ABSTRACT

Title of Dissertation:	CORTICAL AND STRIATAL MECHANISMS OF VALUE-BASED DECISION-MAKING AND THEIR DISRUPTION IN ADDICTION
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For decisions both great and small, the brain utilizes an extensive network that integrates value assessment, reward prediction, and motivation to quickly and efficiently select the most beneficial option while minimizing aversive consequences for ourselves. Numerous psychiatric conditions, in particular drug addiction, can disrupt this network and impair decision-making behavior. It is therefore important to understand the neural underpinnings of decision-making and how neural activity and its associated behavior are disrupted by drugs of abuse. My dissertation will expand on current studies of this circuitry by examining epigenetic and neurophysiological mechanisms of value-based decision-making within two regions of the brain. In my final aim, I explore a new behavioral assay that may be used to study these and other regions relevant for value-based decision-making in the context of another complex behavior.

In my first aim, I have recorded from single neurons in the rat dorsal lateral striatum (DLS) after overexpressing histone deacetylase 5 (HDAC5), an epigenetic enzyme implicated in incubation of craving, in the dorsal striatum (DS). In my second aim I used pharmacological lesion and single-neuron recording combined with cocaine self-administration techniques to study anterior insula, a region well-known for combining internal and external experience but largely under-studied in the context of higher cognitive processes. These studies were conducted while rats performed an odor-guided decision-making task in which the value of rewards were manipulated by either the delay to or the size of the reward across a series of trial blocks. I have found overexpression of HDAC5 in DS promoted inflexible, faster, and automatic behavior in the decision-making task while increasing DLS's response to reward cues- similar to previous studies examining DLS activity and behavior after cocaine self-administration. In my studies of insula, I found recording from this region novel, global signals of reward value that seemed to reflect the overall structure of the behavioral task. Following cocaine-exposure, these signals were diminished while immediate rewards were over-represented on a trial-by-trial basis, leading to steeper discounting of delayed rewards. Additional studies lesioning this region promoted faster reaction times and increased goal-directed behavior. Together, these results provide insights into how drugs of abuse may impair behavioral flexibility and the tracking of long-term changes in reward from multiple mechanisms. However, it is still unknown how these changes in value assessment give rise to complex impairments of behavior. As a first step to addressing this issue, I used a new task to examine how chronic drug use- which disrupts both neural

signals in the corticostriatal circuit and epigenetic enzymes- also impairs the complex ability to delay gratification. This final study replicated well-established findings of drug-induced reversal-learning impairment, but surprisingly did not alter decision-making. This collection of work demonstrates the complexity with which drug exposure alters neural circuitry and value-based decision-making, and additionally shows the importance of utilizing complex behavioral assays to explore the relationship between brain and behavior.

CORTICAL AND STRIATAL MECHANISMS OF VALUE-BASED DECISION-MAKING AND THEIR DISRUPTION IN ADDICTION

by

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Dedication and Acknowledgements

When I first started my doctoral education, I worried – like a lot of people do – that I would not be up for the challenge. But my advisor, Dr. Matthew Roesch, expressed from the very beginning an excitement to work together that meant more to me than he probably could ever know. Thank you, Matt, for everything you have taught me, and for being an inspiring scientist and mentor to me.

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Finally, this dissertation is dedicated to Dylan Hadfield, who started this marathon with me as my boyfriend and crossed the finish line with me as my husband. Thank you for everything, and I love you.

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CHAPTER 1: INTRODUCTION

Effective decision-making is a critical function for everyday life, whether we are deciding what to have for lunch or deciding whether or not to pursue a doctoral degree. It encompasses many cognitive functions, including value assessment, prediction of future outcomes, and behavioral flexibility. Reflective of its complex nature, decision-making requires an expansive neural network encompassing brain regions involved in reward processing, motivation and emotion, and memory. Numerous psychiatric conditions are characterized by the impairment of decision-making and its neural correlates, chief among them being addiction. Thus, the study of decision-making can be crucial in understanding addiction's impact on the brain and potentially discovering new therapeutic interventions. The projects in this dissertation were designed to contribute to this issue by expanding upon what is currently understood of decision-making circuitry. This chapter will review how addiction's impairment of decision-making behavior and neural correlates have been studied, how these methods have evolved to examine more complex cognitive function, and how the projects within this dissertation will add to the existing body of work.

Addiction's Disruption of the Reward-System

Drug addiction is a chronic disease that traps an individual in a cycle of intoxication, withdrawal, and craving. The negative affect and emotional distress of withdrawal quickly transitions recreational drug use into compulsive drug-taking that often cannot be controlled, even in the face of adverse monetary, social, and health

consequences (Koob & Volkow, 2009, 2016; Nora D. Volkow & Li, 2004). The devastating toll addiction takes can clearly be seen in recent drug surveys, which indicate over 70,000 people in the United States had died from drug overdose in 2019 ("Overdose Death Rates | National Institute on Drug Abuse (NIDA)," n.d.).

Initially, drug-taking occurs because of the enhanced pleasure derived from a drug's use. After sustained exposure, drugs take on the homeostatic necessity of driving away the negative affect and emotional distress of withdrawal (Brockett, Pribut, Vázquez, & Roesch, 2018; Di Chiara, 2002; Nora D. Volkow & Morales, 2015). A substantial body of research has been devoted to addiction's dysregulation of the anatomical and neurochemical systems involved in transitioning pleasure-seeking drug use to compulsive use. As a drug of abuse is consumed, dopamine is released in the nucleus accumbens and results in the drug's pleasurable effects (Di Chiara, 2002). As drug use continues, sustained dopamine release then produces phasic changes in firing from medium spiny neurons: D1R-expressing neurons, which contribute to reward learning, are more strongly activated, while D2-expressing neurons, which contribute to aversive learning, are suppressed (Chao, Ariano, Peterson, & Wolf, 2002; R. J. Smith, Lobo, Spencer, & Kalivas, 2013; Nora D. Volkow & Morales, 2015). This altered signaling in turn emphasizes the rewarding properties of the drug. As this process occurs, higher dopamine levels gradually lead to compensatory processes that reduce neurons' excitability, resulting in the need for stronger stimuli (i.e. greater amounts/higher frequency of drug use) to produce the same response in the nucleus accumbens (Dong et al., 2006; Ferrario et al., 2010; Ishikawa et al., 2009; Kalivas & Hu, 2006). It is thought that this combination of

neural changes leads to the craving and compulsive drug use that characterizes addiction.

Incentive salience theory of addiction

The pleasurable nature of drugs of abuse not only changes the physiological balance of how they are processed by the body and mind- they also generate profound changes in the systems that modulate reward learning. This hypothesis is based on years of observing human addicts experience strong cravings from a drug's absence, but also when presented with stimuli associated with drug use (Berridge, 2012; Dayan & Berridge, 2011; Koob & Le Moal, 2005; Robinson & Berridge, 1993; Nora D. Volkow & Morales, 2015; Nora D. Volkow & Li, 2004). These cue-induced cravings are believed to happen because high levels of midbrain dopamine lead to greater incentive salience of both drugs and their associated reward (Berridge, 2012; Robinson & Berridge, 1993). With increased incentive salience, drug-associated cues can signal the presence of drugs but can additionally trigger motivational states that in turn influence behavior (Berridge, 2012).

Importantly, incentive salience is thought to be distinct from regular feelings of desire. Actions fueled by cognitive desire are generally based on a prediction that an action is going to produce an effect that in turn satisfies a need. In other words, an animal "wants" something, pursues it, "likes" it, and the motivational need is met (Berridge & Aldridge, 2008). On the other hand, cues with incentive salience are thought to generate "wanting" regardless of whether or not there is actually a need, and once something is obtained it may not even be "liked" (Berridge & Aldridge,

2008; Robinson & Berridge, 1993). This divorce of "wanting" from "liking" is thought to contribute to the compulsive drug use that develops from the cycle of craving and withdrawal- eventually drugs of abuse are sought out and taken, even though the pleasure derived from them completely disappears.

Cues with greater incentive salience are believed to have the distinct feature that the motivational states ascribed to them makes them attractive to an animal, in ways similar to the reward they actually signal. This characteristic was actually described long before it was studied in the context of drug addiction, using a task called "autoshaping". In an experiment by Brown and Jenkins (1968), pigeons learned that a light was associated with food delivery. However, the subjects expressed this learning in two distinct ways: some, called "goal-trackers", would immediately go to the food trough to wait for their reward; while others, called "sign-trackers", would peck at the light as if it were the food itself. It was hypothesized that these two behaviors signaled two types of motivations that can drive the same learned response: goal-trackers viewed the light as a representation for the relief of hunger, while for sign-trackers the light triggered the motivation for the food-taking response.

These distinctions have contributed to the incentive salience theory of addiction, and continue to be studied in the context of drug addiction. More recent studies of autoshaping have developed the hypothesis that applying incentive salience to cues associated with natural reward may predict the tendency to do the same for cues associated with drugs of abuse. Indeed, rodent studies of autoshaping have found sign tracking to be related to higher likelihood of drug use (Tomie, Grimes, & Pohorecky, 2008), faster acquisition of drug self-administration (Beckmann,

Marusich, Gipson, & Bardo, 2011), and greater preference for drugs of abuse over natural rewards (Tunstall & Kearns, 2015; Vanhille, Belin-Rauscent, Mar, Ducret, & Belin, 2014).

Thus, autoshaping studies have demonstrated that behaviors can be motivated by outcome predictions or can be automatically elicited by cues, and that these behaviors may be tied to drug-seeking behavior. A related line of work that also has become closely intertwined with addiction research is the exploration of how goals and habits shape behavior.

Habit theory of addiction

Habits are beneficial in many ways because they reduce cognitive strain for simple tasks (Malvaez & Wassum, 2018). In the context of addiction, however, drugs are thought to hijack the same neural systems that modulate habit. As a result, drug-taking becomes inflexible and automatic, and therefore harder to stop (Dickinson & Balleine, 2002; Everitt & Robbins, 2016; Sean B. Ostlund & Balleine, 2008). Observations of human drug addicts (Warren K Bickel & Marsch, 2001; Dalley & Robbins, 2017; S. H. Mitchell, 1999; Petry, 2001; Sulzer, 2011; Vuchinich & Simpson, 1998; Yi, Mitchell, & Bickel, 2010) contributed to the idea that addiction may accelerate and strengthen the formation of habits, given how quickly drug-seeking and taking behaviors would transition from controlled taking with the goal of recreation to the compulsory use necessary to prevent the aversive effects of withdrawal (Koob, & Kreek, 2007; Koob & Volkow, 2016).

Devaluation studies in animal models can be used to demonstrate how habits form (Hogarth, 2020; Malvaez, 2020; Malvaez & Wassum, 2018; Sean B. Ostlund & Balleine, 2008). For example, consider a rat that learns to press a lever for a food reward. Learning theory suggests that, as the rat continues to press the lever, the association between lever-pressing and food delivery should be strengthened and the rat should understand that pressing the lever helps achieve the goal of relieving hunger. If the rat is motivated by this goal, devaluation by changing the consequence of lever-pressing (e.g. pressing no longer leads to food) or removing motivation for the reward (e.g. feeding the rat to satiety) should reduce pressing behavior. As the rat continues to be trained, pressing may become habitual, and its pressing response may become an automatic response to the presentation of the lever. In this case, because the outcome is not as strongly tied to the response, lever-pressing should occur regardless of devaluation.

Devaluation has also been used to show inflexibility associated with exposure to drugs of abuse. Studies have largely shown that devaluing a food-associated stimulus reduces its associated behavior while drug-seeking responses are insensitive to these manipulations (Dickinson, Wood, & Smith, 2002; Loughlin, Funk, Coen, & Lê, 2017; Mangieri, Cofresí, & Gonzales, 2012; Miles, Everitt, & Dickinson, 2003). Other studies have also shown that drug addiction enhances habit-learning for natural rewards in addition to drugs of abuse (Corbit, Nie, & Janak, 2012; Schmitzer-Torbert et al., 2015), and makes animals resistant to punishments like footshock (Deroche-Gamonet, Belin, & Piazza, 2004; Pelloux, Dilleen, Economidou, Theobald, & Everitt, 2012; Pelloux, Everitt, & Dickinson, 2007; Vanderschuren & Everitt, 2004) or

unpleasant taste (Hopf, Chang, Sparta, Bowers, & Bonci, 2010; Spanagel, 2009; Wolffgramm, Galli, Heyne, & Thimm, 2000) as they pursue a drug reward.

Thus, there is compelling evidence suggesting addiction involves the hijacking of neural systems that turn drug use into a habitual, compulsive behavior. Devaluation studies in conjunction with autoshaping may also suggest that the attribution of incentive salience to reward-associated cues can contribute to the formation of habit (Amaya, Stott, & Smith, 2020; Keefer, Bacharach, Kochli, Chabot, & Calu, 2020; Nasser, Chen, Fiscella, & Calu, 2015). However, critics of the habit theory note that many of these studies rely on a specific task design in which animals have access to only one action and choice in order to produce such devaluation effects (Hogarth, 2020). Moreover, although work focusing on reward learning and circuitry is critical for understanding addiction's development, it does not fully capture this condition's pervasive nature.

Persistence of craving

Addiction can continue to influence behavior even after a person manages to break through the cycle of craving, withdrawal, and consumption. For example, individuals suffering from addiction are shown to be more susceptible to stress and drug-related cues long after withdrawal symptoms have subsided (Duncan et al., 2007; Heinz et al., 2004; Kreek & Koob, 1998; Langleben et al., 2008; McClernon, Kozink, Lutz, & Rose, 2009), resulting in high rates of relapse even during drug abstinence (Brockett et al., 2018; Werner, Altshuler, Shaham, & Li, 2021; Wikler, 1973).

This phenomenon is mirrored in rodent work using incubation of craving studies, which show that drug-seeking increases (or incubates) after a period of withdrawal. Rats display this behavior across a variety of drug classes, including heroin (Shalev, Morales, Hope, Yap, & Shaham, 2001), nicotine (Abdolahi, Acosta, Breslin, Hemby, & Lynch, 2010), alcohol (Bienkowski et al., 2004), and cocaine (Grimm, Hope, Wise, & Shaham, 2001; Tran-Nguyen et al., 1998), and in fact will show greater drug-seeking responses later compared to early in a withdrawal period. Additional studies have demonstrated important roles of midbrain and striatal regions in the expression of this behavior (Caprioli et al., 2017; X. Li, Zeric, Kambhampati, Bossert, & Shaham, 2015; Pickens et al., 2011; Scheyer et al., 2016; Wolf, 2016). Taken together, these studies demonstrate that the mechanisms of associative learning and motivation are magnified to cement the rewarding properties of drugs. As a result, drug-taking quickly becomes an ingrained behavior, and cravings for drug effects are sustained over long periods of time.

Addiction's Disruption of Decision-Making and Executive Control

Still, there is another factor that may further contribute to addiction's pervasiveness. Alongside changes in motivational and reward systems, behavioral studies show that individuals diagnosed with addiction show poorer decision-making, even for non-drug rewards (Madden, Petry, Badger, & Bickel, 1997; S. H. Mitchell, 1999; Petry, 2001; Vuchinich & Simpson, 1998; Yi et al., 2010). Such findings suggest that there are additional neural changes that result in more systemic deficits in cognitive control that compound the issue of sustained craving. Confirming this idea,

imaging studies of human addicts demonstrate that the expansive network governing decision-making shows long-term changes in function even after drug use has stopped (Bechara, 2005; W.K. Bickel, Pitcock, Yi, & Angtuaco, 2009; Warren K. Bickel, Yi, Landes, Hill, & Baxter, 2011; Warren K Bickel et al., 2007; Jentsch & Taylor, 1999; Kable & Glimcher, 2010; McClure, York, & Montague, 2004).

This work confirms that addiction can induce wide-reaching impairments of executive function, and highlights a need to study addiction in the context of complex cognitive tasks. To this end, researchers have developed a wide array of behavioral assays – comparable across both humans and animal models - that capture aspects of cognitive control, ranging from response inhibition to impulsivity.

Response Inhibition

The ability to stop a response can be studied through Go/No-Go and Stop-Signal tasks, both of which encourage habitual behavior that must then be inhibited or redirected. About 80% of trials in these tasks feature a "go" cue signaling the need for a simple instrumental response for a reward. The remaining 20% of trials feature a "No-Go" cue, where participants must refrain from making the habitual response, or the "go" cue is followed by a "stop" cue in which case the participant must stop the initiated instrumental response (Brockett et al., 2018). Withdrawal from drugs of abuse largely impairs performance on Stop-Signal tasks, across nicotine (Ashare & Hawk, 2012; Harrison, Coppola, & McKee, 2009), methamphetamine (Monterosso, Aron, Cordova, Xu, & London, 2005), and cocaine (Hester & Garavan, 2004; Kaufman, Ross, Stein, & Garavan, 2003; Kelley, Yeager, Pepper, & Beversdorf,

2005). The Roesch lab has additionally developed a Stop-Signal task for rodents, and demonstrated higher rates of failed Stops in rats prenatally exposed to nicotine (Daniel W. Bryden et al., 2016).

