NEURONAL ENCODING OF HABIT-LIKE ALCOHOL SELF-ADMINISTRATION IN THE RAT DORSAL STRIATUM

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ABSTRACT

Rebecca R. Fanelli: Neuronal Encoding of Habit-like Alcohol Self-administration in the Rat Dorsal Striatum
(Under the direction of Donita L. Robinson)

Alcoholism is characterized by persistent drinking that may involve a shift from goal-directed to habitual drinking as behavior becomes engrained and resistant to treatment. Recent evidence suggested these behaviors have distinct anatomical substrates, with the dorsomedial striatum (DMS) implicated in goal-directed behavior, while the dorsolateral striatum (DLS) was required for habit formation. It was unknown, however, whether these regions are differentially activated during habitual alcohol reinforcement and what role specific neurotransmitter systems in the DLS might play in habitual alcohol drinking. The studies described here investigated how DMS and DLS neurons encode cues, actions, and reward deliveries during operant self-administration via extracellular recordings from chronically implanted electrodes. *Our central hypothesis was that the expression of habitual behavior depends on parallel circuits acting in competition, with sensorimotor processing in the DLS exhibiting greater activation and behavioral control during habit-like alcohol self-administration.* First, we characterized dorsal striatal electrophysiology during goal-directed versus habitual models of alcohol self-administration, and found distinct DMS and DLS activation in both models. DLS outcome-related activation was greater for unpredictable reward delivery in the more habitual model. Next, systemic dopamine receptor antagonism reduced alcohol seeking and baseline firing rates without modulating neuronal activation to session-start cues. In the final series of experiments,

neuronal firing patterns were compared in rats self-administering sweetened alcohol versus sweet solutions alone. Alcohol was found to promote DMS activation, while the most habitual rats in the same model showed less DLS outcome activation. Unilateral dopamine receptor antagonism proximal to recording electrodes reduced overall baseline firing rates in the DLS, while bilateral DLS antagonism reduced habit-like reward seeking and phasic DMS neuronal activation during action selection. In sum, these studies suggest that DMS and DLS cooperatively promote reward seeking, and habitual behavior requires DLS dopaminergic activation but not a reduction in DMS activation. These innovative and mechanistic studies significantly advance our understanding of the neural substrates of habitual alcohol seeking and drinking behavior, and elucidate the dependence of behavioral inflexibility on dopamine. Thus, these studies uncover physiological correlates of behavioral resistance to change, providing new avenues for future treatment of alcohol use disorder.

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Experiments described in Chapter 2 began before I joined the laboratory, and were designed by Dr. Robinson and initiated by technicians Dawnya Bohager and Rebecca Reese. Jeffrey Klein, Ph.D., assisted with data analysis on this aim, and provided critical revision on the manuscript. All aims were conducted with the assistance of undergraduate students, including Ian Everitt, Hung-Yu (Hank) Chen, Victoria Burton, Lisa Bowen, Emory Wolf, and Ryan Leite. James Dunville completed an honors dissertation on a project related to Chapter 4, and conducted some initial data analyses. I am extremely grateful to the assistance and engagement of these individuals.

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ACC	Anterior cingulate cortex
AUD	Alcohol use disorder
BEC	Blood ethanol concentration
BLA	Basolateral amygdala
DA	Dopamine
DLS	Dorsolateral striatum
DMS	Dorsomedial striatum
DSM	Diagnostic and statistical manual of mental disorders
FLU	α-flupenthixol
FR5	Fixed-ratio 5
GPe	Globus pallidus externa
GPi	Globus pallidus interna
IRI	Inter-response interval
MSN	Medium spiny neuron
MWU	Mann-whitney U test
NAc	Nucleus accumbens
NP	Non-phasic
OFC	Orbitofrontal cortex
PostEx	Post-excitatory
PostIn	Post-inhibitory
PreEx	Pre-excitatory;
PreIn	Pre-inhibitory

Sac	0.2% saccharin in water
Sac/E	0.2% saccharin + 15% ethanol in water
SCH	SCH23390
SMC	somatomotor cortex
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
STN	Subthalamic nucleus
VI30	Variable-interval 30s
VTA	Ventral tegmental area

CHAPTER 1: GENERAL INTRODUCTION

Background on alcohol use disorders

Over 17.6 million Americans suffer from alcoholism or alcohol abuse (Grant et al. 2004). Alcohol use disorders (AUDs) disrupt performance of responsibilities at home or work, and can lead to social and legal problems (Hasin et al. 2007, NIAAA). In fact, these disorders are diagnosed when an individual's drinking results in distress or harm (NIAAA, American Psychiatric Association 2000). Additionally, alcoholism not only affects the individual and those who depend on him, but also society as a whole, as the economic cost of excessive drinking is estimated at $233.5 billion (Bouchery et al. 2011). AUDs result in loss of workplace productivity (72% of cost) and significant health care expenses (11%), and it also increases law enforcement expenses (9%) and costs from motor vehicle accidents (6%; CDC 2014). Most distressing, excessive alcohol consumption is known to kill about 88,000 people per year (CDC 2014). Clearly, AUD is a significant public health and economic issue, and our research aims to better understand this disorder with the eventual goal of improving treatment and reducing this harm.

Alcoholism was recognized as a disease by the American Medical Association in 1956. The 5th edition of their Diagnostic and Statistical Manual of Mental Disorders (DSM V) has combined symptoms previously described as alcoholism and alcohol abuse, categorizing them as AUDs, which are described as mild, moderate, or severe (American Psychiatric Association 2013). Thus, AUDs are defined by symptoms previously associated with alcoholism: craving or urges to drink, inability to stop drinking, alcohol dependence, and tolerance (American Psychiatric Association 2000). These symptoms are debilitating even the absence of physical

dependence, and while the DSM IV categorized the presentation of symptoms without alcohol dependence as alcohol abuse, this presentation would also be diagnosed as an AUD under DSM V. Significant progress is needed in both recognition and treatment of AUDs, with only a third of sufferers receiving any treatment, and 10% receiving medication, despite surmounting evidence that it is effective (Jonas et al. 2014). Still, only 3 medications are currently approved for the treatment of AUDs, and these treatments have modest effect sizes in clinical trials (Zindel, Kranzler 2014).

Understanding AUD as a neurobiological disorder

In order to uncover novel treatment strategies, we need to understand the etiology of AUDs and other additions. In the field of neurobiology, addiction is broadly conceptualized as a disease of maladaptive learning, in which neural mechanisms of synaptic potentiation are "hijacked" by drugs of abuse (Hyman, Malenka & Nestler 2006, Everitt, Robbins 2005). Substance abuse disorders become chronic, persistent diseases, characterized by cycles of drug taking, abstinence, and relapse. With repetition, a drug-taking behavior becomes habitual or inflexible, contributing to the difficulty of breaking the cycle (Everitt, Robbins 2005, Tiffany 1990, Hilario, Costa 2008). Of those treated for alcoholism, success rates are low, and 80-90% will relapse, even years later (Schuckit 2009).

A canonical component of any learned behavior, cues play a powerful role in addiction (de Wit, Stewart 1981, Everitt, Robbins 2005, Robinson et al. 2014). As Pavlov's dog salivated in response to a bell that had been paired with a food reward (Pavlov 1927), individuals with AUD describe sensations of craving in response to seeing places or people they drank with, or other conditioned stimuli they have associated with alcohol (Litt, Cooney & Morse 2000, reviewed in Drummond 2001). The effect of alcohol-associated cues on the brain was

demonstrated when presentation of alcohol cues produced neural activity that correlated with addiction severity (Filbey et al. 2008, Sjoerds et al. 2014). Furthermore, while additional research is necessary to demonstrate a link between reductions in cue reactivity and treatment success, craving does predict relapse (i.e. Litt, Cooney & Morse 2000).

Instead of actions driven by outcomes, as in flexible, goal-directed behavior, actions driven by stimulus-response associations are inflexible, and resistant to changes in outcome (Belin et al. 2009, Devan, Hong & McDonald 2011). Behavior that is insensitive to change in reward value is defined as habitual behavior (Dickinson 1985, Belin et al. 2009, Devan, Hong & McDonald 2011). Habitual behavior therefore constitutes a key component of alcoholism. When an alcoholic drinks despite negative consequences on his health or responsibilities, this habitual behavior would contribute to a diagnosis of alcoholism (NIAAA , American Psychiatric Association 2000). Some current treatments produce illness upon drinking or blunt alcohol's euphoric effects, but these may be less effective for habitual drinkers (Hay et al. 2013), who drink after arriving in a certain location, or after seeing a preferred beverage, rather than drinking motivated by a desire for alcohol (Tiffany 1990). Therefore, alcoholism is a harmful disease that can become habitual, driven by cues and stimulus-response associations, and resistant to treatment. In this dissertation, I aim to unravel basic mechanisms of alcoholism, characterizing how alcohol drinking becomes habitual, and investigating methods to reverse these processes.

Animal models of addiction

Animal models are critical for the study of alcoholism, permitting the use of invasive techniques and novel treatments that would not be safe for human subjects. However, as addiction is a complex psychological disorder, no one model will encompass all the components of human alcoholism. Nevertheless, the crucial benefit in this simplicity is enabling a controlled

environment for the study of specific exposures. For example, some models employ experimenter-controlled administration of specific amounts of alcohol (through injection, vapor, intragastrically, etc.) in adulthood or in other developmental stages (see reviews: Knapp, Breese 2012, Maurel et al. 2013, O'Leary-Moore et al. 2011, Hellemans et al. 2010, Spear, Swartzwelder 2014). Others allow access to alcohol in the home cage, as in the two-bottle choice procedure (Richter, Campbell 1940), where the animal has access to a bottle of diluted alcohol and another of water, which have been applied to examining animals with genetic or environmental exposure producing susceptibility for altered alcohol drinking (Crabbe et al. 1992, Bell et al. 2014). Models of instrumental behavior, where the animal performs an action to receive an alcohol reward, can be used to investigate motivation to drink (reviewed in Meisch 1982, Green, Grahame 2008). In the studies described in this dissertation, we model alcohol self-administration in rats. Rats were chosen due to their combination of sufficient cognitive abilities and larger brain size compared to mice (facilitating the use of multielectrode arrays), as well as the existence of a significant amount of prior research on rat instrumental behavior and neurobiology.

Operant conditioning was first described as distinct from Pavlovian conditioning by Jerzy Konorsky and Stefan Miller in 1928 (Zielinski 2006). It was Thorndike who then formalized the "law of effect", which theorized that behaviors followed by satisfying consequences are more likely to be repeated, generating the first known learning curves (Thorndike 1911). After Skinner introduced empirical examination of operant behavior with the invention of the operant conditioning chamber (Skinner 1938), animals were trained to self-administer alcohol to assess its reinforcing properties (Meisch 1982, Myers, Tytell 1972). The basis for alcohol self-administration could be ascribed not only to the pharmacological effects of alcohol in the central

nervous system, but also to taste preference (or aversion), or the caloric value of alcohol (Green, Grahame 2008, Cunningham, Fidler & Hill 2000). Nevertheless, two avenues of research have clarified this issue, which are discussed here. First, additional models were employed to confirm the hedonic and reinforcing properties of alcohol (Meisch 1982, Corbit, Janak 2007), and second, the role of other factors was eliminated by direct infusion of alcohol into the brain (Gatto et al. 1994) and devaluation studies (Samson et al. 2004).

The hedonic and motivating properties of alcohol have been demonstrated using Pavlovian techniques such as conditioned place preference and conditioned taste aversion demonstrating "liking" of alcohol (Cunningham, Fidler & Hill 2000, Camarini et al. 2010). Pavlovian-instrumental transfer experiments further demonstrated incentive salience properties of alcohol, as when alcohol rewards were repeatedly paired with a cue, that cue could invigorate instrumental lever-pressing for an alcohol reward (Corbit, Janak 2007). Progressive ratio schedules, in which increasing work (i.e. number of lever presses) is required for the alcohol reward, provide another useful tool, demonstrating that rats are motivated to acquire alcohol (Meisch, Thompson 1973).

In addition to oral self administration, rats will lever press to receive alcohol administered intravenously, intragastrically, or even directly into the ventral tegmental area (VTA, key reward center and source of dopamine in the brain, discussed later) (Gatto et al. 1994, McBride, Murphy & Ikemoto 1999, Meisch, Stewart 1994). Additionally, the pharmacological effects of alcohol were shown to be necessary for self-administration when Samson and colleagues (2004) found that rats trained to self-administer alcohol would reduce responding after ethanol injections were paired with lithium chloride-induced illness, producing an association between interoceptive effects of alcohol and sickness. Reintroduction of the reinforcer after lithium chloride resulted in

initial self-administration, which fell off once doses resulting in postingestive pharmacologic effects were achieved, demonstrating devaluation of alcohol self-administration through manipulation of interceptive effects (Samson et al. 2004). Therefore, maintenance of self-administration is dependent on its interoceptive effects, although initial responding for alcohol may be driven by taste factors.

While oral alcohol self-administration has long been employed to study reinforcement in rodents and monkeys (Meisch 1982, Green, Grahame 2008, McBride, Li 1998), the taste of alcohol can be aversive over 6% wt/vol, and so techniques such as food deprivation have been employed to increase drinking volumes (Meisch, Thompson 1973, Meisch, Henningfield 1977). This strategy confounds interpretation of self-administration, which could be motivated by a desire to obtain calories (Altshuler 1981, Dole, Ho & Gentry 1985). Another approach to encourage alcohol drinking is to employ sucrose substitution, e.g., reducing sucrose and increasing ethanol concentrations in the reinforcer solution over time, without prolonged food or water deprivation (Samson 1986, Shillinglaw, Everitt & Robinson 2014). While doses achieved with self-administration (without prior alcohol experience as in vapor exposure, Gilpin et al. 2009) are typically insufficient to produce dependence even with sucrose fading, dependence is not necessary for a diagnosis of AUD, and furthermore, physical dependence is not always adequate on its own to produce sustained drinking (Freund 1969). Therefore, we utilize sucrose fading or saccharin-sweetened alcohol self-administration to study the effects of alcohol self-administration in the absence of dependence.

Defining habitual behavior

Both goal-directed and habitual alcohol self-administration can be modeled in rodents (Hilario, Costa 2008, Shillinglaw, Everitt & Robinson 2014, Corbit, Nie & Janak 2012,

Mangieri, Cofresi & Gonzales 2012). Habitual behavior can develop with extended training, and it can be produced more quickly with variation in the contingency between the lever-press response and the reward (Dickinson, Nicholas & Adams 1983, Derusso et al. 2010). This was demonstrated when Dickinson and colleagues developed a habit model wherein rats received rewards on a variable-interval schedule, producing a non-linear relationship between the number of lever press responses and reinforcements, such that more frequent or vigorous responding would not directly impact the rate of reinforcement (Dickinson 1985, Dickinson, Nicholas & Adams 1983). Variable interval schedules therefore result in slower, more persistent behavior, with less predictable outcomes reinforcing stimulus-response over response-outcome behavioral strategies. Dickinson and colleagues defined habitual behavior as being resistant to devaluation, when the reinforcer was paired with lithium chloride. Lithium chloride causes the rats to feel ill, and will result in a taste aversion in rats with limited experience with the reinforcer, as previously described in the study by Samson and colleagues (2004). Behavior is tested in a subsequent extinction test, where levers are available but rewards are not delivered so there is no additional learning about reward associations (Colwill, Rescorla 1990, Samson et al. 2004, Mangieri, Cofresi & Gonzales 2012). Rats who were trained on a variable-interval schedule and received lithium chloride paired with the reinforcer responded at similar levels to those who received unpaired treatments, whereas rats trained on a fixed-ratio schedule reduced responding after the lithium chloride pairing (Dickinson, Nicholas & Adams 1983).

Behavioral flexibility can also be evaluated after satiety-specific devaluation, a procedure that revaluates reward seeking after temporarily manipulating reinforcer value by allowing the subject to ingest the reward to satiety. This test procedure has the advantage of allowing for continued self-administration behavior after the devaluation test (Corbit, Nie & Janak 2012, Hay

et al. 2013, Fanelli et al. 2013), so multiple tests can be performed across training (Shillinglaw, Everitt & Robinson 2014, Corbit, Nie & Janak 2012). These procedures compare two sessions in counterbalanced order, one extinction session is preceded by an hour free access to the reinforcer, while the other is preceded by an hour access to maltodextrin as a control for fullness. A significant reduction in responding in the session after access to the reinforcer is interpreted as satiety-specific devaluation, demonstrating goal-directed behavior, while a non-significant difference is interpreted as habitual behavior. In alcohol drinking studies, these satiety-specific devaluation tests may be confounded by the motor impairment subsequent to free access to alcoholic solutions, but this has not prevented habitual behavior from being observed (Corbit, Nie & Janak 2012, Fanelli et al. 2013)

Contingency degradation procedures have also been used to evaluate whether reward-seeking behavior responds flexibly to changes in the response-outcome contingency. After extended training establishing the association between a response and a reward, the animal experiences several sessions when the response is no longer paired with the reward, as rewards are delivered at random intervals (Yin et al. 2005, Shillinglaw, Everitt & Robinson 2014, Fanelli et al. 2013). Extinction sessions before and after the sessions of contingency degradation training are compared to establish whether responding was reduced, which indicates flexible goal-directed behavior. If behavior does not differ between the pre- and post-degradation extinction sessions, behavior is defined as habitual (Yin, Knowlton 2006). While extending contingency degradation can eventually degrade a habit (Braun, Hauber 2012), demonstrating that it is not a permanent state, exposures that have been shown to promote inflexible behavior, such as variable-interval schedules, can produce behavior that is insensitive to this training at time points that rats trained on fixed-ratio (or even variable ratio) schedules show degradation of responding

(Dickinson, Nicholas & Adams 1983). The advantages of contingency degradation are that it directly tests reversibility of stimulus-response associations critical for habitual behavior as would be necessary for treatment of alcoholism, and that extinction tests performed on separate days from training are not effected by intoxication. However, as contingency degradation affects stimulus-response associations, it may prevent future responding and subsequent tests may be confounded.

Does alcohol promote habitual behavior?

One theory of why alcohol addiction is so prevalent and persistent is that alcohol may promote habitual behavior, producing alcohol drinking that is resistant to change (Everitt, Robbins 2005, Belin et al. 2009, O'Tousa, Grahame 2014). Nevertheless, the effect of alcohol on habitual behavior is somewhat unclear, as previous studies have used variable amounts of alcohol exposure and different tests of habitual behavior. Dickinson and colleagues employed the lithium chloride devaluation test to demonstrate that alcohol may facilitate a transition to habitual behavior more quickly than food rewards (Dickinson, Wood & Smith 2002). However, in this experiment rats consumed limited amounts of alcohol (less than 0.3mL 10% EtOH), which the authors acknowledge would not result in pharmacological effects of alcohol. Thus, habitual behavior observed here may represent a floor effect in responding on the alcohol lever, on which the rats did not frequently respond. Two other reports of alcohol's effects on habit defined habit with LiCl devaluation, with different results. Samson and colleagues did not observe habitual drinking in their experiment (2004). However, their self-administration procedure also differed by using a model separating seeking and consumption as rats pressed for 20min access to a bottle (Samson et al. 2004). By pairing lithium chloride with a gavage ethanol dose, they devalued the interoceptive effects of ethanol. Responding was reduced in extinction

tests, and though some responding was maintained in subsequent self-administration sessions, this behavior was only observed before alcohol consumption reached pharmacological levels (taste of alcohol had not been devalued, interoceptive effects had). Nevertheless, Mangieri et al. observed habitual behavior (resistant to LiCl pairing) after 8 sessions in rats pressing levers for access to 10% sucrose/10% ethanol from a sipper tube on a variable-interval schedule, and not in rats drinking sucrose alone or in rats trained on a variable-ratio schedule (Mangieri, Cofresi & Gonzales 2012). These animals drank 0.7-1.2g/kg alcohol and did not have access to alcohol in the home cage, demonstrating that alcohol may promote habitual behavior at sub-dependent doses.

While Corbit and colleagues (2012) used repeated satiety-specific devaluation tests and demonstrated loss of sensitivity to devaluation only in rats self-administering alcohol after 8 weeks , their rats drank alcohol in the home cage for 4 weeks prior to training (and after each self-administration session, around 0.4g/kg/session) and so this increased exposure could be responsible for habitual behavior, rather than alcohol self-administration per se. Indeed, rats self-administering sucrose that also had non-contingent access to alcohol in the home cage (30min access 4hrs after the session) developed more rapid habits for sucrose seeking (Corbit, Nie & Janak 2012). This suggests that long-term alcohol exposure, rather than self-administration experience with alcohol, may be necessary for promotion of habitual behavior.

To date, work from our lab has shown no promotion of habitual behavior by alcohol self-administration alone. When rats responded for 10% alcohol or 10% sucrose on a fixed-ratio schedule over 6 weeks, both groups were goal-directed (Shillinglaw, Everitt & Robinson 2014), while variable-interval self-administration for over 8 weeks produced habitual behavior in both 10% alcohol and 1.5% sucrose groups as measured by satiety-specific devaluation (Hay et al.

2013). Our results using an interval schedule may differ from those of Mangieri et al. because LiCl devaluation may be less likely to detect habit-like behavior in controls, as it produces a novel aversive association rather than only reducing reward value with satiety. Additionally, 1.5% sucrose may be resistant to satiety (Shillinglaw, Everitt & Robinson 2014). Therefore, differences in drinking (our studies produced ~0.5g/kg alcohol intake) and different habit tests may explain differences in the potentiation of habitual behavior by alcohol.

Distinct neuronal circuitry for goal-directed versus habitual behavior

Not only are goal-directed and habitual behavior dissociable at the behavioral level, they can additionally be manipulated independently through interrogation of neural circuits. The dorsal striatum is known to be critical for action selection (Balleine, Delgado & Hikosaka 2007, Da Cunha, Gomez-A & Blaha 2012), and relatively recent studies have shown that lesion of the dorsomedial striatum (DMS, caudate in primates) can prevent flexible, goal-directed behavior (Yin et al. 2005, Corbit, Janak 2010), while lesion of the dorsolateral striatum (DLS, putamen in primates) prevents the formation of habitual behavior (Yin, Knowlton & Balleine 2004).

When rats were overtrained on an interval schedule such that sham operated rats demonstrated insensitivity to LiCl devaluation, Yin and colleagues showed that DLS lesion prior to training prevented this habit formation (2004). Furthermore, DLS inactivation with muscimol during contingency degradation training (one day on an omission schedule, presses result in delay of reinforcement) inhibited habitual behavior, enabling learning of contingency reversal and reduced seeking in extinction on the next day (Yin, Knowlton & Balleine 2006). The roles of the DMS and DLS have also been demonstrated in studies of alcohol self-administration, where goal-directed alcohol self-administration is blocked by DMS inactivation and habitual self-administration is blunted by DLS inactivation (Corbit, Nie & Janak 2012).

The dorsomedial striatum seems to show functional differences between its anterior and posterior regions, supporting the theory that learning broadens engagement of the dorsal striatum from anterior-medial regions into posterior-lateral regions (Miyachi et al. 1997, Corbit, Janak 2010). Repeated inactivation of anterior DMS during short, 3-day training, disrupted goal-directed behavior (Corbit, Janak 2010). However, pre-training excitotoxic lesions of this region did not prevent goal-directed reductions in responding in devaluation and degradation tests after 8 days of training (Yin et al. 2005). Notably, inactivation of anterior DMS following 2 weeks of training (pre-test) did inhibit goal-directed behavior (Corbit, Nie & Janak 2012). Thus, it seems that the anterior DMS is required for goal-directed behavior, unless lesioned prior to the beginning of training, in which case the posterior DMS may compensate after extended training. Posterior DMS is also required for goal-directed behavior in tests of reward devaluation (Yin et al. 2005, Yin, Knowlton & Balleine 2005, Corbit, Janak 2010). Additionally, rats with posterior DMS inactivation during training also failed to acquire novel stimulus-outcome associations in a Pavlovian task, failing to respond less in the presence of a stimulus predicting a devalued outcome, a result also seen after DLS inactivation and not anterior DMS inactivation or in controls (Corbit, Janak 2010). Corbit and her coauthors conclude that compared to the anterior DMS, posterior DMS mediates different aspects of reward-related learning, which are common to response-outcome and stimulus-outcome learning.

Anatomy of the dorsal striatum

The dorsal striatum functions as a component of cortico-basal ganglia circuitry, receiving input from the cortex and projecting to the basal ganglia, through the direct and indirect pathways (**Figure 1.1**; Alexander, Crutcher 1990, Gerfen, Surmeier 2011).

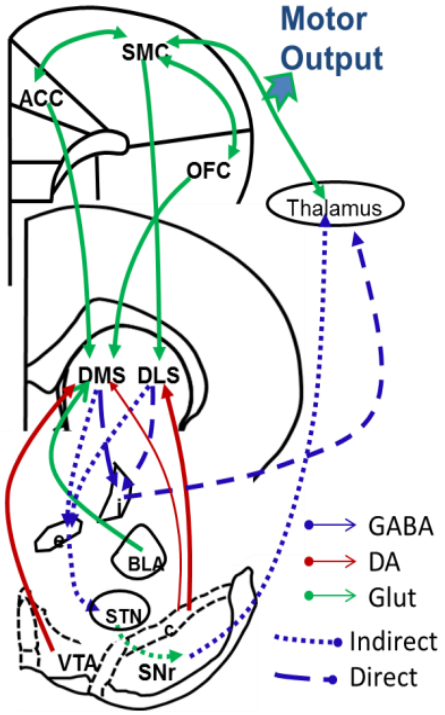

Figure 1.1: Major projections to and from the dorsal striatum. The DMS receives dopaminergic inputs from the VTA (and much less from the SNc), glutamateric input from the BLA as well as from cortical inputs such as the ACC and OFC (among others). Meanwhile, the DLS receives dopaminergic input from the SNc, and cortical input from the SMC. The DMS and DLS send efferent projections through the direct (D1 receptor expressing) and indirect (D2 receptor expressing) pathway MSNs. The indirect pathway extends through the GPe, then the STN, before reaching the SNr, which finally projects to the thalamus. The direct pathway sends inputs through the GPi (or SNr, not shown), directly to the thalamus. The loops are completed as the thalamus has reciprocal connections to the cortex. The convergence between DMS and DLS pathways was unknown. (VTA, ventral tegmental area; SNc, c, substantia nigra pars compacta; BLA, baslolateral amygdala; ACC, anterior cingulate cortex; OFC, orbitofrontal cortex; SMC, somatomotor cortex, MSN, medium spiny neuron; GPe, e, globus pallidus externa; STN, subthalamic nucleus; SNr, substantia nigra pars reticulata; GPi, i, globus pallidus interna; GABA, gamma-aminobutyric acid; DA, dopamine; Glut, glutamate)

The function of the dorsal striatum can be understood through a study of its inputs. While the DMS and DLS are not distinct in rodents, the dorsal striatum demonstrates a gradient in connectivity from anterior-medial to posterior-lateral. The more anterior and medial striatal regions receive predominant cortical input from the associative cortices including the anterior cingulate (ACC) and orbital frontal cortex (OFC; as well as the prelimbic cortex, and medial agranular area, not depicted in Figure 1.1), while the more posterior and lateral areas receive a

large input from the sensorimotor cortex (SMC; Alexander, Crutcher 1990, Balleine, Delgado & Hikosaka 2007). Thus, the DMS integrates information from cortical regions involved in reward value encoding and action selection, while the DLS receives information about cues and motor responses.

From the striatum, GABA-ergic medium spiny neurons (MSNs) expressing dopamine D1-receptors form the direct pathway. Projections from the striatum inhibit neurons in the internal globus pallidus (GPi) and the substantia nigra pars reticulata (SNr, projection not shown in Figure 1.1), which send inhibitory GABA-ergic inputs to the thalamus (Gerfen, Surmeier 2011). The direct pathway thus results in a net increase in thalamic output, in contrast to the indirect pathway, which reduces thalamic activation. MSNs that express the dopamine D2 receptor comprise the indirect pathway, which passes through the external globus pallidus (GPe) and subthalamic nucleus (STN), before projecting to the thalamus, which closes the loop by projecting back to the cortex (Alexander, Crutcher 1990, Gerfen 1988).

Parallel spiraling loops have been shown to ascend from the ventral, to the dorsomedial, to the dorsolateral striatum via midbrain dopamine neurons (Haber, Fudge & McFarland 2000). Nevertheless, lateral integration may occur where the dorsomedial and dorsolateral neurons project to common regions in the internal and external globus pallidus (Nadjar et al. 2006, Balleine, Delgado & Hikosaka 2007). Additional major inputs to these circuits include the amygdala and hippocampus. While the basolateral amygdala (BLA) projects to the DMS, the central amygdala projects to the SNc, which in turn sends dopaminergic efferents to the DLS, positioning the amygdala to control the effect of reward valence and salience on action selection (Balleine, Delgado & Hikosaka 2007). The hippocampus, meanwhile, adds contextual memory

to the computations of the dorsal striatum through its inputs to the amygdala and nucleus accumbens (NAc; Arszovszki, Borhegyi & Klausberger 2014).

Electrophysiological evidence for functional heterogeneity in dorsal striatum

The roles of the DMS and DLS have also been examined in the intact brain, and even during reward-motivated behavior, using electrophysiology. Action potential frequencies of many DMS neurons change in response to conditioned stimuli, and vary according to stimulus-outcome and action-outcome associations. Specifically, extracellular recordings from electrode arrays implanted into the region in rats have shown that firing rates are phasically modulated after cue presentations (Rolls 1994, Kimchi, Laubach 2009). Providing evidence for encoding of stimulus-outcome associations, neuronal responses to cues vary according to the value of the predicted reward in the DMS in rats (Kimchi, Laubach 2009) and in the caudate, the primate analogue of the DMS, in monkeys (Kawagoe, Takikawa & Hikosaka 2004, Kobayashi et al. 2007). These changes cannot be ascribed to changes in behavior, as neuronal activation to cues is altered even prior to changes in behavioral performance (Kimchi, Laubach 2009). Reward anticipation was also demonstrated in the primate caudate (Watanabe, Hikosaka 2005, Kawagoe, Takikawa & Hikosaka 1998), as firing rates prior to the onset of the cue follow reversal of reward contingency. The DMS also encodes action-outcome associations as DMS neurons demonstrate differential patterns of neuronal activity during an action depending on the expected reward outcome (Stalnaker et al. 2010). Activity in the DMS, nevertheless, shows significantly less correlation with motor responses than the DLS (Kimchi et al. 2009). These patterns of DMS activation demonstrate the integration of input about the value of an outcome from the prefrontal cortex, amygdala, and dopaminergic midbrain, to perform flexible action selection according to reward value.

With inputs from sensorimotor cortex, phasic changes in DLS neuronal firing rates correlate with specific motor actions, such as lever presses (West et al. 1990, Carelli, West 1991). DLS neurons do not appear to be modulated by cues alone (Root et al. 2010), but larger modulation of motor encoding is observed when stimuli predicting reward are paired with a motor response, demonstrating the stimulus-response encoding that drives habitual behavior (Stalnaker et al. 2010, Kimchi et al. 2009). Furthermore, increased numbers of neurons are engaged in the DLS as a task is learned (Kimchi, Laubach 2009, Yin et al. 2009, Jin, Costa 2010, Thorn et al. 2010), and cocaine exposure reduces cue-related activation of ventral striatum and increases this cue modulation in the dorsolateral striatum (Takahashi et al. 2007). Thus, in agreement with the previously mentioned inactivation studies, the DMS encoding of outcome associations supports flexible, outcome-dependent behavior, and the DLS encoding of inflexible stimulus-response associations promotes habitual behavior. Overtraining may result in consolidation and a conflicting reduction in the number of DLS-responsive neurons (Carelli, Wolske & West 1997, Tang et al. 2009), but this effect is associated with more efficient task performance rather than habit formation (Tang et al. 2009). This consolidation may not affect studies that use longer courses of treatment in which performance asymptotes, as in our protocols which require over 6 weeks of training. Nevertheless, to control for various exposure effects, including consolidation as well as pharmacological effects of alcohol, we will compare goal- and habit-directed models with equivalent training exposure by training rats on fixed-ratio and variable-interval schedules.

