et al (2015) recently reported that systemic kappa agonist induced conditioned place aversion requires activation of p38 α MAPK signaling in VTA dopamine neurons. While mice did not show kappa agonist induced conditioned place aversion when this signaling cascade was disrupted,

the ability of a kappa agonist to decrease experimenter evoked nucleus accumbens dopamine remained intact. This finding was interpreted as a demonstration that kappa agonist-induced reductions in dopamine do not mediate the aversive properties of KOR activation. However, it is important to note that the confirmation of the effect of KOR activation on dopamine is done in anesthetized animals, and shows that loss of p38 α MAPK signaling does not alter KOR activation-induced blunting of stimulated dopamine release. While this result is convincing, the experiment does not examine the relevant dopamine signal for learning. For the interpretation of these findings, it is important to show that mice lacking p38 α MAPK signaling in VTA dopamine neurons still retain the prolonged reduction in dopamine (below baseline levels) that occurs during place conditioning. Additionally, in order to demonstrate that decreases in dopamine caused by KOR activation are not required for aversive learning, it is prudent to show, in an animal with intact KOR signaling cascades, that maintaining nucleus accumbens dopamine concentrations during conditioning does not impact learning. Importantly, in addition to disrupting aversive learning with a VTA KOR antagonist, the current report describes a similar disruption by maintaining dopamine tone with an intra-nucleus accumbens D2 receptor agonist. This finding suggests that, in our task, the KOR-mediated reduction in dopamine is required for aversive learning. Thus, it is likely that proper aversive learning requires both functional second messenger signaling within VTA dopamine neurons and a reduction in downstream dopamine signaling. Together, the two results are provocative, and indicate that future studies aimed at characterizing

the complex regulation of VTA dopamine neuron activity by aversive stimuli may yield therapeutically useful results.

. The observation that aversion-induced reductions in dopamine signaling are required for punishment learning is consistent with an evolving understanding of the role of different output pathways in the ventral striatum. Studies using fast scan cyclic voltammetry, microdialysis, or computational modeling all estimate that basal nucleus accumbens dopamine concentration is in the low nanomolar range (Parsons & Justice, 1992; Shou Ferrario, Schultz, Robinson, & Kennedy, 2006; Dreyer et al, 2010; Owesson-White et al, 2012; Dreyer & Hounsgaard, 2013). Basal dopamine concentrations in this range would be too low to frequently activate low affinity D1 receptors, but would be expected to more consistently occupy the majority of high affinity D2 receptors in the ventral striatum (Dreyer et al, 2010). Since D1 and D2-expressing MSNs differ not just in the affinity of those receptors for dopamine, but also in their projection targets (Bocklisch et al, 2013; Kupchik et al, 2015), the nucleus accumbens output neurons are well positioned to be differentially sensitive to increases and decreases in dopamine. This notion of differential sensitivity to environmentally-driven directional deviations in dopamine concentration is important for interpretation of the present findings (Figure 3.6). Rewarding stimuli and their predictors cause a transient increase in nucleus accumbens dopamine concentration that preferentially affects low affinity D1-like receptors, resulting in an increased sensitivity to glutamatergic drive to facilitate reward learning. In

contrast, aversive stimuli cause a reduction in naturally occurring dopamine concentration that is preferentially detected by D2 receptors.