Response inhibition can also be examined through reversal learning, which tests the ability to stop acting on a previously learned response and alter behavior to be appropriate for new action-outcome contingencies (Brockett et al., 2018). For example, a participant in a task may learn in a series of trials that pressing one button leads to monetary reward while the other leads to monetary loss. The participant must stop acting on these previously learned associations when the button outcomes are reversed. Similar to other response inhibition tasks, previous exposure to psychostimulant drugs impair performance in these tasks for both humans (Bechara et al., 2001; Grant, Contoreggi, & London, 2000; Rogers et al., 1999) and rats (Burton et al., 2018; Burton, Bissonette, Zhao, Patel, & Roesch, 2017; Calu et al., 2007; Geoffrey Schoenbaum, Saddoris, Ramus, Shaham, & Setlow, 2004; Y. Takahashi, Roesch, Stalnaker, & Schoenbaum, 2007; Vázquez, Pribut, Burton, Tennyson, & Roesch, 2019).

Delay Discounting

Other aspects of cognitive control can be captured by delay discounting, a task that compares a smaller and more immediate reward with a larger reward delivered after a delay. The goal of this task is to manipulate the delay to find the point at which a participant will discount the value of the larger reward, and instead choose to receive the smaller reward (Brockett et al., 2018; A.L. Odum, 2011). Behavioral

studies have demonstrated that, across many drug classes, participants with a history of substance use will select immediate-but-smaller rewards at higher rates compared to controls (Madden et al., 1997; S. H. Mitchell, 1999; Petry, 2001; Vuchinich & Simpson, 1998; Yi et al., 2010). Similarly, participants with a history of nicotine use will also discount the future consequences of continued smoking (Amy L. Odum, Madden, & Bickel, 2002). Other work suggests such discounting may be a product of substance abuse while also predicting it; heroin users with greater rates of delay discounting show greater likelihood of minimizing health risks associated with sharing needles to access a drug sooner (Amy L. Odum, Madden, Badger, & Bickel, 2000), and cocaine users with higher discounting rates are also less likely to successfully maintain abstinence (Verdejo-Garcia et al., 2014; Washio et al., 2011). This bidirectional relationship between reduced cognitive control and drug use has been further supported by rodent studies (Broos, Diergaarde, Schoffelmeer, Pattij, & De Vries, 2012; Mendez et al., 2010; M. R. Mitchell et al., 2014; Perry, Larson, German, Madden, & Carroll, 2005; Simon, Mendez, & Setlow, 2007).

This body of work demonstrates wide-reaching changes in executive function that affects both drug-seeking behavior and decision-making as a whole. These behavioral changes occur alongside lasting and systemic changes to neural function, as previously mentioned, which can be well-explored using neurophysiology techniques in rodents combined with behavioral study. To this end, we have designed a task based on delay discounting. In brief, rats must nose poke into a central port at the onset of house light illumination to receive one of three odor cues (2-Octanol, Pentyl Acetate, or Carvone). The odors instruct rats towards one of two fluid wells

where they could receive a 10% liquid sucrose reward. Two of these odors signal forced-choice trials in which the rat must to go to either the left or right well to receive reward, while the third odor signals a free-choice trial in which the rat can receive a reward from either well. Reward value is manipulated by independently altering reward size and delay to delivery across blocks of ~60 trials

The structure of this task allows us to study building blocks of decision-making like anticipation, prediction errors, and reversal learning alongside their neural correlates. Moreover, we see reliable behavior in both drug-naïve and cocaine-exposed rats, giving us a behavioral foundation from which we can study neural correlates of drug-induced impairments of decision-making. Across studies, we reliably see that rats display preference for high-value rewards across both value manipulations. That is, rats are more accurate, freely choose, and respond faster for small and big rewards compared to long and small rewards (Brockett et al., 2018; Burton et al., 2018, 2017; Burton, Nakamura, & Roesch, 2015; Pribut, Vázquez, Brockett, et al., 2021; Pribut, Vázquez, Wei, et al., 2021; Roesch & Bryden, 2011; Roesch, Takahashi, Gugsa, Bissonette, & Schoenbaum, 2007; Vázquez et al., 2019). After cocaine self-administration, rats exhibit a bias for high-value rewards, especially for immediate rewards, through faster reaction times and higher percent choice for high-value rewards compared to controls (Burton et al., 2018, 2017; Pribut, Vázquez, Brockett, et al., 2021; Roesch et al., 2007; Vázquez et al., 2019). Such findings suggest rats with prior cocaine experience are especially driven towards immediate reward, as is seen in many studies of impulsivity and poor executive

function. The following sections review the circuitry involved in drug-naïve decision-making, and how drugs of abuse impair these networks.

Neurocircuitry of Reward-Guided Decision-Making

Circuit overview

Effective reward-guided decision-making requires the incorporation of value assessment, motivation, salience, and attention. To select the most beneficial option while avoiding aversive events, animals and humans must be able to predict the possible outcome from the actions they take. The orbitofrontal cortex (OFC) plays this role across primates and rodents, with its activity correlating to cues that predict reward (Morrison, Saez, Lau, & Salzman, 2011; Roesch, Calu, Esber, & Schoenbaum, 2010a; Roesch & Olson, 2004; G. Schoenbaum & Eichenbaum, 1995; Geoffrey Schoenbaum, Takahashi, Liu, & Mcdannald, 2011; Wilson, Takahashi, Schoenbaum, & Niv, 2014).

Once the animal makes its selection, the outcome needs to be assessed in comparison to what the animal expected. Starting in the basolateral amygdala (ABL), a region with reciprocal connections to OFC (Churchwell, Morris, Heurtelou, & Kesner, 2009; Ghods-Sharifi, St. Onge, & Floresco, 2009; Winstanley, Theobald, Cardinal, & Robbins, 2004), this region encodes both expected outcomes and also exhibits firing in response to unexpected appetitive or aversive outcomes (Belova, Paton, Morrison, & Salzman, 2007; Roesch, Calu, Esber, & Schoenbaum, 2010b; Tye, Cone, Schairer, & Janak, 2010). These two functions give ABL the role of both

informing OFC's predictions and relaying information on whether or not those predictions were met.

In parallel, the nucleus accumbens (NAc) and midbrain dopamine neurons within the ventral tegmental area (VTA) encode classical prediction error signals to guide reward-learning (Bissonette & Roesch, 2016; Keiflin & Janak, 2015; Koob & Volkow, 2009). The NAc contains neurons that distinctly generate value predictions (Niv & Schoenbaum, 2008; Y. Takahashi, Schoenbaum, & Niv, 2009; Van der Meer & Redish, 2011), and motivate future behavior towards or against appetitive or aversive stimuli, respectively (Bissonette & Roesch, 2016; Floresco, St. Onge, Ghods-Sharifi, & Winstanley, 2008; Stopper & Floresco, 2011). Concurrently, midbrain DA neurons within the VTA strengthen or weaken associations between stimuli and behavior by respectively increasing or decreasing firing if the outcome was better or worse than expected, or maintaining activity when a prediction is met (Roesch et al., 2007; Schultz, 1997, 1999). These calculations are projected to ABL and subsequently to anterior cingulate cortex (ACC), so that attention is increased for subsequent decisions (Daniel W. Bryden, Johnson, Tobia, Kashtelyan, & Roesch, 2011; Hayden, Heilbronner, Pearson, & Platt, 2011).

This collection of information is then sent to dorsal striatum to control the future roles of goals and habits in the execution of behavior. Traditionally, the encoding of goals and habits has been respectively delineated between the dorsomedial striatum (DMS) and the dorsolateral striatum (DLS). This theory is based on findings of prediction-error signaling and activity related to goal-directed control within DMS (McNamee, Liljeholm, Zika, & O'Doherty, 2015; Stalnaker,

Calhoon, Ogawa, Roesch, & Schoenbaum, 2010, 2012; Thorn, Atallah, Howe, & Graybiel, 2010), and studies showing DLS activity being recruited after extended training (K. S. Smith & Graybiel, 2013) and during the process of consolidating complex behavior into more automatic "chunks" (Barnes, Kubota, Hu, Jin, & Graybiel, 2005; Jog, Kubota, Connolly, & Graybiel, 1999; K. S. Smith & Graybiel, 2013; Thorn et al., 2010). Additional support for this idea comes from lesioning these regions (Corbit & Janak, 2010; Gremel & Costa, 2013; Yin, Knowlton, & Balleine, 2004, 2005; Yin, Ostlund, Knowlton, & Balleine, 2005), examinations of the striatum's spiraling connectivity with DA neurons among the rest of the basal ganglia (Haber & Knutson, 2010; Niv & Schoenbaum, 2008; Y. Takahashi et al., 2009; Van der Meer & Redish, 2011), and consistency in findings across rats (Corbit & Janak, 2010; Yin et al., 2004; Yin, Knowlton, et al., 2005; Yin, Ostlund, et al., 2005), primates (Miyachi, Hikosaka, & Lu, 2002; Miyachi, Hikosaka, Miyashita, Kárádi, & Rand, 1997), and humans (Liljeholm, Tricomi, O'Doherty, & Balleine, 2011; McNamee et al., 2015; Tricomi, Balleine, & O'Doherty, 2009).

In summary (see *Figure 1*), information on expectations and whether they were met from OFC and ABL guide value representation and prediction error signals from NAc and VTA DAergic neurons. When outcomes are not as predicted, these and attentional signals from ACC update OFC and NAc predictions, and also shape action-outcome policies maintained within DS. Whether these action-outcome policies are modulated by goals (driven by the predicted outcome) or habits (automatic behavior driven by an associative stimulus) depends on activity within the DMS and DLS, respectively. The Roesch lab has examined how the fine balance

among all of these regions has been disrupted by prior exposure to cocaine, detailed in the following section.

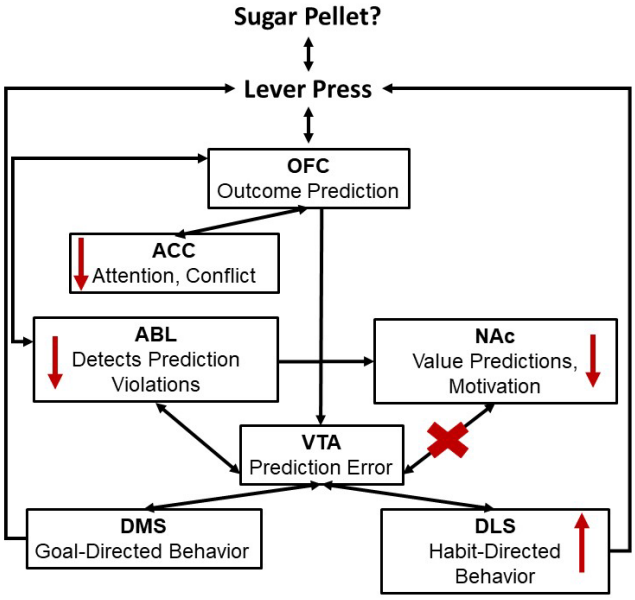

Figure 1: Effective decision-making integrates a wide network of brain regions with functions including executive processing, motivation, reward processing, and associative learning. Exposure to drugs of abuse has been shown to impair functionality across parts of this circuitry (shown in red), which may provide insight into understanding addiction. Black arrows indicate connections among these regions. Red arrows and crosses indicate cocaine-induced impairments in regions' functionality.

Cocaine-induced impairments of decision-making circuitry

Mentioned previously in devaluation and incubation of craving studies, self-administration in animals involves catheterizing a subject and training them on a simple instrumental response (e.g. pressing a lever) to receive an infusion of a drug directly into the bloodstream. The operant response can then be measured to ascertain features of addiction previously mentioned, such as the appetitive nature of the drug itself, persistence of craving, and motivation to work for the drug (Deroche-Gamonet et al., 2004).

The Roesch lab utilizes a 12-day self-administration procedure, in which rats lever press to receive infusions of cocaine for 3 hours every day. Because our interest lies in the long-term effects of drug use on decision-making behavior, we then put the rats through a 1-month withdrawal period. Finally, we combine neurophysiology with the well-established value-based decision-making task based on principles of delay discounting, detailed earlier in this chapter. This combination of techniques has allowed the Roesch lab to study complex decision-making and correlate changes in this behavior to lasting changes in neural activity, corroborating and expanding on the decision-making circuitry described above.

Recall from the previous section that, in drug-naïve decision-making, OFC and NAc guide behavior based on expectations of future outcomes. These predictions can be updated via connections to VTA and ABL (encoding prediction errors), and ACC (modulating attentional changes). Connections to DLS and DMS subsequently influence the formation of action policies and learned associations that can promote more automaticity in behavior. Strikingly, we have observed across studies that there are widespread changes in decision-making circuitry after exposure to drugs of abuse. Even after drug-taking has stopped, we see that outcome prediction is disrupted in that certain rewards – especially immediate rewards – are overrepresented in their value. Attentional mechanisms also fail so that behavior cannot be redirected. As a result of these changes, aberrant action policies are formed so that behavior is inflexibly skewed towards immediate reward.

Specifically, we first see within NAc reduced quantities of neurons that respond to natural reward and their predictive cues. This region is also less capable of

encoding value and other relevant cues for reward receipt (i.e. location or direction where a reward is going to be located). These changes seem to result in disruption of cue processing and reward (Burton et al., 2018).

Downstream of NAc, we also find neural correlates within DLS that previously represented chosen outcome now reflect possible contingencies available during the decision-making task. These signals appear even before the presentation of reward-predictive cues, suggesting DLS is maintaining a representation of preferable rewards and the rat's biases even before the animal knows what rewards are available to it. These changes are related to reductions in behavioral flexibility (Burton et al., 2018, 2017).

Recently, we have also found that previous cocaine exposure reduces signals within ACC, disrupting the relationship between changes in attention and changes in ACC firing. This change was related to overall reduced attention during the decision-making task. From these findings, we suggest ACC is less able to process conflict generated from violated reward expectations, and less able to adjust attention as a means of modifying future behavior after an error (Vázquez et al., 2019).

Taken together, the Roesch lab has uncovered several changes in decision-making circuitry related to impairments of value-based decision-making that are observed even months after prior exposure to cocaine (Figure 1, red notations). Combined with well-documented changes in midbrain DA activity and reward learning (Di Chiara, 2002; Roesch et al., 2007; Saddoris & Carelli, 2014; Saddoris, Sugam, & Carelli, 2017; Saddoris, Wang, Sugam, & Carelli, 2016), our studies suggest that there are additional lasting impairments in maintaining value predictions,

updating action-selection policies, and attention. The combination of these changes indicate impaired cognitive control after exposure to drugs of abuse, and poorer decision-making.

Further Examination of Decision-Making Circuitry

The above work has significantly advanced our understanding of the neural correlates of value-based decision-making, and how these correlates are disrupted by drugs of abuse. My dissertation will expand on our understanding of these research areas in two main projects: the first examining the role of epigenetics in DLS activity, and the second exploring neural correlates of decision-making within the anterior insula and how such correlates are impaired by previous cocaine exposure.

Overexpression of HDAC5 and its role in neural correlates of decision-making

The study of epigenetics has begun to make exciting discoveries on how environmental events affect gene expression and related behavior, especially within the fields of learning and cognition (Malvaez et al., 2018; Malvaez & Wassum, 2018; Peña, Bagot, Labonté, & Nestler, 2014). Of particular importance in this respect are epigenetic enzymes known as histone deacetylases (HDACs). Over time, a wealth of research has implicated HDACs in learning (McQuown & Wood, 2011; Vogel-Ciernia & Wood, 2012), the formation of habits (Malvaez et al., 2018), long-term memory (McQuown et al., 2011), and in psychiatric conditions that can impair cognitive control (X. Li et al., 2018; Peña et al., 2014; Pickens et al., 2011; Renthal & Nestler, 2009; Robison & Nestler, 2011; Rogge & Wood, 2013; Werner et al., 2021).

These studies illustrate HDACs' importance in a wealth of behaviors, but it is not fully understood how HDACs affect neural activity within the circuitry of decision-making.

HDACs are involved in DNA transcription, which occurs when its chromatin structure loosens to give transcription factors access to the DNA strand (Balleine et al., 2021; Dulac, 2010; Turner, 2002; L. Wang et al., 2010). This process can occur through histone acetylation, and is understood to play a role in the functionality of brain regions necessary for learning and memory. For example, higher concentrations of acetylated histones have been found in DMS cells after goal-directed action (Balleine et al., 2021).