While some studies do not find differences between DMS and DLS neuronal activation during a task (Stalnaker et al. 2010, Thorn et al. 2010), these studies employ tasks in which animals must continually rely on both goal-directed and habitual strategies (though Thorn and

coauthors found more cue encoding in DMS and more encoding of trial start and stop in DLS, they did not find regional differences due to changing response or reward values). Possibly bridging these findings, Gremel and colleagues found similar numbers of lever-press-related neurons in DMS and DLS of mice during a habitual task (random interval self-administration) in one context and a goal-directed task (random ratio self-administration) in another context (Gremel, Costa 2013). However, within the DLS, more neurons with firing rates related to lever presses were found later in training, while less DLS and more DMS firing rate modulation in the goal-directed task context (compared to the habit context) *after outcome devaluation* predicted more goal-directed behavior (Gremel, Costa 2013). Therefore, neuronal encoding of action selection in the dorsal striatum may reflect bias for goal-directed versus habitual control over actions.

The studies described in this dissertation will use electrophysiological techniques to examine *in vivo* correlates of habitual alcohol drinking in the dorsal striatum, taking advantage of the ability to record neuronal activity timed with cues and actions in the intact brain. While this technique is limited by the inability to distinguish different types of neurons, the dorsal striatum is composed 90% of medium spiny neurons (MSNs), with only the remaining 10% including cholinergic and GABA-ergic interneurons (Gerfen 1988). Additionally, electrophysiological studies distinguish neurons with physiological characteristics consistent with MSNs (i.e., ≤0.1% of spikes with interspike intervals <1ms and average firing rates <10-12Hz; Kish, Palmer & Gerhardt 1999, Kimchi et al. 2009). These are analyzed separately, though rarely are sufficient numbers of fast-spiking (putative GABA-ergic) neurons recorded for analysis. These factors mitigate the influence of non-MSNs on recordings, although future studies are necessary to compare these findings with those using optogenetic tools to genetically tag and identify

different cell populations. Although alcohol self-administration is known to alter phasic neuronal firing patterns in the nucleus accumbens (Janak, Chang & Woodward 1999), and distinct neurons in that region have been demonstrated to respond to alcohol versus water reinforcement (Robinson, Carelli 2008), the effect of alcohol self-administration in the dorsal striatum is unknown.

Contribution of dopamine to the functions of the dorsal striatum

The importance of dopamine in goal-directed behavior follows logically from that molecule's demonstrated role in motivation (Salamone et al. 2007) and reward prediction (Schultz 2007). Dopamine is also positioned in the circuit to effect dorsal striatal processing, as dopaminergic neurons project to both the DMS and the DLS (Figure 1.1). These projections form tri-part synapses, joining on the same postsynaptic striatal MSNs as glutamatergic inputs from other regions such as the cortex (Surmeier, Carrillo-Reid & Bargas 2011). When dopamine receptors are activated, G-protein signaling increases intracellular calcium, promoting synaptic potentiation, potentially through increased trafficking of AMPA receptors to the membrane (Wolf, Mangiavacchi & Sun 2003, Mangiavacchi, Wolf 2004, Anderson et al. 2008, Wang et al. 2012). One hypothesis for habit formation is that repeated dopamine release and neuronal activation in the DMS may strengthen activation of the DLS, via the spiraling signaling loops through midbrain dopamine neurons, resulting in an eventual shift to habitual behavior (Yin, Knowlton 2006).

Dopamine plays an essential role in the respective functions of both DMS and DLS. In 1987, it was demonstrated that dopamine in the DMS is necessary for a lever-pressing task (Amalric, Koob 1987). When Salamone and colleagues demonstrated that dopamine lesion in NAc disrupts motivation to acquire a reward, as rats would eat freely provided food but not

perform effort-related tasks to acquire a preferred reward, this work demonstrated a specific role for dopamine (Salamone et al. 1991). Similarly, dopamine in the DMS is not required for all voluntary action, but for reward modulation of actions (Nakamura, Hikosaka 2006). Lesion of dopaminergic inputs to the DLS prevents habit formation in rodent models (Faure et al. 2005). In humans, patients with Parkinson's-induced deterioration of dopaminergic neurons show deficits in habit learning (Redgrave et al. 2010).

Furthermore, dopamine in the dorsal striatum may be particularly important in addiction. Antagonism of all dopamine receptors after learning can reverse habit-like, second-order cocaine seeking (Vanderschuren, Di Ciano & Everitt 2005, Belin, Everitt 2008), although no test of habit was conducted in these studies. Nevertheless, Belin and colleagues saw that unilateral dopamine receptor antagonism in the NAc and the contralateral DLS was also sufficient to prevent second-order cocaine taking, demonstrating the importance of the cascading inputs from ventral to dorsal striatum through the midbrain dopaminergic neurons (Belin, Everitt 2008). Cocaine-paired cues have been shown to initially evoke dopamine release in the NAc, but this effect shifts to the DLS after extended experience (Everitt, Robbins 2005, Takahashi et al. 2007, Willuhn et al. 2012). While alcohol self-administration is known to evoke dopamine release in the ventral striatum (Weiss et al. 1993, Doyon et al. 2005, Robinson et al. 2009), its effect in the dorsal striatum is unknown. To determine whether dopamine affects postsynaptic MSNs in the dorsal striatum and habitual alcohol seeking behavior, we utilized dopamine receptor antagonists delivered both systemically and locally into the DLS. This thesis, therefore, proposed to investigate the role of dorsal striatal dopamine in habitual alcohol drinking.

Within the dorsal striatum, dopamine may have a differential impact on the direct and indirect pathways, which express D1 and D2 post-synaptic dopamine receptors, respectively

(McGeorge, Faull 1989, Gerfen, Surmeier 2011). While the D1 direct-pathway neurons seem to initiate movements, the D2 indirect-pathway neurons inhibit competing actions (Freeze et al. 2013, Isomura et al. 2013). In the primate caudate, D1-receptor antagonism reduced the ability of changing reward expectation to modulate performance in monkeys, while D2-receptor antagonism improved this goal-directed behavior (Nakamura, Hikosaka 2006). In studies of alcohol drinking, however, systemic or ventral striatal antagonism of D1 or D2 receptors similarly prevent alcohol self-administration (Dyr et al. 1993, Hodge, Samson & Chappelle 1997; Liu & Weiss, 2002). Both systemic D1- and D2-receptor antagonism reduced alcohol drinking in high-alcohol-drinking rats (Dyr et al. 1993), and antagonism of both receptors in the NAc also reduced alcohol self-administration in the outbred Long-Evans rat strain (Hodge, Samson & Chappelle 1997). Furthermore, both systemic D1 and D2 antagonists reduced cue-induced reinstatement of alcohol drinking (Liu, Weiss 2002). Further supporting a role for D2 receptors in behavioral flexibility, D2-receptor antagonism in the DLS was recently shown to block habitual alcohol drinking behavior after reward devaluation (Corbit, Nie & Janak 2014). Here, we examine the effect of systemic D1 receptor antagonism and local DLS non-selective dopamine receptor antagonism on alcohol drinking and its neuronal correlates. Because of their reciprocal activities, *in vivo* electrophysiology finds that direct and indirect pathway neurons are both active during the same operant events (Gremel, Costa 2013, Isomura et al. 2013). Therefore, electrophysiology records the contributions of both to behavior, and combination with pharmacology can provide insight into pathway-specific dopaminergic mechanisms.

Summary of aims for current studies

The goal of this thesis project was to investigate the neuronal mechanisms of inflexible, habitual alcohol drinking behavior in rats. Inactivation or lesion of the DLS results in a shift

from habitual to goal-directed alcohol drinking behavior (Corbit, Nie & Janak 2012), suggesting parallel processing through the DMS and DLS, but the activity of the DMS and DLS in habitual behavior had not been studied in the intact rat brain. While the DLS may show greater activation in rats during habit-like behavior, alternatives could be that relatively less activity in the DMS produces habitual behavior as cortical associative input is reduced, or that greater activation in both the DMS and DLS occurs in habitual behavior, with DMS remaining active. Furthermore, the specific effect of alcohol was unknown, and given its variable effect on habitual behavior, it was unclear whether this exposure would increase DLS activation. *We hypothesized that expression of habitual behavior depends on the parallel associative and sensorimotor striato-cortical circuits acting in competition, such that the DLS would exhibit greater activation and behavioral control during habit-like alcohol self-administration, which would be dependent on dopamine transmission.* To test our hypothesis, we characterized the patterns of electrophysiological activity in the dorsal striatum during goal-directed versus habitual alcohol self-administration, and self-administration of alcoholic versus non-alcoholic solutions. We also examined the dependence of habit-like behavior and its neuronal correlates on dopamine transmission. The effect of DLS dopamine receptor antagonism to increase or decrease neuronal activity in the DMS would reveal whether these pathways act competitively or cooperatively, respectively. While dopamine D1 receptors are known to be involved in behavioral activation (Freeze et al. 2013), it is unclear whether they retain the ability to modulate habitual behavior, after glutamatergic synapses are potentiated (Yin et al. 2009, Surmeier, Carrillo-Reid & Bargas 2011). Finally, it is unknown if dopamine antagonism in the DLS can prevent habitual alcohol seeking behavior and its neuronal correlates. These studies therefore aimed to improve our understanding of the neurobiological mechanisms underlying alcohol use disorders.

The following chapters describe each of three major experiments. Chapter 2 describes our first study comparing neuronal firing patterns in goal-directed versus habitual models of alcohol self-administration. When we examined neuronal activity around cues and actions, we expected to see greater DLS activation to alcohol-seeking actions in rats modeling habitual alcohol drinking. Conversely, more DMS activation to cues was expected in the goal-directed model. In the same rats, Chapter 3 describes experiments utilizing IP administration of the D1-receptor antagonist SCH23390 (0, 10, and 20μg/kg SCH) prior to self-administration sessions. In Chapter 4, experiments in a second set of rats compared animals self-administering alcoholic and non-alcoholic solutions, to reveal the specific effect of alcohol and uncover whether this exposure also increases DLS activation, allowing alcohol to promote habitual behavior. The impact of alcohol and behavioral flexibility on dorsal striatal electrophysiology were separately analyzed, and we expected that alcohol and habit formation would interact such that the most habit-like rats that were also drinking alcohol would have the greatest DLS activation during alcohol seeking. Next, unilateral DLS infusions of the non-specific dopamine receptor antagonist α-flupenthixol (FLU) was infused mid-session to examine the role of dopamine on individual neuronal firing patterns. Finally, bilateral DLS infusions of FLU were performed prior to a contingency degradation test to explore whether this manipulation would block habitual alcohol seeking and its neuronal correlates in the DMS, increasing DMS neuronal activation. The discussion of this dissertation addresses the impact of these studies on the field, and what our findings in the dorsal striatum can tell us about the neurobiology of alcoholism.

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CHAPTER 2: DORSOMEDIAL AND DORSOLATERAL STRIATUM EXHIBIT DISTINCT PHASIC NEURONAL ACTIVITY DURING ALCOHOL SELF-ADMINISTRATION IN RATS

Introduction

Drug addictions, including alcoholism, are commonly defined by compulsive use despite negative consequences resulting from that use. The drug is initially sought for its rewarding properties; thus, drug-seeking is originally goal-directed (Balleine and Dickinson, 1998; Belin *et al.*, 2009). Later, drug-seeking may transition to a habit that is outcome-independent and persistently elicited by alcohol-associated cues (Adams and Dickinson, 1981; Everitt and Robbins, 2005). Therefore, one aspect of addiction may be maladaptive learning that accompanies a shift from response-outcome representations to habitual, stimulus-response processes as the drug-seeking behavior becomes engrained (Everitt *et al.*, 2001; Hyman, 2005).

The dorsal striatum supports action control, and behavioral reliance on this region differs between goal- and habit-like behavior (Yin and Knowlton, 2006). The dorsomedial striatum (DMS in rodent, caudate in primates) receives input from associative cortices (Alexander and Crutcher, 1990) and is required for goal-directed behavior. Specifically, DMS lesions impair goal-directed reward seeking and promote outcome-independent habitual behavior in rats (Yin *et al.*, 2005; Corbit and Janak, 2010). Conversely, habitual behavior is thought to be dependent on the dorsolateral striatum (DLS in rodents, putamen in primates), which receives input from sensorimotor cortices (Alexander and Crutcher, 1990). For example, DLS lesions can prevent stimulus-response learning and habitual behavior (Yin *et al.*, 2004). Moreover, operant

responding for alcohol is sensitive to DMS manipulation early in training but is interrupted by DLS, but not DMS, manipulation after extended training (Corbit *et al.*, 2012).

Medium spiny neurons (MSNs) encode information about conditioned cues and behavioral responses through phasic fluctuations in their firing rates (Carelli, 2002). DMS neuronal activity correlates with conditioned stimuli (White and Rebec, 1993; Kimchi and Laubach, 2009), while MSNs in the DLS can encode specific motor actions (West *et al.*, 1990; White and Rebec, 1993; Rolls, 1994). However, few studies have directly compared DMS and DLS activity during operant tasks and, to our knowledge, no studies have monitored MSNs in the dorsal striatum during alcohol self-administration.

To address this gap in knowledge, we used extracellular electrophysiology to record DMS and DLS neuronal activity in rats trained to self-administer 10% alcohol under a fixed-ratio (FR) schedule of reinforcement that tends to produce goal-directed behavior (Dickinson, 1985; Yin *et al.*, 2006). Recordings were also made in a second group of rats trained on a variable-interval (VI) reinforcement schedule that produces more persistent and habit-like operant behavior (Yin *et al.*, 2006). Our data support the hypothesis that, consistent with the respective cortical inputs, neuronal firing patterns reflect alcohol-predictive cues in a greater proportion of DMS neurons, while more DLS neurons encode response initiation. Additionally, we predicted that associative DMS activity would predominate during goal-directed behavior, while response-related DLS activity would predominate during habitual behavior. However, the habit-inducing VI model produced greater overlap in neuronal firing patterns between the DMS and DLS, including more DLS post-reinforcement excitations than were observed in FR-trained rats. Moreover, DMS activations triggered by alcohol-associated cues tended to be farther posterior in VI-trained rats.

Methods

Subjects

Male Long-Evans rats (250-300g) were purchased from Charles River (Raleigh, NC, USA) or Harlan (Indianapolis, IN, USA) and individually housed under a 12h light/dark cycle. Except for the initial 5 days of operant training, rats received food and water *ad libitum*. Experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

Behavioral Training

Experiment 1 & 2: General alcohol self-administration procedures

All rats were trained in daily sessions, Monday - Friday, in custom-built Plexiglas operant chambers in sound-attenuating cabinets (MedAssociates, St. Albans, VT, USA) as previously described (Robinson and Carelli, 2008). Briefly, each chamber contained a house light, two levers, two cue lights located above the levers, and two fluid-dispensing cups. Sessions began with the illumination of the house light followed 30s later by extension of the levers into the operant chamber. The first three sessions lasted up to 3h, and all subsequent training sessions were shortened to 30min. To facilitate alcohol self-administration, a sucrose-fading procedure was implemented over the first 20 sessions of training (Hay *et al.*, 2013). Thereafter, alcohol deliveries were limited to a maximum of 25 in a session, after which point the session ended (levers retracted and house light extinguished).

Experiment 1: Fixed-ratio reinforcement schedule

Rats were initially trained on an FR1 schedule (1 lever-press response = 1 fluid delivery) with both levers reinforced, followed by FR3 schedule sessions, as previously described (Hay *et*

al., 2013). After the third session, responses on one lever each session (either right or left) triggered fluid delivery, while the other lever was inactive (responses were recorded but had no consequences). At each reinforced response, 0.1mL of fluid was dispensed into the cup adjacent to the activated lever, and the following events occurred simultaneously and lasted for a 5s period referred to as the 'time-out': the cue light above the lever was illuminated, the house light was extinguished, and the levers were retracted. The reinforcement schedule increased to FR5 by the tenth session. In combination with the 25 alcohol delivery limit, alternation of the active lever each session between left and right prevented overtraining. The time-out period (with lever retraction) was gradually extended to 12s between session 20 and the commencement of electrophysiological recording.

Experiment 2: Variable-interval reinforcement schedule

A second group of rats was trained with sucrose-fading conditions as in Experiment 1, but with different criteria for fluid delivery (Hay *et al.*, 2013). In Experiment 2, the location of the reinforced lever remained fixed throughout training (counterbalanced across animals). In this experiment there was no time-out period; thus, the levers remained extended throughout the session, and upon reinforcement the house light deactivation and cue light illumination always continued for 3.5s. In the first training session, fluid delivery and cue-light illumination occurred on a random-time 60s schedule. The rat was then trained on an FR1 schedule for 1-2 sessions before beginning on a VI7 schedule (VI7: after a variable interval with an average duration of 7s had elapsed, 1 response = 1 fluid delivery). Next, sessions were shortened to 30min and the reinforcement interval lengthened to 30s (VI30) by the 7^{th} session.

Surgery

Surgery was performed after at least 6 weeks of training. Rats were anaesthetized with isoflurane (5% induction, 2% maintenance) and implanted with 16 stainless-steel, Teflon-coated electrodes (NB Labs, Denison, TX, USA; see Robinson and Carelli, 2008). Electrodes were 50µm in diameter and arranged ~0.5mm apart on two 1x8 arrays in an anterior-posterior orientation. One array was aimed at the DMS (0.2 – 2.2mm anterior, 1.7mm lateral, 4.5mm ventral from bregma) and the second at the contralateral DLS (0.2 – 2.2mm anterior, 3.4mm lateral, 4.5mm ventral from bregma), with sides counterbalanced across rats. Rats were monitored after surgery, given 15mg/kg ibuprofen daily for 3 days and allowed a week to recover before returning to the operant chambers.

Electrophysiology

After surgery, rats were habituated to the flexible tether that connected the electrode arrays to the headstage assembly. Training sessions continued in operant chambers equipped for electrophysiological recordings until operant behavior recovered to at least 17 reinforcements in a session (typically 5-6 days); the next session was the electrophysiological recording day. During all sessions on the tether, the chamber remained dark for 15min before session initiation, allowing the experimenter to select a differential reference and discriminate cells from background noise on the microwires. Neuronal activity was recorded using a multichannel acquisition processor (MAP system with SortClient software; Plexon, Inc., Dallas, TX, USA) while video was recorded from an overhead camera. Timestamps from the MAP system to the video and from the MedAssociates software to the MAP system were used to temporally align electrophysiological recordings with behavioral events.

Cell sorting was finalized after the experiment with Offline Sorter software (Plexon, Inc.). Automated clustering based on template analyses and principle component analyses were manually adjusted, guided by signal-to-noise measurements made during data collection (Robinson and Carelli, 2008). Signal-to-noise ratios ≥2, distinct principle component analysis clusters (determined during offline sorting), and physiological characteristics consistent with MSNs (i.e., ≤0.1% of spikes with interspike intervals <1ms and average firing rates <10Hz; Kish *et al.*, 1999; Kimchi *et al.*, 2009) were required for inclusion of neurons in analyses.

Satiety-specific devaluation and contingency degradation testing

Once all electrophysiological experiments were complete, rats were returned to the original training chambers for additional untethered self-administration sessions. In rats that maintained stable lever-press responding after electrophysiological recording, a satiety-specific devaluation test was conducted to assess behavioral flexibility (Hammond, 1980; Yin *et al.*, 2006). To acutely devalue the alcohol reinforcer, rats were given 1h access to 10% alcohol in the home cage to induce satiety for that solution. Lever-press responding was then measured for 10min in the operant chamber under extinction conditions (no consequences of lever presses). To control for drinking a bolus of liquid before the session, rats were given 1h access to 2% maltodextrin (w/v) before an identical extinction test on a separate day (balanced order, 15mL maximum). The two devaluation test days were separated by 2-3 days of maintenance training on the standard FR5 or VI30 reinforcement schedules.

As a second test of behavioral flexibility, contingency degradation training was used to determine the persistence of behavior after complete disruption of action-outcome contingencies (Colwill and Rescorla, 1986; Balleine and Dickinson, 1998). First, a 10min extinction test was performed to assess a baseline level of responding. After two additional standard operant self-

administration sessions, rats underwent three sessions of contingency-degradation training in which the reinforcing solution was delivered into the cup on a random time 30s schedule for 40min, resulting in 51±3 alcohol deliveries. The day after degradation training, the effect of contingency degradation on responding was tested in a 10min extinction session. Reward seeking was compared in the pre- and post-training extinction sessions.

Histology

Rats were anesthetized with ≥1.5g/kg of urethane before 10μA current was applied for 5s to each stainless-steel wire, producing an iron deposit for determination of electrode placement. Rats were perfused, and brains were sectioned and stained as previously described (Robinson and Carelli, 2008).

Data analysis

Description of the recording session: Operant session events are presented as mean ± S.E.M. In rare cases where not all delivered alcohol was consumed, alcohol consumption was calculated from the amount delivered and the amount remaining in the cup at the end of the session. To compare detection of cells in DMS and DLS, the number of MSN cells/wire in each rat was compared by Mann-Whitney U test (MWU; Sigma Plot, Systat Software Inc, San Jose, CA).

Neuronal firing at single events: The average baseline firing rate and coefficient of variance in the 60s before initiation of the operant session were calculated in 0.5s bins. Changes in firing rate at the presentation of cues signaling the start of the operant session (house-light illumination, initial lever extension) were determined by comparison of neuronal firing in the 0.5s bin after the cue to the previous 60s (0.5s signal: 60s baseline firing rate ratio; S:B). The

baseline firing rate, coefficient of variance, and signal-to-baseline ratios were compared across regions (DMS, DLS) by MWU.

Perievent histograms of firing rates were created in NeuroExplorer software (Nex Technologies, Littleton, MA), and population analyses were completed using custom-written programs in MATLAB (MathWorks, Inc., Natick, MA, USA). To illustrate the activity of the population of neurons in the DMS versus DLS, the average firing rates of all neurons in each region were aligned and smoothed with a moving average of 250ms in 50ms steps. Because baseline firing rates varied among neurons, the firing rates of each neuron around intra-session events were normalized before analysis by dividing by the average firing rate across the whole session. This normalization better represented changes in neuronal response magnitude. For further analysis, neurons were split into anterior and posterior positions (1.2 – 2.2mm and 0.2 – 1.2mm relative to bregma, respectively).

Neuronal firing around repeated intra-session events: Spike rates from each neuron around the times of lever responses and cue events were averaged across trials before population analyses were conducted as for single events. There were typically many more non-reinforced than reinforced lever responses under the VI30 reinforcement schedule; thus, to facilitate comparison of neuronal activity, we selected 25 non-reinforced responses that were evenly distributed throughout the session for these analyses.

The firing activity of individual neurons around events that occurred multiple times within each session was classified by calculating z-scores of phasic frequency changes from baseline. For lever-response events, the average number of spikes in two target windows—the 0.5s before and the 0.5s after each event—was compared to a nearby 2s window that was designated as baseline. In Experiment 1 (FR5 model), the baseline was 2-4s before the 1st

response and 8-10s after the 5th response in each series, to compare changes before and after these responses to a baseline outside of the action sequence. In Experiment 2 (VI30 model), baselines were 2-4s before non-reinforced and reinforced responses; as lever responses did not typically occur in bouts, this baseline rarely overlapped with behavioral responding. Neurons with z-scores between -2 and 2 were classified as non-phasic (NP). Those with significant z-scores ($-2 > z > 2$) were classified by the epoch and direction of greatest change as pre-excitatory (PreEx), pre-inhibitory (PreIn), post-excitatory (PostEx), or post-inhibitory (PostIn). In Experiment 1, we also analyzed activity after the lever extension terminating each time-out as a cue of alcohol availability. For this event, the baseline was set as the 2s immediately preceding lever extension, the 0.5s window after the event was analyzed, and cells were classified as PostEx or PostIn.

Behavioral tests: Lever responding during satiety-specific devaluation and contingency-degradation extinction tests was compared within-subjects using a paired t-test. Responding during contingency-degradation training was compared using 1-way RM ANOVA with the Tukey method for multiple comparisons (Sigma Plot).

Results

Experiment 1: Alcohol self-administration with fixed-ratio reinforcement

Twenty-four rats underwent surgery, and 14 completed the protocol for Experiment 1. Rats were trained in 36.5±1.5 sessions to self-administer 10% alcohol on a FR5-reinforcement schedule. On the electrophysiological recording day, rats responded on the active lever 110±4 times, receiving 22±1 alcohol deliveries; inactive lever responses occurred 37±7 times. The average total alcohol consumption was 0.5±0.02g/kg, similar to doses previously reported for a 30min session (Rassnick *et al.*, 1992; Hodge *et al.*, 1997; Robinson and Carelli, 2008). We

recorded 101 neurons that were confirmed to be in the DMS or DLS (**Figure 2.1**) with firing rates ≤10Hz. Rates of detection of these presumed MSNs were similar in the two regions: 0.54±0.10 cells/wire in the DMS and 0.52±0.08 cells/wire in the DLS (*MWU statistic* =96.5, *P*>0.05).

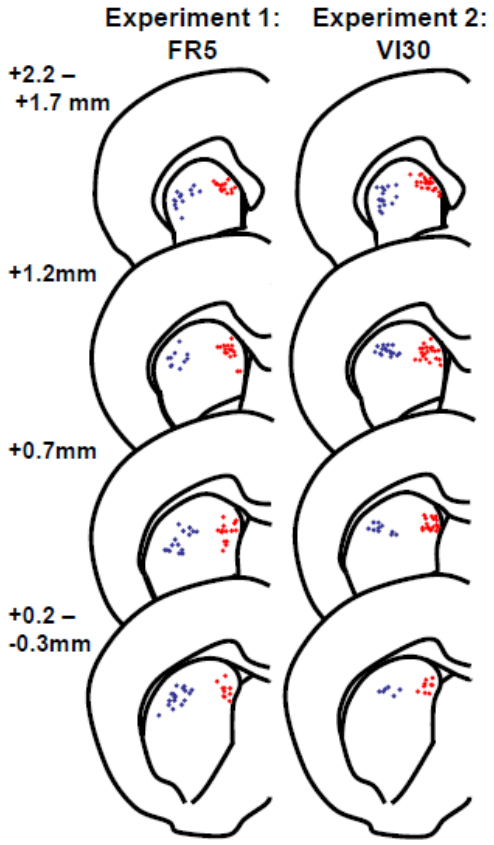

Figure 2.1: Placement of recording electrodes in the dorsal striatum. Dots show the location of DLS (blue) and DMS (red) electrode recording sites in Experiments 1 (left) and 2 (right) as determined by histological analyses. Placements are collapsed onto the left hemisphere and depicted on representative coronal slices with coordinates in mm anterior to bregma (figure adapted from Paxinos and Watson, 1998).

Examples of self-administration behavior and MSN firing patterns from a single FR5-trained rat are shown in **Figure 2.2**. The FR5 schedule produced a fixed contingency between the number of lever responses and reinforcer deliveries (**Figure 2.2A**). The biphasic distribution of inter-response intervals (IRIs, **Figure 2.2B**) demonstrates the fast IRIs exhibited within the 5-

response sequence as well as the longer IRIs imposed by the 12s time-out period. The mean IRI on the active lever for the FR5 rat shown here was 9.3±1.0s, and across all FR5-trained rats mean IRI was 8.7±0.8s. Firing rates of each cell were examined by aligning action potentials around operant events, such as reinforcement at the 5th lever response of a sequence, shown here (**Figure 2.2C**). In these examples, blue triangles indicate lever extension, pink diamonds indicate

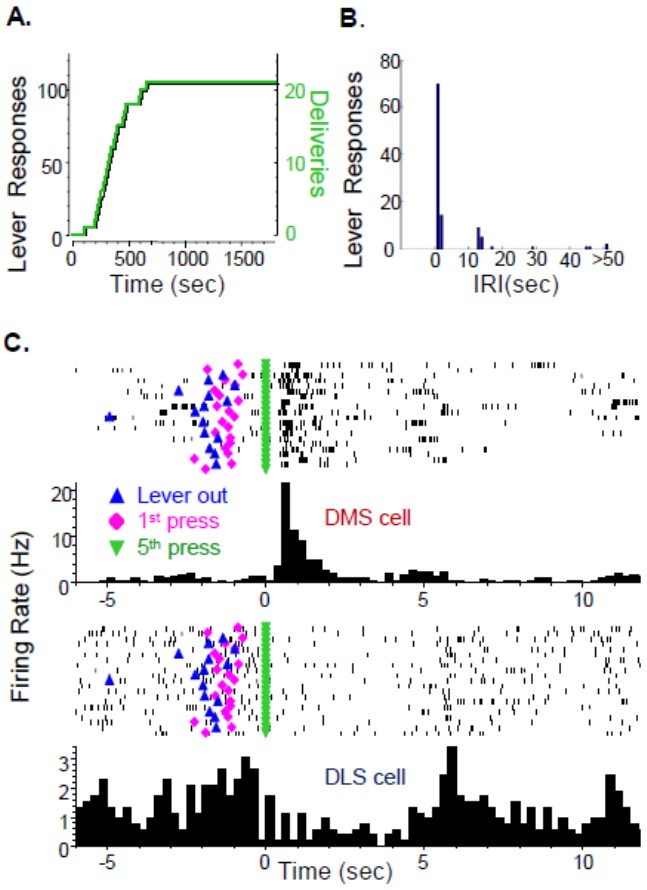

Figure 2.2: FR5-reinforcement schedule: alcohol self-administration behavior and neuronal firing patterns from a representative rat. (A) Cumulative activity plot of lever-press responses (black, left axis) and reinforcements earned (gray/green, right axis) during the recording session. (B) Histogram of IRIs from the FR5 session displayed in panel A. (C) Neuronal activity aligned to each reinforced response during the session shown in panel A from one DMS and one DLS cell. For each cell: (Top) Raster plot in which tick marks (black) represent neuronal action potentials. Behavioral events plotted on the raster are lever extension (upward triangle), 1st response (diamond), and 5th response (downward triangle). (Bottom) Histogram of average firing rate in 250ms bins from all trials; note the different y-axis scales for the two cells.

the 1st lever response, and green triangles indicate the 5th response and reinforcement (alcohol delivery and associated cues). This DMS neuron exhibited increased firing immediately following alcohol delivery. In contrast, the DLS neuron displayed higher firing rates during lever responding than at reinforcement. For this rat, the mean latency from alcohol delivery to start of drinking was 1.2±0.1s and drinking duration was 4.9±0.3s, which corresponded with periods of low firing frequencies in the representative DMS and DLS example cells and a rebound in firing in the DLS neuron at drinking cessation.

Neuronal population activity in DMS versus DLS during FR5 sessions

We first analyzed differences in the basal firing rates and coefficients of variance of DMS and DLS neurons at the start of the session in the 60s before house-light illumination: basal firing rates were 2.1±0.2 in the DMS and 2.4±0.3 in the DLS (*MWU statistic* =1174, $P>0.05$), while the coefficient of variance was 7.9±0.5 in the DMS and 7.9±0.4 in the DLS (*MWU statistic* =1201, $P>0.05$). Therefore, no significant baseline firing differences were found between these regions.