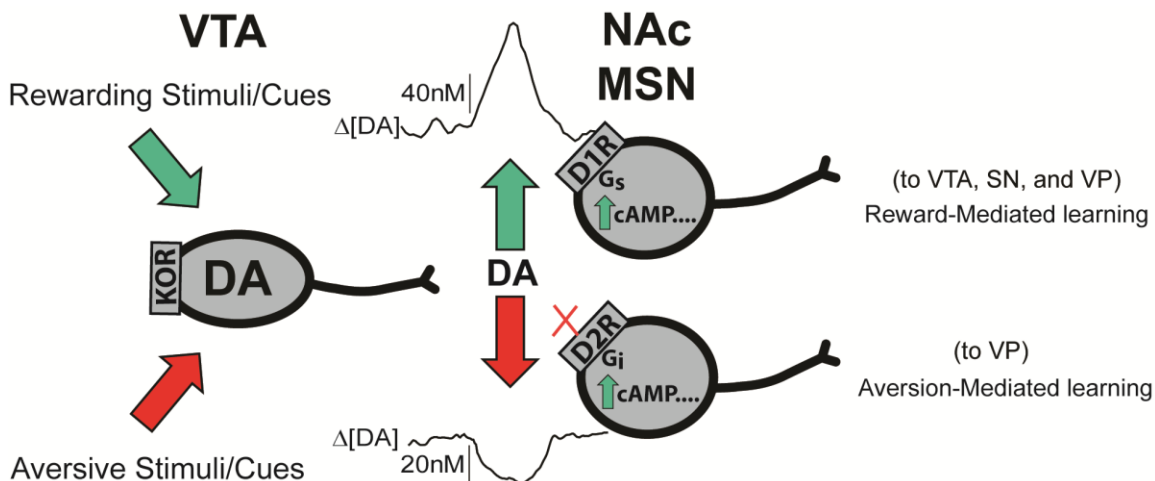


Figure 3.6. Model of striatal signaling during reward and punishment learning. Rewarding stimuli and their predictors elevate dopamine signaling which excites a low-affinity dopamine receptor-expressing output circuit, increasing the sensitivity of these projection neurons to glutamatergic afferents that promote incentivized responding. Alternatively, aversive stimuli promote VTA KOR activation, decreasing naturally-occurring dopamine signaling, and disinhibiting a different, aversion-sensitive output circuit characterized by the expression of high affinity dopamine receptors. The activation of this pathway promotes avoidance learning.

As the frequency of occupancy drops, the inhibitory tone on D2-expressing MSNs is reduced, facilitating avoidance learning. Importantly, even sub-second pauses in dopamine cell firing have been suggested to decrease D2 receptor occupancy (Dreyer et al, 2010). Our studies indicate that a single intraoral infusion of quinine produces a pronounced and sustained decrease in nucleus

accumbens dopamine, taking several seconds to resolve following stimulus offset (Roitman et al, 2008; Wheeler et al, 2011; Twining et al, 2014). These stimulus-driven characteristics are important because the magnitude of bursts and pauses in dopaminergic neuronal activity, and corresponding increases or decreases in terminal dopamine concentration, determines the magnitude of the prediction error signal that guides learning (Bayer, Lau, & Glimcher, 2007).

As a test of the importance of aversion-induced reductions in dopamine, we demonstrated that learning could be prevented downstream by using a D2 receptor agonist to maintain D2 receptor tone during punishment. According to our model, this treatment prevented punishment learning by reducing the capacity of D2-expressing MSNs to detect aversion-induced reductions in extracellular dopamine as they remained occupied by the pharmacological agent. In support of this model, the stimulation of D2-expressing MSNs has recently been linked to the experience of aversive stimuli (Xiu et al, 2014), behavioral suppression (Lobo et al, 2010), social avoidance (Francis et al, 2015), and the ability to learn about aversive outcomes (Porter-Stransky et al, 2013; Danjo et al, 2014; Hikida et al, 2013). Together, these findings support the hypothesis that aversion-induced reductions in nucleus accumbens dopamine are necessary for punishment learning and highlight the importance of decreased dopamine to convey a critical learning signal. This view complies with clinical observations that patients with Parkinson's disease are better at learning to avoid negative outcomes than they are at learning to approach rewards, and these deficits are attenuated by treatment with pro dopaminergic drugs (Frank et al, 2004). Further

study is necessary to determine whether reductions in nucleus accumbens dopamine are causally related to activation of D2-expressing MSNs, as well as provide significant insight into the molecular regulation and downstream targets of this pathway. This level of scrutiny could provide new avenues for therapeutic interventions for related disease states.

CHAPTER IV

GENERAL DISCUSSION

Summary

The studies presented in this thesis provide an evolving understanding of the importance of striatal dopamine signaling in aversion-driven behaviors. This thesis provides the novel demonstration that aversive drug cues that decrease nucleus accumbens dopamine and elicit a negative affective state cause drug seeking. Furthermore, the experiments described here characterize the effect of the aversive drug cue on the dopaminergic response to other drug associated stimuli, and on the activity of striatal neurons. Drug associated cues typically increase striatal dopamine levels. However, prior presentation of the aversive drug cue, and the induction of negative affect, eliminated this response. In this environment of decreased dopamine signaling caused by the presentation of the aversive drug cue, a subset of nucleus accumbens neurons progressively elevate their baseline firing rates. Importantly, this subset of neurons goes on to encode various aspects of reinstatement behavior. Together, these studies suggest that aversion-induced reductions in dopamine activate aversion-sensitive neurons in the nucleus accumbens that promote relapse behavior (Chapter II).

In addition to the characterization of dopamine signaling in the pathological state of drug addiction, experiments presented in this dissertation also scrutinize the significance of reductions in dopamine under more natural conditions. These studies characterize an opioid signaling mechanism by which

aversive stimuli cause reductions in nucleus accumbens dopamine signaling. While studied extensively for their role in aversion-related behaviors, particularly in the limbic system, VTA KORs are an important, but understudied population. Experiments presented here demonstrate that activation of VTA KORs is required for aversion-induced reductions in dopamine. Although pharmacological activation of these receptors has been shown to be aversive and decrease nucleus accumbens dopamine signaling (Chefer et al, 2013), the insight that this mechanism is engaged by environmental aversive events to cause reductions in dopamine is novel and highly significant. Perhaps more importantly, the experiments presented here show that activation of VTA KORs is required for proper avoidance learning caused by punishment; an effect that is attributed to the capacity of KOR activation to mediate aversion-induced reductions in dopamine. In support of this interpretation, data are presented that demonstrate that pharmacologically maintaining D2 receptor activation within the nucleus accumbens during the experience of the aversive stimulus also prevents avoidance learning caused by punishment. Together, these experiments demonstrate how aversive stimuli impinge on mesolimbic dopamine signaling, and describe a role for reductions in nucleus accumbens dopamine as an aversive learning signal that is propagated by changes in D2 receptor occupancy (Chapter III). It is the goal of the following sections to describe an evolving model of differential regulation of striatal output by bidirectional fluctuations in dopamine, and provide support for this model from both preclinical and clinical literature. Finally, this discussion will highlight the utility of this model in

explaining both healthy function and pathological dysfunction in avoidance behavior and learning, and for the examination of novel targets for the development of therapeutics.