As their name suggests, HDACs remove the acetyl groups necessary for acetylation, and result in chromatin compaction that makes DNA less accessible for transcription (Li et al., 2018; Pandey, Ugale, Zhang, Tang, & Prakash, 2008; Penney & Tsai, 2014; Turner, 2002). These enzymes can be classified as either class I or class II HDACs, depending on whether they are located exclusively within the nucleus of the cell, or whether they shuttle between the cytoplasm and nucleus, respectively (Li et al., 2018; Pribut, Vázquez, Wei, et al., 2021). Thus, HDACs are able to serve as a molecular "brake", and their removal can play an important role in cognitive processes. For example, salient events have been found to stimulate the removal of HDACs from the promoter regions of genes and initiate the learning process (McQuown and Wood, 2011; Vogel-Ciernia and Wood, 2012).

HDACs also play a significant role in the hedonic processing of drugs of abuse, as seen in studies examining opioids (Chen et al., 2016; Ferguson et al., 2013),

alcohol (Botia, Legastelois, Alaux-Cantin, & Naassila, 2012; Pandey et al., 2008; Sakharkar, Zhang, Tang, Shi, & Pandey, 2012), and cocaine (Renthal et al., 2007; Taniguchi et al., 2017). As an expansion of this work, the Li lab has explored HDAC5 within dorsal striatum (DS) and its role in the incubation of methamphetamine craving. They first found that HDAC5 concentrations within DS increased during incubation of craving tests for methamphetamine (X. Li, Rubio, et al., 2015). To solidify this relationship, they next used viral techniques to manipulate HDAC5 expression, and found overexpression enhanced incubation of craving (e.g. increased meth seeking after abstinence) while knockdown decreased it (Li et al., 2018).

Taken together, HDACs are clearly necessary for a variety of behaviors modulated by DS, including simpler forms of learning and the expression of craving during addiction. In parallel to this work are studies conducted by the Roesch lab and others examining the lasting changes to both DS circuitry and reward-guided decision-making (Bissonette & Roesch, 2016; Burton et al., 2017, 2015; Lucantonio, Caprioli, & Schoenbaum, 2014; Takahashi et al., 2007). A previous study from the Roesch lab, specifically, has shown neural activity within the DLS that encodes action-outcome policies, and disruptions in such activity is related to behavioral biases for high-value rewards.

Several questions arise from this body of work: how do HDACs influence decision-making and executive functioning? Specifically, do epigenetics influence neural activity in regions significant for decision-making? Furthermore, do epigenetic manipulations produce changes in behavior that may be related to studies of

psychiatric conditions like addiction? These questions are explored in Chapter 2 of my dissertation by overexpressing HDAC5 in DS and recording from DLS neurons as rats perform the reward-guided decision-making task used in the Roesh lab. My findings demonstrate how HDAC5 is related to the formation of action-outcome policies and the execution of decision-making behavior. This work is published in *The Journal of Neuroscience.*

The role of anterior insula in drug-induced impairments in decision-making

Our current understanding of decision-making circuitry effectively demonstrates how the interplay among value-assessment, attention, and motivation is disrupted by drugs of abuse. However, there are other regions that may contribute to how these signals are integrated and how value is subjectively measured, and these regions have yet to be fully uncovered.

One region of interest in this regard is the insula, which is broadly associated with integrating external experience with internal sensation (Naqvi & Bechara, 2009, 2010; Naqvi, Gaznick, Tranel, & Bechara, 2014). Across humans, primates, and rodents, insula spans almost the entire length of the brain, and is subdivided by cell type and anatomy. The insula can be split by both of these distinctions: insula consists of granular, dysgranular, and agranular sections, in reference to the loss of the granule layer in more anterior regions of insula (Naqvi & Bechara, 2009), and can also be split by anterior-posterior distinctions (Gogolla, 2017).

Anatomical distinctions are especially helpful in elucidating the wide array of insula's connectivity and function. Posterior insula (PI) largely receives input from

thalamus, parietal, occipital, and temporal lobes, which allows for the integration of somatosensory, vestibular, and motor stimuli. Anterior insula (AI), on the other hand, has reciprocal connections to ACC, ventromedial prefrontal cortex (vmPFC), and limbic regions like the amygdala and ventral striatum. This subsection of insula also contains large concentrations of D1 receptors (Gaspar, Berger, Febvret, Vigny, & Henry, 1989), corticotropin-releasing hormone receptors (Hurd, Suzuki, & Sedvall, 2001), and μ-opioid receptors (Baumgärtner et al., 2006). This wide connectivity is largely thought to give insula the role of processing conscious experience, as it essentially integrates external experience with emotional, motivational, and cognitive processing (Naqvi & Bechara, 2009).

This collection of evidence has made insula a prime research subject for studying topics ranging from gustation to decision-making, with studies on the latter cementing insula as an important region for higher cognitive function. Neuroimaging studies have demonstrated insula activity related to delay discounting (M. Wittmann, Lovero, Lane, & Paulus, 2010), especially when a decision leads to a worse outcome than expected (Y.-Y. Zhang et al., 2018). Another line of work began implicating insula in addiction, finding activity related to past recollection of drug use (Wang et al., 1999), exposure to drug-associated cues (Bonson et al., 2002), and food cravings (Pelchat, Johnson, Chan, Valdez, & Ragland, 2004). Most notably, an fMRI study found a positive relationship between insula damage and success at quitting smoking, with participants reporting they experienced a “lack of interest” in nicotine that helped them quit (Naqvi, Rudrauf, Damasio, & Bechara, 2007).

From here insula research increased, and rodent studies emerged to further establish insula's role in more complex cognitive function and drug addiction. Studies in PI suggest this region is involved in processing hedonic properties of drugs and craving, as inactivation stops nicotine and alcohol self-administration (Forget, Pushparaj, & Le Foll, 2010; Pushparaj & Le Foll, 2015). AI, on the other hand, seems to be involved in goal-directed behavior (Kesner & Gilbert, 2007; Parkes et al., 2018), and risk-avoidance (Pushparaj et al., 2015), in both drug-naïve rats and in rats exposed to drugs of abuse (Contreras et al., 2012; Seif et al., 2013). These results suggest insula, in particular AI, may play a role in higher cognitive function. In confirmation, neurophysiology studies of AI have shown signals within AI encoding negative prediction errors (Jo & Jung, 2016), consumption and anticipation (Kusumoto-Yoshida, Liu, Chen, Fontanini, & Bonci, 2015; Samuelsen, Gardner, & Fontanini, 2012), and motivation (Mizoguchi et al., 2015; Moschak, Wang, & Carelli, 2018).

As insula research continues to grow within decision-making and addiction neuroscience, it is important to examine how signals observed within insula are involved in complex, reward-guided decision-making and how these signals are affected by exposure to drugs of abuse. Specifically, how does the insula encode value and other elements relevant to decision-making? How might such signals be functionally relevant to decision-making behavior? Chapter 3 of my dissertation addresses these questions by examining neural correlates of AI (referred to as insula from this point forward) and how they are impaired by prior cocaine exposure. The functional relevance of these signals is further explored through lesioning techniques.

Neural recording data is published at *The Journal of Neuroscience*, while lesioning data has been published at *Brain Research*.

Together, the combination of these projects introduce epigenetic factors and new anatomical regions that contribute to reward-guided decision-making. Moreover, my dissertation suggests how these discoveries play a role in both drug-induced impairments of decision-making and how they may be tied to drug relapse. In this dissertation, I present findings suggesting HDAC5, an epigenetic mechanism behind relapse behavior (X. Li et al., 2018; Renthal & Nestler, 2009; Robison & Nestler, 2011; Werner et al., 2021), is involved in striatal changes in neural activity that are related to drug-induced deficits in decision-making (Pribut, Vázquez, Wei, et al., 2021). I additionally present data showing novel activity within the insula that likely encodes global changes in value and satiation (Pribut, Vázquez, Brockett, et al., 2021), and may play a role in the faster responding seen after cocaine self-administration (Pribut, Sciarillo, & Roesch, 2022; Pribut, Vázquez, Brockett, et al., 2021). These findings expand on our understanding of how drugs of abuse induce long-term changes in decision-making, both at anatomical and molecular levels. Finally, I present work demonstrating how we can begin to develop new behavioral tasks to continue efforts in exploring addiction's interactions with optimal decision-making (Chapter 4).

CHAPTER 2: Overexpressing histone deacetylase 5 in rat dorsal striatum alters reward-guided decision-making and associated neural encoding

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ABSTRACT

Accumulating evidence in the past decade implicates histone modifying enzymes, such as class I histone deacetylases (HDACs), in learning and memory, and recently habit formation. However, it is unclear whether HDACs play roles in complex cognitive function. To address this issue, we examined the role of dorsal striatal HDAC5, a class II HDAC, in reward-guided decision-making and associated neural encoding in rats. We first injected adeno-associated virus to overexpress a nuclear-localized HDAC5 in dorsal striatum (DS). We then recorded neural correlates from dorsolateral striatum (DLS) as rats performed two reward-guided choice tasks, in which we manipulated either the size of or delay to reward. During these tasks, rats first learned which of two options led to the better reward, and then reversed those contingencies in a second block of trials. We found that rats with HDAC5 overexpression in DS responded faster and chose higher value reward more often during the first block of trials, but were less able to reverse those contingencies in the second block of trials. At the neural level, HDAC5 overexpression in DS elevated and reduced the number of cells in DLS that increased firing to stimuli and reward, respectively, and shifted encoding toward cues that predicted more immediate reward. These results suggest that the HDAC5 overexpression in DS contributes to inflexible decision-making, demonstrating a role of histone modifying enzymes in complex cognitive function.

INTRODUCTION

Histone deacetylases (HDACs) are a family of epigenetic enzymes that suppress gene transcription by removing acetyl groups from histone proteins (Kouzarides, 2007). Over the past decade, extensive literature has demonstrated that HDACs, primarily class I HDACs (HDAC1, 2 and 3), contribute to synaptic plasticity associated with learning and memory (Mahgoub & Monteggia, 2014; Peixoto & Abel, 2013; Penney & Tsai, 2014; Schmauss, 2017). For example, overexpressing HDAC2 in mouse hippocampus disrupts synaptogenesis, synaptic formation, long-term potentiation and hippocampal-dependent learning and memory formation, while opposite effects are observed in HDAC2-deficient mice (Guan et al., 2009). Emerging evidence recently showed that HDAC3 in rat dorsal striatum (DS) also regulates associative learning, such as habit formation (Malvaez et al., 2018). However, whether HDACs contributes to complex executive cognitive functions, such as reward-guided decision making, is unknown. More intriguingly, how epigenetics influence neural activity at the single cell level during decision-making tasks has not been explored. Dysregulated HDAC function has been linked to aging, neurodegenerative diseases (e.g., Alzheimer's disease) and psychiatric disorders (e.g., drug addiction) (Hwang, Aromolaran, & Zukin, 2017; Peña et al., 2014; Schmauss, 2017; Werner et al., 2021). Therefore, elucidating the role of HDACs in executive cognitive functions can help understand how dysregulated HDAC activity may lead to impaired cognitive functions under these pathological conditions (Forman, Trojanowski, & Lee, 2004; Koob & Volkow, 2009; Murman, 2015; Samson & Barnes, 2013; Nora D. Volkow & Li, 2004; Wyss-Coray, 2016).

Here we examined the role of HDAC5, a class IIa HDAC, in rat DS in reward-guided decision making and associated neural encoding. Like other class IIa HDACs (HDAC4, 7 and 9), HDAC5 can shuttle into the nucleus from cytoplasm upon dephosphorylation in an activity dependent manner (Borrelli, Nestler, Allis, & Sassone-Corsi, 2008; McKinsey, Zhang, Lu, & Olson, 2000). We focused on striatal HDAC5 based on its critical role in regulating drug-related behaviors in rodent models. For example, HDAC5 and its downstream targets in nucleus accumbens (NAc) modulate the rewarding aspect of cocaine (Borrelli et al., 2008; McKinsey et al., 2000; Renthal et al., 2007; M. Taniguchi et al., 2017; Makoto Taniguchi et al., 2012). Work from us and others also demonstrates that HDAC5 in NAc and DS contributes to cocaine and methamphetamine relapse, respectively (X. Li et al., 2018; M. Taniguchi et al., 2017). Such findings suggest that the study of HDAC5 here can provide important insight into our observations that behavioral deficits in decision-making develop after chronic drug use (Brockett et al., 2018; Burton et al., 2018, 2017, 2015; Pribut, Vázquez, Brockett, et al., 2021; Roesch et al., 2007; Stalnaker, Roesch, Franz, Burke, & Schoenbaum, 2006; Stalnaker, Takahashi, Roesch, & Schoenbaum, 2009; Y. Takahashi et al., 2007; Vázquez et al., 2019).

To examine the role of HDAC5 in value-based decision-making behavior, we overexpressed HDAC5 in DS. We additionally recorded from dorsolateral striatum (DLS), a region known for its well-established involvement in forming and governing habits by encoding associative information between stimuli, outcomes and responses (e.g., S-R associations) (Bernard W. Balleine, Delgado, & Hikosaka, 2007; Burton et al., 2017, 2015; Malvaez & Wassum, 2018; Yin & Knowlton, 2006). Our results

showed that HDAC5 overexpression in DS promoted fast, inflexible behavior during reward-guided decision-making, accompanied by enhanced and reduced firing to cues and reward in DLS, respectively. These findings provide the first evidence, to our knowledge, that HDAC5 in DS contributes to impulsive and inflexible decision-making, behavioral deficits previously observed after chronic drug use (Brockett et al., 2018; Burton et al., 2018, 2017, 2015; Pribut, Vázquez, Brockett, et al., 2021; Roesch et al., 2007; Stalnaker et al., 2006, 2009; Y. Takahashi et al., 2007; Vázquez et al., 2019). Therefore, HDAC5 in striatum may serve as a candidate epigenetic target that underlie the impaired cognition associated with drug addiction.

MATERIALS AND METHODS

Subjects

Eighteen Sprague-Dawley rats, both male and female, were obtained at 175-200g from Charles River Laboratories. Seven rats were excluded due to death during surgeries (n = 1) and electrode implantation issues (n = 6). The remaining 11 rats (9 males; 2 females) reported herein refer to those used in statistical analyses. Subjects were tested at the University of Maryland, College Park in accordance with University and National Institutes of Health guidelines.

Reward-guided choice tasks

Prior to surgery, the rats were trained on the Delay/Size choice task (*Fig.* 2.1A) for approximately one month. During the task, rats were trained to nose poke into the central odor port upon illumination of the house light. While in the odor port, the rat was exposed to one of three odor cues (2-Octanol, Pentyl Acetate, or Carvone) that

would direct how to obtain the 10% sucrose water reward. Two odor cues were forced-choices where the rat was instructed to go to either the left or right well to receive reward. The third cue was a free-choice where the rat would be rewarded at either well. The cues associated with each odor were maintained across sessions. Odors were counterbalanced across rats and presented in a pseudorandom sequence with equal distributions of left/right odors and free-choice odor occurring on 7/20 trials. Rats were water deprived to encourage motivation in the tasks. If the incorrect well was selected on a forced-choice trial, the houselights turned off and no reward was delivered. During initial training, rats were first trained to nose poke and then go to the wells for immediate delivery of reward for 1-2 days. After that, we introduced free-choice trials where rats could choose one well or the other to obtain reward. Then, we gradually introduced (2 per day) forced-choice trials. While training them on these contingencies, we progressively increased (100 ms per day) how long rats had to stay in the odor port and fluid wells, until they could remain in the odor port for 1s and wait for delayed rewards up to 7s.

Rats underwent one recording session per day and alternated between two session types: delay and size blocks. At the start of delay sessions, one well was randomly designated to have short delays (500 ms) and the other to have long delays (1000-7000 ms) prior to reward delivery. On long delay trials, the delay increased by 1000 ms each time the long delay well was chosen by the rat during a free-choice trial. The maximum delay time was 7000 ms and could be reduced by 1000 ms on each free choice trial if the long delay well had been chosen <8 times out of the last 10 trials to a minimum delay of 3000 ms. After 60 trials, short and long delay

parameters were switched to opposite wells. At the start of size sessions, one well was randomly designated as the big well and the other as the small well. The small well delivered one 0.05 mL 10% sucrose water bolus; the big well delivered two 0.05 mL 10% sucrose water boluses, the second appearing 500 ms after the first. The delay time before reward delivery was held constant throughout the session at 500ms. After 60 trials, the big and small parameters were switched to the opposite wells.