Next, we compared neuronal activation to conditioned cues signaling the start of the session by plotting the average normalized firing rate of all cells in the DMS and DLS. These population plots showed that the average DMS firing rate increased 4.1-fold compared to the whole session firing rate within 0.25s of the house-light illumination (**Figure 2.3A)**. Similarly, DMS firing exhibited a brief 3.6-fold increase relative to the whole session firing rate immediately after the first lever extension **(Figure 2.3B)**, demonstrating sensitivity to cues of session initiation that were independent of behavior. Comparison of the firing rate in the 0.5s after house-light illumination to the 60s basal firing rate (described above) revealed a significantly greater signal-to-baseline ratio in the DMS (3.4±0.9) compared to the DLS

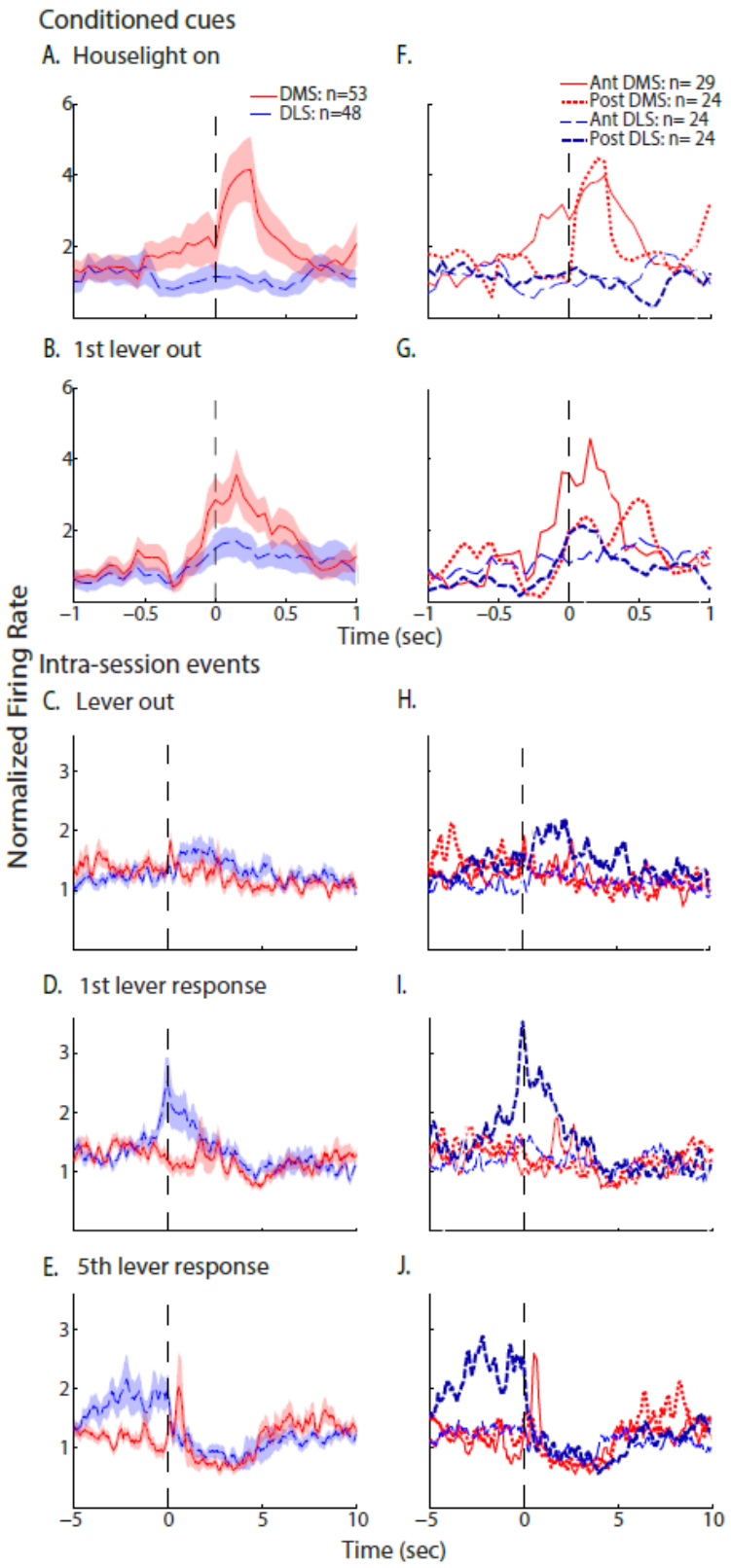
Conditioned cues
A. Houselight on
DMS: n=53
DLS: n=48
F.
Ant DMS: n= 29
Post DMS: n= 24
Ant DLS: n= 24
Post DLS: n= 24
B. 1st lever out
G.
Time (sec)
Intra-session events
C. Lever out
H.
D. 1st lever response
I.
E. 5th lever response
J.
Time (sec)
Normalized Firing Rate

Figure 2.3: Neuronal population activity in the dorsal striatum of FR5-trained rats at start-of-session cues and lever responses. Left: mean normalized firing rate (±SEM shaded) of all neurons in the DMS (red) and DLS (blue) aligned to specific events. Right: the same neurons were divided into two categories by anterior-posterior position (divided at 1.2mm anterior to bregma) and mean normalized firing rates were again plotted relative specific events. Neuronal activity was aligned to single presentations of start-of-session cues: (A, F) house-light illumination and (B, G) initial lever extension. Neuronal activity was aligned to multiple occurrences of operant events: (C, H) lever extension after the 12s time out, (D, I) the 1st of each 5-response sequence, and (E, J) the 5th of each 5-response sequence. Firing rates were binned with a 250ms moving average using 50ms steps; note the different time scales for start-of-session events versus repeated operant events.

(1.0±0.2; *MWU statistic* =965, $P<0.05$). Similarly, after the initial lever extension, the signal-to-baseline ratio was significantly larger in the DMS versus the DLS (2.2±0.3 and 1.3±0.2, respectively; *MWU statistic* =980, $P<0.05$).

We additionally examined events that occurred repeatedly during the self-administration session, including the lever extension cue (12s after each fluid delivery), the 1st lever response in the 5-press sequence, and the combined action, cue and alcohol delivery around the 5th response. When neuronal activity was aligned to all lever extensions in the session, a brief 1.8-fold increase in DMS spike frequency was observed in the population (**Figure 2.3C**). Around the 1st response, however, the DMS showed no apparent change in the population firing rate, while firing increased 2-fold following reinforcement (**Figures 2.3D & 2.3E**). This phasic neuronal activation to the reinforced press was most prominent in the first half of the trials in the session (**Supplemental Figure 2.1,** Appendix 2.1). Similar to the first press activation, no changes were observed in the DMS around inactive lever presses (data not shown). Thus, DMS neurons were most active at alcohol-associated cues of availability and delivery rather than initiation of alcohol-seeking behavior.

Neuronal activity in the DLS differed from that of the DMS around these events. Lever extensions evoked a 1.6-fold elevation in firing rate with a more prolonged DLS activation than

was observed in the DMS; this excitation often encompassed the 1st lever response, as the median interval between the lever extension and the 1st response was 2.3s (**Figure 2.3C**). The 1st response itself was associated with a higher amplitude 2.5-fold increase in firing frequency in the DLS that peaked *before* the lever-press response (**Figure 2.3D**). Moreover, the DLS excitation after the 1st response persisted during the 5-response sequence but not after alcohol delivery (**Figure 2.3E**); indeed, the median interval between the 1st and 5th responses was 2.5s. Pre-response DLS excitations (2-fold increase in firing rates) were also observed before the few inactive lever responses (data not shown). When the first half of trials was compared to the second, there was some increase in excitation across the session (**Supplemental Figure 2.1**). Thus, the predominant response of MSNs in the DLS was a pre-response excitation. Finally, both DMS and DLS demonstrated decreased firing rates after the fluid delivery, extending previous findings of inhibition of MSNs in the nucleus accumbens during reward consumption (Taha and Fields, 2005; Taha and Fields, 2006; Krause *et al.*, 2010) to the dorsal striatum.

As the electrode arrays were positioned in anterior-to-posterior rows, we compared population activity in those neurons anterior and those posterior to 1.2mm bregma (**Figures 2.3F – 2.3J**). This analysis revealed that both anterior and posterior DMS neurons contributed to the DMS activation after house-light illumination, while anterior DMS neurons showed the predominant population changes in firing frequency after the 1st lever extension and the 5th lever response. In contrast, the doubling of DLS firing rates after repeated intra-session lever extensions seen in the entire DLS population was driven selectively by posterior DLS neurons. Similarly, the pre-response excitations around both the 1st and 5th lever responses were primarily driven by posterior neurons.

Firing patterns of individual neurons in DMS versus DLS around FR5 intra-session events

To determine the proportion of MSNs in each striatal region that exhibited particular phasic firing patterns, we classified individual neurons by their firing activity at repeated intra-session events: lever extension, 1st response and 5th response. Specifically, z-scores were used to compare normalized firing rates in the 0.5s bin after lever extension, before lever response, or after lever response to a 2s baseline depicted by the shaded area in **Figure 2.4**, and neuronal activity of the phasically active cells was plotted (nonphasic cells were excluded for clarity). The distribution of neurons across each category of neuronal activity (PreEx: pre-excitatory; PreIn: Pre-inhibitory; PostEx: post-excitatory; PostIn: post-inhibitory; NP: non-phasic) and the proportion of non-phasic neurons are displayed in pie charts on each graph. When we examined changes in firing after intra-session lever extensions, we found only PostEx phasic patterns in the DMS, comprising 28% (15/53) of the recorded neurons (**Figure 2.4A**). Similar to the DMS, 30% of DLS neurons displayed significantly different firing frequencies after the lever extension, although only 17% (8/48) of DLS neurons were PostEx while 13% (6/48) were PostIn (**Figure 2.4B**).

Around the 1st lever response of the 5-response sequence, 44% of DMS neurons (23/53) and 65% of DLS neurons (31/48) exhibited significant changes in firing rates. All firing patterns were observed in the DMS and DLS at the 1st response, but the regions differed in the proportions of neurons demonstrating each category of neuronal activity. Consistent with the population frequency plots, excitations were prominent in the DLS, where 21% of cells (10/48) exhibited a brief, 5-fold PreEx firing activity and 27% of cells (13/48) showed a more sustained PostEx pattern (**Figure 2.4D**). In contrast, the predominant phasic activity in the DMS was

PostIn (11/53 neurons, or 21%; **Figure 2.4C**). Nevertheless, a subset of DMS neurons (6/53) exhibited PostEx activity that showed similar timing and amplitude to the DLS PostEx pattern.

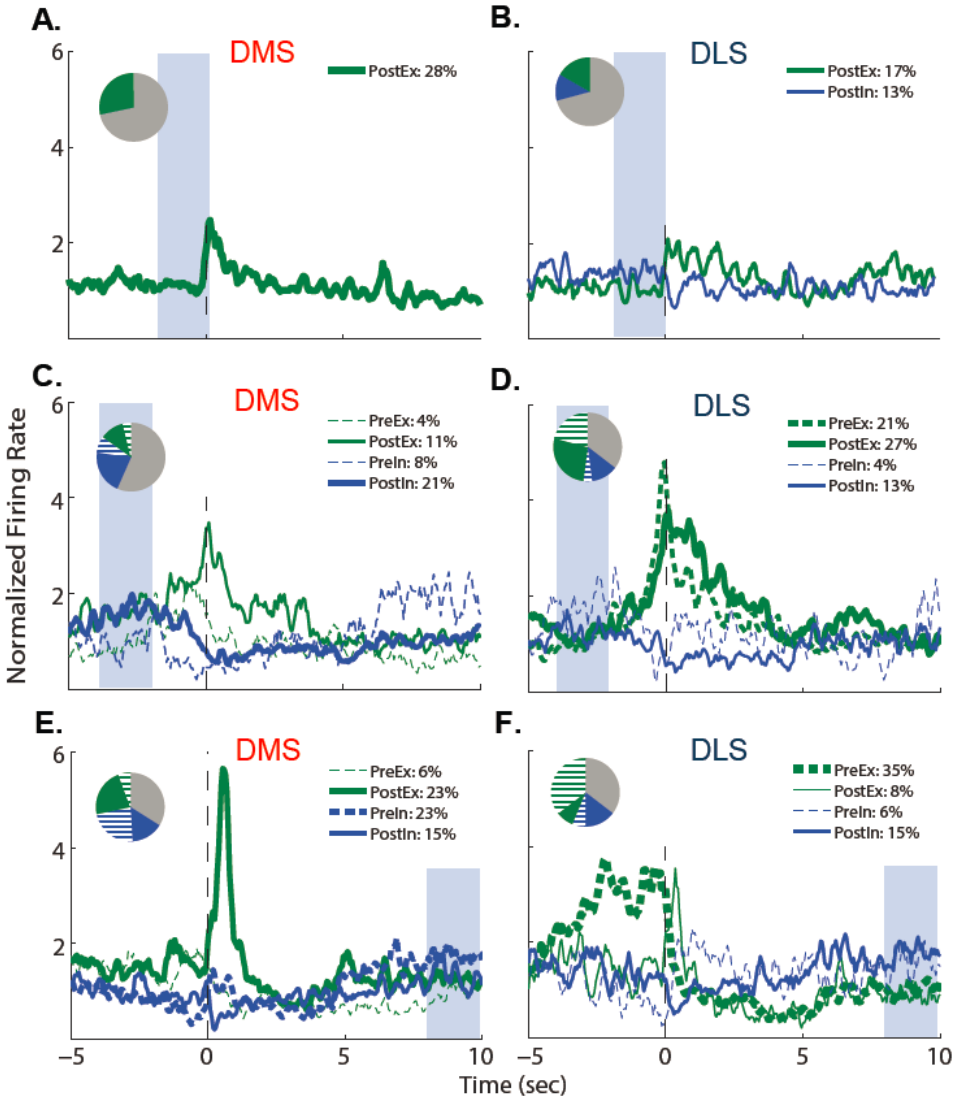

Figure 2.4: Distribution of specific firing patterns of dorsal striatal neurons of FR5-trained rats around repeated operant events. DMS (top) and DLS (bottom) neurons were classified by the epoch and direction of significant changes in firing rate to each repeated intra-session event (PreEx: pre-excitatory; PreIn: Pre-inhibitory; PostEx: post-excitatory; PostIn: post-inhibitory; NP: non-phasic; see methods for category criteria): (A, D) lever extension after the 12s time out; (B, E) the 1st of each 5-response sequence; (C, F) the 5th of each 5-response sequence. Line thickness is proportional to the number of neurons in each category, such that thicker lines represent greater proportions of neurons; NP neuronal activity is not shown. Firing rates were binned with a 250ms moving average using 50ms steps. Inset: pie charts display the proportions of cells in each category (see legend for color key; NP neurons shown in gray).

Overall, the DLS had a higher proportion of phasically active cells around the 1st response and these cells showed excitations time-locked to the lever response event, while the DMS had fewer phasically active cells and these tended to exhbit inhibitions that were less closely time-locked to the action.

When aligned to the 5th response, which initiated cue onset and alcohol delivery, we found that 67% of DMS and 64% of DLS cells demonstrated significantly altered firing rates. The highest magnitude of frequency change observed after categorization in either region was the PostEx activity after the 5th response in the DMS, with 12 of 53 cells (23%) reaching on average 5.6 times their whole-session firing rate (**Figure 2.4E**). This change was brief and time-locked to the reinforced lever-press response. In contrast, the predominant neuronal firing pattern in the DLS was a prolonged, 3- to 4-fold PreEx pattern exhibited by 17 of 48 neurons (35%; **Figure 2.4F**). This DLS excitation appeared to be a continuation of the firing activity that began at lever-response initiation and continued through the 5-response sequence; indeed, 24 of the 29 DLS neurons that were significantly excited ±0.5s around the 5th response also showed significant excitations ±0.5s around the 1st response. However, a small subset of DLS 5th response PostEx neurons (4/48) displayed brief excitations whose timing matched the DMS PostEx neurons, although with diminished amplitude.

Thus, while similar specific firing patterns were observed in the DMS and DLS, these regions differed in the proportions of neurons displaying these patterns. The DMS exhibited less phasic activity around the 1st lever response but distinct excitation after the 5th response and lever extension, while the DLS excitations appeared to persist throughout the action sequence.

Experiment 2: Alcohol self-administration with variable interval reinforcement

In Experiment 2, 21 rats underwent surgery and 16 rats completed successful electrophysiological recordings. These rats were trained to self-administer 10% alcohol over 35.3±1.5 weeks on a VI30-reinforcement schedule. On the electrophysiological recording day, rats responded on the active lever 117±16 times for 22±1 alcohol reinforcements, resulting in average total alcohol consumption of 0.5±0.02g/kg; inactive lever responses occurred 1±1 times. During these sessions, we recorded 102 neurons confirmed to be in the DMS or DLS with firing rates ≤10Hz (**Figure 2.1)**. Detection rates were 0.61±0.10 presumed MSNs/wire in the DMS and 0.42±0.07 MSNs/wire in the DLS (*MWU statistic* =86, *P*>0.05), for a total of 61 DMS and 41 DLS neurons.

Examples of self-administration behavior and MSN firing patterns from a single VI30-trained rat are shown in **Figure 2.5**. Notably, alcohol delivery in the VI30 reinforcement schedule is less contingent on the rate of lever responding, as illustrated by the divergence of the cumulative activity plots in **Figure 2.5A**. The IRI for this rat was 9.7±1.1s, while the mean IRI in Experiment 2 was 11.3±0.7s. Compared to the FR5 schedule, the VI30-reinforcement schedule generated slower lever-press behavior, with a smoother IRI distribution (**Figure 2.5B**). Neuronal firing rates were examined by aligning action potentials around operant events, such as the firing around the reinforced response in the representative cells in **Figure 2.5C**. The DMS neuron displayed here demonstrated increased firing rates after reinforced responses, while the predominant change in the DLS cell was an excitation before reinforced lever-press responses. Both cells were less active during drinking periods, and the DMS cell exhibited a rebound excitation after drinking, which was initiated 0.8±0.03s after alcohol delivery and was sustained for the following 6.4±0.3s during the session shown here.

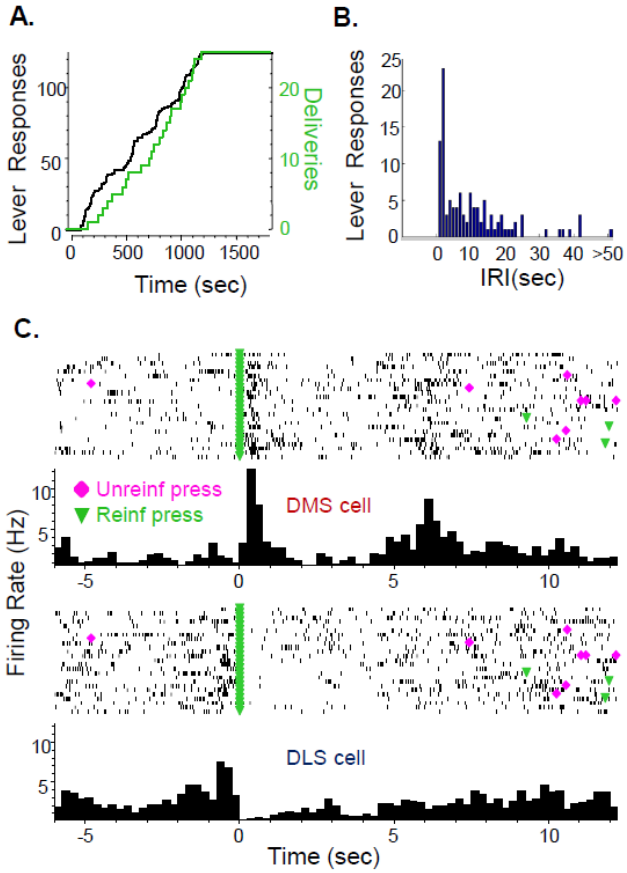

Figure 2.5: VI30-reinforcement schedule: alcohol self-administration behavior and neuronal firing patterns from a representative rat. (A) Cumulative activity plot of lever responses (black, left axis) and reinforcements earned (gray/green, right axis) by a VI30-trained rat during the recording session. (B) Histogram of IRIs from the VI30 session displayed in panel A. (C) Neuronal activity aligned to each reinforced response during the session shown in panel A from one DMS and one DLS cell. For each cell: (*Top)* Raster plot in which tick marks (black) represent neuronal action potentials. Behavioral events plotted on the raster are non-reinforced (diamond) and reinforced (triangle) lever responses. (*Bottom)* Histogram of average firing rate in 250ms bins from all trials.

Population neuronal activity in DMS versus DLS during VI30 sessions

To analyze whether there were differences in the basal firing of each region, we compared spike frequency in the 60s before house-light illumination at the start of the session. Before normalization, the basal firing rate was 2.9±0.3 in the DMS and 2.3±0.2 in the DLS (*MWU statistic* =1131, *P*>0.05). The coefficient of variance was 5.1±0.4 in the DMS and 5.6±0.7

in the DLS (*MWU statistic* =1129, *P*>0.05). There were no significant differences in the basal firing or coefficient of variance between the DMS and DLS.

We expected that lever-press responses and the appearance of cues and reinforcers would be encoded by fluctuations in the firing rates of MSNs, but that there would be less of this phasic activity in the DMS of VI30-trained rats compared to FR5-trained rats. Therefore, we compared the population activity in the DMS and DLS around session-initiation cues, as well as non-reinforced and reinforced responses. Specifically, we analyzed average, normalized neuronal firing rates by aligning spike timing to either session-initiation cues, reinforced responses or 25 non-reinforced responses that were evenly distributed throughout the session. (Repeated lever extensions were not present in this model due to the lack of a time-out period.) **Figures 2.6A & 2.6B** illustrate that population activity in the DMS increased to around three times its whole-session firing rate after the session-initiation cues of house light illumination and initial lever extension, while the DLS showed a more modest increase to both cues that was less time-locked. However, no significant difference in the signal-to-baseline ratio of these regions was discovered when the ratio of the firing rate in the 0.5s after either event was compared to the 60s basal firing rate (1.3±0.2 in the DMS and 1.2±0.3 in the DLS at the house light, *MWU statistic* =1112, *P*>0.05; 1.5±0.5 in the DMS and 1.4±0.3 in the DLS at the 1st lever; *MWU statistic* =1141, *P*>0.05).

Around lever-press responses, a brief 1.8-fold increase over the baseline firing rate was observed in the DMS immediately after reinforced responses but not after non-reinforced responses, consistent with an association of neuronal activity to cues and alcohol delivery rather than lever responses per se (**Figures 2.6C & 2.6D**). The DLS showed a modest ramping of firing rate leading up to either type of lever response that peaked at approximately 1.5-fold increase

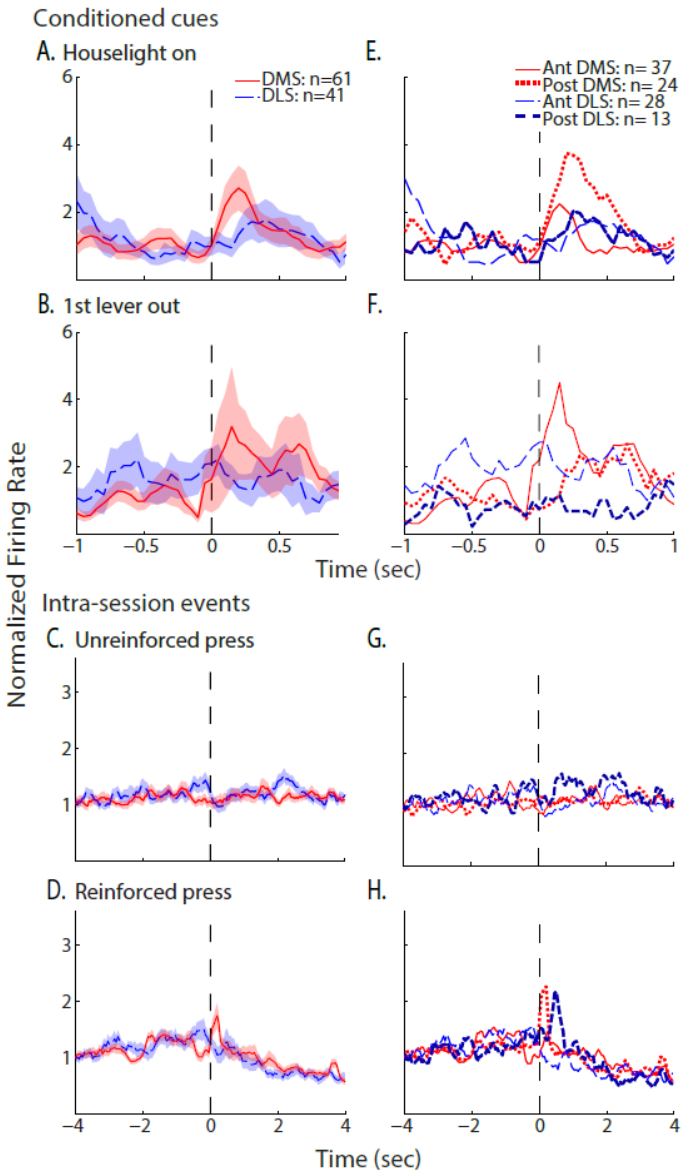

Figure 2.6: Neuronal population activity in the dorsal striatum of VI30-trained rats at start-of-session cues and lever responses. Left: mean normalized firing rate (±SEM shaded) of all neurons in the DMS (red) and DLS (blue) aligned to specific events. Right: the same neurons were divided into two categories by anterior-posterior position (divided at 1.2mm anterior to bregma) and mean normalized firing rates were again plotted relative specific events. Neuronal activity was aligned to single presentations of start-of-session cues: (A, E) house-light illumination and (B, F) initial lever extension. Neuronal activity was aligned to multiple occurrences of operant events: (C, G) non-reinforced (non-reinf.) lever responses and (D, H) reinforced lever responses. Firing rates were binned with a 250ms moving average using 50ms steps; note the different time scales for start-of-session events versus repeated operant events.

over baseline before returning to basal rates. Inactive lever presses were too infrequent for

analysis. Firing rate changes were larger in the first half of trials in the DMS, with no consistent change in DLS activation (**Supplemental Figure 2.2**). Finally, the population activities of both the DMS and DLS were diminished in the seconds following alcohol delivery, consistent with drinking-associated inhibition, as observed in Experiment 1. Overall, phasic firing patterns in both DMS and DLS neuronal populations at all events were smaller in amplitude than those in FR5-trained rats.

Dividing the neurons along the anterior-posterior axis (as in Experiment 1) revealed a higher amplitude response in the posterior DMS at the house-light cue and in the anterior DMS at the 1st lever extension (**Figures 2.6E & 2.6F**). Moreover, while the delayed excitation to house-light illumination was expressed across the DLS, the broad excitations that spanned ±1s around the initial lever extension were driven by posterior DLS neurons. At reinforced responses, DMS excitations were predominantly in the posterior cells (**Figure 2.6H**). The posterior DLS cells also exhibited brief increases in firing rates after reinforced responses, although this activity was delayed by 0.5s relative to the DMS excitation, similar to the DLS response to the house-light cue. However, the anterior-posterior analysis did not reveal sub-regional variation in the discharge activity at non-reinforced responses or during drinking (**Figures 2.6G & 2.6H**).

Individual neuronal firing patterns around VI30 lever response

To assess the distribution of phasic firing patters at reinforced versus non-reinforced responses, we categorized individual cells by their firing activity (**Figure 2.7**). Again, all firing patterns were observed in both DMS and DLS after non-reinforced and reinforced responses, but their proportions varied by region at each event. At non-reinforced responses, 55% of DMS cells exhibited significant changes in firing rates, including PreEx (10/61 cells), PostEx (13/61 cells),

and PostIn (9/61 cells). In the DLS, the total proportion of phasically active cells was also 55%, but the predominant activity pattern was PreEx at 29% (12/41 cells), consistent with population activity. The largest amplitude of firing frequency change at non-reinforced responses in the DMS was a 2-fold change in PostEx cells, while the DLS peak phasic firing activity within 0.5s of the lever response was a 2.4-fold increase in PreEx cells.

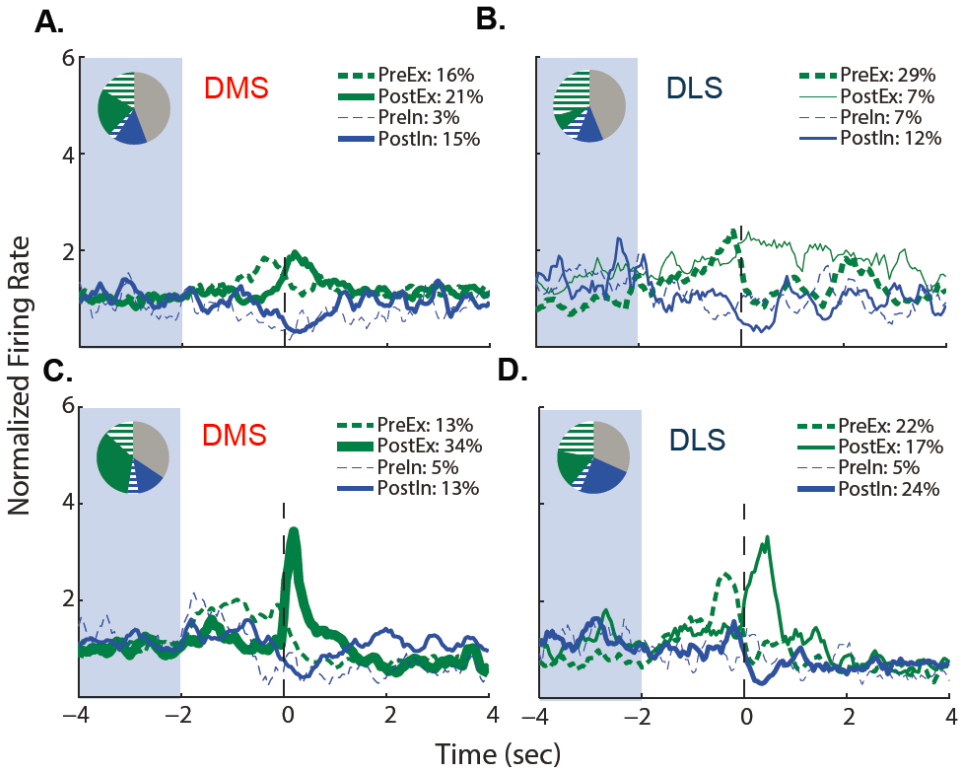

Figure 2.7: Distribution of specific firing patterns of dorsal striatal neurons of VI30-trained rats around repeated operant events. DMS (top) and DLS (bottom) neurons were classified by the epoch and direction of significant changes in firing rate to each repeated intra-session event (PreEx: pre-excitatory; PreIn: Pre-inhibitory; PostEx: post-excitatory; PostIn: post-inhibitory; NP: non-phasic; see methods for category criteria): (A, C) non-reinforced lever responses; (B, D) reinforced lever responses. Line thickness is proportional to the number of neurons in each category, such that thicker lines represent greater proportions of neurons; NP neuronal activity is not shown. Firing rates were binned with a 250ms moving average using 50ms steps. Inset: pie charts display the proportions of cells in each category (see legend for color key; NP neurons shown in gray).

More neurons exhibited significant changes in discharge rates around the reinforced responses as compared to non-reinforced responses: 65% of DMS and 68% of DLS cells. In the DMS (**Figure 2.7C**), 34% of neurons (21/61) were classified as PostEx and exhibited a 3.4-fold increase in firing rate. Interestingly, similar proportions of all other categories of neuronal activity were seen in the DMS around non-reinforced as reinforced responses, suggesting these DMS neurons encoded both lever-press responding and reinforcement-associated events in the VI30 model. In the DLS (**Figure 2.7D**), firing patterns included PreEx (9/41), PostEx (7/41) and PostIn (10/41). The PostEx activity in the DLS was less robust than in the DMS, with half the percentage of neurons classified as PostEx, but with similar amplitude and timing. Thus, lever responses and reinforcement were encoded in both regions, albeit with variable activity patterns. Again, the predominant pre-response DLS excitation and post-cue excitation in the DMS were smaller in magnitude in VI30-trained versus FR5-trained rats.

Satiety-specific devaluation and contingency degradation testing

Once all electrophysiological recordings were completed, satiety-specific devaluation and contingency degradation were used on rats with stable lever-press behavior to test whether behavior was goal-directed and dependent on action-outcome associations or habit-like and controlled by stimulus-response associations. First, satiety-specific devaluation of 10% alcohol tested whether alcohol-seeking behavior (i.e., lever responses) was reduced by 1h of home-cage access to 10% alcohol compared to access to a control fluid (2% maltodextrin). If pre-exposure and satiety for alcohol resulted in less lever responding during extinction versus pre-exposure to the control solution, the rat was considered goal-directed and sensitive to changes in reward value. Rats consumed 6.6±0.5mL of alcohol (4.62±0.35kcal; for a dose of 1.3±0.1g/kg) or 8.4±0.7mL (0.67±0.056kcal) of maltodextrin before a 10min extinction session (no cues or

alcohol deliveries). Lever responses were compared between the two extinction sessions **(Table 2.1**): paired t-tests demonstrated that FR5-trained rats decreased responding by 43% in the extinction session after alcohol pre-access compared to maltodextrin pre-access (t_{12}=2.58, P<0.05). Similarly, VI30-trained rats decreased pressing after devaluation by 34% (t_{14}=2.52, P<0.05). Thus, rats under both training schedules demonstrated alcohol-seeking behavior that was sensitive to satiety-specific devaluation.