Dopaminergic Neurotransmission within the Nucleus Accumbens

The findings presented here that reductions in nucleus accumbens dopamine may contribute to relapse and are required for punishment learning, are consistent with contemporary views of how bidirectional fluctuations in dopamine may regulate different ventral striatal output pathways. Central to this view is the notion that basal concentrations of dopamine serve as a contrast point by which increases or decreases in concentration produce a distinct physiological response (Arbuthnott & Wickens, 2007). This is possible because the GABA-ergic medium spiny projection neurons of the nucleus accumbens are heterogeneous, and most MSNs express either Gs-coupled D1-like, or Gi-coupled D2-like dopamine receptors (Gerfen et al, 1990). While both receptor subtypes have high- and low-affinity states, it is thought that striatal D1 receptors are typically in the low affinity state ($\sim 1\mu\text{M}$) while D2 receptors are in the high affinity state ($\sim 10\text{nM}$) (Richfield, Penney, & Young, 1989; Rice & Cragg, 2008; Sulzer, Cragg, & Rice, 2016). Although this is difficult to verify, it is corroborated by observations of a high rate of tonic D2 receptor activity (Bertran-Gonzalez, et al, 2008; Svenningsson et al, 2000). Estimates of time-averaged naturally occurring nucleus accumbens dopamine concentration in an awake, resting rat using fast scan cyclic voltammetry are approximately 20-30nM (Owesson-White

et al, 2012), which are fairly consistent with estimates resulting from microdialysis measurements ranging from 5-18nM (Parsons and Justice, 1992; Shou et al, 2006). These estimations are corroborated by computational models that estimate a moment-to-moment concentration of up to 67nM (Dreyer et al, 2010; Dreyer & Hounsgaard, 2013). Midbrain dopamine neurons are tonically active and have firing rates of 1-5Hz when not engaged in burst firing (Cooper, 2002). Given the restriction of the dopamine transporter, the main mechanism of uptake, to presynaptic sites, its low density of expression, and its relatively slow uptake kinetics, it is thought that the majority of the thousands of dopamine molecules released per single quantal release event spill over into the extra synaptic space (Rice & Cragg, 2008). Accounting for this, it has been suggested that a single quantal dopamine release event can influence the occupancy of D2 receptors at 20-100 individual synapses (Arbuthnott & Wickens, 2007). Thus, the consistent presence of a low nanomolar concentration of dopamine would be expected to frequently occupy the majority of D2 receptors in the ventral striatum. Indeed, modeling data indicates that tonic firing of midbrain dopamine neurons results in the occupancy of 75% of nucleus accumbens D2 receptors and only 3.5% of D1 receptors, while even sub-second pauses in firing reduce D2 receptor occupancy (Dreyer et al, 2010). Together, these estimates describe a basal extracellular striatal environment with persistent occupancy of the majority of D2 receptors and very low occupancy of D1 receptors by dopamine.

An important outcome of dopamine receptor signaling is the indirect modulation of neuronal activity through alterations in the sensitivity to

glutamatergic drive. Given the opposing G-protein coupling of D1 and D2 receptors on nucleus accumbens MSNs, dopamine receptor activation differentially alters neuronal excitability. D1 receptors are G_s/G_{olf} coupled and activation of these receptors leads to activation intracellular PKA. D1-mediated activation of PKA has been shown to phosphorylate AMPA and NMDA receptor subunits to promote surface expression of these receptors in the striatum (Snyder et al, 2000; Hallett, Spoelgen, Hyman, Standaert, & Dunah, 2006). As a result, D1 receptor activation in MSNs enhances synaptic currents evoked by NMDA receptor activation (Cepeda, Buchwald, & Levine, 1993). In addition, D1 receptor-dependent PKA phosphorylation of $GABA_A$ receptors has been shown to reduce their function (Flores-Hernandez et al, 2000). D1 receptor activation also alters the activity of various voltage-gated ion channels that control membrane excitability. D1-induced activation of PKA phosphorylates L-type Ca^{2+} channels which augments the response of intrasomatic current injection (Surmeier et al, 1995; Hernández-López, Bargas, Surmeier, Reyes, & Galarraga, 1997). D1 receptor stimulation also inhibits the activity of several voltage gated K^+ channels (Surmeier et al, 2007). Together, these studies show that, through a combination of several mechanisms, activation of D1 receptors enhances membrane excitability and increases sensitivity to excitatory synaptic neurotransmission.