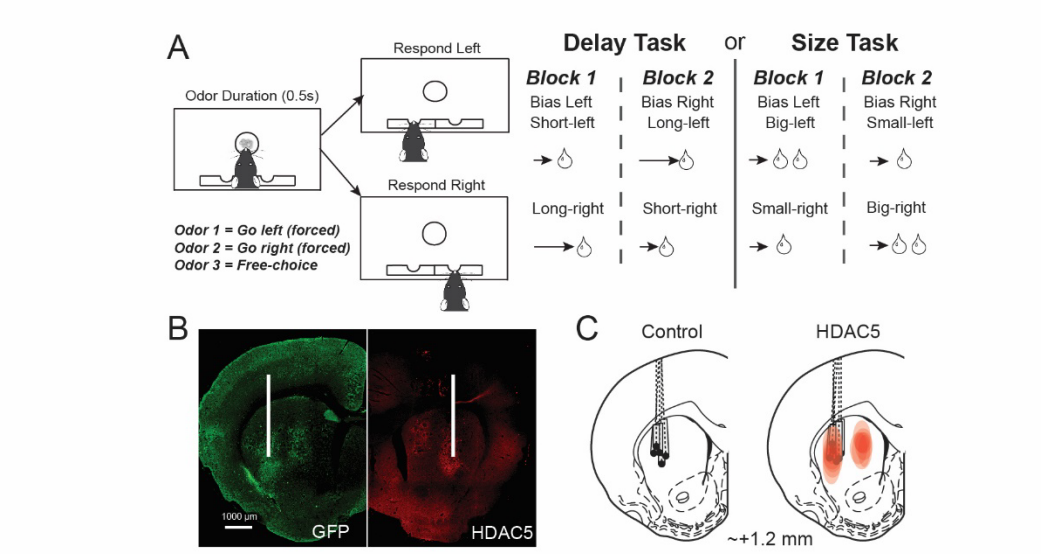

Figure 2.1: Reward-guided decision-making task, virus injections/expressions and recording sites.
(A) Schematics of the reward-guided decision-making task, showing the sequence of events in a single trial (left) and the sequence of blocks for delay and size tasks. **(B)** Representative images of GFP expression from control rats and HDAC5 immunostaining from HDAC5 group. White lines indicated placement of electrodes. **(C)** Electrode placements in control and HDAC5 rats, and virus spread in HDAC5 rats (red area in the far right diagram). Note the overlap between the recording area and virus expression in HDAC5 rats.

Adeno-associated virus (AAV) injections

We used AAV2 that shows mostly neuronal tropism (Haery et al., 2019). Both AAV2-mHDAC5 ($5x10^{e12}$ viral particles/ml) and AAV2-GFP ($4x10^{e12}$ viral particles/ml) are driven by CMV promoters. AAV2-mHDAC5 expressed a

dephosphorylated mutant of HDAC5 (S259A/S279A/S498A or 3SA) that was primarily localized to the nucleus (M. Taniguchi et al., 2017). AAV2-GFP expressed green fluorescence proteins (GFP) and was used as the control condition. Detailed plasmid maps are available upon request. The *in vivo* validation of HDAC5 overexpression by AAV2-mHDAC5 and comparisons to baseline expression of HDAC5 were previously demonstrated by Li et al (X. Li et al., 2018). (*Fig. 2.1*B).

Rats received either bilateral AAV2-mHDAC5 (n=6: 5 males and 1 female) or AAV2-GFP injections (n=5: 4 males, 1 female) as described previously (X. Li et al., 2018). Briefly, each hemisphere received a total of four injections (0.75 μl/injection), with two injections aiming at the dorsomedial striatum (DMS) and the other two injections aiming at DLS. We used the following coordinates from Bregma: DMS: anterior posterior(AP), +1.2 mm; medial lateral (ML), ±2.6 mm (6° angle); dorsal ventral (DV), -4.0 mm and -5.0 mm. DLS: AP, +1.2 mm; ML, ±3.8 mm (6° angle); DV, -5.0 mm and -6.0 mm. We delivered the AAVs by Hamilton syringes (32 gauge) at a rate of 0.375 μl/min. After each injection, we left the injection needle in place for an additional minute to allow diffusion. After the final injection, we filled the drilled hole with bone wax. It is noted that we overexpressed HDAC5 in the entire DS. This is based on previous observations that HDAC5 manipulations need to be administered to the entire DS, as those targeting the DLS or DMS alone are ineffective in modulating drug seeking (X. Li et al., 2018).

Electrode implantation

Immediately after virus injections, we implanted unilateral drivable electrodes (bundles of 10-25 µm-diameter FeNiCr wires, cut at an angle so that wires are at different lengths) in the DLS (AP, +1.2 mm, ML, 3.2 mm, DV, -3.5 mm) for subsequent single-unit recordings, and we counterbalanced the hemispheres of the electrode implantations (*Fig. 2.1*C).

Single-unit recording

Procedures were the same as described previously (D. W. Bryden & Roesch, 2015). Wires were screened for activity daily; if no activity was detected, the rat was removed and the electrode was advanced 40 or 80 µm to reach new cells. Otherwise, a session was conducted and the electrode was advanced at the end of the session. Neural activity was recorded using four identical Plexon Multichannel Acquisition Processor systems. Signals from electrode wires were amplified 20x by an op-amp headstage, located on the electrode array. Immediately outside of the training chamber, the signals were passed through a differential preamplifier (Plexon, PBX2/16sp-r-G50/16fp-G50) where single unit signals were amplified 50x and filtered at 150-9000 Hz. Single unit signals were then sent to the Multichannel Acquisition Processor box, where they were further filtered at 250-8000 Hz, digitized at 40 kHz and amplified at 1-32x. Waveforms (>2.5:1 signal-to-noise) were extracted from active channels and recorded to disk by an associated workstation with event timestamps from the behavior computer.

Experimental Design and Statistical Analyses

Behavior during the reward-guided choice tasks were analyzed by calculating percentage choice of a particular valued condition (i.e. short, long, large, small) on free-choice trials, reaction times on free-choice trials (i.e. odor offset to odor port exit), and movement time on free-choice trials (i.e. odor port exit to fluid well entry). Like previous studies (Brockett et al., 2018; Burton et al., 2018, 2017; Pribut, Vázquez, Brockett, et al., 2021; Vázquez et al., 2019), percent choice analyses included the first and last 10 trials in their respective reward categories to examine changes in behavior after a block switch. We additionally examined behavior in each session to determine whether there were any transient changes to behavior during the recording period (Vaidya, Pujara, Petrides, Murray, & Fellows, 2019).

Behavioral analyses were computed for each individual session, and averaged across sessions for HDAC5 and control groups. We took the minimal number of sessions collected from one rat, and then used that same number of sessions for each rat split over the entirety of recording. Thus, each rat contributed the same number of sessions to the behavioral analyses. For reaction and movement times, we used a repeated measures analysis of variance test with factors for group (Control vs HDAC5), task (Delay vs Size), and session as a within-subjects measure. For percent choice, we used a repeated measures ANOVA test with factors of group (Control vs HDAC5), phase (first vs last 10 trials), block (1st or 2nd), and session as a within-subjects measure. Bonferroni corrected post hoc t-tests were used to further examine significant interaction terms.

Single units were sorted using template matching software in Offline Sorter (Plexon, Dallas, TX). Timestamps and event markers were extracted from the file using Neuroexplorer (Nex Technologies, Colorado Springs, CO. Data was analyzed using RStudio (Boston, MA) and MATLAB (MathWorks, Natick, MA). Analysis epochs were calculated by taking the total number of spikes and dividing by time. Baseline firing activity was taken 1 s before odor onset. Increasing- and decreasing-type neurons were designated based on whether firing increased or decreased significantly relative to the baseline (Wilcoxon; $p<0.05$). The odor cue epoch was taken 100 ms after odor onset until well-entry. The reward epoch of 1 s encompassed 250 ms before reward delivery to 750 after reward delivery. This epoch has been used previously (Burton et al., 2018, 2017; Pribut, Vázquez, Brockett, et al., 2021; Vázquez et al., 2019) to capture firing related to the anticipation and delivery of reward. The epoch captures activity immediately preceding reward delivery without overlapping with movement-related firing even at the shortest delays (i.e., 500 ms) and captures firing related to the multiple sucrose boli delivered during large reward trials. Relationships between neural firing and behavioral activity were determined with regression tests for each neuron, separately. Specifically, regressions were performed on trial firing rates and reaction times collected during each recording session, as opposed to averaging across trials.

Histology

Rats were deeply anesthetized with isoflurane and perfused transcardially with 500 mL of 0.01 M phosphate buffered saline (PBS). Brain tissue was then fixed with

500 mL of 4% paraformaldehyde (PFA) for 1 h before being transferred into 30% sucrose PBS solution. Once the brains sunk, they were sectioned into 30 μm slices using a Leica cryostat and stored in cryoprotectant at -80 ºC. For HDAC5 immunohistochemistry, the sections were washed for 10 minutes in PBS and then incubated for 1 h at room temperature in blocking buffer (2% BSA in PBS with 0.3% Triton-x100). The sections were incubated next with a primary antibody against HDAC5 (sc-133106, 1:500, Santa-Cruz, TX, RRID: AB_2116793) in blocking buffer overnight at room temperature. After washing the sections 3 times in PBS (5 min each), they were incubated with the secondary antibody Alexa 594-labeled anti-mouse (R37121, 1:200, Thermo Fisher Scientific, MD, RRID: AB_2556549) in blocking buffer for 1 h at room temperature. Finally, the sections were washed in PBS and mounted on glass slides (Fisherbrand™ Superfrost™ Plus Microscope Slides, Cat #12-550-15) that were air-dried and cover-slipped with Fluormount G (Electron Microscopy Sciences).

RESULTS

HDAC5 overexpression in DS decreased reaction time during both delay and size tasks

Our first analyses examined reaction time, defined as the time taken to exit the central nose port after presentation of the odor stimulus, across delay (8 sessions/rat) and size (6 sessions/rat) tasks (*Fig. 2.2A, C*). We analyzed data using a repeated measures ANOVA with factors of group (Control vs HDAC5) and task (Delay vs Size), and session as a within-subjects measure. A significant main effect of task

demonstrated that all rats exhibited significantly faster reaction times during size tasks ($F(1,146) = 11.005$, $p = 0.001$, *ANOVA*). Furthermore, a significant main effect of group showed that HDAC5 rats were also faster overall across both task manipulations ($F(1,146) = 4.345$, $p = 0.039$, *ANOVA*). There was no significant effect of session ($F(1,146) < 0.01$, $p > 0.05$, *ANOVA*), nor were there any significant interactions ($F(1,146) \leq 1.734$, $p \geq 0.881$, *ANOVA*).

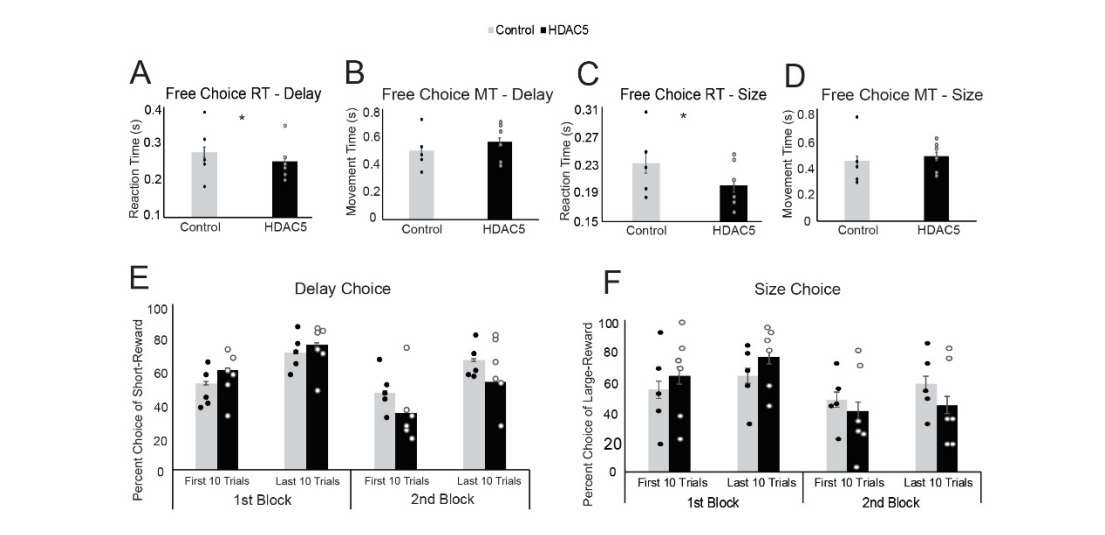

Figure 2.2: HDAC5 overexpression decreased reaction time and made choice behavior less flexible

Dots represent individual rat averages. **(A)** Reaction time (RT; odor offset to odor port exit) averaged over all correct trials and recording sessions. We took the minimal number of sessions collected from one rat, and used that same number of sessions for each rat split over the entirety of recording for the Delay Task (control rats' n's = 162, 123,111, 49, 40 and HDAC5 rats' n's = 153, 132, 118, 63, 36, 35). **(B)** Movement time (MT; odor port exit to fluid well entry). **(C-D)** Same as A-B during performance of the Size task (control rats' n's = 103, 98, 63, 46, 45 and HDAC5 rats' n's = 143, 114, 99, 61, 33, 28). **(E)** Percent choice of short-delay trials during blocks 1 and 2. Within each block, trials were split into first and last 10 free-choice trials. Post-hoc t-tests for significant interactions between group and block were averaged across these early and late periods of a session. **(F)** Same as E during performance of the Size task.

Based on the assumption that rats leave the odor port once they have made their choice selection, one interpretation of the reaction time result is that rats with HDAC5 overexpression are making faster decisions than control rats. However, an alternative

interpretation is that HDAC5 rats simply exhibit enhanced motor responses in general. To address this issue, we also measured movement time as defined as the time from odor port exit to the fluid well as a general reflection of movement speed (*Fig. 2.2B, D*). We found no significant main effects of group ($F(1,146) = 2.426$, $p = 0.122$, *ANOVA*), task ($F(1,146) = 0.989$, $p = 0.322$, *ANOVA*), or session ($F(1,146) < 0.01$, $p > 0.05$), nor were there any significant interactions ($F(1,146) \leq 2.426$, $p \geq 0.122$, *ANOVA*). Thus, HDAC5 overexpression selectively decreased reaction time but caused no general motor enhancement.

HDAC5 overexpression in DS promoted inflexible behavior

Decisions governed under habitual control are thought to be under the control of model-free systems that do not take into account task structures (e.g., frequent reversals), thus allowing animals to respond without deliberations and to develop associative behaviors more strongly and quickly. There is a tradeoff, however, with behavioral flexibility. In line with these theories, we found that HDAC5 rats formed stronger associations in the first block of trials that were difficult to reverse in the second block of trials.

Our next set of analyses (*Fig. 2.2E* and *2.2F*) examined the percent choice on free-choice trials for high value choices (i.e., short-delay or large-reward), broken down into first or last 10 trials for blocks 1 and 2 (Note there were roughly 20 free-choice trials per block that were randomly interleaved with forced-choice trials). We used a repeated measures ANOVAs to analyze these data with factors of group

(Control vs HDAC5), task (Delay vs Size), phase (First 10 vs. Last 10 trials within a block), and block (1st vs 2nd). Session was examined as a within-subjects factor.

For both Delay (*Fig. 2.2E*) and Size Tasks (*Fig. 2.2F*), we observed a significant main effect of phase ($F(1,584) = 45.543$, $p < 0.001$, ANOVA), indicating that all rats selected high-value rewards significantly more during the end of a trial block compared to the beginning. A main effect of block additionally showed all rats selected high-value rewards significantly more in the first compared to the second block of trials ($F(1,584) = 35.161$, $p < 0.001$, ANOVA), likely because rats were overriding previously learned reward contingencies they had acquired in the first block. There was no significant main effect of session ($F(1,584) < 0.01$, $p > 0.05$, ANOVA) or task ($F(1,584) = 0.004$, $p = 0.950$, ANOVA). There was significant interaction between task and phase ($F(1,584) = 5.724$, $p = 0.017$, ANOVA), however Bonferroni-corrected post-hoc t-tests indicated no significant differences between either the first 10 trials of delay and size tasks ($t(306) = -1.176$, $p = 0.240$, t-test), nor the last 10 trials of delay and size tasks ($t(306) = 2.172$, $p = 0.031$, t-test). Thus, all rats generally chose more high-value rewards by the last 10 trials and during the first block, and there were no significant differences between tasks.

Interestingly, although there was no significant main effect of group ($F(1,584) = 0.063$, $p = 0.803$, ANOVA), we did observe a significant interaction between group and block ($F(1,584) = 12.745$, $p < 0.001$, ANOVA). Bonferroni-corrected post-hoc t-tests indicated that HDAC5 rats selected high-value rewards significantly more than control rats during block 1 ($t(306) = 2.366$, $p = 0.019$, t-test), but subsequently selected high-value rewards significantly less than control rats during block 2 (t(306)

= -2.743, $p = 0.006$, t-test). All other interactions were not significant ($F(1,584) < 0.001$, $p > 0.05$). Together, these results suggest that rats generally performed best by the end of the first block and needed to re-learn reward contingencies in the second block. This contrast in performance between the first and second block was significantly amplified by HDAC5 overexpression, across both delay and size tasks.