Table 2.1. Behavioral characterization of sensitivity to changes in reward value or action-outcome contingency in rats trained to self-administer 10% ethanol on FR5- or VI30-reinforcement schedules. Alcohol seeking was evaluated as lever responses (±S.E.M.) during brief, 10min extinction tests or during contingency degradation training.

Satiety-specific devaluation test	**Experiment 1 FR5, n=13**	**Experiment 2 VI30, n=15**
2% maltodextrin	105±19	40±5
10% ethanol	59±13 [a]	26±5 [a]
Contingency degradation training	**Experiment 1 FR5, n=12**	**Experiment 2 VI30, n=12**
Day 1	134±34	182±28
Day 2	78±18	147±34
Day 3	58±16 [b]	93±22 [b]
Contingency degradation test	**Experiment 1 FR5, n=12**	**Experiment 2 VI30, n=12**
Pre-degradation	104±18	46±8
Post-degradation	28±5 [c]	35±6

[a] significant effect of pre-access solution on extinction, paired t-test, P<0.05
[b] significant effect of training day, 1-way RM ANOVA, P<0.05
[c] significant effect of degradation training on extinction, paired t-test, P<0.05

The outcome of the satiety-specific devaluation test may have been affected by transferring the rats back to their training chambers (disrupting a habitual response), greater caloric content of the alcohol versus the control solution, or by the intoxicating effects of alcohol (slowing lever-press behavior). Thus, we used an additional test to distinguish goal-directed from

habit-like behavior – contingency degradation training – that tested behavior in the absence of alcohol. We compared the number of responses during brief extinction sessions before versus after three sessions of contingency degradation training (fluid deliveries were made on a random time 30s schedule, independent of lever responses). Fewer lever responses in the post-training session would indicate goal-directed behavior that was sensitive to changes in reward contingency. Both FR5-trained and VI30-trained rats decreased responding during the contingency degradation sessions when feedback was provided (**Table 2.1;** 1-way RM ANOVA, FR5: $F_{2,22}$=4.28, P<0.05; VI30: $F_{2,22}$=7.85, P<0.01). This decrease in response rate persisted into the post-training test only in FR5-trained rats, demonstrating sensitivity to prior conditioning (t_{11}=4.28, P<0.001). VI30-trained rats showed no significant effect of degradation training on alcohol seeking during extinction (t_{11}=1.95, P>0.05). VI30 rats responded more slowly than FR5 rats in all conditions; while this difference is well known and expected (Dickinson, 1985; Hilario et al., 2007; Mangieri et al, 2012; Hay et al., 2013), a floor effect cannot be ruled out. Nevertheless, contingency degradation indicated that behavior remained goal-directed in FR5-trained rats after all self-administration sessions, as expected (Yin, 2006), while VI30-trained rats demonstrated less flexible operant behavior, specifically in response to changing action-outcome contingencies.

Discussion

Maladaptive stimulus-response learning resulting in habit formation likely contributes to the persistent drinking and susceptibility to relapse that characterize alcoholism (Everitt and Robbins, 2005). Previous research suggests that alcohol self-administration in rats can become habitual and resistant to changes in reward value, and alcohol exposure may facilitate habit formation (Dickinson *et al.*, 2002; Corbit *et al.*, 2012; Mangieri *et al.*, 2012). However, the

specificity of neuronal activity in dorsal striatum engaged by alcohol self-administration had not previously been investigated. Thus, the present study recorded neuronal activity in DMS and DLS in rats trained on one of two operant reinforcement schedules that produce distinct behavioral patterns (response sequences versus single responses) and differences in behavioral flexibility (differential sensitivity to contingency degradation). A major finding was that the predominant phasic firing patterns of each region occurred in response to distinct events: excitations in the DMS were largely time-locked to alcohol delivery and alcohol-predictive cues, while DLS excitations primarily occurred prior to lever responses. Indeed, these regional specificities were observed in both behavioral models despite distinct alcohol-seeking patterns. Parallels with previous studies of dorsal striatal activation during instrumental behavior, discussed below, suggest common processing for alcohol and non-drug rewards. We additionally report novel evidence of differential encoding of conditioned cues in the two operant models. In the VI30-habit model, the DMS and DLS neurons exhibited more similar response patterns and the population response amplitudes were reduced as compared to the neuronal activity observed in FR5-trained rats. Moreover, putative MSNs in the DMS that responded to alcohol delivery and associated cues were more anterior in FR5-trained rats and more posterior in VI30-trained rats. These findings provide evidence that differential dorsal striatal encoding of alcohol-conditioned behavior accompanies differences in response contingencies that affect behavioral flexibility.

DMS activation to cues in two models of alcohol self-administration

Alcohol-associated cues are known to promote alcohol-seeking behavior (Epstein *et al.*, 2006; Corbit and Janak, 2007). We observed higher amplitude excitations to alcohol-associated cues in the DMS versus DLS of FR5- and VI30-trained rats, consistent with our hypothesis based on the region's associative connectivity, including reciprocal connections to the prefrontal cortex

and midbrain dopamine neurons (McGeorge and Faull, 1989; Haber *et al.*, 2000; Voorn *et al.*, 2004). Our findings additionally agree with previous neurophysiological studies that found greater percentages of DMS/caudate than DLS/putamen neurons are activated by reinforcement-related stimuli (West *et al.*, 1990; Carelli and West, 1991; White and Rebec, 1993; Rolls, 1994). The present study extends these observations to alcohol self-administration, suggesting that this is a common response to drug and non-drug rewards, although results of ongoing research will be necessary to directly compare alcoholic and non-alcoholic self-administration in the same model. The attenuation in both experiments of phasic DMS activation at reinforcement in later trials indicates that some aspect of neuronal encoding of reinforcement changes within session, such as reward value (satiety). In contrast, DMS activation time-locked to the lever-extension cue was undiminished across the session, arguing against alternative explanations such as decreased general arousal or a pharmacological effect of increasing alcohol concentrations.

Also in agreement with the expectation that FR5-trained rats would show predominant DMS activation, DMS excitation to start-of-session and reinforcement cues was of greater amplitude in rats on the FR5 versus the VI30 schedule. The diminished amplitude of neuronal firing patterns, accompanied by a greater proportion of neurons activated by cues in the VI30-trained rats, may be subsequent to habit formation in that group. However, the present study is limited due to its between-subjects design, and the differences in operant behavior necessitate caution in direct comparison of neural data from FR5 and VI30 schedules. An alternative explanation is that the reduced magnitude of the phasic firing patterns in the VI30-trained rats is directly associated with decreased expectancy of reinforcer and subsequently decreased arousal, which may be integral to the habit-promoting nature of the schedule. However, this explanation

does not necessarily account for the diminished DMS firing to start-of-session cues, as they predicted alcohol availability equally between the two groups.

DLS activation to lever responses during alcohol self-administration

The activity of the DLS at lever responses is consistent with DLS connectivity to sensorimotor cortex and DLS encoding of specific motor actions, including forelimb movements required for lever responses (West *et al.*, 1990). Notably, the DLS population excitation associated with lever responses was of higher amplitude in FR5- versus VI30-trained rats, in contradiction to our hypothesis. While this may be due to reduced goal-directed behavior under the VI30 schedule, a more parsimonious explanation is the differential response requirements: the FR5 schedule required 5 lever responses for each reinforcer delivery, while the VI30 model required a single response after a time delay. Moreover, Jin and Costa (2010) showed that MSN phasic activity encoding the start and stop of an FR8 response sequence emerged with learning, particularly in the DLS and to a lesser extent in the DMS; this is consistent with DLS population activity at the 1st and 5th responses observed in Experiment 1. Additionally, DLS activation was associated with inactive lever responses as well as 2nd, 3rd, and 4th active lever responses, though these correlates could not be isolated due to their temporal proximity to other responses. Thus, phasic firing patterns in the DLS associated with operant responses may be common to drug and non-drug rewards.

Studies from West and colleagues have shown diminished DLS phasic activation during a motor task (Carelli *et al.*, 1997; Tang *et al.*, 2007; Tang *et al.*, 2009), which is apparently associated with more efficient task performance rather than habit formation (Tang *et al.*, 2009). In contrast, Kimchi and colleagues demonstrated elevated proportions of phasically active DLS neurons during the development of habitual responding (Kimchi *et al.*, 2009). An advantage of

the present study was that rats in the goal-directed and habitual models had the same amount of instrumental training, and we did not observe a greater proportion of phasic DLS neurons in the VI30 model overall.

Functional gradient from anterior DMS to posterior DLS

Previous studies observed an anterior-medial to posterior-lateral gradient of behavioral plasticity (Miyachi *et al.*, 1997; Corbit and Janak, 2010) and support the hypothesis that striatal control of behavior shifts from DMS to DLS with habit formation, but less is known regarding subregional shifts within the DMS. Goal-directed, action-outcome behavioral control clearly depends on the posterior DMS (e.g., Yin *et al.*, 2005; Corbit and Janak, 2010), and consistent with this finding, we observed phasic activation of relatively posterior DMS neurons to alcohol-associated cues in FR5-trained rats. In contrast, the role of the anterior DMS is less clear. Yin *et al.* (2005) found that permanent, pre-training lesions of the anterior DMS did not disrupt goal-directed behavioral control when tested after 8 days of training, while Corbit and Janak (2010) disrupted goal-directed behavior with repeated, acute inactivation of the anterior DMS over 3 days of training. Furthermore, post-training, pre-test inactivation of the anterior DMS disrupted goal-directed behavior after 2 weeks of training (Corbit *et al.*, 2012). While these apparently discrepant findings might simply be due to different lesioning techniques, another interpretation is that the anterior DMS is important for goal-directed behavioral control, but the posterior DMS can compensate for anterior DMS lesions if the rats are trained long enough in the absence of anterior DMS activity. In the present study, anterior DMS neurons displayed phasic firing patterns associated with start-of-session and reinforcement cues in FR5-trained, but not VI30-trained, rats. The persistent activation of the anterior DMS in the FR5-trained rats supports a role for this structure in goal-directed behavior that is sustained after extended training, while the lack

of anterior DMS activation in VI30-trained rats suggests that anterior DMS contributions diminish with habit formation. Finally, posterior DMS activity was also apparent in the VI30-trained rats, and we suggest that this sustained activity may be related to the ability of habitually trained rats to exhibit goal-directed behavioral control after DLS lesions (Yin et al., 2004). Future studies monitoring neuronal activity across a broader anterior-posterior range of the DMS and throughout the duration of training are needed to fully interpret these results.

The DLS excitations we observed at lever responses were predominately posterior in both VI30- and FR5-trained rats. The more posterior DLS electrodes overlapped with areas that have previously been shown to inhibit habitual behavior when lesioned (Yin et al., 2004), indicating that our electrodes were placed in a region linked to habit control. Moreover, the fact that the phasic firing patterns were similarly positioned in both reinforcement models suggests that the neural activity is related to well-learned motor responses (Miyachi et al., 1997) that may not manifest as habits during goal-directed behavior (e.g., in FR5-trained rats), but may be expressed as habits when DMS activity is reduced due to lesion (Yin et al., 2005, Corbit and Janak, 2010). One intriguing possibility is that alcohol itself may facilitate DLS activity, or more broadly, a shift from anterior DMS to posterior DLS neuronal activation, as alcohol exposure can promote habit formation (Corbit et al., 2012). This possibility will be addressed in Chapter 3 by comparison of groups self-administering alcoholic and non-alcoholic rewards.

VI schedule of reinforcement reduces functional heterogeneity in the dorsal striatum

Although population activity differed between DMS and DLS at operant events, we found that firing patterns of individual neurons overlapped between the regions. Interestingly, the 1st-response activity in the FR5 model showed the most discrepancy in activation between striatal regions, with more phasic neurons in DLS (65%) than in DMS (44%; compared to 55%

in each region in the VI30 model). We observed a greater degree of overlap in DMS and DLS firing patterns in the VI30 model, where twice as many DMS cells exhibited pre-excitatory activation to non-reinforced responses (characteristic response of DLS) and DLS cells displayed more brief post-reinforcement excitation (characteristic response of DMS) in the VI30- than in the FR5-trained rats. This finding may reflect increasing involvement of the DLS with development of habits or well-learned behaviors observed in other studies (Yin *et al.*, 2009; Kimchi *et al.*, 2009), and it extends these findings with observations of persistent involvement of DMS neurons.

The present data extend our knowledge of neuronal encoding during alcohol seeking by revealing regionally specific activity in dorsal striatum during two alcohol self-administration models that differ in behavioral flexibility. Future studies can address whether alcohol accentuates habit-related response patterns that are common to drug and non-drug rewards, and extend the correlative measurements reported here to mechanistic by using local pharmacology or optogenetic manipulations to disrupt regional phasic firing patterns. Human-subject studies confirm that differential activation of striatal regions accompany different aspects of reward learning and habit expression (Jenkins *et al.*, 1994; Tricomi *et al.*, 2009; Vollstadt-Klein *et al.*, 2010) as well as response to alcohol-associated cues (Filbey *et al.*, 2008). Dorsal striatal signaling, thus, is important for understanding the processes involved in reward-related learning and, by extension, addiction.

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CHAPTER 3: DOPAMINE D1 RECEPTOR BLOCKADE IMPAIRS ALCOHOL SEEKING WITHOUT REDUCING DORSAL STRIATAL ACTIVATION TO CUES OF ALCOHOL AVAILABILITY

Introduction

Cues can play a powerful role in addiction, triggering craving, drug-seeking, and relapse (Volkow et al. 2006, Corbit, Janak 2007, Le, Shaham 2002). In human functional MRI studies, alcohol cues activate both ventral and dorsal striatum (Filbey et al. 2008). In ventral striatum of rodents, alcohol-associated cues can trigger increases in neuronal firing rates (Janak, Chang & Woodward 1999, Robinson, Carelli 2008) as well as dopamine release (Weiss et al. 1993, Gonzales, Weiss 1998, Howard et al. 2009). Less is known of the neurobiology of dorsal striatal activity in response to alcohol-associated cues. However, the dorsal striatum receives spiraling, feed-forward input from the ventral striatum via midbrain dopamine neurons (Haber et al. 2000), and the dorsal striatum is known to be essential for updating reward value and for action selection (Haber, Fudge & McFarland 2000, Yin, Knowlton 2006, Devan, Hong & McDonald 2011).

The dorsal striatum is functionally heterogeneous, with the dorsomedial striatum (DMS, homologous to the primate caudate) required for learning relationships between actions and outcomes and the dorsolateral striatum (DLS, homologous to the primate putamen) necessary for stimulus-response associations, becoming increasingly engaged later in learning (Yin, Knowlton & Balleine 2005, Yin, Knowlton & Balleine 2006, Kimchi et al. 2009, Corbit, Nie & Janak 2012). These functions also depend on dopamine. Systemic D1 receptor antagonism with SCH23390 blocks the reinforcing effects of cocaine and reduces motivated behavior (Koob, Le

& Creese 1987, Weissenborn et al. 1996, Liu, Weiss 2002). In the DMS, antagonism of D1 receptors reduces the ability of a reward to modulate behavior (Nakamura, Hikosaka 2006). Additionally, interruption of the dopaminergic inputs to the DLS can prevent habit formation (Faure et al. 2005) and reduce habit-like cocaine seeking (Belin, Everitt 2008). Therefore, we hypothesized that dopamine transmission via D1 receptors in the dorsal striatum may directly modulate excitatory neuronal activation to alcohol-associated cues while reducing alcohol seeking.

To investigate engagement of the dorsal striatum by alcohol cues and during alcohol seeking, we previously performed *in vivo* extracellular electrophysiology during alcohol self-administration in rats and monitored neuronal firing patterns (Fanelli et al. 2013). We found that the DMS predominantly demonstrated phasic excitations to cues, while the DLS was activated around lever-press responses. Start-of-session cues elicited phasic activation of both DMS and DLS neurons and behavioral approach responses. Since the D1-expressing direct-pathway neurons in the striatum express D1 receptors and contribute to initiation of behavior while D2-expressing indirect-pathway neurons inhibit behavior (Freeze et al. 2013), we expected that D1 receptor antagonism would blunt the observed dorsal striatal activation. The present study tested the effect of the D1-like receptor antagonist SCH23390 (SCH) in rats with continued, stable operant behavior, from which DMS and DLS neuronal activity during typical self-administration training sessions was previously reported. SCH was administered prior to alcohol self-administration sessions during which we used electrophysiology to record neuronal activity in the DMS and DLS. Antagonism of D1-like receptors inhibited alcohol-seeking behavior and reduced basal firing rates without preventing neuronal excitations to alcohol-associated cues, suggesting an uncoupling of phasic neuronal encoding and behavioral responses. As addiction

can result in a hypodopaminergic state (Koob 2009, Morikawa, Morrisett 2010), enhanced signal to baseline ratios seen here after D1 receptor antagonism may be important for processing and adaptive learning in addiction.

Methods

Subjects

Adult male Long-Evans rats (250-300g) were purchased from Charles River (Raleigh, NC, USA) or Harlan (Indianapolis, IN, USA). Rats were individually housed under a 12h:12h light:dark schedule and received food and water *ad libitum* except for the first 5 days of operant training, when they were water restricted for 23hrs/day. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

General alcohol self-administration procedures

Rats underwent sucrose-fading procedures in order to self-administer 10% w/v ethanol. Rats were trained in one 30-min session each day, Monday - Friday, in custom-built Plexiglas operant chambers in sound-attenuating cabinets (MedAssociates, St. Albans, VT, USA) as previously described (Fanelli et al. 2013). Rats initially entered operant chambers 5min prior to the session start, and this habituation period was lengthened to 15min by the time of electrophysiological recordings, to allow the experimenter to set recording parameters and choose a differential reference. Important for this study, sessions began with the illumination of the house light and extension of the levers into the operant chamber 30s later; these stimuli signaled the start of the operant session and predicted alcohol availability. Reinforcer deliveries of 0.1mL were paired with a cue light located above the response levers.

Rats were trained to respond on an FR5 (every fifth lever-press response = 1 fluid delivery) or a VI30 schedule (after a variable interval averaging 30s has elapsed, the first response = 1 fluid delivery). Responses on only one lever (either right or left) triggered fluid delivery and cue light illumination, while the other lever was inactive (responses were recorded but had no programmed consequences). More details can be found in our previous report (Fanelli et al. 2013). Sessions ended after 25 reinforcements were earned or after 30 min, whichever came first.

Surgery

After at least 6 weeks of training, rats that maintained stable self-administration behavior were implanted with 16 stainless-steel, Teflon-coated electrodes (50µm diameter, 500µm apart; NB Labs, Denison, TX, USA) as previously described (Fanelli et al. 2013). Oriented anterior to posterior with electrodes linearly aligned, electrode arrays targeted the DMS (+0.2 to +2.0mm AP, ±1.7mm ML, -4.5mm DV from bregma) and the contralateral DLS (+0.2 to +2.0mm AP, ±3.4mm ML, -4.5mm DV), with sides counterbalanced across rats. After surgery, rats were given 15mg/kg ibuprofen daily for 3 days and allowed a week to recover.

Electrophysiology

Next, rats were habituated to the tether connecting the electrode arrays to the headstage assembly in operant chambers identical to the training chambers except that they were equipped for electrophysiological recordings. Recordings were analyzed from sessions acquired after operant behavior stabilized. Neuronal activity was recorded using a multichannel acquisition processor (MAP system; Plexon, Inc., Dallas, TX, USA). Neural activity was recorded simultaneously from the 16 electrodes (Sort Client software; Plexon, Inc.; for a complete description, see Fanelli et al. 2013). Briefly, a differential reference electrode was designated on

each array. Cell sorting was finalized after the experiment with Offline Sorter software (Plexon, Inc.). Automated clustering based on template analyses and principle component analysis (PCA) was manually adjusted, guided by observations made during data collection (Robinson, Carelli 2008, Fanelli et al. 2013). Signal-to-noise ratios ≥2 (online), distinct PCA clusters (offline), and physiological characteristics consistent with MSNs (i.e., ≤0.1% of spikes with interspike intervals <1ms and average firing rates <10Hz; Kimchi et al. 2009, Kish, Palmer & Gerhardt 1999) were required for inclusion of neurons in analyses.

SCH23390 effects on self-administration and neuronal activity

After initial electrophysiological recording of the baseline operant session as previously reported (Fanelli et al. 2013), electrophysiological data were recorded during operant sessions after administration of SCH23390 (Sigma-Aldrich, St. Louis, MO) or vehicle. Only rats that maintained stable lever-press behavior through the baseline recording session were included in this study. Inclusion criteria required that rats receive > 65% of the 25 available reinforcements prior to SCH testing (excluded after receiving < 65% for 3 consecutive sessions). SCH was dissolved in saline vehicle to achieve concentrations of 0, 10 or 20µg/kg in a final injection volume of 0.3 - 0.6mL. Doses were selected that were reported to reduce behavioral responses to cues associated with cocaine and not food-associated cues (Weissenborn et al. 1996). Rats received SCH doses (i.p.) 30min prior to start of session in a counter-balanced order, with a habituation injection of saline (0.9%) administered on a day prior to the first test. Specifically, as early experiments found that 20µg/kg often affected operant behavior on subsequent days, the majority of rats received saline and 10µg/kg SCH in randomized order, followed by the 20µg/kg dose. SCH test sessions were separated by at least two regular operant sessions.

Histology

Rats were anesthetized with ≥1.5g/kg of urethane (50% w/w in saline) before 10μA current was applied for 5s to each wire. Rats were perfused, and brains sectioned and stained as previously described to confirm electrode placement (Robinson, Carelli 2008).

Data analysis

Perievent histograms of firing rates were created using NeuroExplorer (Nex Technologies, Littleton, MA), and population analyses were completed using custom-written programs in MATLAB (The MathWorks, Inc., Natick, MA, USA). The average firing rates of all neurons in each region were aligned to each event and smoothed with a moving average of 250ms in 50ms steps. Normalized firing rates were calculated through division of each bin by the mean whole-session firing rate. Firing rates around start-of-session events are averaged for each cell, then within each region, and presented as mean ± S.E.

The average number of spikes in a target window—the 0.5s after an event (signal) — was compared to a baseline calculated from the 60s prior to the start of the session (i.e., prior to houselight illumination). The two start-of-session cues were expected to have similar effects on neuronal activity, and this was confirmed by Wilcoxon Signed Rank Test (**Supplemental table 3.1,** appendix 3.1); consequently, light and lever presentations were treated as two observations of the same event (cue signal). Neuronal activity from FR5- and VI30-trained rats were compared for firing rate (raw, non-normalized) and coefficient of variance in the baseline, as well as firing rates in the signals (averaged and individually) with 2-way ANOVA, and main effects were examined with Holm-Sidak posthoc multiple comparison method. These analyses yielded no significant effects of group (**Supplemental table 3.2**); consequently, the groups were combined for subsequent neuronal activity analyses. The effect of SCH dose on signal and baseline in DMS and DLS were tested by parametric multivariate regression analysis [GENMOD

procedure, with a Poisson-distribution regression model with repeated measures (RM) and using a log transform of time to account for differences in the time window for signal versus baseline]. Main effects, interactions and pairwise contrasts were compared with the Wald test (SAS, SAS Institute Inc., Cary, NC). Cell detection rates in each rat and brain region were compared by 2-way RM ANOVA. The proportion of individual neurons showing altered firing rates around events was calculated using z-scores comparing phasic frequency in the 0.5s after the cues to the prior 60s baseline. A significant change in firing rate occurred when $|Z| \geq 2$.

Operant behavioral data are presented as mean ± S.E. Latency to the first press, lever press responses, and reinforcements earned were compared across sessions with the Friedman ANOVA on ranks with repeated measures (Sigma Plot, Systat Software Inc, San Jose, CA). Posthoc contrasts were made with the Tukey test for multiple comparisons. Spearman rank order correlation examined the relationship between behavioral measures (latency to the first press, lever presses, and EtOH deliveries earned) and the number of action potentials during the signal and the baseline epochs (Sigma Plot).

Results

In order to investigate the contribution of D1 receptor activation to dorsal striatal neuronal firing in response to alcohol-associated cues, we administered 0, 10, and 20µg/kg SCH i.p. to 26 rats in a within-subject design (1 rat ceased tolerating the tether and did not undergo the 20µg/kg dose). For this and the previous study, 24 FR5 rats underwent surgery, 14 completed baseline recordings (Fanelli et al., 2013), and 11 rats met subsequent performance criterion and were included in this study. For the VI30 group, 21 rats underwent surgery, 16 completed baseline recordings (Fanelli et al., 2013), and 15 met performance criterion and were included in this study. We first tested whether training schedule affected firing rates at baseline and at

stimulus presentations. As shown in **Supplemental Table 3.2**, there was no effect of group on these firing rates, so data were combined across training groups. We next determined the effects of SCH on the number of putative MSNs detected and their basal firing rates. While fewer cells were detected per electrode wire in the DLS than the DMS (main effect of region: $F_{1,24}$=8.64, P<0.01), SCH did not significantly alter the number of neurons detected (main effect of dose: $F_{2,24}$=0.93, P=0.4, and dose by region interaction: $F_{2,24}$=0.53, P=0.6). The number of cells recorded per rat on a given day ranged from 1 to 9 neurons; see **Figure 3.1** for total cell numbers after each dose.

As the primary goal of this study, we analyzed phasic firing changes to stimuli associated with the operant session that were not contingent on the animals' behavior. Specifically, all alcohol self-administration sessions began with the illumination of the house light followed 30s later by the extension of the operant levers into the chamber, providing cues of alcohol availability that were independent of behavioral activity. Average neuronal firing rates around the presentation of these two cues (averaged across cue type) in the DMS and DLS across SCH doses are shown in **Figure 3.1** (a smaller time window is displayed in the insets, plotted as normalized firing rates). Firing rates increased at cue presentation (at time 0) compared to the frequency before and after, and these cue-related increases were larger in the DMS. Increases after cue presentation appear larger after SCH treatment, and baseline appears lower after both doses, particularly in the DLS.

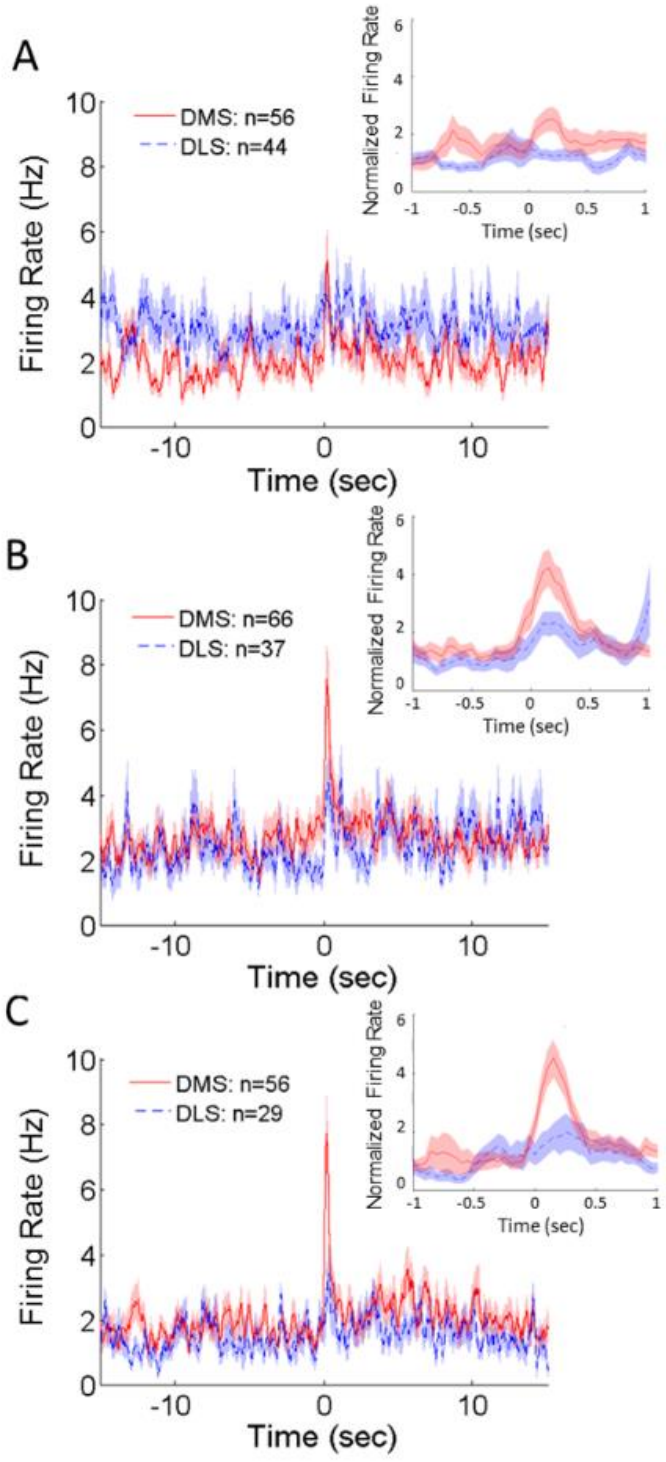

Figure 3.1: Neuronal firing rates (Hz) in the DMS (red, solid line) and DLS (blue, dotted line) aligned to start-of-session cues (at 0 s), averaged across the two cues. Mean firing rates (± SEM shaded) recorded during self-administration sessions following (A) saline, (B) 10µg/kg SCH, and (C) 20µg/kg SCH. Insets display a 2s window to focus on cues (at 0 s), with the firing rate of each neuron normalized to the whole session firing rate.

For statistical analysis, we compared firing frequency of DLS and DMS neurons during the 60s baseline period (B, immediately before the start of session) to the signal firing frequency (S, the 0.5s after each of the 2 cue onsets, entered as 2 observations of the same variable), as shown in **Figure 3.2A**.

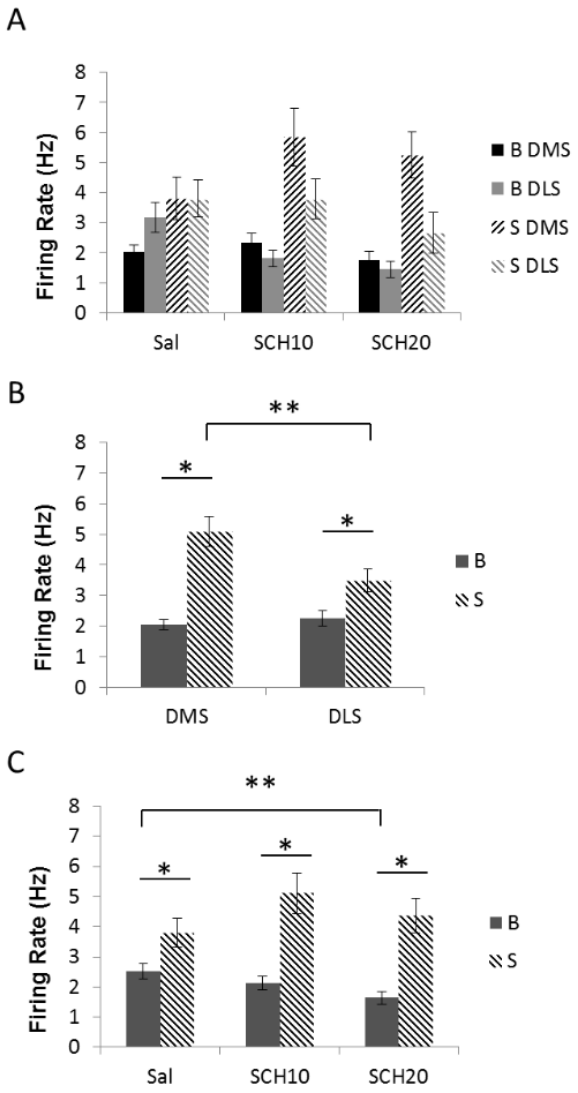

Figure 3.2: Mean neuronal firing rates during baseline in the 60s prior to cue onset (B, solid bars) and the mean signal in the 0.5s after each of the two cue onsets (S, hashed bars) are displayed (A) for the DMS (black) and DLS (grey) and grouped by dose: saline (Sal), 10µg/kg SCH (SCH10), and 20µg/kg SCH (SCH20). (B) Collapsed across dose, signal firing rates were greater than baseline (P<0.0001*), and the DMS signal was greater than the DLS signal (P<0.05**). (C) Collapsed across region, signal firing rates were significantly greater than baseline (P<0.001*), and baseline firing rates were significantly reduced under SCH20 (P<0.005**).