Contrary to the role of the D1 receptor, D2 receptors are $G_{i/o}$ coupled and activation of these receptors negatively regulates intracellular PKA signaling. Activation of D2 receptors promotes trafficking of AMPA receptors out of the

synapse through dephosphorylation and reduces AMPA and NMDA receptor mediated synaptic currents (Cepeda et al, 1993; Håkansson et al, 2006; Higley & Sabatini, 2010). D2 receptor activation also regulates a series of voltage gated ion channels to decrease membrane excitability. For example, D2 receptor stimulation inhibits depolarizing intracellular Ca^{2+} influx via PKA-dependent and independent mechanisms, and enhances hyperpolarizing K^+ efflux (Greif, Lin, Liu, & Freedman, 1995; Higley & Sabatini, 2010; Surmeier, Carrillo-Reid, & Bargas, 2011), the net effect of which is to maintain the cell in a hyperpolarized state. These effects of D2 receptor stimulation are supported by the observation that D2-expressing MSNs show enhanced excitability in dopamine depleted mice (Peterson, Goldberg, & Surmeier, 2012). Importantly, PKA increases following D2 receptor inactivation occur rapidly (Yamaguchi et al, 2015), suggesting that activation of these G-protein coupled receptors may modulate excitability on a time scale suitable for responsivity to stimulus driven events. Together, these studies support the view that activation of D2 receptors in the striatum is inhibitory through reductions in membrane excitability and sensitivity to excitatory input.

Model of Striatal Signaling

The differential effects of nucleus accumbens dopamine receptor activation, differences in projection targets of D1 and D2-expressing MSNs, and the likelihood of frequent D2 receptor occupancy by basal dopamine concentrations together describe differential striatal circuitry that is perfectly

adapted to be oppositely regulated by bidirectional fluctuations in dopamine concentration. The model presented throughout this thesis reflects this understanding to describe the importance of mesolimbic dopamine for approach and avoidance behaviors and learning (Figure 4.1).

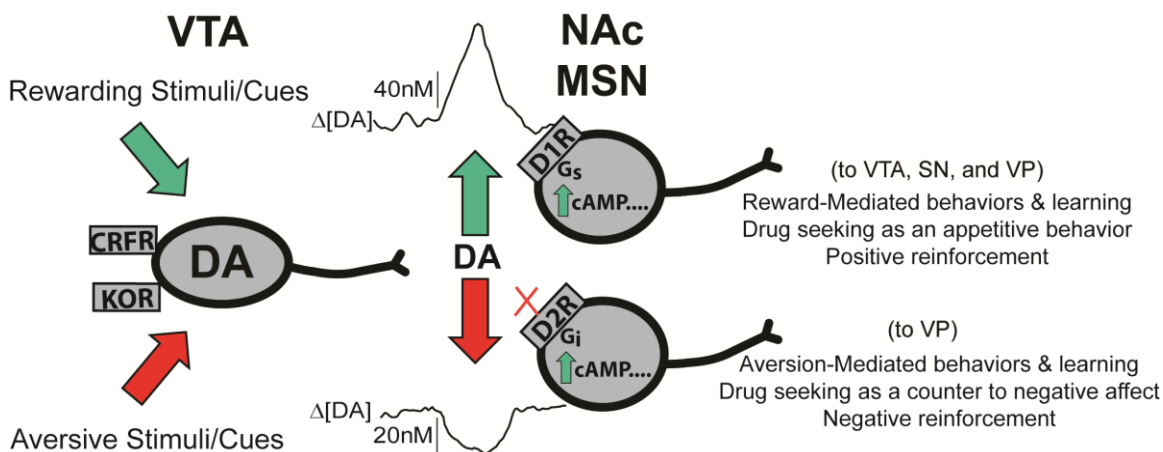


Figure 4.1. Evolving model of regulation of drug-seeking and learning by striatal circuits. Rewarding stimuli increase nucleus accumbens dopamine concentrations, which increases sensitivity of D1-expressing medium spiny neurons to excitatory drive. Activation of this pathway is critical for reward learning and drug-seeking as an appetitive behavior. Aversive stimuli decrease dopamine via intra-VTA activation of CRF and/or kappa opioid receptors. Aversion-induced reductions in dopamine are associated with the emergence of negative affect, and increase the sensitivity of D2-expressing medium spiny neurons to excitatory drive. Activation of this pathway promotes aversion learning and drug-seeking through negative reinforcement.

Rewarding stimuli and reward-associated cues increase nucleus accumbens dopamine concentrations, which activates low affinity D1 receptors. Activation of these receptors increases membrane excitability and sensitivity to glutamatergic inputs that activate this pathway that promotes approach behaviors and reward learning. Conversely, aversive stimuli decrease nucleus accumbens dopamine

which reduces the frequency of D2 receptor occupancy. The loss of D2 receptor-driven inhibitory tone increases membrane excitability and sensitivity to glutamatergic inputs on D2-expressing MSNs. Activation of this pathway promotes avoidance behavior and aversive learning.