HDAC5 overexpression in DS elevated the number of neurons that increased firing to reward predicting stimuli

During the Delay Task, we recorded from the DLS: 485 neurons in control rats (n's = 162, 123,111, 49, 40) and 537 neurons in HDAC5 rats (n's = 153, 132, 118, 63, 36, 35). In control rats, 14% (n's = 66; 31, 17, 10, 7, 1) and 39% (n's = 189; 89, 54, 17, 15, 14) of neurons significantly increased and decreased firing during odor cue sampling, respectively (*Fig. 2.3A*; gray). In HDAC5 rats, 26% (n's = 137; 83, 14, 14, 12, 10, 4) and 38% (n's = 205; 88, 56, 28, 16, 9, 8) of neurons significantly increased and decreased firing, respectively (*Fig. 2.3A*; black). The frequency of increasing to decreasing neurons was significantly higher in HDAC5 rats (X^2= 12.5; $p = 0.0004$, X^2), as was the frequency of increasing to total cells recorded ($X^2 = 14.7$; $p = 0.0001$, X^2). The counts of decreasing cells did not significantly differ between groups ($X^2 = 0.01$; $p = 0.91$, X^2). We also found no significant difference in baseline activity in either increasing ($t(171) = -0.002$, $p = 0.999$, *unpaired t-test*) or decreasing cells ($t(392) = 0.250$, $p = 0.802$, *unpaired t-test*).

During the size task, we recorded from the DLS: 355 neurons in control rats (n's = 103, 98, 63, 46, 45) and 478 neurons in HDAC5 rats (n's = 143, 114, 99, 61, 33,

28). In control rats, 19% (n = 67; 38, 10, 8, 6, 5) and 29% (n = 102; 44, 26, 15, 9, 8) of neurons significantly increased and decreased firing during odor cue sampling, respectively (*Fig. 2.3B*; gray). In HDAC5 rats, 30% (n = 142; 88, 18, 13, 10, 9, 4) and 38% (n = 181; 92, 39, 27,9, 8, 6) of neurons increased and decreased firing, respectively (*Fig. 2.3B*; black). The frequency of increasing to total cells recorded was significantly higher in HDAC5 rats ($X^2 = 7.3$; $p = 0.007$, X^2) than control rats, whereas the frequency of decreasing cells did not significantly differ between groups ($X^2 = 3.5$; $p = 0.06$, X^2). We found no significant difference in baseline activity in either increasing ($t(203) = -1.273$, $p = 0.204$, *unpaired t-test*) or decreasing cells ($t(159) = 1.246$, $p = 0.215$, *unpaired t-test*). Taken together, overexpressing HDAC5 in DS elevated the counts of DLS neurons that increased firing during sampling of reward-predicting stimuli across tasks.

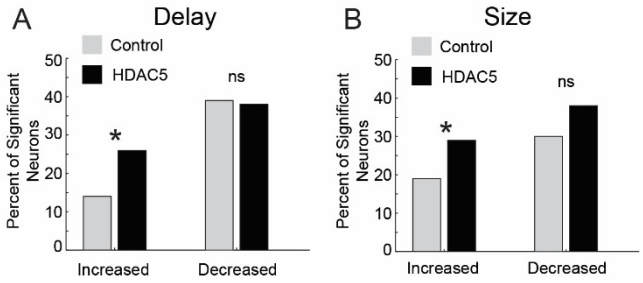

Figure 2.3: HDAC5 overexpression elevated the count of neurons that increased firing during stimulus presentation
Percent of cells that significantly increased or decreased firing during odor sampling (odor onset to port exit) compared to baseline (1 s epoch starting 1 s before odor onset; Wilcoxon, $p < 0.05$) during the Delay (Control: 14% increased, 39% decreased; HDAC5: 26% increased, 38% decreased) **(A)** and Size (Control: 19% increased, 29% decreased; HDAC5: 30% increased, 38% decreased) **(B)** Task.

HDAC5 overexpression in DS increased the number of neurons that fired more strongly for short-delay reward

Previously, we have shown that neurons in DLS will fire more strongly for certain directions (e.g. left or right), and for certain outcomes (e.g. short, long, big, or small) with similar distributions of each. Here, we examined temporal firing of increasing and decreasing neurons, and quantified this selectivity during cue sampling. We sorted firing into preferred (i.e. towards the neuron's response field; *Fig. 2.4* left panels) and non-preferred directions (i.e. away from the neuron's response field; *Fig. 2.4* right panels), and preferred and non-preferred outcomes, based on the direction and outcome that elicited the highest firing rate. For example, if a neuron fired the strongest to cues that predicted short delayed reward for responses made in the left direction, 'short delayed reward' was designated as the 'preferred outcome' and 'left' was designated as the response made 'into' that cell's response field. In this example 'long delayed reward' would be the 'non-preferred' outcome and 'right' would be the response made 'away' from the response field. This procedure simply allows us to average firing across all neurons, so that we can examine temporal changes in firing to task events.

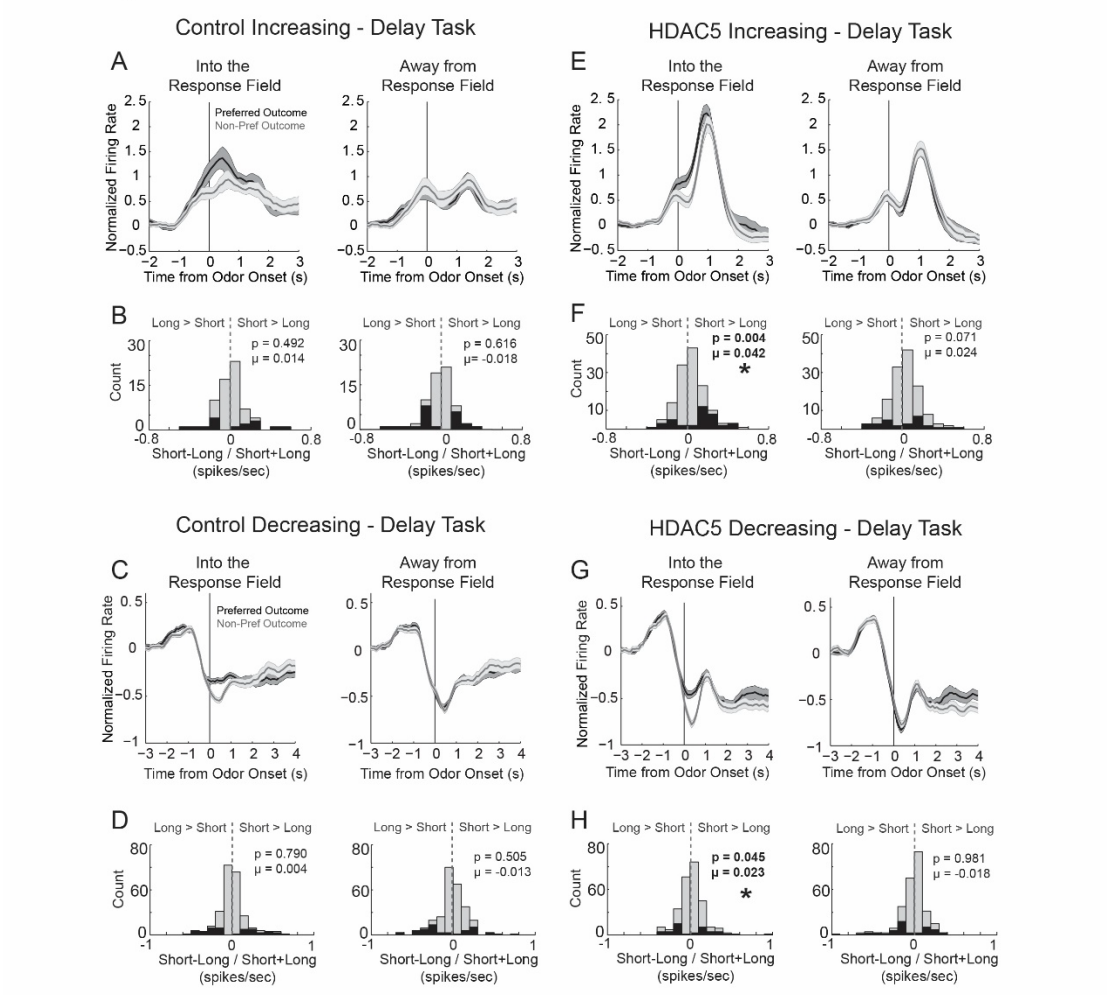

Figure 2.4: HDAC5 overexpression increased movement-related firing and the proportion of cells firing to short-delay predicting cues.
(A) Average firing over all control cells that increased firing during odor sampling (increasing n = 66; decreasing n = 189) aligned to odor onset. We sorted firing into preferred (i.e. towards the neuron's response field) and non-preferred directions (i.e. away from the neuron's response field), and preferred and non-preferred outcomes, based on the direction and outcome that elicited the highest firing rate. **(B)** Distribution of delay indices computed for neuron during the odor epoch (short-long/ short+long). Gray bars reflect the distribution of indices across the entire population of neurons, while black bars represent neurons that exhibit a 'preference' for short- or long-delay trials, firing significantly more (above zero; short > long) or firing significantly less (below zero; long > short) for cues that predicted short delayed reward. **(C, D)** Same as A, B except for cells that decreased firing during the odor epoch (n = 189). **(E-H)** Same as A-D with data from HDAC5 rats (increasing n = 137; decreasing n = 205).

Fig. 2.4A and *E* illustrate normalized firing – aligned to odor onset – for neurons that increased firing in control and HDAC5 rats during delay blocks. Data were

normalized by z-scoring. As defined, increases in firing were present during odor sampling. Interestingly, while firing in control rats peaked near the end of odor sampling (500 ms after its onset) prior to the onset of the movement, firing in HDAC5 rats exhibited a more dramatic rise after odor presentation (> 500 ms after odor onset) during initiation of the movement, which likely reflected accelerated responses as described above (see *Fig. 2.2*) and further analyzed below by showing correlations between firing rate and reaction time at the level of single neurons.

To quantify selectivity during the delay task, we plotted the normalized difference between short and long (delay index = short – long /short + long) for each neuron for control and HDAC5 rats. Gray bars reflect the distribution of indices across the entire population of neurons, while black bars represent neurons that exhibit a 'preference' for short- or long-delay trials, firing significantly more (above zero; short > long) or firing significantly less (below zero; long > short) for cues that predicted short delayed reward (*Fig. 2.4B*). Examining activity across odor-responsive cells from control rats, we found distributions of delay indices were not shifted significantly above or below zero (into: $p = 0.492$, $\mu = 0.014$, Wilcoxon; *Fig. 2.4B*, left; away: $p = 0.616$, $\mu = -0.018$, Wilcoxon; *Fig. 2.4B*, right). Interestingly, in HDAC5 rats, the preference for neurons to increase firing to short delayed reward increased. Unlike controls, the distribution of delay indices in HDAC5 rats were significantly shifted above zero for movements made into to the response field ($p = 0.004$, $\mu = 0.042$, *Wilcoxon*; *Fig. 2.4F*, left), indicating that the majority of neurons tended to fire more strongly for cues that predicted short delayed reward. Similar results were present for

neurons that decreased firing during odor presentation (i.e., *Fig. 2.4H*, left; $p = 0.045$, $\mu = 0.023$, *Wilcoxon*)

These analyses were repeated for neurons responsive to odor cues during size blocks (*Fig. 2.5*). Across conditions, response profiles were similar to those observed during delay blocks; however, unlike delay manipulations, shifts in selectivity distributions (size index; large – small/ large + small) did not differ between groups, suggesting the 'size' encoding was not altered by HDAC5 overexpression beyond there being fewer task-related neurons overall. For increasing-type cells, none of the distributions--for either control or HDAC5 rats--were significantly shifted (*Fig. 2.5B.* into: $p = 0.194$, $\mu = -0.030$, *Wilcoxon*, left; away: $p = 0.881$, $\mu = 0.019$, *Wilcoxon*, right; *Fig. 2.5F*. into: $p = 0.120$, $\mu = 0.017$, *Wilcoxon*, left; away: $p = 0.790$, $\mu = -0.002$, *Wilcoxon*, right). This was also observed in neurons that decreased firing for responses made into the response field (Control, *Fig. 2.5D*, left: $p = 0.606$, $\mu = 0.005$, *Wilcoxon*; HDAC5, *Fig. 2.5H*, left: $p = 0.781$, $\mu = 0.015$, *Wilcoxon*). For responses made away from the response field, distributions in both groups were shifted in the negative direction (*Fig. 2.5D*, right; $p = 0.005$, $\mu = -0.041$, *Wilcoxon*; *Fig. 2.5H*, right; $p = 0.042$, $\mu = -0.024$, *Wilcoxon*).

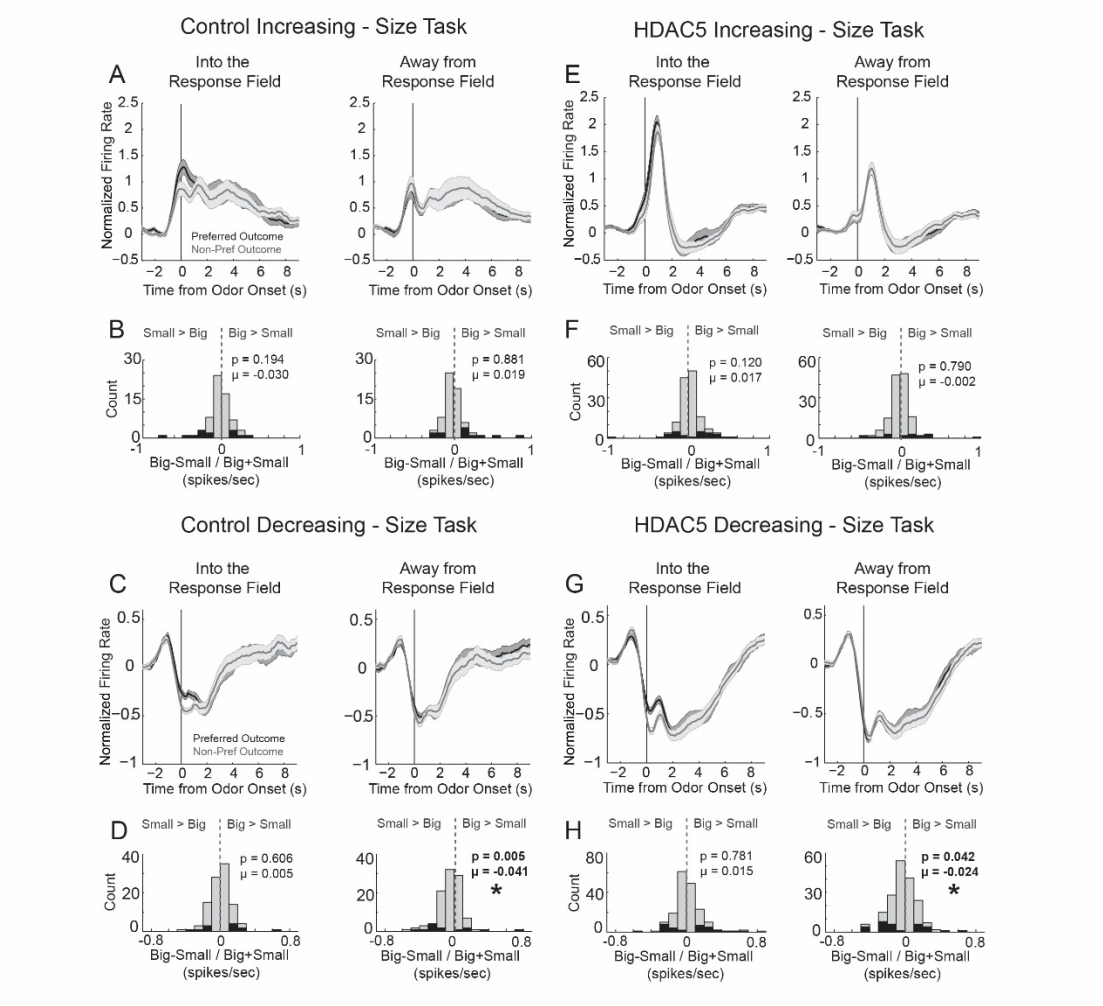

Figure 2.5: HDAC5 overexpression in dorsal striatum increased movement-related firing during the Size Task.
(A) Average firing over all control cells that increased firing (increasing n = 67; decreasing n = 102) during odor sampling aligned to odor onset. We sorted firing into preferred (i.e. towards the neuron's response field) and non-preferred directions (i.e. away from the neuron's response field), and preferred and non-preferred outcomes, based on the direction and outcome that elicited the highest firing rate. **(B)** Distribution of size indices computed for neuron during the odor epoch (large-small/ large+small). Gray bars reflect the distribution of indices across the entire population of neurons, while black bars represent cells with significant differences between large and small (Wilcoxon; $p < 0.05$). **(C, D)** Same as A, B except for cells that decreased firing during the odor epoch (n = 102). **(E-H)** Same as A-D with data from HDAC5 rats (increasing n = 142; decreasing n = 181).