The GENMOD model of firing rates by time, brain region and dose yielded significant interactions of time by region (χ^2=9.59, P<0.005), and time by dose (χ^2=15.39, P<0.001), with no significant interaction of dose by region or 3-way interaction. To follow up on the time by region interaction, we collapsed across dose and compared firing rates in DMS and DLS in the signal and baseline. Firing rates were greater in the signal than in the baseline when collapsed across dose (main effect of time, χ^2=171.37, P<0.0001, see * in **Figure 3.2B**), and the signal was greater in the DMS than the DLS (χ^2=5.81, P<0.05, see ** in **Figure 3.2B**). To follow up on the time by dose interaction, we collapsed across region and compared firing rates during baseline and signal by SCH dose. Signal firing was significantly higher than baseline in all conditions (P<0.001, see * in Figure 2C). SCH treatment reduced basal firing activity, with a significant reduction in firing rate at 20µg/kg SCH (posthoc Sal vs SCH20, χ^2=9.31, P<0.005 see ** in **Figure 3.2C**). In contrast, SCH did not alter phasic excitations, as the firing rate during the signal was similar in all drug conditions (P>0.1).

To assess the effect of SCH on the proportion of neurons with phasically altered firing rates after start-of-session cues, we averaged responses to the two cues, again treating them as trials, and categorized each neuron's phasic activity by evaluating changes in firing with z-score statistics. The proportion of cells with significantly increased firing rates after the cues almost tripled after either SCH dose versus Sal (**Table 3.1**). Together, these data indicate that D1 receptor blockade generally reduced firing frequency during baseline, but not at cue onset, and thereby increased the relative excitation to non-contingent, predictive cues.

Table 3.1: Percent of individual neurons with significantly altered firing rates after cue presentations. Percent of total units in each region with $|Z| \geq 2$ for z-score comparison of the firing rate in the 0.5s after the cues (averaged responses to light and lever cues) to the 60 baseline prior to the first cue. In parentheses, number of significant neurons over total neurons recorded. Note that all neurons with significant changes in firing rate were excited, rather than inhibited.

SCH dose, µg/kg	DMS	DLS
0	11% (6/56)	5% (2/44)
10	30% (20/66)	14% (5/37)
20	32% (18/56)	14% (4/29)

After presentation of the predictive cues, the D1 receptor antagonist significantly lengthened the latency to the first press (**Table 3.2**; χ^2=15.6, P<0.001). Both SCH doses produced significantly longer latency compared to vehicle in posthoc contrasts (all P's < 0.05). Furthermore, there was a significant negative correlation between the number of action potentials in the 60s BL and the latency to the first press (Spearman rank order correlation, R=-0.309, P<0.05; Figure 3A); that is, the lower the basal firing rate of the dorsal striatal neurons, the longer the latency for a rat's initial lever press. No such relationship was found between the press latency and S, the neuronal activity after the start-of-session cues (R=-0.164, P>0.1).

Table 3.2: Behavioral measures from alcohol self-administration sessions after systemic SCH23390. Latency to the first press (s), active lever responses, EtOH deliveries earned, and inactive lever responses during alcohol self-administration sessions 30 min after administration of 0, 10 or 20µg/kg SCH.

SCH dose, µg/kg	Latency (s)	Active responses	EtOH deliveries	Inactive responses
0	36.0 ± 20.2	120 ± 15	22 ± 1	21 ± 6
10	297.9 ± 101.0[a]	31 ± 5[a]	9 ± 1[a]	6 ± 2[a]
20	566.5 ± 147.8[a]	15 ± 4[a]	5 ± 1[a]	6 ± 3

[a] P<0.05 versus 0 µg/kg dose

Active lever responding was significantly reduced by SCH (**Table 3.2**; χ^2=38.2, P<0.001). Post-hoc comparisons found that lever presses at each SCH dose significantly differed from vehicle (all P's < 0.05), with 75% and 88% reductions after 10 and 20μg/kg SCH, respectively. SCH also significantly reduced reinforcements earned (Table 2; χ^2=41.57, P<0.001), and posthoc contrasts found that reinforcements after SCH10 and SCH20 were significantly lower than after saline (all P's<0.05). Finally, inactive lever responses were also attenuated by SCH (χ^2=8.9, P<0.05), with inactive lever presses after SCH10 significantly fewer than after saline (P<0.05), though greater variability at SCH20 prevented a significant reduction from being detected at this dose (**Table 3.2**). These behavioral responses were also similar between rats trained on FR5 and VI30 schedules (**Supplemental table 3.3**). However, because the VI schedule results in a well-established reduction in the rate of reinforcements at baseline (Fanelli et al. 2013, Dickinson 1985), the effect of SCH on the number of reinforcements earned was less significant in this group (**Supplemental table 3.3**).

Active lever responding also significantly correlated with basal firing rates in the 60s prior to session start (R= 0.25, P=0.04; **Figure 3.3B**). As with latency, no correlation was found between active presses and neuronal firing rates after the cue signals (S; Ps>0.1). Inactive lever responding did not correlate with basal or signal neuronal activity, nor did the number of EtOH deliveries earned (Ps>0.05; **Figure 3.3C-D**).

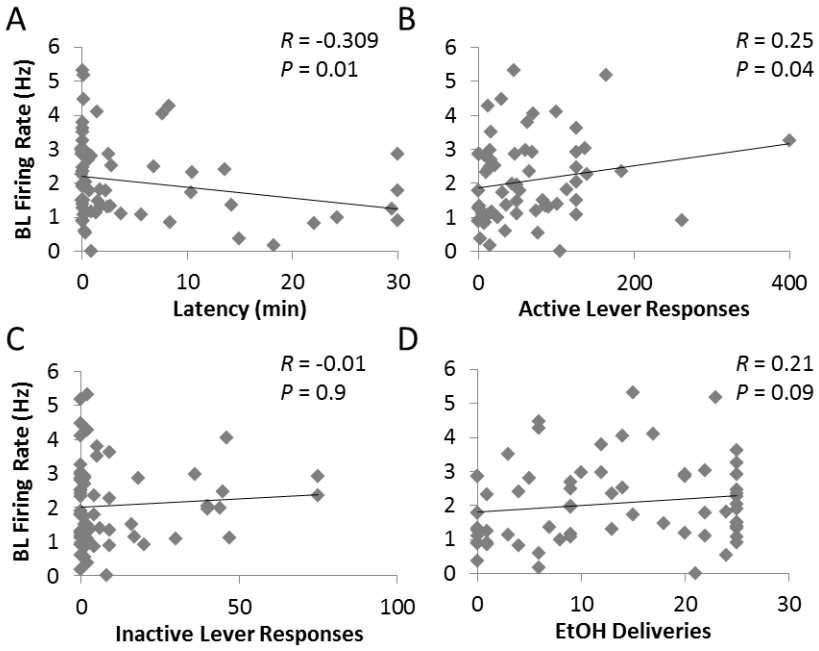

Figure 3.3: Baseline firing rates (BL, averaged per rat) significantly correlated with (A) latency to the first lever press and (B) the number of active lever responses during the alcohol self-administration sessions (*P*s<0.05). Therefore, alcohol seeking was slower to initiate and reduced in operant responses in rats with lower firing rates in the 60s prior to session start. (C, D) Baseline firing rates did not correlate with inactive lever responses or with EtOH deliveries earned during a session.

Discussion

We report here that systemic treatment with SCH23390 reduces both basal firing rates of DMS and DLS neurons and alcohol-seeking behavior without attenuating neuronal activation to alcohol-associated cues in rats with extensive alcohol self-administration experience. This treatment reduced inactive responding in a manner consistent with a reduction in effort, as hypothesized. However, we predicted that SCH and interruption of dopamine D1 transmission would reduce neuronal excitations to cues, but an increase in the proportion of significantly excited cells was observed. These findings suggest that dopamine modulates dorsal striatal neuronal activity by altering signal to baseline ratio, but is not necessary for neuronal excitations to well-learned cues. Furthermore, as observed in the correlation between the reduction in firing

and measures of alcohol seeking after D1-like receptor antagonism, blocking dopamine transmission may disrupt the link between cue recognition and the initiation of drug-seeking behavior.

Dopamine receptor blockade, systemically or in the ventral striatum, has long been known to suppress ethanol self-administration (Dyr et al. 1993, Hodge, Samson & Chappelle 1997). Reward-predictive cues generate dopamine release in the ventral striatum (Roitman et al. 2004, Day et al. 2007, Howard et al. 2009), suggesting that the actions of cues on craving and self-administration (Le, Shaham 2002, Volkow et al. 2006, Corbit, Janak 2007) may also depend on dopamine. For example, tonic dopamine measured by microdialysis increases after rats trained to self-administer alcohol are transferred to the operant chamber prior to ethanol availability (Weiss et al. 1993, Howard et al. 2009). Furthermore, phasic dopamine release in the ventral striatum has been measured proximal to neurons that showed phasic changes in firing rates (Cacciapaglia, Wightman & Carelli 2011, Cheer et al. 2005, Belle et al. 2013). Therefore, we hypothesized that dopamine release in the dorsal striatum may provide a mechanism for neuronal activation to reward-associated cues.

Since dopamine release to reward-associated cues has been demonstrated to initiate reward seeking (Steinberg et al. 2013), we expected that dopamine receptor blockade would increase latency to lever-press for alcohol and reduce alcohol seeking. Reward-associated cues are known to initiate reward-seeking behavior (Flagel, Akil & Robinson 2009, Berridge, Robinson 2003, Cardinal, Everitt 2004), and latency to behavioral response has been studied as a measure of behavioral motivation (Wise, Raptis 1985, Morita et al. 2013, Salamone, Correa 2002, Blackburn, Phillips & Fibiger 1987, Liu, Weiss 2002). Given the involvement of the dorsal striatum in action selection and reward seeking (Haber, Fudge & McFarland 2000, Yin,

Knowlton 2006, Devan, Hong & McDonald 2011, Balleine, Delgado & Hikosaka 2007), and previously reported correlations between dorsal striatal activation and behavior (Fanelli et al. 2013, Hassani, Cromwell & Schultz 2001, West et al. 1990, Kawagoe, Takikawa & Hikosaka 1998), we expected that neuronal responses to predictive cues in the dorsal striatum would be related to response latency and lever responding. Therefore, we analyzed neuronal responses to cues signaling the initiation of the operant session: houselight illumination and lever extension. These cues evoked similar responses in the dorsal striatum, as previously reported (Fanelli et al. 2013). We found that SCH reduced basal firing rates and not cue-evoked excitations. Moreover, lever-press latency was longer and active lever presses were reduced after SCH administration, and these measures were significantly correlated with basal, but not cue-evoked, firing rates in the dorsal striatum.

Since D1-expressing direct pathway neurons initiate behavior (Freeze et al. 2013) and increases in striatal firing rates caused by stimulation of dopamine neurons is inhibited by the D1-like receptor antagonist SCH (Gonon 1997), we treated rats with SCH prior to alcohol self-administration sessions. However, while SCH reduced basal neuronal firing, it did not prevent phasic activity to alcohol-associated cues. These data agree with prior studies demonstrating reductions in basal firing rates by SCH (Burkhardt, Jin & Costa 2009; Cheer et al. 2005), and extend to the dorsal striatum the finding that SCH delivered into the nucleus accumbens increases signal to baseline of phasic excitations through a reduction in baseline (Cheer et al. 2005). Why, then, is phasic dopamine release co-localized with phasically active medium spiny neurons (Cacciapaglia, Wightman & Carelli 2011, Cheer et al. 2005, Belle et al. 2013)? One likely explanation is that phasic excitations of firing, such as these activations to well-learned alcohol-associated cues, are facilitated by dopamine release but are primarily glutamatergic.

Moreover, the relatively slow time scale of dopamine's actions would promote plasticity and synaptic potentiation of fast glutamatergic synapses (Mangiavacchi, Wolf 2004; see review: Surmeier, Carrillo-Reid & Bargas 2011), rather than an instantaneous modulation of phasic firing. Future studies are necessary to determine whether extended dopaminergic blockade and reduction of basal firing rates would eventually result in reduced neuronal activation to cues, or whether it would prevent further plasticity under conditions requiring behavioral adaptation, such as changes in contingency or reward value. Importantly, the results described here demonstrate that phasic changes in neuronal firing rate are not necessary for changes in subsequent reward-seeking behavior; thus, dopamine may modulate behavior, as by maintaining baseline firing rates, independent of glutamatergic input to alcohol-predictive cues.

Nevertheless, there are a few caveats that are important to discuss. The use of a systemic antagonist treatment raises the possibility that effects were not specific to the dorsal striatum. For example, generalized dopamine blockade may cause non-specific motor impairment (1mg/kg; Gimenez-Llort); though 10µg/kg SCH was previously shown to reduce behavioral responses to cocaine-associated but not food-associated cues (Weissenborn et al. 1996). Herein, as inactive lever presses were reduced by SCH, there may have been a general motor effect impairing operant responding. SCH may also effect dopaminergic neurons in the midbrain, as it has been shown to increase dopaminergic output of the substantia nigra pars compacta (Carlson, Bergstrom & Walters 1986, Radnikow, Misgeld 1998), where dopamine release would have an amplified effect on D2 receptors (given D1 receptor blockade). D2 receptor activation would, therefore, increase autoreceptor function in addition to activation of the inhibitory indirect pathway, generally reducing movement. SCH thus may indeed reduce dopamine contributions to the dorsal striatum as well as more generally throughout the brain (Belle et al. 2013, Glovaci,

Caruana & Chapman 2014), and compromised dopamine transmission is associated with deficits in initiating voluntary motor behavior without an external stimulus (Jahanshahi 1998, Choi, Balsam & Horvitz 2005). Another possible extra-striatal mechanism is the antagonism of D1 receptors in the PFC that may reduce top-down inhibition of striatal cue activation (reviewed in Feil et al. 2010), thereby maintaining phasic, excitatory input to cues that no longer produce an effective behavioral response. Indeed, this may explain the increase in cue-induced neuronal activation after SCH. Supporting this mechanism, muscimol inactivation of the mPFC can increase cue-induced excitations in firing rates in the VTA (Jo, Lee & Mizumori 2013). Additionally, SCH effects may occur through other receptors, such as D5 receptors (Bourne 2001) that can colocalize with GABA receptors (another possible mechanism of increased cue responses seen here; Liu et al., 2000). SCH is also a 5HT2 and 5HT1C receptor agonist, though with 10-fold lower affinity (Bourne 2001). Future studies utilizing optogenetic approaches can elucidate the specific role of striatal D1 direct pathways in dorsal striatal encoding of alcohol-associated cues. Thus, while the specific role of dorsal striatal D1 receptors is unclear, we find it interesting that this systemic manipulation was not sufficient to blunt dorsal striatal cue responses.

The specificity of SCH to affect the direct pathway may account for the reduction in alcohol-seeking behavior observed here, which is not reflected in the behavior of individuals with alcohol use disorder who may be in a hypo-dopaminergic state (Koob 2009, Morikawa, Morrisett 2010). Indeed, systemic D1 antagonism can increase tonic DA levels as measured by microdialysis in the DMS (Kurata, Shibata 1991), presumably resulting in enhanced D2 receptor activation. The direct (D1) and indirect (D2) pathways act in parallel, with neurons of each pathway firing in synchrony, such that D1-expressing neurons activate specific action pathways

while D2-expressing neurons deactivate competing pathways (Isomura et al., 2013; Yin et al., 2009). Thus, it is possible that the neuronal excitations to alcohol cues observed here may emanate from D2-expressing MSNs. However, this would not explain the increase in the proportion of responsive neurons, since we would also expect D2-expressing indirect pathway neurons to have been active at baseline. Nevertheless, the reduction in alcohol-seeking behavior shown here may result from tipping the scales between the D1/D2 pathways, as blocking only the D1 pathway would result in predominance of the D2 inhibitory pathway. Future investigations will manipulate D2 receptor activation, as antagonism of D2 receptors may reduce alcohol seeking (Weissenborn et al. 1996, Corbit, Nie & Janak 2014) while exerting bidirectional effects on neuronal activity in the dorsal striatum, since pre- and post-synaptic D2 receptors differ in function (Seeman, Van Tol 1994, De Mei et al. 2009, Beaulieu, Gainetdinov 2011, Anzalone et al. 2012). We expect that higher doses of D2 antagonist, which might effect less efficient post-synaptic D2 receptors, would not affect dorsal striatal response to cues, replicating the effects observed in this study. Meanwhile, lower doses of D2 antagonist may have a greater impact on high-efficiency pre-synaptic receptors, resulting in increases in dopamine neuronal activity and increases in neuronal activation to cues in the dorsal striatum.

Activation to alcohol-associated cues was found in both medial and lateral regions of the dorsal striatum. In a previous experiment, we observed increased population activity in both DMS and DLS to noncontingent, start-of-session cues (Fanelli et al. 2013), and that finding is replicated here. These phasic activations were significantly larger in the DMS, where prior studies have identified neuronal activity related to associative processing (Rolls 1994, White, Rebec 1993). While similar neuronal activation might have been evoked by any novel stimulus, previous reports found that dorsal striatal neuronal excitation to a reward-predictive stimulus is

amplified to be detectable at the population level only after extended training (Kimchi et al. 2009), whereas habituation would be expected to the repeated presentation of neutral stimuli. Future studies can examine the development of cue-evoked excitations during acquisition and maintenance of operant self-administration. DMS activity observed here may, therefore, reflect encoding of the association of these cues with the initiation of the alcohol self-administration sessions, consistent with the role of the DMS in flexible, goal-directed behaviors (Yin et al. 2005).

Previous studies have demonstrated that DLS activation is related to motor behavior (West et al. 1990) and the DLS is required for habitual behavior (Yin, Knowlton & Balleine 2006), defined as actions driven by stimulus-response associations (Devan, Hong & McDonald 2011, Belin et al. 2009). Therefore, DLS activation seen here may reflect the ability of anticipatory cues to initiate habit-like approach behavior. While the inhibition of operant behavior was too profound to examine other motor responses here, studies are underway to examine the effect of local dopamine antagonists delivered into the DLS, unilaterally and bilaterally, on dorsal striatal activity around explicit motor responses such as unreinforced compared to reinforced VI30 lever press responses.

In conclusion, the finding that systemic dopamine D1 receptor antagonism reduced alcohol seeking without affecting phasic cue-related activity has implications for studies of addiction and motivated behaviors. While the electrophysiological data demonstrate that dopamine is not acutely necessary for neuronal activation to conditioned stimuli, the behavioral data suggest that dopamine is important in linking these responses to behavioral activation. Studies of clinical populations with addiction disorders have shown that striatal reactivity to alcohol cues correlates with addiction severity (Filbey et al. 2008), and the reduction in D2

receptor availability in the dorsal striatum in response to cocaine-associated cues correlated with self-reported craving (Volkow et al., 2006). The results of this study suggest that activation of the D1-expressing direct pathway may be responsible for cue-induced drug seeking.

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CHAPTER 4: DOPAMINE RECEPTOR ANTAGONISM REDUCES HABITUAL ALCOHOL SEEKING AND DORSAL STRIATAL NEURONAL ACTIVATION SPECIFIC TO ALCOHOL

Introduction

A goal-directed behavior is controlled by association of an action with its outcome and can adapt flexibly to changes in outcome value. Eventually, however, repeated performance can result in the development of inflexible, habitual behavior (reviewed in: Dickinson 1985, Yin, Knowlton 2006, Hilario, Costa 2008, O'Tousa, Grahame 2014). Habitual behavior is controlled by sensorimotor circuits including the dorsolateral striatum (DLS in rodents, putamen in primates; Yin, Knowlton & Balleine 2004, Tricomi, Balleine & O'Doherty 2009). In contrast, distinct associative circuits are engaged by goal-directed behavior, which include the dorsomedial striatum (DMS in rodents, caudate in primates; Yin, Knowlton & Balleine 2005, Corbit, Janak 2010). While the automatic control of behavior by sensorimotor circuits conserves cognitive resources, behavior that no longer responds to changes in reward value can be maladaptive, as in the case of addictive behavior. Indeed, alcohol use disorder is characterized by drinking despite negative consequences (American Psychiatric Association 2013). Understanding the contribution of alcohol to dorsal striatal activation during habitual behavior is, therefore, important for future prevention and treatment of addiction.

Previous research has shown that alcohol can promote the transition to habitual seeking (Dickinson, Wood & Smith 2002, Corbit, Nie & Janak 2014, Mangieri, Cofresi & Gonzales 2012, Corbit, Nie & Janak 2012, but see Samson et al. 2004). While the effect of alcohol in the dorsal striatum is unknown, DLS dopamine is necessary for habit formation (Faure et al. 2005)

and for habit-like second order responding for cocaine (Belin, Everitt 2008). Furthermore, dopamine release time-locked to cocaine-associated stimuli increases in the DLS over the course of cocaine use (Willuhn et al. 2012), potentially increasing DLS-mediated, habitual control over behavior. Alcohol may have similar effects on dorsal striatal dopamine, given that dopamine receptor antagonism systemically or in the ventral striatum reduces alcohol self-administration (Dyr et al. 1993, Hodge, Samson & Chappelle 1997, Fanelli & Robinson submitted [Chapter 4]), and alcohol is known to elicit dopamine release in the ventral striatum (e.g., Gonzales, Weiss 1998, Robinson et al. 2009). Therefore, a possible mechanism for alcohol to promote habit is through dopamine transmission in the DLS.

In a previous study, we recorded neuronal activation with extracellular electrophysiology in the DMS and DLS during alcohol self-administration (Fanelli et al. 2013). While the DMS showed time-locked, phasic changes in neuronal firing rates around the time of cue presentation and reinforcement delivery, DLS neuronal activity occurred primarily to lever press responses. We also compared a fixed-ratio 5 (FR5) schedule and a variable-interval 30s schedule (VI30), because the latter schedule, which has a lower perceived contingency between action and outcome, promotes habit formation (Dickinson, Nicholas & Adams 1983, Mangieri, Cofresi & Gonzales 2012). The VI30 schedule resulted in more posterior DMS and overall greater DLS encoding of reinforcement delivery (Fanelli et al. 2013). A subsequent study of the effect of dopamine on start-of-session cues showed that systemic dopamine D1 receptor antagonism reduced alcohol seeking as well as basal firing rates of dorsal striatal neurons, but not neuronal activation to the cues (Fanelli & Robinson submitted, Chapter 4). However, the effect of alcoholic versus nonalcoholic rewards on dorsal striatal encoding of habitual behavior and the role of dopamine therein remains unknown.

To address this, we trained rats to self-administer saccharin (Sac) or saccharin + ethanol (Sac/E) on a VI30 schedule to test the effect of alcohol on habitual seeking behavior, dorsal striatal encoding, and the capacity of dopamine receptor antagonism to reverse these effects. Satiety-specific devaluation tested progress of habitual behavior before surgery. We used extracellular electrophysiology to record firing rates of individual DMS and DLS neurons under 3 conditions: during self-administration, after unilateral infusion of the non-specific DA antagonist α-flupenthixol proximal to the DLS electrodes, and after bilateral infusion of α-flupenthixol following contingency degradation testing of habitual behavior. We hypothesized that alcohol would promote habitual behavior and that rats self-administering alcohol would express greater DLS activation. Furthermore, dopamine receptor antagonism was predicted to reduce this DLS activation when administered unilaterally, and to inhibit habitual behavior when administered bilaterally. Finally, if DMS and DLS are indeed acting in competition, DLS dopamine antagonism would increase DMS activation during self-administration.

Methods

Self-Administration Training

Male Long-Evans rats (250-300g) were purchased from Harlan (Indianapolis, IN, USA) and housed individually with *ad libitum* food and water except during initial operant training. Experimental procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

A timeline of all experimental stages is displayed in **Table 4.3**. Self-administration training was conducted in Med Associates operant chambers (St Albans, VT, USA) as previously

described (Fanelli et al. 2013), and as detailed in the Supplemental Methods (Appendix 4.1).

Table 4.3. Timeline for self-administration training and all experimental stages.

Experiment stage	**Day (week)**	**Session details**
Training	d1-20 (week 1-4)	increasing work requirements and %alcohol
Maintenance	d21-31; 33-34 (week 5-7)	VI30 (30min)
Satiety-specific Devaluation	d32, 35 [42, 45*] (week 7 [&9*])	5min extinction after 1hr solution pre-access
EPhys Chamber Habituation	d36-40 (week 8)	VI30 (30min)
Surgery & Recovery	d41-45 (week 9)	
Sessions with electrophysiological recordings		
Self-administration	d46-49 (week 10)	VI30 (30min)
Unilateral Infusions	d50-55 (wk 10-11)	1hr VI30, infusion after 10^{th} reinforcement in sessions 48hrs apart, intermediary sessions were standard VI30 (30min)
Pre-extinction Test	d56 (week 11)	10min extinction & 40min VI30
Contingency Degradation	d57-58 (week 11-12)	RT30
Post-extinction Test 1	d59 (week 12)	10min extinction & 40min VI30
Contingency Degradation	d60 (week 12)	RT30
Post-extinction Test 2	d61 (week 12)	10min extinction & 40min VI30

*repeated 2wks later if not habit-like in week 7 (see Methods), all procedures from surgery onward delayed 1wk

delivery. Rats were trained to self-administer either 0.2% saccharin (Sac) or 0.2% saccharin/15% ethanol (w/v) solution (Sac/E) on a VI30 schedule (after a variable interval with an average duration of 30s had elapsed, 1 response = 1 fluid delivery), according to the timeline in

Supplemental table 4.1. Inactive lever press responses were recorded but had no programmed consequences. After lever press acquisition, sessions were limited to 30min, and after the 19th session, rats were limited to a maximum of 25 reinforcements per session to better equate reinforcement history across rats. Blood ethanol content (BEC) was determined from a tail blood sample collected after the final self-administration session prior to surgery. In order to assess behavioral flexibility, a 2-part satiety-specific devaluation test was conducted on week 7 as previously described (Chapter 2, Hay et al. 2013). Briefly, rats were allowed 1hr free access to 2% maltodextrin (malto) or their reinforcer solution (in counterbalanced order, separated by 2 VI30 sessions), prior to a 5min extinction test. All rats were transitioned to operant chambers equipped for electrophysiological recordings at the start of week 8. If self-administration behavior was found to be flexible during week 7 (>20% reduction in responding after free access to reinforcer solution compared to after maltodextrin), an additional satiety-specific devaluation test was conducted during week 9 (N= 4 Sac, 10 Sac/E).

Surgery and electrophysiology

Rats were anesthetized with isoflurane and two 26-gauge guide cannulae were stereotaxically implanted bilaterally directly above the DLS (+0.2mm AP, ±4.0mm ML, -3.5mm DV from bregma). Additionally, 16 Teflon-coated electrodes (50-μm diameter) were implanted. A linear 8-electrode array was aimed at the DMS (+0.2mm AP, ±1.7mm ML, -4.5mm DV from bregma) in an anterior-posterior orientation, and a circular 8-electrode array, encircling the guide cannula, was aimed at the ipsilateral DLS (+0.2mm AP, ±4.0mm ML, -3.5mm DV from bregma) with the left/right side counterbalanced across rats. Following surgery, rats were given 15mg/kg of ibuprofen daily for 3 days and were allowed 1 full week of recovery before returning to the operant chambers.

Rats were habituated to a flexible tether that connected to both electrode arrays. This tether remained connected for all future operant sessions, which were run 6d/week. Before the initiation of each session, the operant chamber remained dark for 15min, allowing for the selection of a differential reference. Neuronal activity was measured using a multichannel acquisition processor (Plexon, Inc., Dallas, TX, USA), as described in (Fanelli et al. 2013) and the Supplemental Methods.

Unilateral Microinfusions

All post-surgery infusions were administered into the DLS through a 33-gauge injector extending 1-2mm past the cannula and level with the tips of the adjacent electrodes. Unilateral infusions were performed during the operant session, starting after the 10^{th} reinforcement. The fluid line screwed onto the guide cannula, allowing the rats to move freely around the chamber during infusions. On the sham day, injector needles with empty fluid lines were inserted into the guide cannulae and removed after 12min. In counter-balanced order, we infused 0.5µL phosphate-buffered saline (VEH, pH = 7.4), 5µg (5FLU), or 15µg (15FLU) α-flupenthixol dissolved in VEH at a rate of 0.05µL/min (10min total; du Hoffmann, Kim & Nicola 2011). Injector needles remained in the guide cannulae for another 2min to allow for diffusion and then were removed to curtail leaks. These sessions were extended to a maximum of 40 reinforcements or 1hr to maximize recording of reinforcements in the post-infusion epochs. One standard VI30 session (25 reinforcements/30 min) occurred between each infusion day.

Contingency Degradation Testing and Bilateral Pre-Session Infusions

The contingency degradation procedure was adapted from a prior protocol (Fanelli et al. 2013, Shillinglaw, Everitt & Robinson 2014) and outlined in **Table 4.3** (d56-d61). A second

post-degradation test was added to allow analysis of the within-subject effect of FLU. Infusions were performed bilaterally and only DMS recordings were made; therefore, pre-session infusions were used with a faster infusion rate (0.25μL/min for 2min; needles removed after another 2min). On day 1, a bilateral sham treatment was performed (PRE/Sham test), in which injectors were inserted into both DLS cannulae and the rat was loosely held by the handler for 4min. A 10-min extinction test then established pre-degradation baseline performance, and was immediately followed by 30min of self-administration on a VI30 reinforcement schedule to maintain behavior. All post-degradation sessions likewise included a 10min extinction and a 30min VI30 period, allowing comparison of reinforced behavior after contingency degradation and bilateral infusions. On days 2 and 3, sessions of contingency degradation training were performed in which action-outcome contingencies were disrupted by deliveries of the reinforcer on a random time 30s schedule (RT30) for 30 min. On day 4, the first of two post-degradation extinction/VI30 sessions was conducted following a pre-session bilateral infusion of either VEH or 15FLU (counter-balanced). Day 5 consisted of a third degradation training session, and day 6 of the second post-degradation VEH or FLU infusion and extinction/VI30 session.

Following the conclusion of experiments, all rats were euthanized and brain tissue was analyzed to verify electrode placement (Robinson, Carelli 2008).

Data Analysis

Behavioral data are presented as mean± SEM and statistics were calculated with Sigma Plot (Systat Software Inc, San Jose, CA) by using Mann-Whitney U tests or RM ANOVA. For analysis of behavioral flexibility, change scores were calculated for devaluation and degradation, respectively: (control-devalued)/control or (pre-post)/pre. Lever-press responding after satiety-

specific devaluation determined behavioral flexibility after training, prior to surgery. Lever press responding during extinction sessions following contingency degradation training evaluated the influence of alcohol and dopamine receptor antagonism on behavioral flexibility. Behavior during habit tests was compared across solution and devaluation or degradation exposure by using 2-way RM ANOVA with the Holm-Sidak method for multiple comparisons. The effect of unilateral infusion on lever presses was similarly examined with 2-way RM ANOVA (Sigma Plot, Systat Software Inc, San Jose, CA).

All electrophysiological results discussed here were recorded during the VI30 schedule and were aligned to lever-press events with NeuroExplorer software (Nex Technologies, Littleton, MA, USA) and analyzed with custom-written programs in MATLAB (MathWorks, Inc., Natick, MA, USA). Firing rates were averaged across trials, and since there were typically many more non-reinforced than reinforced lever responses, we selected 25 non-reinforced trials evenly distributed throughout the session to compare with reinforced trials. The firing rate of each neuron was normalized by dividing by its average firing rate across the whole session (except for unilateral infusion sessions, when firing rates were normalized to the average prior to infusion). Neuronal activity in the population of neurons in each region were displayed by averaging across neurons in each region, and smoothing with a moving average of 250ms in 50ms steps. The firing rates of neurons in the DMS or DLS in the second before or after selected unreinforced or reinforced lever presses were compared between groups with a Mann-Whitney U test (MWU; Sigma Plot).