Experiments from our lab have contributed to the understanding of specific aspects of this model. We have demonstrated that aversive stimuli impinge on mesolimbic dopamine signaling through activation of VTA CRF receptors, and activation of these receptors is required for aversion-induced reinstatement of cocaine seeking (Twining et al, 2014). In addition we have shown that an aversive, cocaine-predictive cue decreases dopamine, causes reinstatement of cocaine seeking, and activates a subpopulation of aversion-sensitive striatal neurons (Wheeler, Robble et al, 2015; Chapter II). According to our model, it is likely that these aversion-sensitive neurons are D2-expressing MSNs. Subsequent experiments scrutinized critical components of the model outside of the pathological state of drug addiction. Novel findings presented here show that, under normal conditions, aversive stimuli decrease nucleus accumbens dopamine signaling through activation of VTA KORs, and VTA KOR activation is required for avoidance learning caused by punishment. Importantly, we demonstrated that maintaining nucleus accumbens D2 receptor activation during the experience of the aversive stimulus also prevents punishment learning. This manipulation indicates that a decrease in D2 receptor occupancy, caused by aversion-induced reductions in dopamine, is required for avoidance learning caused by punishment (Chapter III). According to the model, intra nucleus

accumbens shell quinpirole prevents avoidance learning by maintaining inhibitory tone on D2-expressing MSNs. This last finding is significant, as the link between aversion-induced reductions in dopamine, activation of D2-expressing MSNs, and avoidance learning remains largely uncharacterized.

Evidence that Reward-Evoked Dopaminergic Neurotransmission Leads to Activation of D1-Expressing Medium Spiny Neurons that Drive Reward-Related Behaviors.

The recent advent of advanced techniques such as chemo- and optogenetics has allowed for significant advances to our understanding of how dopamine may modulate the activity of discrete striatal output pathways. Optogenetic activation of VTA dopamine neurons is sufficient to cause a conditioned place preference (Ilango et al, 2014), and supports self-stimulation behavior in rats (Witten et al, 2011). In addition, activation of VTA dopamine neurons during reward omission attenuates extinction learning (Steinberg et al, 2013). These studies provide a causal link between elevated dopamine signaling and positive reinforcement and reward learning. Downstream, optogenetic activation of D1-expressing MSNs has been shown to be sufficient to cause conditioned place preference (Kravitz et al, 2012), while blockade of neurotransmitter release from D1-expressing MSNs prevents cue-mediated reward learning (Yawata et al, 2012). These studies indicate that activation of D1-expressing MSNs is both necessary and sufficient for reward learning. This pathway also has been examined for its importance in cocaine addiction. Blocking neurotransmission in D1-expressing MSNs demonstrated the

requirement of activation of this pathway for the acquisition and expression of cocaine conditioned place preference and cocaine-induced locomotor sensitization (Hikida, Kimura, Wada, Funabiki, & Nakanishi, 2010; Hikida et al, 2013). The necessity of these neurons for locomotor sensitization to cocaine has been further clarified by work from Bocklisch et al (2013), indicating that this behavior is mediated specifically by the D1-expressing MSNs that project to the VTA and act to disinhibit VTA dopamine neurons in response to cocaine. The *in vivo* activity pattern of this specific pathway has also been associated with drug-seeking behavior. In an elegant study, Calipari and colleagues (2016) recorded the activity of nucleus accumbens D1-expressing neurons during the acquisition, extinction, and reinstatement phases of cocaine conditioned place preference. They found that these neurons were activated by cocaine, and following conditioning these neurons exhibited their strongest activation immediately prior to entry into the paired side. These responses decreased with extinction, and were potentiated during cocaine-primed reinstatement. In accordance with other reports, pathway specific chemogenetic inhibition prevented acquisition and expression of cocaine conditioned place preference. Together, these studies support the widely accepted role for reward-induced increases in nucleus accumbens dopamine, and subsequent activation of D1-expressing MSNs in both reward seeking and learning.

Evidence that Reduced Dopamine Signaling Leads to Activation of D2-Expressing Medium Spiny Neurons that Drive Aversion-Related Behaviors.

While the role of increased dopamine and D1-expressing MSN activation in reward has been extensively studied, the involvement of dopamine signaling in aversion has received less examination. With regards to the model presented here, the view that aversive stimuli, through reductions in dopamine, activate D2-expressing MSNs to cause avoidance behaviors and avoidance learning is relatively novel and thus is less substantiated. However, support for this function of this pathway has begun to accumulate in recent years. As discussed previously and replicated by the experiments presented in earlier chapters, aversive stimuli and their predictors decrease nucleus accumbens dopamine as measured by fast scan cyclic voltammetry. Optogenetic inhibition of VTA dopamine neurons causes real time and conditioned place aversion, and this effect requires D2 receptor expression in the nucleus accumbens (Llango et al, 2014; Danjo et al, 2014). In addition, optogenetic stimulation of VTA dopamine neurons, and maintaining D2 receptor tone pharmacologically both prevent changes in behavioral preference caused by reward omission (Porter-Stransky et al, 2013; Stopper, Maric, Montes, Wiedman, & Floresco, 2014). These observations indicate that reductions in dopamine are aversive, and that they facilitate learning through D2 receptor signaling.