Firing of single neurons was correlated with reaction time

To better understand the relationship between behavior and changes in firing, we examined the correlation between firing rate and reaction time for each single neuron.

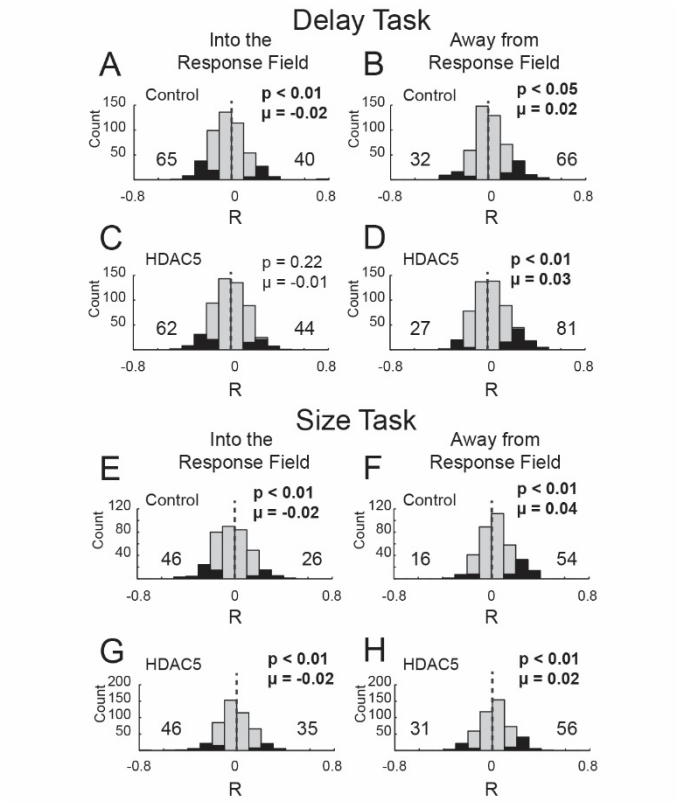

Figure 2.6: Firing in dorsolateral striatum was correlated with reaction time. **(A-D)** Distribution of R values for within cell correlations between firing rate and reaction time in control **(A, B)** and HDAC5 **(C, D)** rats for movements made into (A, C; left panels) and away from (B, D; right panels) from the response field.

Overall, firing tended to be negatively correlated with reaction time for movement made into a cell's response field and positively correlated for movements made away from a cell's response field, suggesting that increases and decreases in firing promoted and attenuated choices to be made into the cell's response field, respectively. For movement into the cell's response field, all distributions were shifted in the negative direction (Control delay: *Fig. 2.6A*, $p < 0.01$, $\mu = -0.02$, *Wilcoxon*; HDAC5 delay: *Fig. 2.6C*, $p < 0.22$, $\mu = -0.01$, *Wilcoxon*; Control size: *Fig. 2.6E*, $p < 0.01$, $\mu = -0.02$, *Wilcoxon*; HDAC5 size: *Fig. 2.6G*, $p < 0.01$, $\mu = -0.02$, *Wilcoxon*), whereas for movement away from the cell's field, all distributions were shifted in the positive direction (Control delay: *Fig. 2.6B*, $p < 0.05$, $\mu = 0.02$, *Wilcoxon*; HDAC5 delay: *Fig. 2.6D*, $p < 0.01$, $\mu = 0.03$, *Wilcoxon*; Control size: *Fig. 2.6F*, $p < 0.01$, $\mu = 0.04$, *Wilcoxon*; HDAC5 size: *Fig. 2.6H*, $p < 0.01$, $\mu = 0.02$, *Wilcoxon*).

HDAC5 rats had fewer reward-responsive neurons

The above analyses suggest that HDAC5 overexpression elevated firing to stimuli prior to and during the initiation of the behavioral response, and firing during the sampling of stimuli was correlated with reaction time at the single neuron level. During these analyses, we noticed that--while firing was increased during presentation of odors and subsequent responding--firing during the delivery of reward appeared to be lower in HDAC5 rats than control rats (*Fig. 2.5A* and *2.5E*). Based on this observation, we examined reward-related activity by asking how many neurons significantly increased firing during the anticipation and delivery of reward (reward epoch; $p < 0.05$; *Wilcoxon*). We found that 21% (n = 102; 37, 37, 16, 7, 5) cells in control rats increased firing to reward, whereas only 10% (n = 54; 17, 14, 13, 4, 4, 2,) increased responding after HDAC5 overexpression (*Fig. 2.7A*; $X^2 = 16.7$, $p = 4.4E\text{-}5$, X^2). However, there were no differences in baseline activity between these two populations of cells (t(75) = -0.963, *p* 0.338, *unpaired t-test*).

The average firing of these neurons aligned to reward delivery is illustrated for both control and HDAC5 rats in *Fig. 2.7B* and *C*. As defined, firing increased over long delays until reward was delivered. Likewise, for size manipulations, we found 29% (n = 104; 49, 16, 14, 13, 12,) neurons from control rats increased during the reward epoch, whereas only 10% (n = 49; 19, 13, 7, 5, 5, 0) were observed after HDAC5 overexpression (*Fig. 2.7D*; $X^2 = 32.4$, $p = 1.26E\text{-}8$, X^2). Notably, consistent with the analysis during odor sampling described above, firing of HDAC5

overexpressed ‘reward’ neurons also displayed prominent cue and movement-related firing prior to reward (*Fig. 2.7C*, HDAC5 (~-3s); *Fig. 2.7F*, HDAC5 (~-1s)).

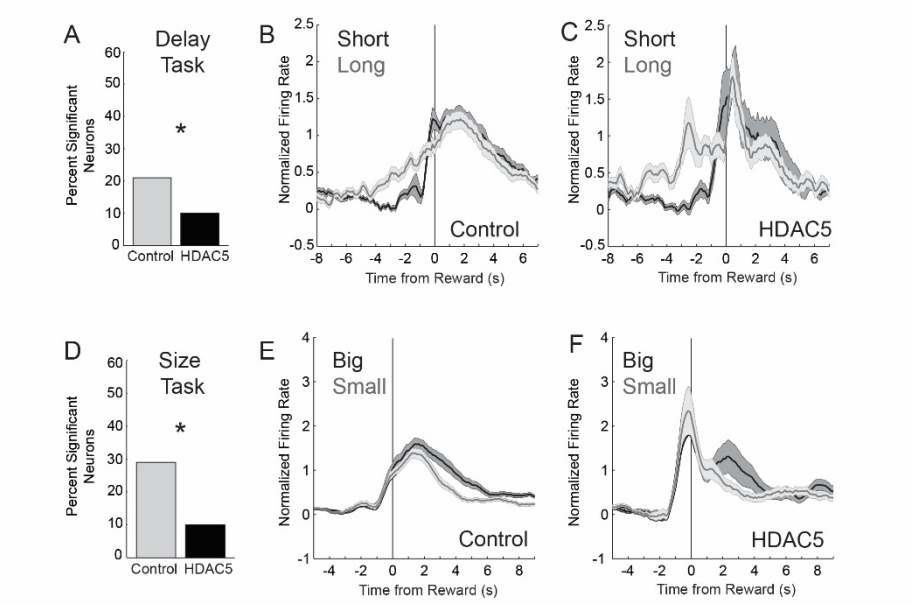

Figure 2.7: HDAC5 overexpression reduced counts of neurons that increased firing to reward delivery.
(A) Percentage of neurons that increased firing during the reward epoch (1 s; 250 ms before to 750 ms after reward delivery). **(A, C)** Average firing of neurons that increased firing during the reward epoch in control (n = 102, 21%) and HDAC5 rats (n = 54, 10%) in the Delay Task. Firing is aligned to reward delivery. **(D-F)** Same as A-C with data from the Size Task (control = 104, 29%; HDAC5 = 49, 10%).

DISCUSSION

Here we examined the effects of DS HDAC5 overexpression on reward-guided decision-making and associated neural correlates in DLS. Our main findings are as follows: at the behavioral level, rats with HDAC5 overexpression showed decreased reaction time and inflexible behavior on both delay and size tasks; at the neural level, we observed an increase in the numbers of neurons that fired more to reward-predicting stimuli and cues that predicted short delayed reward, but a decrease in the number of neurons that were responsive to anticipation and reward delivery following HDAC5 overexpression.

We observed a compelling increase and decrease in the selection of high-value rewards during the first and second block of trials in our HDAC5 rats. There are a number of potential explanations for this result; it is possible that learning in the first block of trials interfered with performance in the second block, or rats may have had trouble acquiring new associations once reward contingencies reversed in the second block. While it is difficult to pinpoint the exact cause of our findings, we interpret these results to be evidence of behavioral inflexibility, based on previous studies examining the roles of HDACs and dorsal striatum in habit (Malvaez et al., 2018; Malvaez & Wassum, 2018). Decisions governed under habitual control are thought to be under the control of model-free systems that do not take into account task structures (e.g., frequent reversals), thus allowing animals to respond without deliberations and to develop associative behaviors more strongly and quickly. There is a tradeoff, however, with behavioral flexibility. Here, the decrease in high-value reward selection may indicate such an inability to modify behavior once reward contingencies change.

Further support of this hypothesis may also come from the faster reaction times seen in our HDAC5 rats. In previous studies (Brockett et al., 2018; Daniel W. Bryden, Johnson, Diao, & Roesch, 2011; Burton et al., 2018, 2017; Pribut, Vázquez, Brockett, et al., 2021; Vázquez et al., 2019) and our current study, we have used reaction time (i.e. how quickly rats leave the odor port after odor presentation) as a measure of how quickly rats decide which reward well to approach. Importantly, we observed no significant difference in movement times (i.e. how quickly rats enter the fluid well after odor port exit) in our HDAC5 rats, indicating our reaction time

findings were not just a reflection of enhanced motor output. Taken together, our behavioral findings hint a relationship between behavioral inflexibility and HDAC5 overexpression, although future studies will be needed to further explore other potential roles of HDAC5 in learning and memory mechanisms.

Most importantly, our findings suggest HDAC5 may be involved in abnormal decision-making behavior, a relationship that has thus far been largely unexplored. To date, only one other study has used a classic conditioning procedure to examine the role of HDAC3 in the formation of habitual behavior (Malvaez et al., 2018). This study, conducted by the Wassum lab, found that suppressing HDAC3 function--either through pharmacological or viral approaches, in either DLS or DMS--facilitates habit formation, while potentiating HDAC3 function through viral-mediated HDAC3 overexpression in either dorsal striatal subregions prevents habit formation. Overall, these data indicate that, in DS, HDAC3 negatively regulates habit formation. In contrast, we showed that HDAC5 overexpression in DS led to faster inflexible behavior, implicating a positive role of HDAC5 in regulating habitual behavior.

However, direct comparison between these two studies should be made with caution. Although all HDACs generally suppress gene expression, HDAC3 and HDAC5 belong to Class I and Class IIa HDAC, respectively, and they differ in many aspects that determine their distinct functions--such as structures, the protein complexes they form, downstream targets, cellular and tissue localization, enzymatic activities and substrate specificities (De Ruijter, Van Gennip, Caron, Kemp, & Van Kuilenburg, 2003; Seto & Yoshida, 2014). A question for future research is what

cellular mechanisms- and differential effects on the regulation of downstream targets- lead to their distinct roles in habit and decision-making behavior.

Our single-unit recording data also provides the first evidence, to our knowledge, of a relationship between epigenetic mechanisms and the way in which neurons respond to different cues and reward, suggesting potential, dynamic connections amongst epigenetic events, neural activity, and changes in behaviors. With our current dataset, it is unclear whether HDAC5 overexpression produced changes in neural activity and subsequently behavior, or epigenetic changes produced behavioral changes that in turn altered patterns of neural activity within the DLS. However, we speculate that a finely orchestrated adaption of gene expression across several classes of molecules (e.g., ion channels, glutamate receptors, transcription factors), as well as alterations in intracellular signaling pathways, would be required to modify the encoding properties of neurons. These relationships will be elucidated in future studies.

An intriguing application of our data is that HDAC5 and other epigenetic enzymes may serve as a critical link between psychiatric disorders and associated cognitive impairment. For example, we and others have previously studied how drugs of abuse disrupt normal decision-making and lead to impulsive, habitual behavior (Burton et al., 2018, 2017; Mendez et al., 2010; Pribut, Vázquez, Brockett, et al., 2021; Vázquez et al., 2019). Our lab has also shown that prior cocaine experience- even long after its acute effects have worn off- altered activity within DLS. These results are remarkably similar to the current study. A robust body of work has also examined HDACs in relation to drug addiction (see (Renthal & Nestler, 2009;

Robison & Nestler, 2011; Rogge & Wood, 2013; Werner et al., 2021) for in-depth review). In particular, we have previously shown that dysregulated HDAC5 in DS is relevant in an animal model of methamphetamine relapse (X. Li et al., 2018), leading to the question of whether or not the mechanisms between relapse and drug-induced impairments in decision-making are at all shared. While addressing this question is beyond the scope of our current study, we are interested in future work where these issues are studied in tandem.

One limitation in the present study is that we overexpressed HDAC5 in both DMS and DLS; therefore, whether behavioral effects observed here require manipulations of the entire DS or specific sub-regions is unknown. However, based on our previous finding that decreased methamphetamine seeking is only observed after HDAC5 knockdown in both DMS and DLS, but not in either subregion alone (X. Li et al., 2018), we speculate that behavioral effects observed here require HDAC5 overexpression in the entire DS. Regarding neural correlates--although we focused on examining DLS encoding here, we hope to include the DMS in future studies, in light of emerging evidence implicating DMS in habitual control (Malvaez & Wassum, 2018; Stalnaker et al., 2010; Vandaele et al., 2019).

In sum, we demonstrated that rats with HDAC5 overexpression in DS demonstrated inflexible behaviors and altered associated neuronal encoding in DLS. Our findings contrast with the recent examinations on the role of HDAC3 in DS in habit formation (Malvaez et al., 2018), indicating distinct mechanisms underlying decision-making and habitual control across different HDACs. Interestingly, our results were in line with previous observations of decision-making impairments after

chronic cocaine use (Brockett et al., 2018; Burton et al., 2018, 2017, 2015; Pribut, Vázquez, Brockett, et al., 2021; Roesch et al., 2007; Vázquez et al., 2019). Such findings may suggest that HDACs could be a critical link between psychiatric disorders and associated cognitive impairment. Further studies will focus on the causal relationship amongst epigenetics, neural activity, and observed behavior, and how HDAC5 impacts executive control and neuronal encoding in the context of drug addiction in order to further our understanding of HDACs' potential utility in psychiatric treatments.

CHAPTER 3A: Prior cocaine exposure increases firing to immediate reward while attenuating cue and context signals related to reward value in the insula

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ABSTRACT

The insula contributes to behavioral control and is disrupted by substance abuse, yet we know little about the neural signals underlying these functions or how they are disrupted after chronic drug self-administration. Here, male and female rats self-administered either cocaine (experimental group) or sucrose (control) for twelve consecutive days. After a one-month withdrawal period, we recorded from insula while rats performed a previously learned reward-guided decision-making task. Cocaine-exposed rats were more sensitive to value manipulations and were faster to respond. These behavioral changes were accompanied by elevated counts of neurons in the insula that increased firing to reward. These neurons also fired more strongly at the start of long delay trials, when a more immediate reward would be expected, and fired less strongly in anticipation of the actual delivery of delayed rewards. Although reward-related firing to immediate reward was enhanced after cocaine self-administration, reward-predicting cue and context signals were attenuated. In addition to revealing novel firing patterns unique to insula, our data suggests changes in such neural activity likely contribute to impaired decision-making observed after drug use.

INTRODUCTION

The insula has recently gained traction as a key contributor to relapse and drug-seeking behaviors, and as a potential therapeutic target for addiction. Work in humans has shown that insula is activated during the presentation of drug cues and recollection of past drug use (Bonson et al., 2002; Wang et al., 1999). Further, it has been shown that damage to insula can promote drug abstinence (Naqvi et al., 2007). Rodent work has supported these ideas by showing that disruption of insula function reduces the ability of drug-associated cues and contexts to drive drug-seeking (Campbell et al., 2019; Cofresí et al., 2019; Contreras et al., 2012; Contreras, Ceric, & Torrealba, 2007; Forget et al., 2010; García, Simón, & Puerto, 2013; Gil-Lievana et al., 2020; C. L. Li, Zhu, Meng, Li, & Sui, 2013; Pelloux, Murray, & Everitt, 2013; Pushparaj & Le Foll, 2015; Seif et al., 2013; R. Zhang et al., 2019).