Activation of individual neurons to operant events was analyzed by calculating z-scores comparing the firing rate in the second before or the second after the event to a 2s baseline beginning 14s previously (determined as a time when firing frequency typically equaled the

whole-session firing rate, thus normalized firing rate=1). Neurons with significant z-scores ($-2 > z > +2$) were grouped into a category based on the period of greatest change (pre-press or post-press) and direction of change (excitatory or inhibitory), resulting in 5 categories: pre-excitatory (PreEx), pre-inhibitory (PreIn), post-excitatory (PostEx), post-inhibitory (PostIn), and non-phasic (NP). For each response category, the number of neurons detected per wire in each rat was compared between reinforcers (or groups based on behavioral flexibility) by MWU (Sigma Plot). The relationships between neuronal firing rate and performance on the degradation test were analyzed with Spearman Rank Order Correlations (Sigma Plot). Peri-event activity plots are displayed for the 10 most and the 10 least habitual rats, as determined by degradation score. Electrophysiological differences between groups were further analyzed by comparing anterior to posterior DMS neurons, with the division at +1.2mm anterior to bregma (Fanelli et al. 2013) using a 2-way ANOVA.

DLS neuronal activity after unilateral infusions proximal to the electrodes was analyzed from units on wires without a significant reduction in firing rate after PBS infusion, as a precaution to control for potential mechanical displacement of electrodes by the infusion. If no unit was detected on a wire on the PBS infusion day, that wire was not analyzed for the other treatment days. To analyze bilateral infusion sessions, population peri-event firing graphs and graphs of neuronal firing within each z-score category of activation were plotted as before.

Results

Satiety-Specific Devaluation: confirmation of habit-like behavior

Thirty-one rats (16 Sac & 15 Sac/E) developed stable operant self-administration. On the final session prior to surgery, Sac/E rats achieved BECs of 62±7mg/dl, consuming 0.84±0.02g/kg alcohol. Satiety-specific devaluation tested behavioral flexibility after 7 and/or 9 weeks of self-administration. While the Sac/E group exhibited somewhat greater reductions after reinforcer access, only a main effect of pre-access solution was observed at this time point, thus neither group demonstrated habit-like behavior (2-way RM ANOVA; $F_{1,14}$=12.27 , P<0.005). Analysis of the final devaluation test for each rat by RM ANOVA confirmed that both groups exhibited habit-like reward-seeking behavior prior to surgery (2-way RM ANOVA; $F_{1,14}$=12.27 , P<0.005*; **Figure 4.1)**.

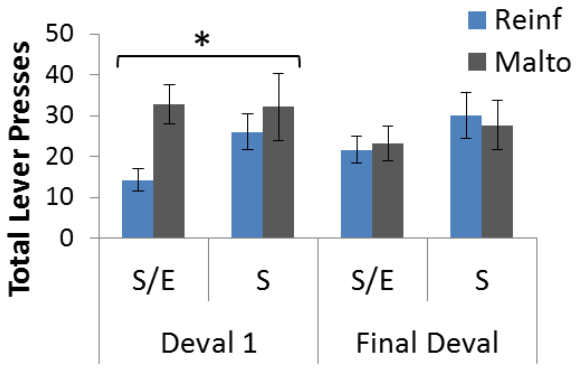


Figure 4.12: Lever responses (±SEM) under extinction conditions following 1hr free access to either the reinforcer (Reinf) or maltodextrin (Malto). Significantly fewer presses after reinforcer access indicate goal-directed behavior after the first devaluation in both groups (2-way RM ANOVA, P<0.005). All rats showed habit-like behavior in the final devaluation before surgery.

Electrophysiological recordings were analyzed from electrodes histologically confirmed to be in the DMS or DLS (**Figure 4.2**). On the initial recording day, Sac rats made 3±1 inactive and 105±15 active lever-press responses, receiving 24-25 reinforcements (15/16 rats received all

25). Sac/E rats responded at similar levels, with 6±4 inactive and 91±10 active responses, receiving 17-25 reinforcements (9/15 rats earned 25) and consuming 0.76±0.03g/kg ethanol.

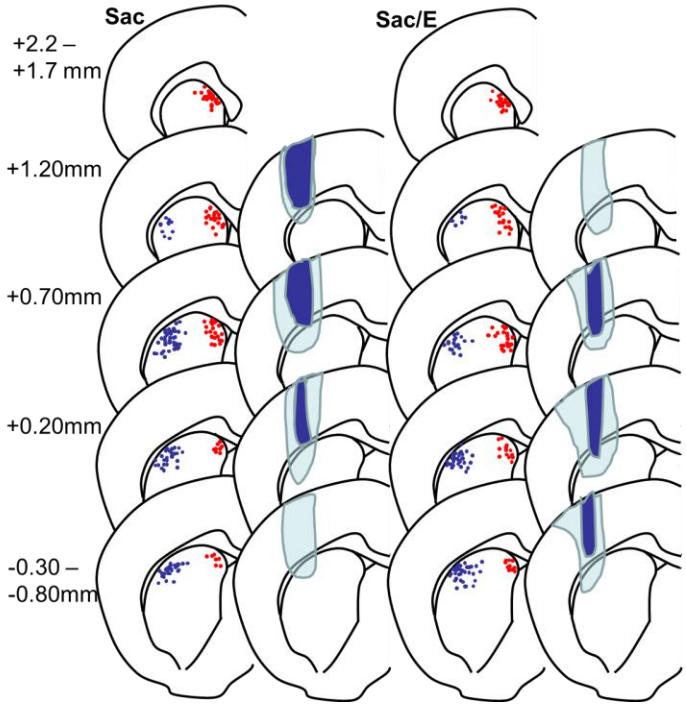


Figure 4.12: Final electrode placements in Sac and Sac/E rats in the DMS (red dots) and DLS (blue dots) on coronal slices with anterior/posterior position displayed relative to Bregma (adapted from Paxinos, Watson 1998). The area of damage from cannulae placements across all rats is lightly shaded, and an example of damage from one Sac and one Sac/E rat is displayed in dark shading.

Ps>0.05). However, compared to only 9% of Sac DMS neurons, 21% of Sac/E DMS neurons showed significant PreEx activation (more PreEx neurons/wire/rat in Sac/E than Sac; *MWU*=68.5, *P*<0.05). This alcohol-associated amplification in the proportion of DMS pre-response selective neurons was also observed before reinforced lever presses (**Figure 4.3D-F**; *MWU*=55.5, *P*<0.01). Sac rats showed significantly more DMS PreIn than Sac/E rats (unreinforced: MWU=70.5, P<0.05; reinforced: MWU=83.0, P<0.05), although few inhibitory neurons were observed overall. Unlike unreinforced presses, increased firing rates immediately

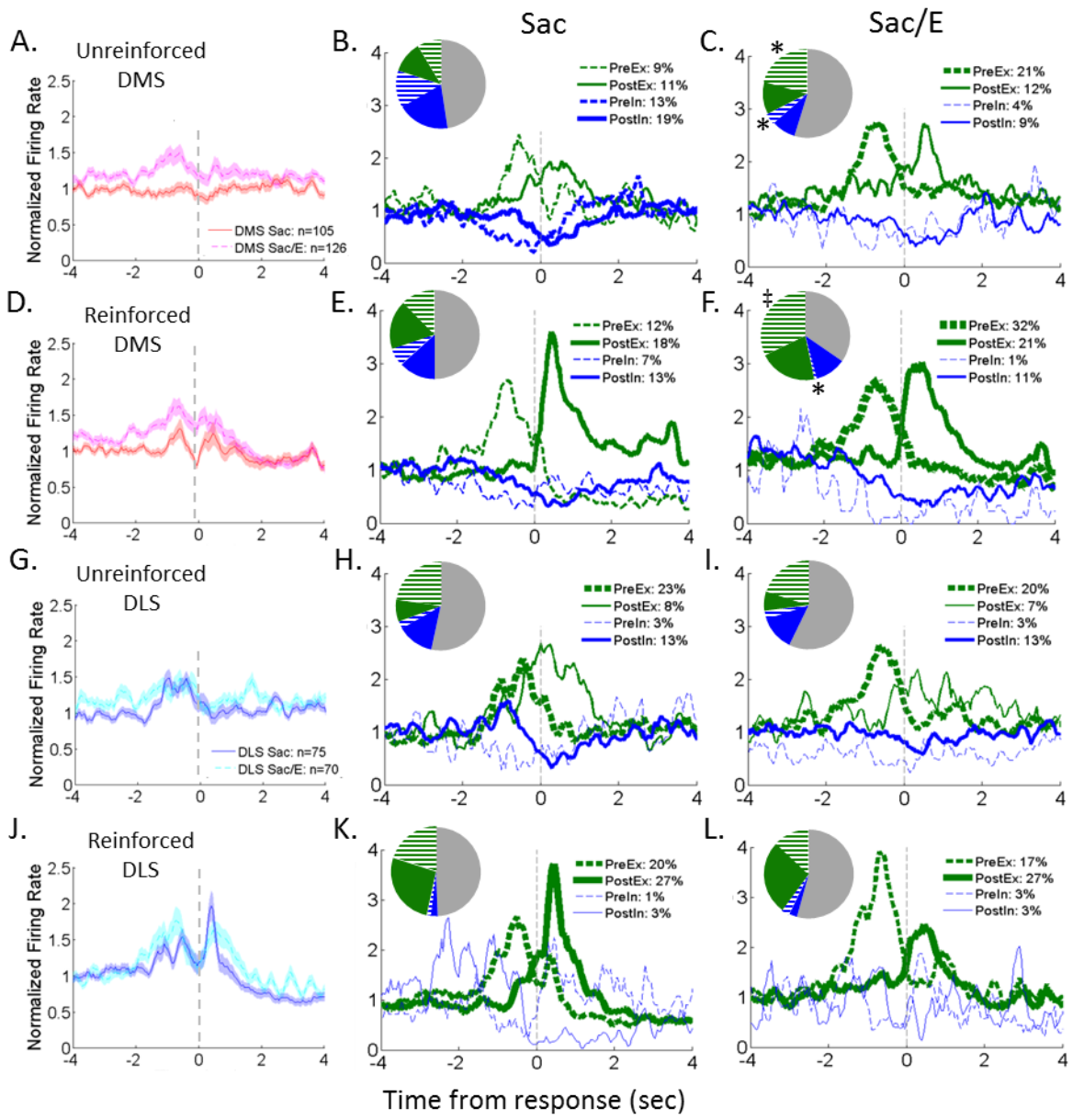


Figure 4.13: DMS and DLS electrophysiological activity in rats self-administering Sac or Sac/E around unreinforced or reinforced press responses. (A) Population firing rates in DMS (mean, SEM shaded) were more altered in SacE than Sac rats around unreinforced presses and (B-C) the proportion of neurons in the PreEx and PostIn categories of phasic changes significantly differed between reinforcer groups (#neurons/wire/rat; PreEx: P<0.05*; PreIn: P<0.05*). (D-F) The same analyses are displayed for the DMS around reinforced presses (P<0.01‡", P<0.05*"), and (G-I) for the DLS around unreinforced and (J-L) reinforced presses. No differences between reinforcer groups were observed in the DLS. Categories of phasic activity defined by significant excitation or inhibition in the second before or after a lever press (PreEx: pre-excitatory; PreIn: Pre-inhibitory; PostEx: post-excitatory; PostIn: post-inhibitory; NP: non-phasic). Line thickness is proportional to the percentage of cells in a category. Pie charts display proportion in each category, with NP units in gray. N's indicate number of neurons in a subregion.

followed reinforced presses in DMS neurons of both Sac and Sac/E rats. No differences in PostEx or PostIn were observed between reinforcers.

In the DLS, both Sac and Sac/E rats displayed increases in neuronal firing prior to unreinforced lever-press responses (**Figure 4.3G-I**). No differences were observed in DLS firing patterns between groups, either in firing rates of neurons with each activity pattern or in proportion of neurons exhibiting each firing pattern. When comparing DLS activity around reinforced presses (**Figure 4.3J-L**), the average amplitude varied between Sac and Sac/E groups, but these differences were not significant due to variability across individual neurons (PreEx: MWU=2272.0, P=0.16; PostEx: MWU=2492.0, P=0.6). Moreover, the proportions of neurons exhibiting specific firing patterns were nearly identical between groups. Thus, while we expected alcohol to increase DLS activation, we found instead more DMS pre-press excitations and fewer pre-press inhibitions in rats drinking alcohol compared to saccharin. As unreinforced and reinforced presses evoked similar neuronal activity pre-press, and reinforced presses resulted in additional activation post-press, subsequent analyses were confined to reinforced presses.

The relationship between behavioral flexibility and neuronal encoding of self-administration was examined by Spearman Rank correlation of the proportion of cells in each activity category around a reinforced press (per wire per rat) and the degree of habitual responding as indicated by the degradation and devaluation tests. DLS reinforced press PostEx (#detected/wire/rat) was found to correlate with the percent change on the contingency degradation test (R=0.43, P<0.05; **Figure 4.4A**). No such relationship was found between neuronal activation and the earlier satiety-specific devaluation test (i.e., DLS PostEx: R=-0.08, P=0.69). Neuronal firing rates were plotted for the 10 most habitual and the 10 least habitual rats, by contingency degradation score, collapsing across reinforcers (**Figure 4.4B-E**).

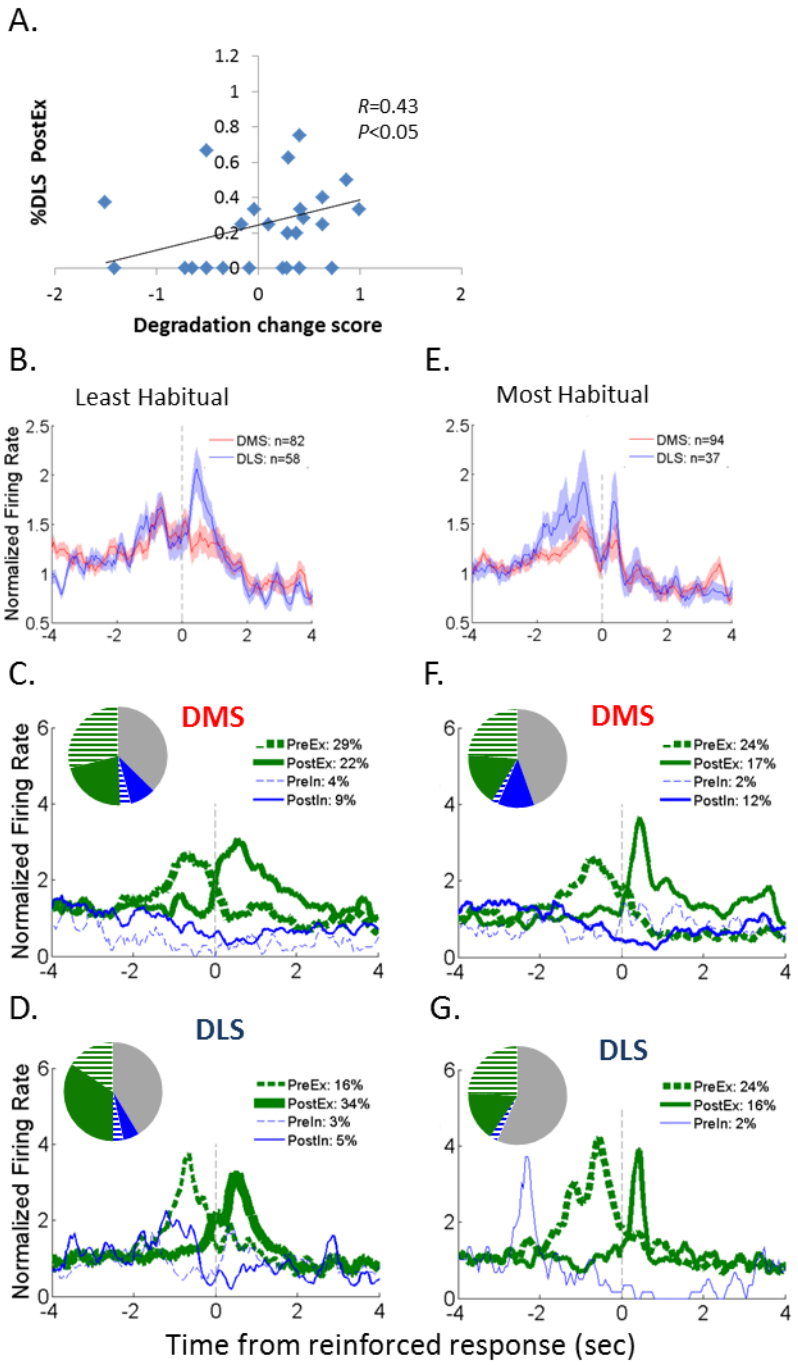


Figure 14.4: The same electrophysiology data from figure 4.2 are shown organized by behavioral flexibility. (A) Proportion of PostEx neurons (per wire per rat) in the DLS significantly correlated with the contingency degradation change score: # lever presses (pre-post)/pre; R=0.43, P<0.05. (B) Mean firing rates of DMS and DLS neurons (±SEM shaded) in the 10 least habital rats, by degradation score. (C-D) Mean firing of neurons categorized by significant excitation or inhibition in the second before or after a lever press, as in Figure 2. (E) For the 10 most habitual rats, firing rates in the DMS and DLS populations and (F-G) firing rates categorized by phasic response type are also shown. The most habitual rats trended toward fewer PostEx neurons (P=0.08).

Pie charts display proportion in each category, with NP units in gray. N's indicate number of neurons in a subregion. (PreEx: pre-excitatory; PreIn: Pre-inhibitory; PostEx: post-excitatory; PostIn: post-inhibitory; NP: non-phasic)

These groups showed no difference in lever-press responding during this VI30 self-administration session. The most habitual rats performed 105±20 active presses and earned 24±0 reinforcements, while least habitual rats performed 79±9 active presses and earned 23±1 reinforcements (MWU, *P*s>0.05). While minimal differences were observed across these groups in the DMS (**Figure 4.4B-C**), there was a trend in the DLS for rats with stronger habit-like behavior to exhibit less PostEx selectivity following a reinforced response than rats with more flexible behavior (**Figure 4.4D-E**, *MWU*=24.5, *P*=0.08). Therefore, while alcohol did not alter DLS neuronal selectivity, stronger habitual behavior marginally correlated with reduced outcome encoding in the DLS.

When neuronal activity was compared between anterior and posterior DMS, no significant effect of position was found on the differences in neuronal activity between reinforcers (**Supplemental figure 4.1A-F**). When the data were organized by habit strength, the 10 least habitual rats exhibited marginally more phasic activity in anterior DMS (**Supplemental figure 4.1G-L**).

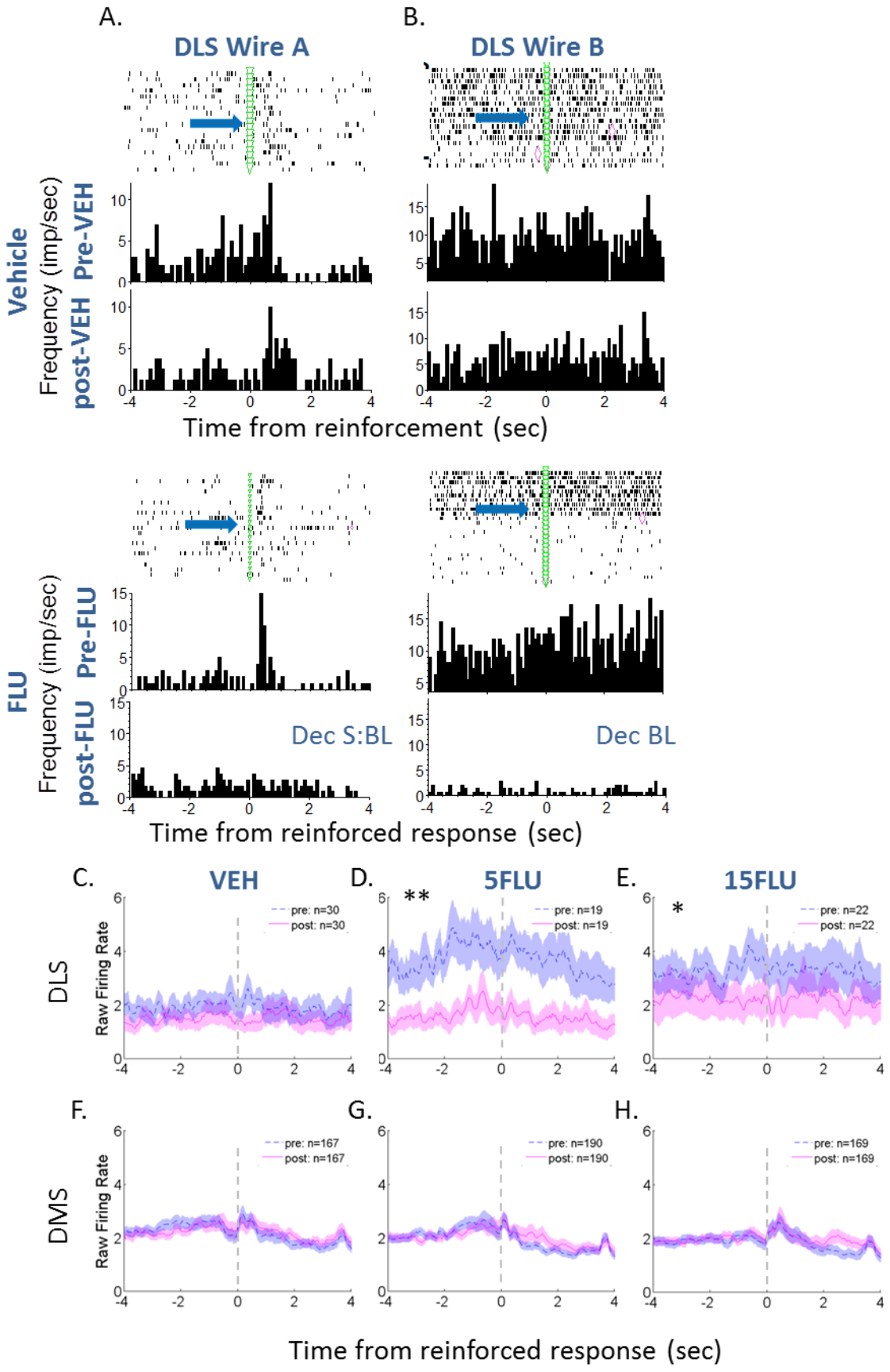
Unilateral dopamine receptor antagonism disrupts DMS/DLS balance

To examine the role of dopamine on DLS activity, we infused α-flupenthixol (FLU) unilaterally into the DLS mid-way through a self-administration session, allowing analysis of local effects on individual neurons. One Sac and two Sac/E rats were excluded due to cannula misplacement outside the DLS or lack of DLS wires. Lever-press behavior during unilateral infusion sessions was unaltered by infusion of 5 or 15FLU (2 -way RM ANOVA; $F_{3,26}$=2.16,

P=0.1); therefore, the effects of unilateral infusion are not the consequence of behavioral change. Changes in firing rates were observed closely following the time of unilateral FLU infusion, and these were found on wires that also recorded neurons with no change in activity after vehicle infusion (**Figure 4.5A-B**). Individual neurons displayed a variety of effects after proximal FLU infusion (**Figure 4.5A-B**), including both increases and decreases in phasic activation around lever presses and increases and decreases in tonic, basal firing rates**.** However, the population effect was a reduction in baseline firing rate in DLS, but not DMS. In the DLS, a significant depression of baseline firing rates was observed after infusion of FLU on electrodes with a unit that displayed no reduction in firing on PBS infusion sessions (5FLU *MWU*=62, *P*<0.001;or 15FLU *MWU*=141.0,*P*<0.05; not PBS *MWU*=338.0, *P*=0.099; **Figure 4.5C-E**). After unilateral DLS infusion of FLU, firing rates in ipsilateral DMS appeared unchanged (**Figure 4.5F-H)**, and no difference was observed in baseline firing rates in the DMS (MWU *Ps*>0.1).

Bilateral DLS dopamine receptor antagonism reduces habitual alcohol seeking and related DMS activation

Bilateral infusions of 15FLU or VEH prior to a contingency degradation test of habitual behavior allowed examination of the impact of DLS dopamine receptor antagonism on habitual behavior and its neuronal correlates. Three Sac rats and three Sac/E rats were not included in this experiment due to technical issues with cannulae. A significant reduction in lever presses was observed across the first two days of contingency degradation training, demonstrating that the rats recognized the degraded response-outcome contingency (main effect of day, $F_{2,12}$ =5.43, *P*<0.05; no effect of group or interaction; **Figure 4.6A**). The critical test of habit was comparison of reward seeking under extinction conditions before and after the degradation training. Comparing lever pressing across all 3 extinction tests, changes in operant responding were small


A.
DLS Wire A
B.
DLS Wire B
Vehicle
Frequency (imp/sec)
Pre-VEH
post-VEH
Time from reinforcement (sec)
FLU
Pre-FLU
post-FLU
Dec S:BL
Dec BL
Time from reinforced response (sec)
C.
VEH
D.
5FLU
E.
15FLU
DLS
Raw Firing Rate
pre: n=30
post: n=30
pre: n=19
post: n=19
pre: n=22
post: n=22
F.
G.
H.
DMS
pre: n=167
post: n=167
pre: n=190
post: n=190
pre: n=169
post: n=169
Time from reinforced response (sec)

but

Figure 4.16: Effect of unilateral infusion of FLU into the DLS. (A-B) Examples of DLS neuronal activity around reinforced presses. FLU or vehicle was infused after 10 trials (blue arrow). For each cell: (Top) Raster plot of neuronal spikes (black) around each reinforced press (green triangle). (Bottom) Histogram of average firing rate (Hz) from trials before and after the infusion. (C-E) Baseline firing rates were significantly reduced in the DLS after FLU (5FLU, $P<0.001$**;or 15FLU, $P<0.05$*; not PBS, $P=0.099$). Raw firing rate (Hz) displayed before (pre) and after (post) infusion of vehicle (VEH), 5µg FLU (5FLU), or 15µg FLU (15FLU), from the population of neurons in the DLS and (F-H) DMS.

consistent. A 2-way RM ANOVA found a main effect of reinforcer solution (Sac/E rats lower than Sac rats, $F_{2,24}=24.19$, $P<0.001$) and treatment ($F_{2,24}=7.18$, $P<0.005$), with no significant interaction ($F_{2,24}=2.62$, $P=0.093$; **Figure 4.6B**). Collapsed across solution, lever responding during the post- degradation extinction test after VEH infusion was not significant compared to the pre-test, confirming habitual behavior in both Sac and Sac/E rats (posthoc PRE vs VEH, $P=0.129$). However, habitual behavior was blocked by the 15FLU infusion, which significantly attenuated reward seeking in the post-degradation extinction test compared to the pre-test (posthoc PRE vs FLU, $P<0.005$). No order effect was observed (no effect of session order in 3-way ANOVA of reinforcer by dose by order, P's>0.5). After each 10min extinction period, behavior during the 30min VI30 period showed similar effects of FLU. 2-way RM ANOVA found a main effect of reinforcer ($F_{2,24}=8.08$, $P<0.05$), and a main effect of treatment ($F_{2,24}=8.08$, $P<0.05$), with posthocs revealing a significant reduction in active lever responding between PRE and FLU only ($P<0.05$; **Figure 4.6C**). Moreover, neither inactive lever responses nor the latency to the first press were significantly altered by FLU (2-way RM ANOVA; P's>0.1).

DMS neuronal firing rates during VI30 self-administration were altered by both contingency degradation (Post/VEH) and ipsilateral DLS dopamine receptor antagonism (Post/15FLU) compared to the pre-test (PRE/Sham), especially in Sac/E rats (**Figure 4.7A-F**).

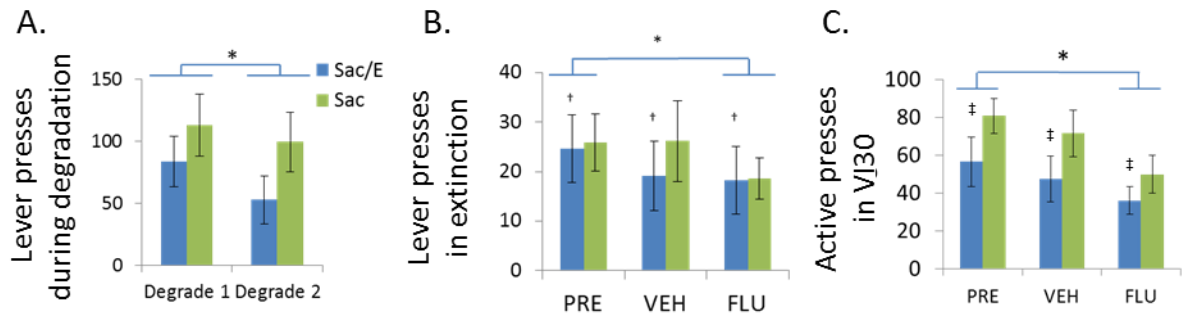


Figure 4.16: Effect of contingency degradation and FLU on lever responses. (A) Lever press responses reduced across 2 sessions of contingency degradation (effect of session, $P<0.05$*). (B) Responses in extinction prior to degradation (PRE), after degradation and PBS (VEH), or after degradation and 15µg FLU (FLU; 2-way RM ANOVA effect of solution $P<0.001^{\dagger}$, and treatment $P<0.01$; collapsed across solution, PRE vs. FLU, $P<0.05$*). (C) Active lever responses in the VI30 period of the post-degradation sessions followed the same pattern as the extinction tests, with a significant reduction in active responding between PRE and FLU (main effect of solution, $P<0.05^{\ddagger}$, main effect of treatment, $P<0.05$; posthoc PRE vs. FLU, $P<0.05$*).

When analyzing the effect of session and solution on firing in each response category, the only significant difference observed was the effect of FLU on the proportion of PreEx cells. Specifically, within-subject comparison of the number of PreEx cells (per wire per rat) across the 2 reinforcers and 3 sessions revealed a main effect of session ($F_{2,41}=5.47$, $P<0.01$). Posthoc comparisons collapsed across reinforcer found that PreEx activation was reduced by bilateral 15FLU compared to PRE/sham (Fisher LSD $P<0.005$). While the average amplitude of PreEx activation was higher after FLU, the difference was not significant (effect of session: $F_{2,58}=2.07$, $P=0.14$). No difference was observed between Sac and Sac/E rats. Furthermore, only a main effect of session and no effect of habit was found in DMS when PreEx cells were analyzed by individual differences in habit strength (as defined previously; **Supplemental Figure 4.2**). Overall, bilateral FLU in the DLS allowed contingency degradation to reduce reward-seeking behavior and produced a corresponding reduction in DMS phasic activation. While unilateral DLS dopamine receptor manipulation altered DLS activity, the reduction in ipsilateral DMS phasic firing only after bilateral FLU suggests that DMS activation reflects behavioral responses.