Downstream, aversive stimuli have recently been shown to preferentially activate D2-expressing neurons in the nucleus accumbens (Xiu et al, 2014), and optogenetic activation of this pathway causes a conditioned place aversion

(Kravitz et al, 2012). These findings indicate that this pathway is sensitive to aversive stimuli and involved in avoidance learning. Indeed, using a cell type specific inducible tetanus toxin to block neurotransmission, Hikida et al (2010) demonstrated that mice lacking neurotransmission in D2-expressing MSNs are unable to learn to avoid a chamber previously paired with an electric foot shock. In an important study, this group also examined the importance of dopamine receptor signaling in this avoidance learning task. They demonstrated that unilateral induction of tetanus toxin in D2-expressing MSNs did not affect avoidance learning. However, site specific infusion of a D2 receptor agonist on the functionally intact side did prevent learning in these mice (Hikida et al, 2013). Importantly, this treatment did not disrupt immediate avoidance induced by the shock, indicating that the effect was specific to learning from the aversive experience. Furthermore, aversive conditioning in this task elevates PKA in D2-expressing MSNs specifically, and the magnitude of this elevation predicts future avoidance of the shock paired chamber. This aversion-induced PKA elevation is required for learning, as inhibiting PKA on the intact side of mice with unilateral neurotransmission blockade in D2-expressing MSNs prevents learned avoidance (Yamaguchi et al, 2015). Given that foot shock has a largely inhibitory effect on midbrain dopamine neurons through increased GABA transmission (Tan et al, 2012), these results suggest that aversion-induced reductions in D2 receptor occupancy are required for avoidance learning. This interpretation is further supported by the observation that D2 receptor agonists suppress cue-induced avoidance behavior, an effect that is only observed once striatal D2 receptor

occupancy reaches 70% (Wadenberg, Kapur, Soliman, Jones, & Vaccarino, 2000). These effects are consistent with reports of the responsiveness of this neuronal pathway to other aversive events. Social defeat stress causes increased excitatory signaling in D2-expressing MSNs, and optogenetic activation of these neurons enhances social avoidance caused by defeat (Francis et al, 2015). Furthermore, models of neuropathic pain that involve nerve injury cause reductions in nucleus accumbens dopamine and enhanced excitability of D2-expressing MSNs. Optogenetic activation of this pathway enhances behavioral indices of neuropathic pain, while optogenetic inhibition has the opposite effect (Ren et al, 2016). Together, these reports support the model that, through reductions in striatal dopamine concentrations, a range of aversive stimuli activate D2-expressing MSNs to cause both avoidance behaviors and learning.

Clinical Evidence for the Role of Dopamine Signaling in Reward and Aversion Learning

The studies reviewed here, as well as the data presented in this dissertation, characterize a striatal system that is a critical regulator of reward and avoidance learning through distinct dopaminergic output pathways. In light of the present finding that D2 receptor activation reduces punishment sensitivity (Chapter III), consideration of this pathway could shed light on shared anatomical dysfunction present in diverse behavioral disorders involving punishment insensitivity (Luman et al, 2008; Fairchild et al, 2009; de Ruiter et al, 2009). This

model may also provide a potential mechanism for symptoms observed in clinical conditions involving dopamine dysfunction. Patients with Parkinson's disease are markedly better at learning to avoid negative outcomes than they are at learning to approach positive outcomes. Correspondingly, the pro-dopaminergic drug, L-dopa, in combination with administration of a D2 receptor agonist interferes with avoidance learning and enhances reward learning in this patient population (Frank et al, 2004). Similar effects are also observed in patients with Parkinson's disease following treatment with a D3 receptor agonist (Cools et al, 2006). The influence of reduced dopamine signaling on aversion sensitivity is not unique to individuals afflicted with Parkinson's disease. Healthy individuals with low baseline dopamine synthesis capacity learn more effectively from unexpected punishment, compared to unexpected reward, and this is altered by treatment with a D2 receptor agonist (Cools et al, 2009). In addition, dietary depletion of dopamine precursors enhances punishment-based reversal learning in females (Robinson, Standing, DeVito, Cools, & Sahakian, 2010), a gender-specific effect that may involve the higher dopamine synthesis capacity of females (Laakso et al, 2002).