Although insula is clearly involved in drug addiction, it is less clear how it contributes to normal behavioral control, or how those mechanisms might be disrupted by chronic drug abuse. Traditionally, insula has been described as an interoceptive center (Craig, 2002, 2010; Damasio et al., 2000; Naqvi & Bechara, 2010), but is also thought to contribute to functions related to reward processing and decision-making (Burke & Tobler, 2011; Droutman, Read, & Bechara, 2015; Mesulam & Mufson, 1982; Ongur & Price, 2000; Preuschoff, Quartz, & Bossaerts, 2008; Rogers-Carter & Christianson, 2019). Consistent with these functions, recent work has shown that firing in insula correlates to the anticipation and delivery of both positive and negative outcomes (Guillem, Kravitz, Moorman, & Peoples, 2010; Jo & Jung, 2016; Kusumoto-Yoshida et al., 2015; Mizoguchi et al., 2015; Moschak et al.,

2018; Samuelsen et al., 2012; Vincis, Chen, Czarnecki, Chen, & Fontanini, 2020; M. K. Wittmann et al., 2020). Here, we ask if outcome-related neural correlates in insula are disrupted by chronic cocaine self-administration in rats performing a reward-guided decision-making task.

During this task (*Fig. 3.1A, B*) rats use reward-predicting cues to guide choice behavior between two options, the values of which are manipulated across two contexts: in one, rats choose between an immediate and delayed reward; in the other, rats choose between a large and small reward with delays to reward held constant. In both contexts, rats learn which option yields the preferred outcome, and maintain those expectations during delays to reward and across trial blocks while following forced-choice rules. We have previously shown that rats prefer immediate over delayed reward, and large over small reward, and that rats are more motivated during 'size' block contexts (Burton et al., 2018, 2017; Roesch & Bryden, 2011; Roesch et al., 2007; Vázquez et al., 2019). Further, we have shown that rats that previously self-administered cocaine are more sensitive to value manipulations and exhibit faster reaction times months after drug exposure (Brockett et al., 2018; Burton et al., 2018, 2017; Vázquez et al., 2019).

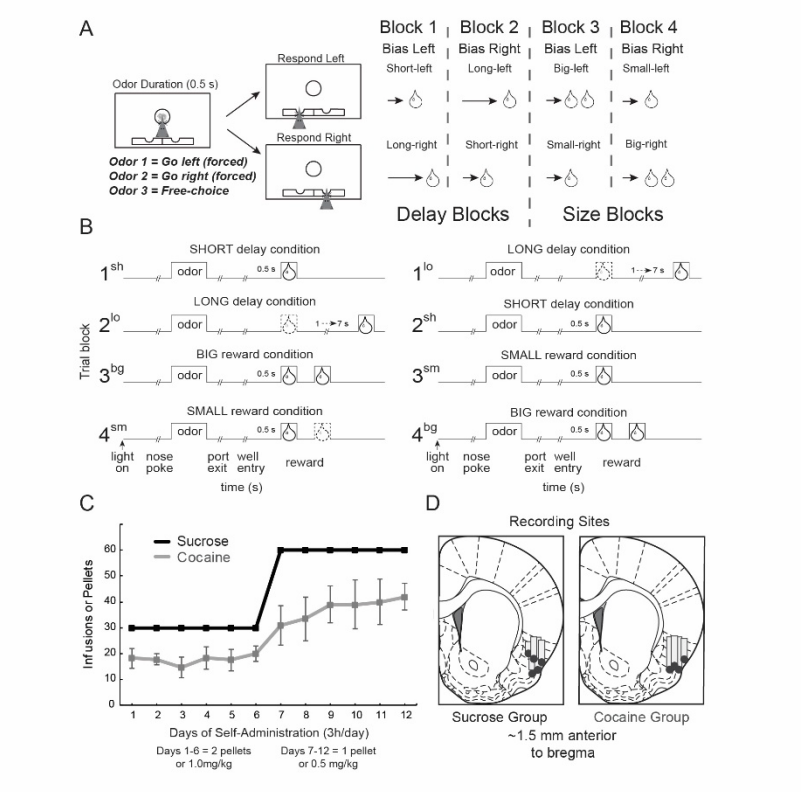

Figure 3.1: Reward-guided decision-making ask, self-administration data, and recording sites
(A) Task schematic, showing an example trial (left panels) and block sequence in a session (right). Rats nose-poked in a central port for 0.5s to receive an odor with a duration of 0.5s. Odors would instruct them to respond to fluid wells below where they could receive liquid sucrose rewards after 500-7000ms. (**B)** Summary of block structure. During a recording session, one fluid well would be arbitrarily designated as short (delivering reward after a short 500ms delay) and the other designated as long (delivering reward after a 1-7s delay). Contingencies were reversed after ~60 trials (Block 2). In Block 3, delays to reward were held constant at 500ms, but the well designated as long in the previous block now offered 2 boli (i.e. big reward), while the other well offered only 1 (i.e. small reward). Contingencies switched once again in Block 4. Each block shift was unsignaled so that rats needed to learn through behavior that reward values had changed. **(C)** Average lever-press rates for Control (black) and Cocaine-exposed (gray) rats for each day of self-administration. Data points represent each day. Bars on the cocaine-exposed group represent standard error of the mean (SEM). **(D)** Electrode placement for each rat, verified by histology.

Given insula's role in addiction and its dysregulation following drug use, we hypothesized that functional signals related to reward processing would be disrupted by chronic cocaine self-administration. We found reward delivery-related signals in

cocaine-exposed rats were enhanced, while context- and cue-related firing was reduced. Further, encoding of immediate delivery of reward was more prominent in cocaine-exposed rats. These results suggest heightened reward responding and lower cue and context selectivity in insula may contribute to increased impulsivity and poor decision-making observed after chronic drug use (Brockett et al., 2018; Burton et al., 2018; Mendez et al., 2010; Roesch et al., 2007; Simon et al., 2007; Vázquez et al., 2019).

MATERIALS AND METHODS

Subjects

Nine Long-Evans rats (weight: 175-200g, 3 females; 6 males) rats were obtained from Charles River Laboratories. Subjects were held and tested at University of Maryland, College Park according to university and NIH guidelines.

Odor-guided Delay/Size choice task

Rats were trained on the Delay/Size task (summarized in *Fig. 3.1A, B*) for one month prior to surgery. In brief, subjects needed to nose poke into a central port during house light illumination to receive one of three odor cues (odors were 2-Octanol, Pentyl Acetate, or Carvone). These odors instruct rats which direction to move to receive a 10% liquid sucrose reward from wells located on either side of the central port. Two of these odors instructed rats to go either left or right for reward (forced-choice), while the third odor indicated that rats could receive a reward from either well (free-choice). Forced-choice odors were counterbalanced across rats, and

were presented in a pseudorandom sequence with free-choice odor presented on 7/20 trials. Incorrect well-selection during a forced-choice trials resulted in no reward delivery.

During each recording session, reward value was independently manipulated across four blocks of 60 correct trials (Trial summary in *Fig. 3.1A*; Block summary in *Fig. 3.1B*). During the first two blocks, one well was randomly designated to deliver reward immediately (500ms delay; 0.05ml) while the other well delivered sucrose with delays that would gradually increase (1000-7000ms). Delay contingencies would then be switched at the start of the second block, so that the well previously containing higher-valued reward (short delay) now carried the longer delay. During the final two trial blocks, delays on both wells were held constant at 500ms, and reward value was instead manipulated by size. At the start of the third block, the well that previously carried the long delay condition now delivered two boli of sucrose solution. Value contingencies were again switched at the fourth block (i.e., well previously containing large reward now contained small reward, and vice versa). Rats were water deprived to increase motivation to complete the task. We first removed water bottles at noon the day before training began. After a session, rats were given water for 20 minutes. Because testing took place Monday through Friday, rats were given ad lib access to water after Friday's session and then had water bottles removed Sundays at noon. This deprivation schedule has been used in our previous studies, and has been shown to increase motivation in the behavioral task without detriment to the rats' health (Burton et al., 2018, 2017; Roesch & Bryden, 2011; Roesch et al., 2007; Vázquez et al., 2019).

Surgery

All subjects were implanted with catheters in the jugular vein for self-administration and drivable electrodes (+1.5AP, +/-5.0ML, -5.0DV from brain surface) for single-unit recordings (control = 5 and cocaine = 4). Catheters were made from SILASTIC tubing (0.02x0.037 in; Dow Corning, Midland, MI) with a modified G 5-up cannula (Plastics One, Roanoke, VA). The cannula traveled through the fascia layer over the shoulder and was cemented on top of the skull. Electrodes (bundles of 10-25µm-diameter FeNiCr wire) were implanted unilaterally during this same surgery, with hemispheres counterbalanced across rats (Cocaine = 2 left, 2 right; Control = 1 left, 4 right).

Self-Administration

After one week of recovery, rats underwent a 12 d self-administration protocol in Med Associates operant chambers (St. Albans, VT). Animals in the cocaine group could press a lever for an infusion of cocaine, while control animals pressed for sucrose pellets. Cocaine dosage was calculated by weight, with separate doses calculated for males and females to account for weight differences. On days 1-6, rats self-administered 1 mg/kg dosage of cocaine (experimental group) or 2 sucrose pellets (control) per lever press for a maximum of 30 infusions or for a 3 h time limit. For the final 6 days, the dosage of cocaine was halved to 0.5mg/kg, and only 1 sucrose pellet was delivered per lever press, for a maximum of 60 presses. This

procedure allowed us to assess increases in drug-seeking when doses are cut in half to maintain the desired level of drug intake.

Importantly, rats were trained on the Delay/Size task before cocaine self-administration occurred. No behavioral or recording data were collected during cocaine exposure, or during the 1-month withdrawal period. Self-administration procedures expose rats to cocaine prior to recording during the Delay/Size task in order to determine how drug exposure impacts brain and behavior in the long term. These procedures are consistent with work establishing that continuous access to high cocaine doses evokes drug-taking and drug-seeking behaviors that are consistent with promoting symptoms of addiction (Allain & Samaha, 2019), and have been shown to change behavior and neural signals in other brain regions (Brockett et al., 2018; Burton et al., 2018, 2017; Calu et al., 2007; Vázquez et al., 2019).

Single-unit recording

Procedures were the same as described previously (D. W. Bryden & Roesch, 2015). Wires were screened for activity each day; if no activity was detected, the rat was removed and the electrode assembly was advanced 40 or 80μm. Otherwise, a session was conducted, and the electrode was advanced at the end of the session. Neural activity was recorded using four identical Plexon Multichannel Acquisition Processor systems (Dallas, TX). Signals from electrode wires were amplified 20x by an op-amp headstage located on the electrode array. Immediately outside the training chamber, the signals were passed through a differential pre-amplifier (Plexon Inc., PBX2/16sp-r-G50/16fp-G50) where single unit signals were amplified 50x and

filtered at 150-9000Hz. The single unit signals were then sent to the Multichannel Acquisition Processor box, where they were further filtered at 250-8000Hz, digitized at 40kHz and amplified at 1-32x. Waveforms (>2.5:1 signal-to-noise) were extracted from active channels and recorded to the disk by an associated workstation with event timestamps from the behavior computer.

Experimental Design and Statistical Analyses

Behavior in the recording task was analyzed by calculating the percent of correct responses on forced-choice trials (i.e. the amount of trials the animal correctly responded to the side corresponding to the directional odor cue), the percent of trials rats chose a particular valued condition (short, long, large, small) on free-choice trials, and reaction times (odor offset to odor port exit). These calculations included all trials in their respective categories, including those following a block switch. Behavioral analyses were computed for each individual session (separated by cocaine and control groups), and then averaged across sessions for each group. Conducting analyses across sessions—instead of across individual subjects—provides a better reflection of the neural correlates corresponding to behavior. Importantly, the main behavioral findings described in this paper have been replicated in three different studies (Burton et al., 2018, 2017; Vázquez et al., 2019). Multi-factor analysis of variance factors included group (control vs. cocaine), reward value (high vs. low), and value manipulation (size vs. delay). Post hoc between subjects t-tests ($p<0.05$) were used to determine differences between the cocaine and control groups.

Single units were analyzed in Offline Sorter using template matching software (Plexon), and were exported to NeuroExplorer (Nex Technologies, Colorado Springs, CO) and Matlab (MathWorks, Natick, MA). Again, all rewarded trials were analyzed, from both free and forced-choice trials, including trials that immediately followed a block switch. Neural activity was analyzed in three epochs: baseline firing rate, odor cue onset, and reward onset. All were calculated by dividing the total number of spikes by time. Baseline activity was taken 1 s before odor presentation, the odor epoch was taken 100ms after odor onset until port exit, and the reward epoch was taken 250ms before sucrose delivery to 1s after reward delivery. This epoch was designed to capture activity related to reward expectancy and delivery. Importantly, there was no overlap between epochs, even during short delay (500ms) conditions. To analyze neural activity during these epochs, we normalized firing rates so that high and low-firing cells could be analyzed together. We determined significant changes in firing rate by taking difference scores for each neuron, and plotting the number of neurons with significant differential firing from baseline (demonstrated through Wilcoxon tests, $p < 0.05$). Relationships between neural firing and behavioral activity were determined using regression analyses.

Histology

Upon completion of recordings, subjects were deeply anesthetized via isoflurane and perfused transcardially with 200ml of 0.9% saline, followed by 500ml of 4% paraformaldehyde (PFA). Brains were held in 4% PFA until ready for slicing, at which time they were placed in a 20% sucrose in PBS until the brains had sunk.

We then sectioned brains at 50 µm and used a Cressyl violet stain to confirm electrode placement (*Fig. 3.1D*).

RESULTS

Cocaine exposure produced a response bias towards high-valued rewards

Behavioral data from 416 cocaine recording sessions (n's for each rat = 128, 115, 89, 84,) and 315 control sessions (n's = 81, 75, 74, 72, 13; *Fig. 31D*) were analyzed using a multi-factor ANOVA across dependent variables of percent choice, percent correct, and reaction times on forced- and free-choice trials. The ANOVA factors consisted of group (control vs cocaine), value (high vs low), and block (delay vs size).

Analyzing free-choice trials (*Fig. 3.2A*) found a significant main effect of value ($F(1,5841) = 1077.58$, $p < 0.001$, *ANOVA*); selection of high value rewards was significantly greater in both delay ($t(1461) = -53.458$, $p < 0.001$, *unpaired t-test*) and size blocks ($t(1461) = -21.551$, $p < 0.001$, *unpaired t-test*). Cocaine rats' stronger preference for high value rewards is further illustrated in the inset showing the difference between high (i.e., short; big) and low (i.e., long; small) value free-choice responding for control and cocaine-exposed rats (*Fig. 3.2A*; inset; $t(707) = -5.026$, $p < 0.001$, *unpaired t-test*).

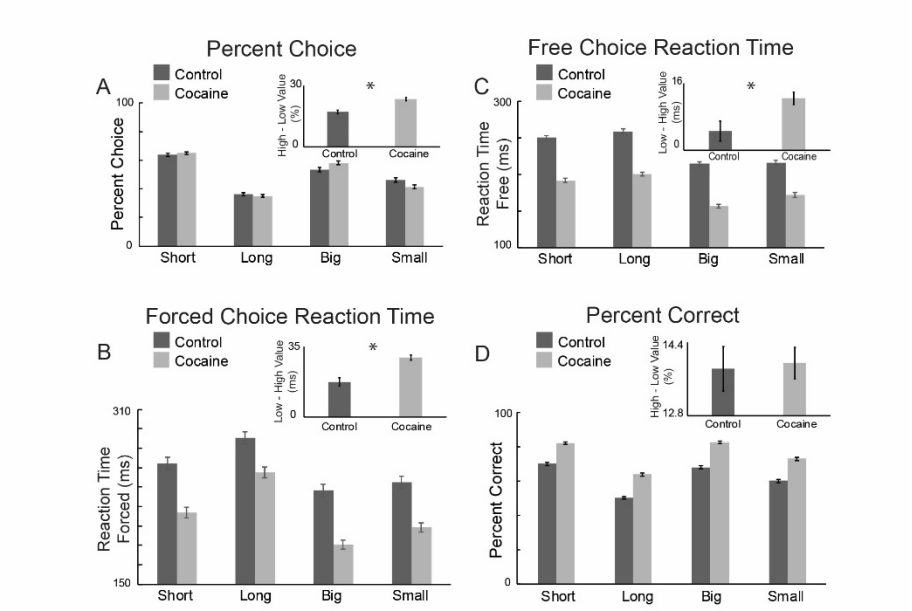

Figure 3.2: Cocaine exposure produced faster reaction times and response biases for high-valued rewards
All error bars indicate SEM. **(A)** Percent choice on all free-choice trials in each value manipulation. Results were averaged across animals and weighted by the number of recordings performed to provide a better representation of the behaviors observed as related to the neural analysis (controls, dark bars; cocaine, light bars). Inset: Response bias (percent choice high – low value rewards). **(B)** Reaction time (odor port exit – odor onset) on forced-choice trials for each value manipulation. Inset: Response bias (reaction time low – high value rewards) **(C)** Reaction time for free-choice trials. Inset: Response bias, calculated as in B. **(D)** Percent correct on all forced-choice trials. Inset: Response bias, calculated as in A.