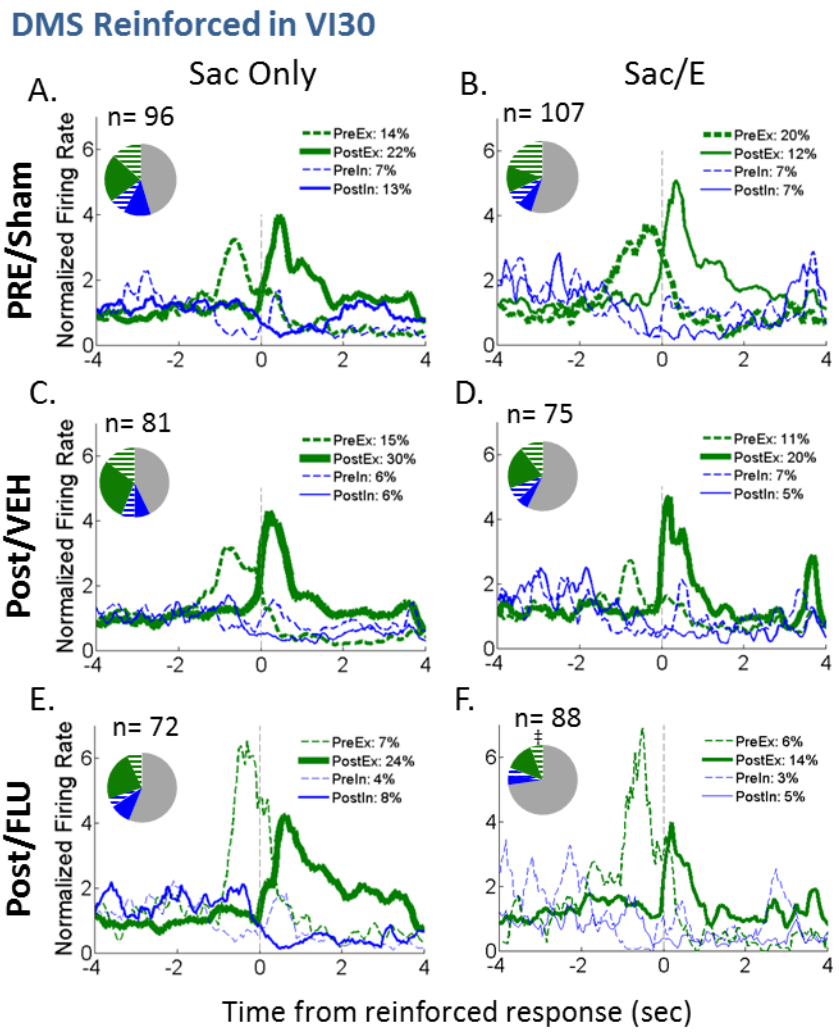


Figure 4.17: Neuronal activity around reinforced lever press responses during the VI30 period of sessions following extinction testing, organized by reinforcer solution. Mean firing of neurons categorized by significant excitation or inhibition in the second before or after a lever press (PreEx: pre-excitatory; PreIn: Pre-inhibitory; PostEx: post-excitatory; PostIn: post-inhibitory; NP: non-phasic), as in Figure 2, for Sac and Sac/E rats. (A-B) Neuronal activity was similar to baseline recordings when reinforcements were re-introduced following contingency degradation pre-test (PRE/sham), (C-D) PreEx was non-significantly reduced in Sac/E rats after degradation post-test with DLS infusion of PBS (Post/VEH), (E-F) and significantly fewer PreEx neurons were found post-test with infusion of 15µg FLU (Post/FLU). #PreEx/wire/rat was significantly reduced in Post/FLU compared to Pre/Sham (main effect of session $P<0.01$; posthoc $P<0.005^{\ddagger}$).

Discussion

The experiments described here demonstrate that dopamine contributes to habitual alcohol drinking and its neuronal correlates. We also found that alcohol and the degree of habit had distinct effects on neuronal firing patterns in the dorsal striatum, and may independently promote alcohol seeking through different neural circuits. Rats drinking alcohol had increased DMS activation prior to a lever press, after a moderate exposure of around 60mg/dl for 10-11 weeks of drinking alcohol, suggesting that social drinking in humans may impact neural circuitry. DLS activity varied by behavioral flexibility, rather than by reinforcer solution, with more habit-like rats showing less phasic neuronal activity to reinforcement. This finding supports the definition of habitual behavior as independent of the outcome, and highlights that habitual behavior is a continuous variable. Dopamine antagonism with FLU, unilaterally administered into the DLS, reduced basal firing rates of nearby neurons without altering behavior. Finally, bilateral FLU infusion enabled limited contingency degradation training to block habitual alcohol drinking, and furthermore reduced pre-press neuronal activation in the DMS, reversing the effect of alcohol on the dorsal striatum.

Previous studies support the role of alcohol in the DMS. The current results agree with the findings of *ex vivo* studies where repeated ethanol exposure (2g/kg i.p. once/day for 7 days) increased NMDAR-mediated LTP in the DMS (Wang et al. 2010, Wang et al., 2012). Increased pre-press excitations seen here could be attributed to motor, motivation or reward expectancy encoding. Previous studies have found reward expectation encoding in the DMS, as DMS neuronal firing to a cue tracks the magnitude of the associated reward (Kimchi, Laubach 2009, Stalnaker et al. 2010). Since DMS activity prior to reward delivery is sensitive to varying reward magnitudes (Hassani, Cromwell & Schultz 2001, Cromwell, Schultz 2003). Therefore,

expectation of reward may be increased in Sac/E rats, which could be related to the finding that dopamine activity in dorsal striatum correlated with craving in addiction (Volkow et al. 2006). Future studies can investigate whether DMS pre-excitations in alcohol drinking rats vary with changes in reward expectation, rather than being activated by any motor response.

In contrast, habit formation and skill consolidation have been demonstrated to increase DLS activity during skill performance (Kimchi et al. 2009, Yin et al. 2009). Our previous study of dorsal striatal electophysiology recorded from rats self-administering alcohol on FR5 and VI30 schedules, with only the VI30 schedule producing behavior that was insensitive to contingency degradation (Fanelli et al. 2013). Based on the current findings, previously reported increases in DLS post-press excitations in VI30- compared to FR5-trained rats was likely due to the reduced predictability of reward in VI30, as the current study recorded sufficient neurons to compare across the habit spectrum in the same schedule and found that habit correlated with reduced DLS post-excitations. Reduced expectation of reward in the VI30 schedule may therefore increase dopamine release to a reward, according to the reward prediction error theory of dopamine function (Schultz, Dayan & Montague 1997), and may explain why VI30 schedules promote faster formation of habitual behavior. Once habitual behavior is established and independent of reward outcome, however, dopamine release and DLS post-reinforcement excitations may be reduced, and this hypothesis is currently being investigated in the lab.

Dopamine receptor blockade in the DLS reduced habit-directed behavior. While we observed no significant reduction in reward-seeking behavior after contingency degradation paired with vehicle infusion, there were individual differences: some rats reduced pressing under this condition (despite all rats being habitual prior to surgery), resulting in the range of habit strength used in analyses herein. It has long been acknowledged that “inflexible” habits can be

degraded (Dickinson 1985, Dickinson et al. 1996, Braun, Hauber 2012). Nevertheless, contingency degradation only significantly reduced alcohol and saccharin seeking when performed in combination with FLU infusion. To examine the pharmacological effect of FLU infusion on DLS neuronal activity, we performed mid-session, unilateral infusions into the DLS. After FLU infusion, but not PBS, we observed a reduction in tonic firing rates of DLS neurons in the absence of behavioral change. This result extends prior reports of similar effects of systemic dopamine receptor antagonists (Burkhardt, Jin & Costa 2009), and further demonstrates a diverse effect on phasic responses, which can be further studied with genetic approaches to distinguish between cell types. We did not observe a dose effect, as 5FLU and 15FLU did not produces significantly different effects on neuronal firing, which may be attributed to the local nature of the recordings, as the effective dose on any particular neuron would be dependent on the location relative to the cannula. We conclude that dopamine receptor blockade in the DLS reduces the expression of habitual behavior by reducing DLS firing rates. Dopamine may, therefore, act to increase tonic firing in the DLS, increasing synaptic plasticity in the region (Surmeier, Carrillo-Reid & Bargas 2011, DePoy et al. 2013), and supporting the DLS control of habitual behavior.

Another novel finding of this study was the reduction in phasic DMS activity after DLS dopamine receptor antagonism. The reduction in DMS activation was observed specifically in pre-press excitations, which had been promoted by alcohol, demonstrating that DLS dopamine receptor antagonism can reverse the effects of alcohol on the dorsal striatum. Since DMS activity prior to reward delivery is sensitive to varying reward magnitudes (Cromwell, Schultz 2003, Stalnaker et al. 2010), attenuation of pre-press responses may represent a reduction in expectation of reward probability or value as a result of dopamine receptor blockade.

Most theories of DMS and DLS parallel processing suggest that these circuits work in antagonism to each other, with a shift to habitual behavior resulting in reduced goal-directed processing (Hilario, Costa 2008, Daw, Niv & Dayan 2005), leading to our original hypothesis that FLU in the DLS would conversely increase DMS activation. However, the observed reduction in DMS activation, which was not observed after unilateral dopamine antagonism, reflects the reduction in alcohol seeking. It furthermore suggests that DMS and DLS may act additively rather than antagonistically to promote behavior. Instead of shifting from DMS to DLS, neuronal activation is maintained in the DMS while spreading into the DLS, similar to the maintenance of dopamine activity in the nucleus accumbens during goal-directed behavior and engagement of DMS dopamine release (Brown et al. 2011). Furthermore, DMS, DLS, and substantia nigra pars compacta (SNc) all showed elevated cFos expression in rats trained to habitually self-administer nicotine (Clemens et al. 2014). This framework also explains how lesion of the DLS in habitual rats results in immediate goal-directed behavior (Yin, Knowlton & Balleine 2004, Corbit, Nie & Janak 2012), uncovering DMS-driven behavior rather than causing a shift to the DMS, which would be expected to take time to re-learn.

These studies provide a novel framework for independent DMS and DLS processing of reward-directed behaviors. Furthermore, while alcohol and habit-promoting repetition of behavior may independently encourage persistent drinking, a combination of pharmacological and behavioral treatments may likewise target differential neural pathways and contribute additively to the reversal of alcoholism. Human studies demonstrate that the dorsal striatum is more activated by action selection and alcohol-related cues in individuals with alcohol use disorder (Sjoerds et al. 2013, Sjoerds et al. 2014), and that dorsal striatal dopamine transmission is altered in addiction (Volkow et al. 2006). Additional research is necessary to determine

whether behavioral and pharmacological interventions in human alcoholics can reduce dorsal striatal processing changes induced by alcoholism and predict treatment outcomes.

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CHAPTER 5: GENERAL DISCUSSION

Summary of findings: physiological correlates of behavioral resistance to change

The studies described in this dissertation investigated the neuronal mechanisms of inflexible, habitual alcohol drinking. Prior reports suggested that as behavior evolved from goal-directed to habitual, neuronal encoding shifted from the DMS to the DLS (Yin, Knowlton 2006, Hilario, Costa 2008), and we therefore expected that DLS activation would be amplified in habit models that result in reduced behavioral flexibility. In addition, since alcohol use disorder is characterized by inflexible drinking in the face of negative consequences (American Psychiatric Association 2013), and alcohol can promote habit formation (Corbit, Nie & Janak 2012, Mangieri, Cofresi & Gonzales 2012), we hypothesized that alcohol drinking also increases DLS activation. Using *in vivo* extracellular electrophysiology, we characterized DMS and DLS activity during goal-directed and habit-like alcohol self-administration models: FR5 and VI30 reinforcement schedules, respectively. We observed more excitation in firing rate in response to reinforced presses in the posterior DMS and in the DLS of VI30-trained rats. When we blocked dopamine D1 receptors systemically, we found reduced basal neuronal activity across the dorsal striatum, as well as reduced alcohol seeking, but no effect on neuronal firing excitations to cues. These studies provided a detailed analysis of dorsal striatal activation during goal-directed and habitual alcohol seeking.

Next, we compared rats drinking alcohol sweetened with saccharin to those drinking saccharin solutions alone, to examine whether alcohol-directed habits are differentially encoded; differences in neural mechanisms underlying habits for alcoholic and nonalcoholic reward may

explain the persistence of alcohol use disorders. We found that while DLS activation correlated with behavioral flexibility, rats drinking alcoholic solutions had similar DLS activation to those drinking non-alcoholic solutions. Instead, alcohol drinking increased associative, DMS processing. We next hypothesized that dopamine would provide the mechanism for increased DLS activation in habit. Bilateral infusion of the non-selective dopamine receptor antagonist α-flupenthixol (FLU) into the DLS reduced neuronal activity in the DMS and also reduced habitual drinking of either saccharin or sweetened alcohol. Therefore, we contribute to the field a novel model in which DMS and DLS processing contribute additively to behavior. This dissertation, thus, uncovered mechanisms of habit that can be targeted in future treatments for alcoholism. In this final discussion of all experiments in this dissertation, I will address these implications, the limitations of our experiments, and what they can tell us about the neurobiology of habitual alcohol drinking.

Discussion of overall findings

In all studies, we observed predominantly excitatory responses to cues and actions during alcohol self-administration in both the DMS and DLS. The finding that most modulation in the region produces increases in firing rate is supported by the primarily glutamatergic projections to the dorsal striatum from cortex (Figure 1.1; Gerfen, Surmeier 2011) and previous electrophysiological studies in the dorsal striatum (i.e. Kimchi et al. 2009). An advantage of utilizing electrophysiological techniques was that we were able to observe that responses in DMS and DLS were not all-or-nothing. Rather, both regions were active (to varying degrees) during self-administration, as had been previously shown in learning-related tasks (Yin et al. 2009, Kimchi, Laubach 2009, Jin, Costa 2010, Thorn et al. 2010). Additionally, we found that DMS neurons were predominantly activated in response to cues, while DLS neurons were more

activated around actions, as had been reported during tasks such as treadmill locomotion, conditioned avoidance and learning a T-maze (West et al. 1990, White, Rebec 1993). Therefore, our general findings of the roles of DMS and DLS activation during alcohol self-administration agree with previous findings. To our knowledge, our studies included here were the first studies to compare DMS and DLS activation during alcohol self-administration.

Electrophysiological comparisons between groups in the studies reported here are more difficult to interpret. In Chapter 2, FR5 rats showed greater amplitude of firing frequency changes, but it was unclear whether these are due to the greater number of presses in an action sequence or because their behavior is more flexible. Since increases in neuronal firing rate in dorsal striatum have been associated with the start and stop of action sequences (Jin, Costa 2010, Thorn et al. 2010), and since no difference in the amplitude of firing rate changes was observed across the spectrum of habit-like behavior in Chapter 4, we interpret the amplitude differences in Chapter 2 to the difference in the action sequences. The correlations of electrophysiology and habit score in Chapter 4 were posthoc analyses, however, so it is unclear what factors predisposed rats to develop inflexible behavior more quickly. Future studies can record electrophysiology across habit formation, or contrast typical self-administration exposure with exposures that have been shown to promote faster habit, such as with home cage drinking (Corbit, Nie & Janak 2012) or stress (Taylor et al. 2014). The current electrophysiological approach of chronically implanted electrodes resulted in fewer neurons detected over time; thus, longitudinal studies of habit formation will need movable electrodes to maintain recordings across a longer period.

An apparent discrepancy in our findings was that we observed greater DLS post-press excitations in VI30 than in FR5 rats (Figures 2.4 & 2.7), while analysis of variability within

VI30 habit-like rats in Chapter 4 found reductions in post-press excitations in rats with less sensitivity to contingency degradation training (Figure 4.3). However, this can also be explained by the different comparisons in each study. In Chapter 2, VI30 rats had lower expectation of receiving a reward than FR5 rats, and unexpected reward delivery would result in a reward prediction error and dopamine release, which could account for increases in firing rate (Owesson-White et al. 2009, Belle et al. 2013). In contrast, only one schedule of reinforcement was examined in Chapter 4; therefore, no differences in expectation occurred. Thus, our data suggest that the effect of habit, controlling for reinforcement schedule, is likely to reduce post-press DLS excitations, which is in agreement with a habit model in which behavior is independent of its outcome (Yin, Knowlton 2006, Everitt, Robbins 2005).

Amplification of DMS pre-press activation by alcohol has implications for alcohol drinking, especially in social drinkers. In the experiments described in Chapter 4, rats self-administered alcohol for 10-11 weeks, reaching post-session blood ethanol concentrations (BECs) of approximately 60mg/dl. This exposure most closely models heavy social drinking, as it is greater than "moderate drinking" levels of 1-2 drinks per day, but does not qualify as "binge drinking" defined at 80mg/dl BEC or 4-5 drinks per day (NIAAA). Importantly, this exposure level was enough to promote DMS activation. Considering that DMS activation can increase DLS activation (Haber, Fudge & McFarland 2000), it is plausible that the alcohol-induced amplification of DMS activity could increase future drinking, eventually leading to unhealthy and habitual drinking patterns. However, the significance of DMS activation prior to a motor response is not clear from these studies. These increases in neuronal firing rates were associated with lever pressing for alcohol (outcome-dependent), and were reduced by FLU in the DLS after contingency degradation training, so we propose that they may be encoding expectation of

reward or motivation to receive the reward. In other studies, lesion of dopamine neurons projecting to posterior DMS with 6-OHDA prevented promotion of faster operant behavior after cues indicating larger reward size, without blocking stimulus-response learning for reward versus no reward (Calaminus, Hauber 2009). Additionally, DMS neurons, including response-selective units, encode reward magnitude signaled by a cue (Kimchi, Laubach 2009, Stalnaker et al. 2010). However, these neuronal responses could have been encoding stimulus-response associations and not response-outcome expectations. To determine whether DMS pre-motor firing patterns encode expectation for specific actions, future experiments could manipulate reward value, as in a discriminative stimulus task, when a cue could signal responding on one lever will result in larger reward. Subsequently, blockade of pre-press excitations (as with FLU or optogenetics) would be expected to reduce preference for the lever associated with larger rewards. Pre-access to solutions could separately manipulate motivation in a goal-directed model.

In these studies, we did not directly compare unsweetened alcohol with a sweet reinforcer. We also did not control for caloric value by comparing alcohol to a caloric reinforcer such as sucrose. Chapters 2 and 3 examined only rats drinking alcohol (all solutions in water), and Chapter 4 compared rats drinking saccharin (Sac) to those drinking saccharin with alcohol (Sac/E). We chose to use Sac to increase alcohol intake, to avoid the reduction of BECs by sucrose, and because Sac does not produce escalation in consumption seen with addictive substances and sucrose (Beeler et al. 2012). Previous studies have found that Sac self-administration evokes dopamine release into the NAc, but dopamine responses diminished after exposure, an effect that was not observed with sucrose reinforcement (Beeler et al. 2012). However, other studies have demonstrated that Sac induces more Pavlovian conditioned

approaches and is preferred over intravenous cocaine or heroin (Madsen, Ahmed 2014). Therefore, saccharin may have unique reinforcing effects versus caloric sweet rewards. We would expect that a compound reinforcer, Sac/E, would be more reinforcing and would promote a more habit-like phenotype. Comparison of our studies with alcohol and sweetened alcohol in VI30-trained rats (Chapters 2 and 4) suggests the possibility that Sac may indeed increase the rate of habit formation, as rats in the first study were sensitive to satiety-specific devaluation at a later time point. However, devaluation procedures were performed after electrophysiological recordings (with the added stress of repeated tethering) and switching animals to a different operant chamber. Another obstacle in direct comparison, rats in the first study (Chapters 2 and 3) received SCH treatment prior to devaluation testing. Rats in the first study also had DLS arrays in a linear alignment that extended across an anterior-posterior range, and recorded more anterior DLS neurons, explaining apparent reductions in DLS activation compared to Sac/E rats. Therefore, experimental design was too different across studies for direct comparisons. Future studies can compare alcohol alone with an isocaloric sucrose solution, in comparison to the reinforcing properties of saccharin and sweetened alcohol.

Perhaps the greatest unresolved question in these studies is whether alcohol promotes habit formation. In the experiments described here, we did not observe *promotion* of habitual behavior by alcohol self-administration. Additional studies in our lab also did not show an effect of alcohol on habit (Hay et al. 2013), though other groups have found selective habit formation in alcohol-drinking rats (see Chapter 1; i.e. Corbit, Nie & Janak 2012, Mangieri, Cofresi & Gonzales 2012). In Chapter 4, rats consumed larger doses of alcohol (~1g/kg) compared to our previous studies (~0.5g/kg;), yet satiety-specific devaluation still demonstrated goal-directed behavior for both Sac and Sac/E rats at week 7 and habit-like behavior for both groups after 7-9

weeks of training. If anything, alcohol-drinking rats trended toward being less habitual (Figure 4.1). Both groups also showed habit-like behavior after contingency degradation training under the PBS infusion condition. In comparison, Mangieri et al. reported habitual behavior after just over one week of training in alcohol-drinking rats (~1.2g/kg), and not sucrose-drinking rats, by using LiCl reward devaluation (Mangieri, Cofresi & Gonzales 2012) and omission training (contingency reversal; Mangieri, Cofresi & Gonzales 2014). Other studies found habit promotion with satiety-specific devaluation after extended alcohol exposure, with habitual behavior after 12 weeks alcohol experience including home cage access. In that study, rats were not habitual after 4 weeks home cage plus 4 weeks operant alcohol exposure (Corbit, Nie & Janak 2012). Indeed, not all alcohol exposures result in habit-like behavior (Samson et al. 2004). Therefore, we may have seen no difference in habit because we did not test habit in the window between habit formation with saccharin and with our alcohol doses. Regardless, if we had used extended home cage alcohol exposure to promote faster habit-like behavior over a control group with no alcohol access, alcohol-drinking rats may have had differential DLS activation compared to control rats, but this neuronal activity would still have been related to habitual behavior, and would not be selective to alcohol. We have shown here that saccharin, when it promotes habit, also promotes DLS activation. Therefore, regardless of the effect of alcohol on habit, DLS activation correlates with habit formation.

Defining the role of dorsal striatal dopamine in alcohol self-administration

Our ability to interpret the role of dopamine in the dorsal striatum is also hindered by similarities between Sac and SacE rats. We found that habitual alcohol self-administration was dependent on dopamine transmission in the DLS. FLU did not reduce inactive lever responses (though few were observed), nor did it have a significant effect on latency to press, suggesting

that FLU did not reduce all motor behavior. However, DLS infusion of FLU also reduced habit-like self-administration of Sac, and therefore the effects of FLU were not specific to alcohol. Since targeting DLS dopamine transmission might therefore prevent adaptive habits (such as looking left before crossing the street), this finding limits the usefulness of this mechanism as a potential therapeutic target. Nevertheless, the role of dopamine may still provide insight into habit mechanisms in the dorsal striatum; alternative methods for modulating dorsal striatal activation to reverse the effects of alcohol will be discussed below.

A recent investigation in our lab recorded phasic changes in dopamine concentration in the DMS and DLS in rats self-administering alcoholic and non-alcoholic solutions on a VI30 schedule (Shnitko et al., submitted). Shnitko and colleagues measured dopamine release with fast-scan cyclic voltammetry (FSCV), and they observed increases in dopamine after reinforced presses in the DLS. In the DMS, dopamine release prior to lever presses was greater in rats self-administering alcohol, in support of our findings reported here. Shnitko et al. also found that dopamine release in the DLS did not differ between rats self-administering alcoholic and non-alcoholic solutions. Other studies have found co-localized changes in neuronal firing and dopamine release in the NAc (Owesson-White et al. 2009, Belle et al. 2013, Cheer et al. 2007). However, only 12% of NAc cells increased firing rates in response to dopamine application alone, while 70% increased firing in response to electrical stimulation, demonstrating the impact of other neurotransmitters and inputs that would have been elicited by electrical stimulation (Belle et al. 2013). Therefore, future studies using combined FSCV and electrophysiology in the DLS across learning could determine the extent of dopamine release and other modulators required to increase neuronal firing rates. In the meantime, our FSCV study supports our findings of DMS activation by alcohol, co-activation of DMS and DLS during self-administration on a

habit-promoting schedule, and the contribution of dorsal striatal dopamine to reward-seeking behavior.

Taken together, results from Chapters 3 and 4 suggest a role for D2 receptors in habit reversal, although they did not test this directly. While systemic D1 receptor antagonism did not reduce cue-evoked neuronal activity in the dorsal striatum, some neurons were observed with reductions in phasic reinforced-press activity after FLU infusion (Figure 4.5). Caveats for each study were discussed within each chapter; for example, SCH could have reduced inhibition on the cortex, thereby preventing the observation of reduced cue responses, and future studies can directly compare locally infused D1 and D2 antagonists. Furthermore, extracellular recordings cannot distinguish neuronal subtypes, and it is possible that the reductions in phasic activity were recorded in inhibitory interneurons that are modulated by D2 receptors (Bracci et al. 2002, Wieland et al. 2014). However, the blockade of phasic activity by FLU suggests that D2 mechanisms may support cue-evoked responses in habitual behavior. In support of this interpretation, D2 receptor antagonists have been shown to block habitual alcohol drinking (Corbit, Nie & Janak 2014). Moreover, D1 receptor antagonism may not affect cue responses because habit formation results in LTP, producing synapses that are excitable by glutamatergic inputs alone. Meanwhile, antagonism of D2 receptors (Gi-coupled, inhibits adenylyl cyclase) may permit dopamine release onto non-potentiated synapses, reduce inactivation of the indirect pathway, or prevent accurate timing of dopamine release or indirect pathway inactivation. Indeed, alcohol increases LTP in the DMS and reduces inhibition in the DLS (Wang et al. 2012, Wilcox et al. 2014). D2 receptor expression is significantly greater in the more lateral striatum (Yin et al. 2009, Joyce, Loeschen & Marshall 1985). Corbit and colleagues have hypothesized that since potentiation was found in D2- and not D1-expressing neurons after extended training

(Yin et al. 2009), as behavior becomes habitual it may become independent of D1 receptors as glutamatergic synapses are strengthened, but more sensitive to activation of D2 receptors (Corbit, Nie & Janak 2014). In support of this hypothesis, D1 antagonists have been shown to be less effective to change behavior in cocaine-dependent rats (Ramoa et al. 2013). However, alcohol dependence has also been found to potentiate reductions in drinking caused by D1 and D2 antagonists (Liu, Weiss 2002), and some studies find that direct and indirect pathways act in parallel to promote action selection (Isomura et al. 2013, Gremel, Costa 2013). Studies to distinguish between pre- and post-synaptic D2 mechanisms could clarify this issue. Additional research is also necessary to understand the relative contributions of down-regulation in striatal D2 receptors with addiction (Moore et al. 1998) and of potentiation of indirect pathway in skill acquisition (Yin et al. 2009) to the sensitivity of addictive phenotypes to pharmacological manipulations. Thus, while the study of systemic SCH was confounded by a lack of regional specificity, when combined with the findings of the unilateral infusions, they suggest that dopamine D2 receptors in the DLS may be an important future target for understanding neural dynamics of habit formation.

Parallel, cooperative pathways through the DMS and DLS both contribute to alcohol drinking

The model we propose to account for our findings is represented in Figure 5.1 (below). When behavior is driven by a desire and motivation to obtain alcohol, associative cortical input and dopamine release may converge on MSNs in the DMS to increase goal-directed behavior. In contrast, when a behavior is independent of the reward, and is instead driven by a stimulus-response association, sensorimotor inputs to the DLS may drive habit-like behavior. While these theories had been proposed (Yin, Knowlton 2006), previous models based on lesion studies suggested that neuronal activity shifted from the DMS to the DLS in habit (Hilario, Costa 2008).

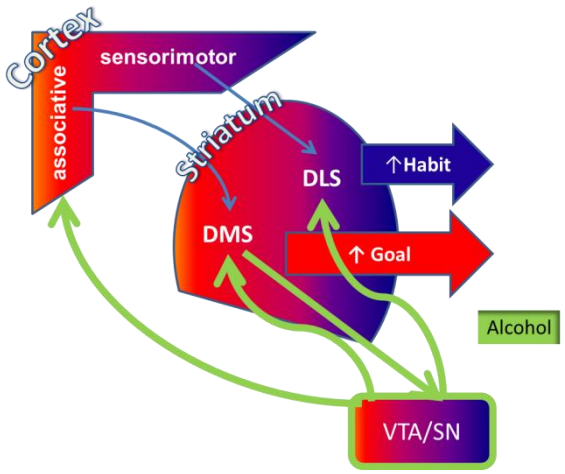

Figure 5.1: Parallel processing of goal-directed and habitual behavior in the dorsal striatum. DMS and DLS are both activated during alcohol drinking behavior. The effects of alcohol on dopamine transmission may increase DMS and DLS activation, amplifying both goal-directed and habitual seeking, such that drinking will be driven both by a desire for alcohol and by stimulus-response associations.

Since DLS lesion produces goal-directed behavior (Yin, Knowlton & Balleine 2004), previous theories suggested that DLS activation would be dominant in habitual behavior and DMS activation would be reduced (Yin, Knowlton 2006, Hilario, Costa 2008). In contrast, we find that DMS activation parallels activation in the DLS, as DMS processing continues, unaltered, in more compared to less habitual behavior (Figure 4.3), and is reduced when DLS activation is blunted by dopamine receptor antagonism (Figure 4.7). A recent study measured increased cFos expression in the DMS, DLS and SNc after extended (compared to short-term) nicotine self-administration (Clemens et al. 2014). They also demonstrated in another cohort of animals that extended training produced habitual behavior as measured by LiCl devaluation. One caveat of that study, it is important to note that there was no motor control as rats self-administering saline performed fewer lever responses, thus striatal activation could be related to movement rather than habit. However, increased cFos expression can be speculated to represent increases in gene transcription caused by increased cellular activity seen here, and further supports the idea that

habit causes a dorsolateral spread, rather than a shift, in activation. This functional organization may explain why individuals with substance use disorders can sometimes demonstrate automatic drug-taking in response to external cues, and sometimes have highly goal-directed behavior, performing unusual and focused behavior to acquire drug.

The results described here support the theory that habitual behavior is driven by both DMS and DLS activation during action selection (Thorn et al. 2010), but if both regions contribute to behavior, how do we select an action strategy? One possible mechanism for this switching could be relative DLS activation at the time of action selection. In a novel task, behavior may be driven by action-outcome processes as a result of trial and error, choosing the strategy that works best (Balleine, O'Doherty 2010). Failure to perform a task would result in a negative prediction error and, thus, reduced dopamine release (Schultz, Dayan & Montague 1997, Schultz 1998), preventing a switch to habit-like behavior, and promoting goal-directed behavior that can flexibly explore novel approaches. In contrast, a behavior repeatedly performed correctly may promote habit formation. Work by Graybiel and colleagues found greatest DMS activation in the middle block of sessions, when one task was learned and the other was not, suggesting that DMS gates access to DLS (Thorn et al. 2010), and both must be active during habit. Additionally, Gremel and colleagues found that inactivating the OFC can prevent goal-directed behavior, suggesting that the prefrontal cortex also plays a role to determine whether goal-directed or habitual strategies are employed (Gremel, Costa 2013). Indeed, prefrontal areas would necessarily calculate decision-making strategies in goal-directed behavior, when the approach to obtain the desired outcome must be continually updated. Human research has found that the inferior lateral prefrontal and frontopolar cortex acts as an “arbitrator”, determining whether goal-directed or habitual strategies will be employed (Lee, Shimojo & O'Doherty 2014).

Future studies can employ optogenetic manipulation to activate inputs from cortical control regions to the dorsal striatum, and post-behavioral fluorescence microscopy or *in vitro* slice studies can investigate the connectivity of this region.

Thus, dopamine may play a role in promoting goal-directed or habitual behavior, providing input on reward prediction error as well as motivational signals from the NAc (via midbrain spiraling loops). Indeed, dopamine prediction signals would explain the increased DLS activation in VI30 compared to FR5 rats, as the VI30 schedule results in less predictable reward (Figures 2.4 & 2.7). A study of thousands of striatal neurons found action-specific reward prediction error encoding only in DMS fast-spiking interneurons, suggesting that these signals may modulate wider patterns of activation since FSIs synapse on multiple MSNs (Stalnaker et al. 2012). This mechanism would explain how dopamine could increase excitation in response to reward value, since dopamine inputs themselves impact both D1 and D2 receptors, which are in 1:1 ratios, and therefore should produce observed reward encoding in the striatum (Isomura et al. 2013, Stalnaker et al. 2012). Additionally, dopamine modulation of interneurons might provide a mechanism for dopamine to affect cortical input to the striatum, and selection of goal-directed versus habitual behavior.