Reduced dopamine has also been shown to affect reward learning. In a probabilistic reward task, healthy individuals learn a response bias towards the option that yields the largest net reward. The development of this response bias, indicative of proper reward learning, is abolished in individuals treated with a low dose of the D2 receptor agonist, pramipexole (Pizzagalli et al, 2008). Low doses of D2 receptor agonists have been shown to decrease dopamine release, inhibit

midbrain dopamine neuron activity, and reduce blood flow to the nucleus accumbens (Piercey, Hoffmann, Smith, & Hyslop, 1996; Schmitz, Benoit-Marand, Gonon, & Sulzer, 2003; Chen, Choi, Andersen, Rosen, & Jenkins, 2005). This response profile is attributed to selective activation of presynaptic D2 autoreceptors (Samuels, Hou, Langley, Szabadi, & Bradshaw, 2006), which have a higher affinity for dopamine than the postsynaptic receptor (Cooper, Bloom, & Roth, 2003). Individuals treated with the dopamine agonist also reported increased negative affect, which aligns with our findings that negative affect and reductions in nucleus accumbens dopamine co-occur (Wheeler et al, 2011; Chapter II). Importantly, perturbations in reward learning in the probabilistic reward task caused by dopaminergic manipulations have also been shown in animals. Pramipexole abolishes normal response bias in rats, while treatment with the pro-dopaminergic drug, amphetamine, enhances reward learning (Der-Avakian, D'souza, Pizzagalli, & Markou, 2013). Importantly, deficits in reward learning in the probabilistic reward task are also observed in patients with depression (Pizzagalli, Iosifescu, Hallett, Ratner, & Fava, 2008), a mood disorder which is associated with reductions in striatal dopamine (Lambert, Johansson, Ågren, & Friberg, 2000). The parsimonious explanation of these results is that a low dopamine state reduces the capacity of rewarding stimuli to activate D1-expressing MSNs to promote reward learning. Taken together with the data presented here (Chapter III), clinical studies indicate that both normal and dysfunctional avoidance learning in humans are mediated by aversion-induced reductions in dopamine that signal through disinhibition of D2 receptor-

expressing MSNs. Further study could provide significant insight into the molecular regulation and downstream targets of this pathway, and new avenues for therapeutic interventions for related disease states.

Building a Conceptual Framework

A likely explanation of the preclinical and clinical results reviewed here is that basal nucleus accumbens dopamine concentration serves as a point of contrast that determines the signaling efficacy of stimulus-driven increases or decreases in concentration. States of low basal nucleus accumbens dopamine concentration lower the threshold for aversion-induced reductions in dopamine necessary to engage an aversion-sensitive, D2 receptor-expressing MSNs output pathway that promotes avoidance learning. At the same time, a state of reduced dopamine increases the threshold for reward-induced elevations to activate a separate, D1-expressing MSN output pathway to promote reward learning. This enhanced sensitivity to aversive events, and blunted sensitivity to rewards explains why patients with Parkinson's disease show both enhanced avoidance learning and deficits in reward learning, and why these same perturbations in learning are caused by dietary dopamine precursor depletions. Conversely, states of elevated basal dopamine have the opposite effect on sensitivity to rewards and aversive events, which is consistent with the observations that pro-dopaminergic drugs enhance reward learning and reduce disease-related enhancements in avoidance learning.

This view of the importance of striatal dopamine in guiding outcome-sensitive behavior is closely aligned with the proposed involvement of dopamine in the economics of decision making. States of reduced dopamine are associated with decreased effort due to an increased sensitivity to effort-based costs (Niv et al, 2007). If costs are viewed as aversive, it follows that states of reduced dopamine would enhance cost sensitivity and blunt the effectiveness of rewards to motivate behavior. This may account for the decreased initiation of effort in patients with Parkinson's disease (Chong et al, 2015). This view also accounts for why states of increased dopamine are associated with increased effort in humans and rodents (Wardle et al, 2011; Hamid et al, 2015), as the perceived aversive nature of vigorous behavioral output is reduced and reward value is simultaneously enhanced.

Implicit in this argument is the idea that states of reduced dopamine signaling and the emergence of a negative affective state are linked. Although it has not been demonstrated that a negative affective state requires a reduction in striatal dopamine, numerous observations support the idea that the two are related. The incidence of depression is higher in patients with Parkinson's disease than it is in the general population (Reijnders et al, 2008), and drugs that decrease dopamine increase negative affect in healthy human subjects (Pizzagalli et al, 2007). Furthermore dopamine precursor depletion selectively enhances the affective component of pain (Tiemann, Heitmann, Schulz, Baumkötter, & Ploner, 2014). In other words, states of reduced dopamine make the subjective experience of pain more unpleasant, without altering the sensory

experience of pain intensity. Given that decreased dopamine signaling facilitates increased activation of D2-expressing MSNs to promote avoidance, it may be the case that induction of a negative affective state may be sufficient to promote avoidance behavior through negative reinforcement. In other words, according to the model presented here, negative affect may promote avoidance behavior in order to alleviate such an aversive state. The relationship between decreased dopamine, negative affect, and avoidance requires further examination, as it holds relevance for the treatment of numerous neuropsychiatric conditions.