Conclusions: implications and impact of the current work

The studies in this dissertation have described neuronal encoding of goal-directed and habitual seeking for alcoholic and non-alcoholic rewards. We discovered that DMS and DLS processing differ in their predominant sensitivities, but have overlapping response patterns, and both are activated during the strategies of reward seeking studied here. Alcohol and habit formation have dissociable effects on dorsal striatum, with alcohol increasing associative DMS processing and habit increasing motor responses in the DLS. Nevertheless, treatments that reduce

one pathway, such as by reducing habitual reward seeking, also reduce activation of the other. Four main avenues for future studies will be (1) to characterize reinforcers such as saccharin, sucrose, and drugs of abuse for their inherent and preference-related habit-promoting properties, (2) to understand the microcircuitry within the dorsal striatum and which neuronal cell types are responsible for the patterns shown here, (3) to investigate the mechanism by which dopamine affects DLS and DMS activation, and (4) to decipher the connectivity of the corticostriatal circuits and integration of DMS and DLS processing. Further research in human subjects can also determine whether habitual drinkers, with consistent drinking patterns, might preferentially respond to treatments affecting plasticity rather than drugs affecting reward processing (Hay et al. 2013). For example, one current avenue of research is BDNF-inhibiting miR206 (Tapocik et al. 2014), which might target habitual behavior given BDNF elevations in DLS after habit-like drinking (Jeanblanc et al. 2009). Additionally, cannabinoid signaling is necessary for habit formation (Hilario et al. 2007), and their antagonism reduces alcohol self-administration (Malinen, Hyytia 2008). Opioid transmission and their downstream effectors may provide a third mechanism for reducing drinking, as these receptors may also reduce dorsal striatal excitation through LTD (Atwood, Kupferschmidt & Lovinger 2014). Furthermore, treatment strategies targeting habitual behavior may be useful for treating other addictions, especially given our finding that DLS FLU reduced habitual saccharin drinking. Therefore, this dissertation may have far-reaching implications for the study of inflexible behavior, uncovering novel mechanisms for future treatment.

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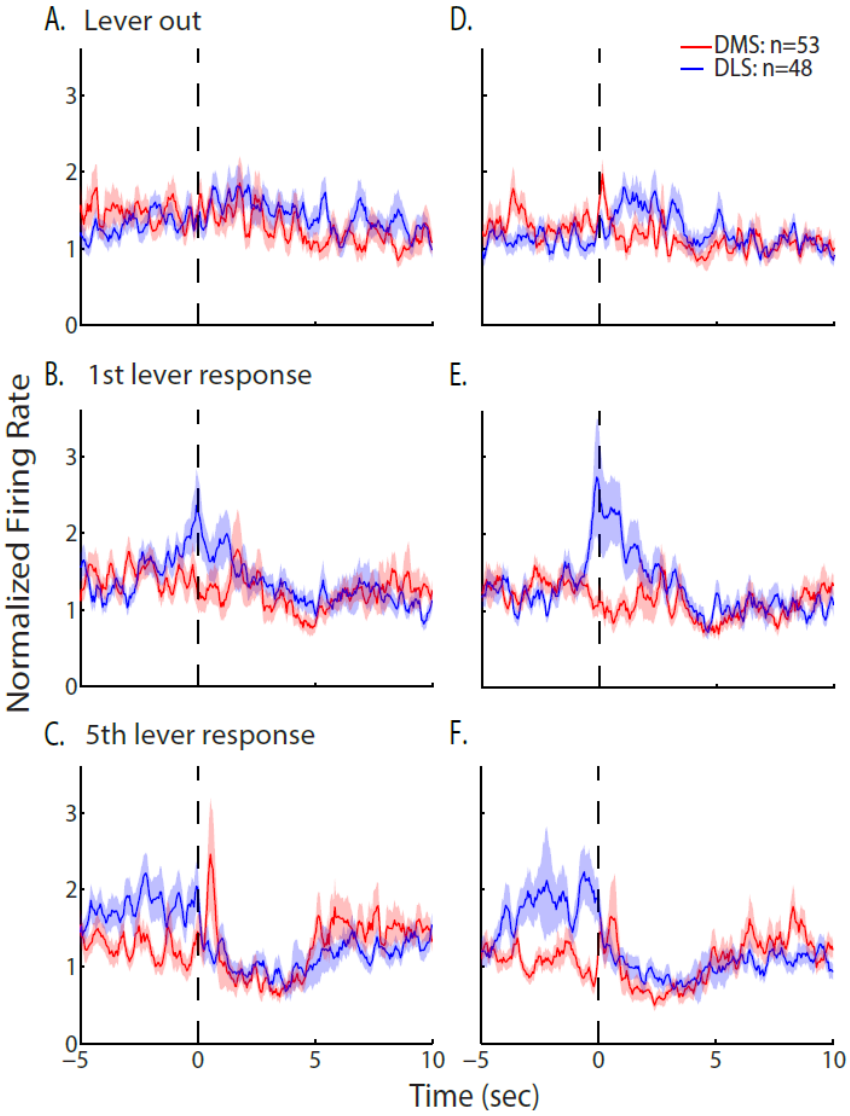
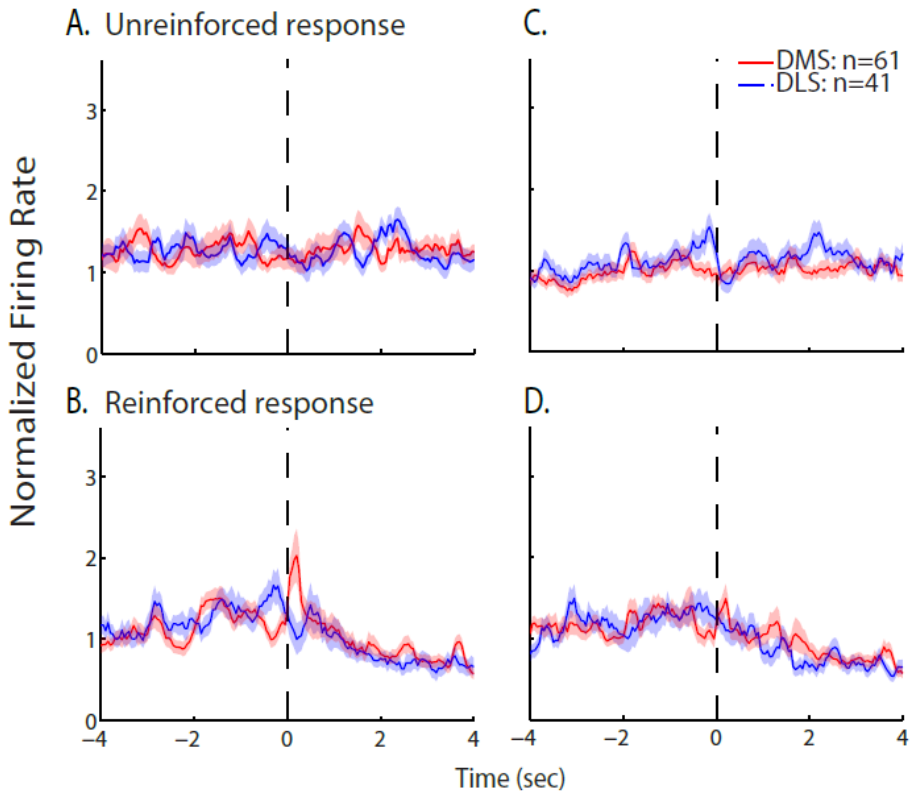
APPENDIX 2.1: NEURONAL ACTIVITY CHANGES ACROSS SESSIONS



Supplemental figure 2.1: **Neuronal firing activity in the dorsal striatum of FR5-trained rats (Experiment 1) aligned to cues and lever responses early versus late in the session.** Left: mean normalized firing rate (±SEM shaded) of all neurons in the DMS (red) and DLS (blue) aligned to the earlier half of occurrences of each event. Right: mean normalized firing rates aligned to the later half of occurrences of each event. If there were an odd number of trials, the middle trial was included in the "late" category. Neuronal activity was aligned to operant events: (A, D) lever extension after the 12s time out, (B, E) the 1st of each 5-response sequence, and (C, F) the 5th of each 5-response sequence. In all plots, firing rates were binned with a 250ms moving average using 50ms steps. DMS excitation following 5th lever responses was reduced by 32% in the second half of trials, but DMS excitation to the lever extension increased by 15%.

DLS firing rates increase in amplitude by 16% around the 1st response and appear broader. However, when aligned to the 5th lever response, DLS firing is more similar across the session (<10% increase).



Supplemental figure 2.2: Neuronal firing activity in the dorsal striatum of VI30-trained rats (Experiment 2) aligned to lever responses early versus late in the session. Left: mean normalized firing rate (±SEM shaded) of all neurons in the DMS (red) and DLS (blue) aligned to the earlier half of occurrences of each event. Right: mean normalized firing rates aligned to the later half of occurrences of each event. If there were an odd number of trials, the middle trial was included in the "late" category. Neuronal activity was aligned to operant events: (A, C) unreinforced lever responses, and (B, D) reinforced lever responses. In all plots, firing rates were binned with a 250ms moving average using 50ms steps. DMS excitation to reinforced responses diminished by 25% from the first to second halves of the session. DLS firing rates at lever-press responses were more similar over time; excitations in the second half of the session were 15% larger before unreinforced responses and 18% smaller before reinforced responses versus the first half.

APPENDIX 3.1: BREAKDOWN BY CUE TYPE AND TRAINING SCHEDULE

Supplemental table 3.4: Comparison of neuronal activation to the two start-of-session cue types. At the start of operant sessions, the houselight was illuminated followed 30s later by extension of levers into the chamber. We compared neuronal firing upon presentation of each cue to determine whether the neural response was fundamentally different between the two cues or whether the response was similar and the cues could be combined in the statistical analysis. Firing rates in the 500ms after each cue presentation are displayed in Hz (mean ± SEM) and recorded after saline, 10µg/kg SCH and 20µg/kg SCH injections (i.p.) in rats with extended alcohol self-administration experience on FR5 or VI30 schedules. The effects of these session-start cues on neuronal activity were compared by Signed Rank Test within each group (repeated measure, as activity to both cues was recorded from each unit). Overall, we observed similar neuronal activation after light and lever cues (in 10 of 12 comparisons below). Different neuronal responses to the cues were observed in only two conditions: in DMS of VI30 rats under 10 µg/kg SCH, and in DLS of FR5 rats under 20 µg/kg SCH. Thus, as there was no general trend for either cue to be more effective within a region, we treated the two stimuli as repeated trials of the same event (i.e., cue) in our GENMOD analysis to increase the robustness of the analysis.

DMS	FR5		VI30	
SCH dose, µg/kg	Light cue	Lever cue	Light cue	Lever cue
0	4.5±1.9	3.0±2.4	3.4±1.2	4.2±1.4
10	6.6±1.4	8.4±1.8	3.6±1.2	5.6±1.5[a]
20	5.4±1.7	5.4±2.1	4.7±1.2	5.6±1.5

DLS	FR5		VI30	
SCH dose, µg/kg	Light cue	Lever cue	Light cue	Lever cue
0	2.4±1.1	4.2±1.3	3.9±0.8	4.2±1.0
10	4.3±1.1	5.7±1.4	2.4±0.9	3.7±1.1
20	0.8±1.2	4.3±1.5[b]	2.8±1.0	2.7±1.2

[a] W=293.0, P<0.01

[b] W=35.0, P<0.05

Supplemental table 3.5: Comparison of the effect of SCH on neuronal firing rates in rats trained on FR5 and VI30 schedules. In this study, rats were trained to self-administer alcohol on FR5 and VI30 schedules which are known to generate different operant behavioral strategies (e.g., rates of responding). The dependent measure was neural activity at baseline and upon presentation of the start-of-session cues that were presented independently of behavior. Nevertheless, we compared neural activity between FR5- and VI30-trained rats to determine whether it was fundamentally different between the two groups or whether neuronal activity was similar and the groups could be combined in the statistical analysis. Baseline firing rate (Hz; mean ± SEM) in the 60s prior to the start of the session is presented after saline, 10µg/kg SCH and 20µg/kg SCH injections (i.p.). Neuronal firing frequency was recorded from rats trained to self-administer alcohol on FR5 or VI30 schedules. Separate 2-way ANOVA were run on baseline firing rates, coefficients of variance, and signal firing rates in DMS and DLS to compare group and dose effects. No effects of training schedule were observed in any of the analyses. Specifically, no differences were found in the basal firing rates (30s before presentation of the first cue), in the firing-rate variability during the baseline period (measured with coefficient of variance), nor in the firing rate in the 500ms after cue presentation (average of both cue types). Only significant effects of SCH treatment were observed on baseline firing measures (P<0.05).

DMS

SCH dose, µg/kg	Baseline Firing Rate		Coefficient of Variance		Signal Firing Rate	
	FR5	VI30	FR5	VI30	FR5	VI30
0	2.1±0.6	2.0±0.3	7.9±1.0	9.2±0.6	3.8±1.7	3.8±1.0
10	2.9±0.4	2.1±0.3	8.9±0.8	7.2±0.6	7.5±1.2	4.7±1.0
20	1.4±0.5	2.0±0.4	7.3±0.9	7.2±0.7	5.5±1.6	5.2±1.1

DLS

SCH dose, µg/kg	Baseline Firing Rate		Coefficient of Variance		Signal Firing Rate	
	FR5	VI30	FR5	VI30	FR5	VI30
0	2.9±0.6	3.2±0.4	7.1±0.8	8.2±0.6	3.3±1.0	4.1±0.8
10	2.9±0.6	1.4±0.5	8.4±0.8‡	6.8±0.6‡	5.0±1.1	3.0±0.8
20	1.3±0.7*	1.6±0.6*	5.3±0.9‡	5.7±0.7‡	3.2±1.2	2.8±1.0

*main effect of treatment, $F_{2,104}$=4.0, P<0.05; posthoc Sal vs. SCH20, P<0.05
‡main effect of treatment, $F_{2,104}$=5.1, P<0.01; posthoc Sal vs. SCH20 and SCH10 vs. SCH20, Ps<0.05

Supplemental table 3.6: Effect of SCH on alcohol-seeking behavior in rats trained to self-administer alcohol on FR5 and VI30 schedules. In this study, rats were trained to self-administer alcohol on FR5 and VI30 schedules. We compared the effects of SCH in the two groups on multiple aspects of operant behavior: latency to the first lever press (s), number of active lever responses during the session, and number of EtOH deliveries earned (mean ± SEM). Behaviors were measured during operant sessions after i.p. injections of either saline (0), 10 (SCH10) or 20μg/kg SCH (SCH20) and analyzed with repeated-measures, 2-way ANOVA. Only main effects of treatment on latency and active responding were observed. Latency was reduced by either SCH10 or SCH20, while active responding was dose-dependently reduced. There was a significant interaction of SCH treatment and training schedule on EtOH deliveries earned. Specifically, VI30 rats earned more reinforcements than FR5 rats under SCH10, producing a dose-dependent effect of SCH in the VI30 group, while both doses of SCH reduced reinforcements similarly in the FR5 group.

Training group	SCH, μg/kg	Latency (s)	Active lever responses	EtOH deliveries
	0	62.8 ± 50.2	104 ± 10[c]	21 ± 2
	10	388.8 ± 207.6[b]	22 ± 8[c]	4 ± 1[d,e]
FR5 N=11	20[a]	221.9 ± 107.9[b]	12 ± 5[c]	2 ± 1[d,e]
	0	18.7 ± 8	132 ± 24[c]	23 ± 1[f]
	10	239.5 ± 103.2[b]	37 ± 7[c]	13 ± 2[d,f]
VI30 N=15	20	796.7 ± 214.1[b]	17 ± 5[c]	7 ± 2[d,f]

[a] N=10
[b] P<0.001 main effect of treatment, $F_{2,47}$=1.43; posthoc Ps<0.01 vs. Sal
[c] P<0.001 main effect of treatment, $F_{2,47}$=1.93; posthoc Ps<0.01 between all doses
[d] Training schedule x treatment interaction, $F_{2,47}$=5.9, P<0.005; posthoc Ps<0.05 between reinforcement schedules after SCH10 and SCH20
[e] Training schedule x treatment interaction [d] ; posthoc Ps<0.001 vs.saline within group
[f] Training schedule x treatment interaction [d]; posthoc Ps<0.001 between all doses within group

APPENDIX 4.1: SUPPLEMENTAL MATERIALS FOR CHAPTER 4

Supplemental Methods

Self-administration training

Supplementary table 4.1 describes specific self-administration training procedures. All rats experienced increasing intervals between reinforcements. Sac/E rats also received gradually increasing concentrations of alcohol. Operant chambers contained two levers on the front wall, with two cups between them into which reinforcements were delivered. Either the left or right lever was assigned as the active lever (counterbalanced across rats), which remained constant throughout all experiments. On the first day in the operant chambers, rats received reinforcements on a random-interval schedule, independent of lever presses, to habituate them to the chamber and the delivery of saccharin into the cups. Sessions were shortened to 30min after day 3. The VI30 schedule was typically applied by session 5, and 15% alcohol (w/v) was

Supplemental table 4.1: Self-administration training schedule with increasing reinforcement intervals and ethanol concentrations.

Day [a]	Sac group	Sac/E group	Session length	Reinfocement schedule [b]
1	0.2% Sac	0.2% Sac	60 min	Random deliveries
2	0.2% Sac	0.2% Sac	60 min	FR1
3	0.2% Sac	0.2% Sac	60 min	VI7
4	0.2% Sac	0.2% Sac / 2.5% E	30 min	VI15
5	0.2% Sac	0.2% Sac / 2.5% E	30 min	VI30
6-7	0.2% Sac	0.2% Sac / 2.5% E	30 min	VI30
8-11	0.2% Sac	0.2% Sac / 5% E	30 min	VI30
12-15	0.2% Sac	0.2% Sac / 10% E	30 min	VI30
16-17	0.2% Sac	0.2% Sac / 12.5% E	30 min	VI30
18-19	0.2% Sac	0.2% Sac / 15% E	30 min	VI30
20+	0.2% Sac	0.2% Sac / 15% E	30 min or 25 deliveries	VI30

Ethanol (E); Saccharin (Sac); [a] Sessions run Monday – Friday; [b] A single lever (left or right) was assigned for the duration of the study

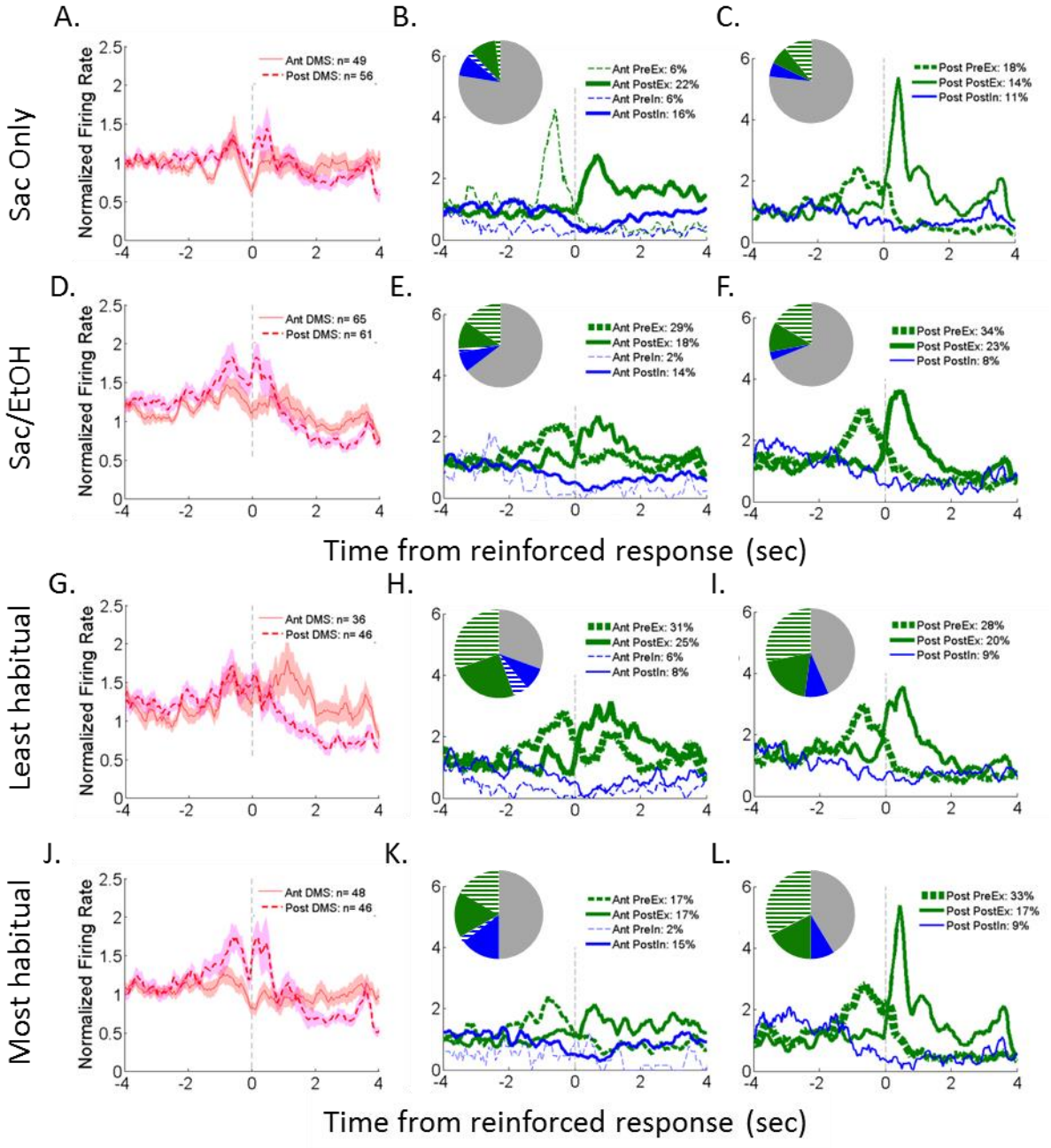
achieved in Sac/E rats by session 18, but increases in schedule and solution were delayed if rats received less than 17 reinforcements in a session. If the criterion was reached in the following session, then reinforcement interval or alcohol concentration was increased, alternately, until the final VI30s schedule and 15% alcohol (in Sac/E rats) was stably self-administered. Two sessions after these final parameters were applied (typically session 20, at most by the end of week 6), reinforcements were capped at 25 per session, to better equate reinforcement history between rats. Rats who received fewer than 17 reinforcements 3 days in a week were excluded from the study. Satiety-specific devaluation was first performed in the third week after reinforcements were capped (typically in week 7 on days 32 and 35), and the schedule for all subsequent tests proceeded as described in **Table 4.1**.

Electrophysiology

Timestamps from the Med Associates software to the MAP system were used to temporally align cell firing with behavioral events. An oscilloscope was used for the purpose of recording signal-to-noise ratios for each channel displaying a possible cell unit. Following each session, cell sorting was finalized through a combination of both automated and manual cluster selection using Offline Sorter software (Plexon, Inc.). Template analysis, principal component analysis, and signal-to-noise ratios were incorporated during cluster segmentation. Only units displaying a distinct cluster and physiological characteristics consistent with MSNs (i.e. $\leq 0.1\%$ of spikes with interspike intervals < 1ms and average firing rates < 10Hz; (Kish, Palmer & Gerhardt 1999) were included in neuronal analysis.

Supplemental Results

We assessed how phasic firing activity in DMS neurons varied by anterior-posterior placement of the electrodes. When neuronal activation categories were compared between DMS neurons anterior and posterior to +1.2mm from bregma, a 2-way RM ANOVA yielded a significant main effect of reinforcer solution on the number of PreEx and PreIn neurons detected, as SacE rats exhibited more of these pre-press activations in both DMS subregions (PreEx: $F_{1,56}$=9.87, P<0.005; PreIn: $F_{1,56}$=5.17, P<0.05; **Supplemental figure 4.1A-F**). However, no effect of anterior-posterior position was found. Likewise, no significant anterior-posterior differences were seen when comparing the 10 most and 10 least habitual rats, based on degradation scores (**Supplemental figure 4.1G-L**). While the difference did not reach significance, the least habitual rats had more activity in the anterior DMS (31% PreEx, compared to 17% in most habitual rats), in agreement with previous studies showing more anterior activation in goal-directed FR5-trained rats (Fanelli et al. 2013). This finding is consistent with the differences between anterior and posterior DMS in the source of cortical inputs (Haber, Fudge & McFarland 2000), as well as functional differences in reward-motivated behavior, with anterior DMS being more associated with processing required early in learning (Miyachi et al. 1997, Yin et al. 2005, Corbit, Janak 2010). Lesion of anterior DMS can be overcome with extended training, suggesting its role in flexible behavior (Yin et al. 2005), while posterior DMS shows more overlap in function with the DLS (required for stimulus-oucome association) (Corbit, Janak 2010).

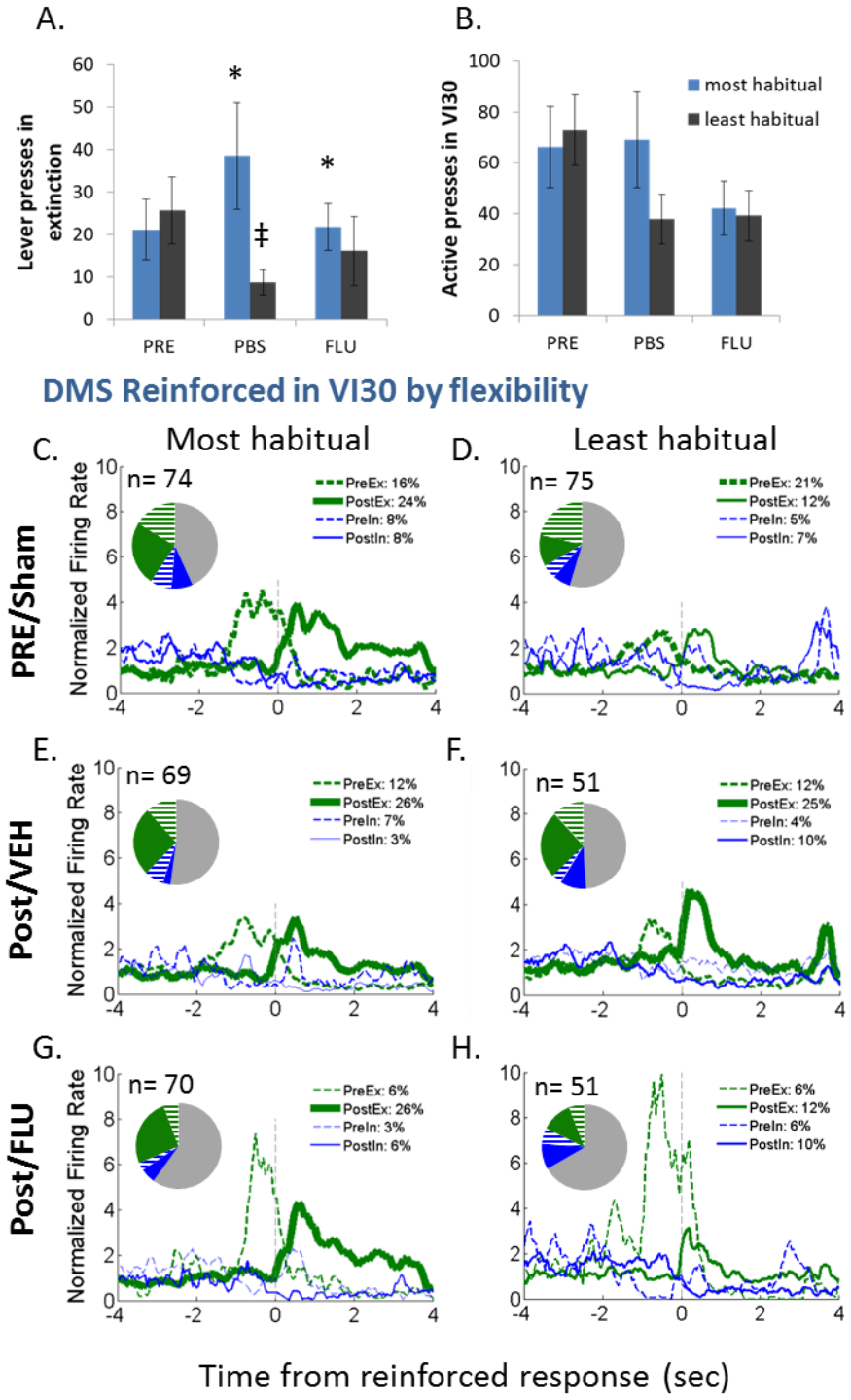


Supplemental figure 4.1: Mean normalized firing rates of anterior and posterior DMS neurons around reinforced press responses. Neuronal activity is displayed for the population of cells in a region (left; shading displays SEM), and categorized by activity type (middle and right). Panels A-F compare Sac to Sac/E rats. Sac/E rats had more PreEx neurons in both anterior (Ant) and posterior (Post) DMS, but there was no significant main effect of location. Panels G-L compare the most and least habitual rats. While no significant effect of location was observed between most and least habitual rats, the least habitual group showed more anterior DMS activation. Mean firing of neurons were categorized using z-scores comparing 1s before and after a reinforced press to a 2s baseline (PreEx: pre-excitatory; PreIn: Pre-inhibitory; PostEx: post-excitatory; PostIn: post-inhibitory; NP: non-phasic). Line thickness is proportional to the percentage of cells in a category. Pie charts display the proportion of neurons in each category, with gray being NP units. N's indicate the number of neurons recorded in a subregion.

We evaluated the effects of contingency degradation and bilateral FLU between the 10 most and 10 least habitual rats (**Supplemental figure 4.2**). These groups were defined by their performance during the 10-min degradation extinction tests; therefore, least habitual rats showed a reduction in responding in Post/VEH, while the most habitual rats did not. A significant interaction was found between flexibility and dose in extinction lever responding ($F_{2,36}$=8.63, P<0.001). Posthoc analyses show that the most habitual rats showed a significant difference between PRE and PBS as well as between PBS and FLU (Ps<0.05), while the least habitual rats significantly reduced responding only under PBS, compared to PRE. Thus, the most and least habitual groups were significantly different under PBS (P<0.05), but showed similar behavior under Post/FLU. In the VI30 reinforcement period that followed extinction testing within each session, no significant differences were found between behavioral flexibility groups (**Supplemental figure 4.2A-B**).

After extinction testing, electrophysiology was analyzed during the subsequent VI30 period in the same session (**Supplemental figure 4.2C-H**). As with Sac and SacE rats, the most and least habitual groups both showed a reduction in the number of PreEx neurons detected in the Post/FLU condition (main effect of test session, $F_{2,17}$=5.90, P<0.01). Posthoc comparisons found significant differences between PRE/Sham and Post/PBS (P<0.05) as well as PRE/Sham and Post/FLU (P<0.005). Therefore, since Post/PBS significantly differed from PRE/Sham and no difference was observed between Post/PBS and Post/FLU, differences in PreEx neurons in this analysis may be due to contingency degradation. No effect of behavioral flexibility was observed on the number of PreEx neurons detected; the degree of behavioral flexibility did not predict the neural response to treatment. Nevertheless, only a modest reduction in the number of PreEx neurons was observed in the most habitual rats in the Post/PBS condition (from 14% to

12%), therefore the least habitual rats were likely driving the effect of contingency degradation observed here. FLU in DLS may be more effective in altering DMS activation in less habitual behaviors. A potential explanation is that the role of D1 receptors may be reduced later in habit learning as synaptic potentiation increases the capacity of glutamatergic inputs to lead to action potentials without coincident dopaminergic innervation (Ramoa et al. 2013, Surmeier, Carrillo-Reid & Bargas 2011). Future studies can explore whether D2 receptor antagonists are more effective to change behavior and underlying neural signaling in more habitual rats.



Supplemental figure 4.2: Lever presses and neuronal activity during the VI30 portion of degradation test sessions organized by behavioral flexibility. (A) Analysis of lever presses in extinction revealed a significant interaction between test session and behavioral flexibility between most and least habitual rats ($P<0.001$). Most habitual group was defined by the 10 rats with the least reduction in lever responding between PRE and PBS, resulting in a significant increase after PBS, and a significant reduction by FLU(*Ps<0.05). Most habitual rats

significantly differed from least habitual rats only under PBS ($^{‡}P<0.05$). (B) Active lever responding in the VI30 period did not significantly differ between groups. (C-H) Mean firing rate of neurons categorized using z-scores comparing the second before and after a reinforced press to a 2s baseline for the most and least habitual rats (PreEx: pre-excitatory; PreIn: Pre-inhibitory; PostEx: post-excitatory; PostIn: post-inhibitory; NP: non-phasic). Reinforcements following contingency degradation pre-test (PRE), degradation post-test with DLS infusion of PBS (VEH), or post-test with infusion of 15µg FLU.

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