Relapse as an Avoidance Behavior

This mechanistic description of how aversive events and aversive states motivate avoidance behavior through negative reinforcement may have particular relevance for relapse in drug addiction. Drug addicts exhibit specific and persistent neuroadaptations, such as reduced striatal D2 receptor expression and increased dopamine transporter function (Staley et al, 1994; Malison et al, 1998; Little et al, 1999; Volkow & Fowler, 2000), that indicate a basal striatal environment of decreased dopamine signaling. Furthermore, numerous drug-induced neuroadaptations have been characterized that contribute to a hyperactive response to stressful events (Koob & Le Moal, 2005; Volkow, Koob, McLellan, 2016). In human addicts, drug associated cues and stressful events evoke a negative affective craving state which predicts relapse (Paliwal et al, 2008). In addition, stressful stimuli preferentially activate striatal regions in addicts compared to nondrug users (Sinha et al, 2005). Thus it is possible that

long term drug-induced neuroadaptations result in decreased dopamine and enhanced excitability of D2-expressing MSNs in the striatum. This state would lead to heightened sensitivity to aversive events, or cues that induce a negative affective state, as the low dopamine tone would reduce the threshold required for these stimuli to cause a response. Thus, through negative reinforcement, relapse can be seen as an avoidance behavior. In this case, the drug seeking act would be an attempt to alleviate an aversive event or cue-induced negative affective state. It is worth noting that the persistence of the observed neuroadaptations and hyper-activity to stress is not necessary for the view that relapse may be conceptualized as an avoidance behavior. Since aversive events promote avoidance in healthy individuals, it may simply be that drug addicts preferentially turn toward drug seeking as a coping mechanism simply because this behavior is part of a relatively thin behavioral repertoire. Recent reports from our lab (Twining et al, 2014; Chapter II) have demonstrated that aversive stimuli and aversive drug cues evoke a negative affective state, and cause drug seeking in an environment of persistent reductions in dopamine. Furthermore, aversive drug cues activate a subpopulation of striatal neurons, which, according to the model presented here, are likely to be D2-expressing MSNs that promote avoidance. Finally, more recently collected data from our lab (that are logical extensions of the data presented here) indicate that selective reductions in dopamine increase both drug taking and drug seeking. Thus, our data are consistent with the classical interpretation (Soloman & Corbit, 1974) that relapse is the manifestation of avoidance caused by a negative affective state. Future studies are required to

determine if decreased dopamine is the mechanism by which aversive stimuli activate D2-expressing MSNs, and if the activation of this pathway is required for aversion-induced drug seeking.

Conclusions

The experiments presented in this dissertation describe an emerging view that ventral striatal dopamine signaling is a critical regulator of affective state and motivated behavior. The mesolimbic dopamine system has been extensively studied for its role in reward processing and learning, while the capacity of this system to process aversive events and guide subsequent learning has remained relatively uncharacterized. The results of the present studies significantly contribute to the view that the ventral striatum is well positioned to modulate both approach and avoidance behavior based on stimulus-driven fluctuations in dopamine concentration, and the effect of those changing dopamine levels on distinct and opposing output pathways. The view that basal dopamine levels are critical in determining the effectiveness of reward and aversion-induced rapid increases and decreases in concentration has important implications for both motivation and learning. By determining the threshold required for these stimuli to engage their respective output pathways, striatal dopamine concentrations appear to have the capacity to bias behavior in either direction. Furthermore, this system may represent a common pathway capable of explaining aberrant reward and aversion sensitivity and learning in a wide array of behavioral and neurodegenerative disorders. Further, examination of this pathway may be of

importance for fully characterizing and treating drug relapse. Classical views of drug addiction, corroborated by reports from human addicts, have focused on the role of negative reinforcement as a motivator of drug taking and seeking, but neurological evidence of this has remained elusive. The data presented here indicate that aversion-induced reductions in dopamine and the related negative affective state are important motivators of avoidance behavior through activation of an aversion-sensitive output pathway. Although evidence is mounting in support of this view, the full understanding of the bidirectional modulation of motivation and learning by striatal dopamine requires further study. With the advent of advanced techniques in neuroscience, future experiments will be able to further characterize the capacity of a changing striatal dopaminergic environment to modulate downstream signaling. Integrating these tools with sensitive behavioral measures should also add clarity to the relationship between reductions in dopamine and the emergence of a negative affective state; and whether negative affect can motivate avoidance through negative reinforcement. Finally, it will be important to determine the necessity of activation of the aversion-sensitive striatal output pathway to control relapse behavior specifically, and avoidance behavior generally, as well as characterize the relevant inputs that drive this pathway in the face of decreased dopamine. The characterization of this dopaminergic circuit is an exciting prospect as it may explain dysregulation in motivation and learning across numerous disease states, and offer novel and precise targets for the development of new therapeutics.

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