The exaggerated response bias in cocaine rats toward high-valued reward was also observed in reaction times. Main effects of value and block during forced-choice trials demonstrated all rats were faster for high-valued rewards (*Fig. 3.2B*; $F(1,5841) = 69.26$, $p < 0.001$, *ANOVA*), and were faster for size compared to delay blocks ($F(1,5841) = 209.49$, $p < 0.001$, *ANOVA*). There was also a main effect of cocaine, indicating that rats that had self-administered cocaine were altogether faster compared to controls ($F(1,5841) = 276.87$, $p < 0.001$, *ANOVA*). Finally, cocaine-exposed rats showed a stronger response bias for high vs low value rewards (*Fig. 3.2B*, inset; significant interaction between group and value $F(1,5841) = 4.7$, $p = 0.030$, *ANOVA*;

t(543) = 4.856, $p < 0.001$, *unpaired t-test*). Thus, cocaine-exposed rats exhibited an exaggerated response bias toward high-valued rewards, as well as faster reaction times.

Similarly, reaction times during free-choice trials (*Fig. 3.2C*) were faster for high-valued rewards (Main effect of value; F(1,5717) =11.05, $p < 0.001$, *ANOVA*) and during size blocks compared to delay blocks (Main effect of block; F(1,5717) = 186.14, $p < 0.001$, *ANOVA*). Rats that self-administered cocaine were also significantly faster compared to controls overall (Main effect of group: F(1,5841) = 443.5, $p < 0.001$, *ANOVA*), particularly for high-valued rewards (*Fig. 3.2C*; inset; t(537) = 2.733, $p = 0.006$, *unpaired t-test*).

Altogether, these results replicate previous findings that rats demonstrate a preference for high-valued rewards through their choices and reaction times, and that responding is biased towards high-valued rewards in animals previously exposed to cocaine (Brockett et al., 2018; Burton et al., 2018, 2017; Vázquez et al., 2019). Interestingly, in contrast to previous studies, we found that cocaine-exposed rats were overall more accurate on forced-choice trials (*Fig.3.2D*). There were significant main effects of block and value, with rats being more accurate during size blocks (F(1,5841) = 90.09, $p < 0.001$, *ANOVA*) and for high-valued rewards (F(1,5841) = 945.27, $p < 0.001$, *ANOVA*). An additional main effect of group showed cocaine rats altogether followed forced-choice rules more accurately compared to controls (F(1,5841) = 866.17, $p < 0.001$, *ANOVA*), more strongly adhering to stimulus-response (S-R) contingencies (i.e., odor 1 = go left; odor 2 = go right).

Cue-related value-encoding was attenuated after cocaine exposure

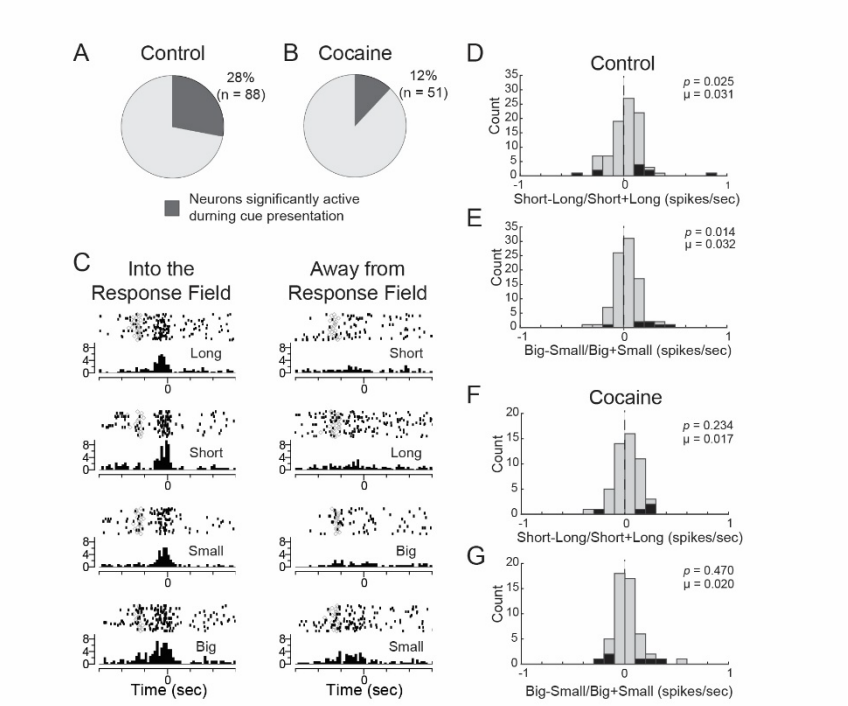

Figure 3.3: Cocaine exposure reduced cue-induced activity and value-encoding from odor-responsive cells
Selective vs non-selective odor-responsive cells from **(A)** control and **(B)** cocaine-exposed rats. Selectivity was defined by significantly greater firing rates during the odor analysis epoch (100ms after odor onset to odor port exit) compared to the baseline analysis epoch (1s before odor onset; *Wilcoxon*, $p < 0.05$). **(C)** Raster plots from an odor-responsive example cell. Activity is aligned to odor unpoke (i.e. when the rat leaves the odor port), with each row displaying activity for each trial-type. **(D)** Distribution of value analysis (short – long/ short + long; big – small/ big + small) for cells taken from control rats, comparing firing rates for short and long-delay rewards. **(E)** Similar analysis as D, comparing firing rates for big and small rewards during blocks where reward size is manipulated. **(F, G)** Same analyses as D, E, but for cells from cocaine-exposed rats. Black bars represent counts of neurons with firing that significantly differed between trial types ($p < 0.05$, *Wilcoxon*).

We first asked if cocaine exposure altered the counts of insula neurons that were responsive during odor sampling (odor epoch: 100 ms after odor onset to port exit) compared to baseline (1s before odor onset; $p < 0.05$, *Wilcoxon*). In controls, 28% (n = 88, out of total 315 cells) of neurons were responsive during odor sampling (*Fig. 3.3A*), whereas only 12% (n =51, out of total 416 cells) were responsive in rats

that self-administered cocaine (*Fig. 3.3B*). Both counts were significantly greater than expected by chance alone (control: $\chi^2 = 617.371$, $p < 0.001$, *chi-square*; cocaine: $\chi^2 = 135.51$, $p < 0.001$, *chi-square*), and the frequency of cells that increased firing in controls was significantly higher compared to cells from cocaine-exposed rats ($\chi^2 = 18.395$, $p < 0.001$, *chi-square*). Thus, rats that had self-administered cocaine had fewer cue-responsive neurons compared to controls. An example of a cue-responsive neuron is illustrated in *Figure 3.3C*. This neuron fired more strongly for odor cues that predicted higher value reward for behavioral responses to be made into the cell's response field (i.e., the cell's preferred response direction, or direction that elicited the strongest firing; left panels in *Fig. 3.3C*).

To quantify value selectivity during the odor epoch, we computed the difference between firing for high and low-valued rewards in each neuron during this epoch (i.e., odor onset to port exit) for delay (delay index = short – long / short +long) and size blocks (size index = big – small / big + small), for responses made into each cell's response field. For controls, there were significant positive shifts in delay ($p = 0.025$, $\mu = 0.031$, *Wilcoxon*; *Fig. 3.3D*) and size ($p = 0.014$, $\mu = 0.032$, *Wilcoxon*; *Fig. 3.3E*) indices, indicating a higher frequency of neurons with stronger firing rates to cues that predicted high-valued reward within size and delay trial blocks (black bars represent counts of neurons with firing that significantly differed between trial types; $p < 0.05$, *Wilcoxon*). In rats that self-administered cocaine, there were no significant shifts in either delay ($p = 0.234$, $\mu = 0.017$, *Wilcoxon*, *Fig. 3.3F*) or size indices ($p = 0.470$, $\mu = 0.020$, *Wilcoxon*, *Fig. 3.3G*), however neither were significantly different from control distributions (delay: $z = -0.754$, $p = 0.451$; size: $z = -0.889$, $p = 0.374$,

Wilcoxons). Thus, we conclude that cocaine self-administration diminished value-encoding during odor sampling by reducing the counts of odor-responsive neurons (*Fig. 3.3A* vs *B*), and only slightly attenuating outcome selectivity in those that remained (*Fig. 3.3D, E* vs *Fig. 3.3F, G*).

Immediate rewards were more strongly represented after cocaine exposure

Our next analyses examined neurons whose activity increased during the anticipation and delivery of reward. In controls, 46% (n = 144) of neurons increased firing during the reward epoch (*Fig. 3.4C*; reward epoch: 250ms before reward delivery to 1s after reward delivery) compared to baseline ($p < 0.05$, *Wilcoxon*. $\chi^2 = 1568.594$, $p < 0.001$, *chi-square*). In rats that self-administered cocaine, we found an 18% increase in the counts of reward-responsive neurons (*Fig. 3.4D*; 64%; n = 265). The proportion of reward-responsive neurons in cocaine-exposed rats was significantly greater than the proportion observed in controls ($\chi^2 = 6.454$, $p = 0.011$, *chi-square*). Single-cell examples from this neuronal population are illustrated in *Figure 3.4A* and *B*. Many neurons fired in anticipation of reward across long delays, exhibiting sustained firing from well-entry until reward delivery (*Fig. 3.4A*). However, other neurons seemed to fail in representing reward across a long delay (*Fig. 3.4B*). These cells exhibited increases in firing after well-entry, at the time when reward would have been delivered on the majority of trials (i.e., after 500 ms), followed by a decrease in firing during the remainder of the delay. To determine response patterns across the entire population of reward responsive neurons, we

plotted average firing aligned to both well-entry (*Fig. 3.4E,F*) and reward delivery (*Fig. 3.4G,H*) for actions made into the response field.

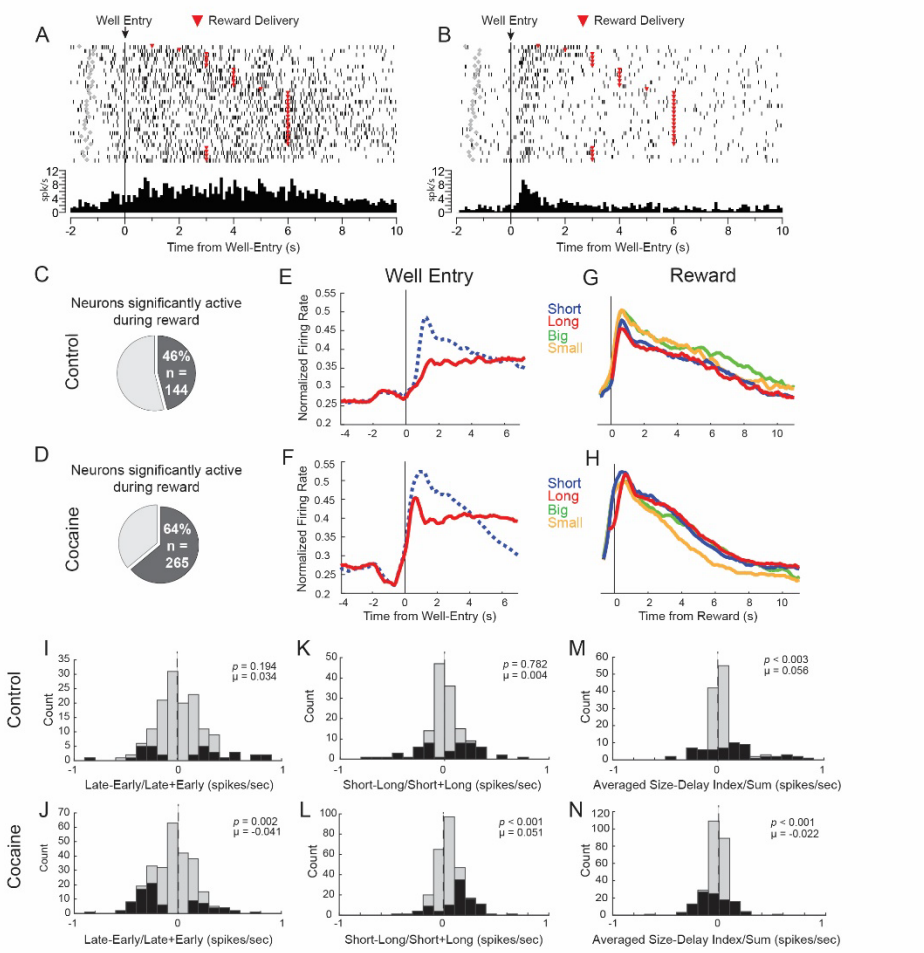

Figure 3.4: Cocaine exposure reduced delay-representation and activity was discounted for long-delay rewards in reward-responsive cells
(A) Single neuron example during a long-delay trial in which activity is sustained during the delay period. Activity is aligned to well-entry (time 0, black line). **(B)** Single neuron example during a long-delay trial in which activity initially increases after well-entry, and subsequently decreases, failing to maintain reward-representation throughout the long delay. **(C)** Percentage of reward-selective cells from control and **(D)** cocaine-exposed rats, as defined by significantly greater firing during the reward analysis epoch (250ms before reward to 1s after reward delivery) compared to baseline (1s before odor onset; *Wilcoxon*, $p < 0.05$). **(E)** Normalized firing rates for neurons that increased firing rates during the reward epoch from control (n = 144) and **(F)** cocaine-exposed rats (n = 265). Activity is aligned to fluid well entry, comparing short (blue dotted line) and long-delay (red line) rewards. **(G)** Normalized firing rates for cells from control and **(H)** cocaine-exposed rats, aligning activity for short (blue), long (red), big (green), and small (orange) rewards to reward delivery. **(I)** Distribution of value indices for cells taken from control and **(J)** cocaine-exposed rats, comparing firing rates during the first and last 500ms of long delay trials. **(K)** Distribution of delay indices (short – long/ short + long) for cells taken from control and **(L)** cocaine-

exposed rats, comparing firing rates during the reward epoch. **(M)** Distribution of context indices reflecting changes in global value (size block – delay block/ size block + delay block) comparing average activity during size and delay blocks during the post reward delivery epoch (1-2 s after reward delivery), for control and **(N)** cocaine-exposed rats. Black bars represent counts of neurons with firing that significantly differed between trial types ($p < 0.05$, *Wilcoxon*).

Aligning activity to well-entry, we found that insula neurons from control rats exhibited sustained elevated firing during long delays, starting at well-entry (*Fig. 3.4E*; red). In controls, anticipatory firing after well-entry for longer delays (red) rose less rapidly than for rewards delivered after a short delay (blue dashed). This was not true after cocaine exposure (*Fig. 3.4F*). Instead, early activity during long delays tracked firing as if on a short delay trial, peaking around the time when the more immediate reward would have been delivered before dropping to sustained levels. Thus, qualitatively it appeared that firing after cocaine exposure held on to the expectation that reward would be delivered after a shorter delay (which occurs on 3 (short; big; small) of the 4 trial-types).

To quantify this observation, we computed the difference in activity during the first and last 500ms of the delay period on long-delay trials (Late – Early / Late + Early; early = first 500ms after well-entry; late = last 500ms before reward). These two time points encompass non-overlapping activity early and late in the delay period, when rats stay in the fluid well before reward delivery. In controls, we observed no significant shift in the distribution, demonstrating that both early and late expectancy-related firing were similarly represented across longer delays ($p = 0.194$, $\mu = 0.034$, *Wilcoxon, Fig. 3.4I*). However, for rats that self-administered cocaine, this distribution was significantly shifted below zero ($p = 0.002$, $\mu = 0.041$, *Wilcoxon, Fig. 3.4J*) and significantly different from the control distribution ($z = -2.866$, $p =$

0.004, *Wilcoxon*), indicating that cocaine-exposed rats had a higher frequency of neurons firing more strongly at the start compared to the end of long delays.

These results indicate that in cocaine-exposed rats, neural correlates of immediate reward expectancy were maintained during performance of long-delay trials. These findings also suggest that cocaine exposure impairs the ability of insula neurons to maintain expectancy-related firing from early to late in delay trials. Such a lack of firing might lead to a reduced or discounted neural representation of delayed rewards compared to those delivered after 500ms (i.e., short-delay trials). Overall, this result suggests that immediate rewards are better represented in insula after cocaine exposure. Indeed, examining average firing aligned to reward delivery (*Fig. 3.4G, H*) suggests that anticipatory firing for immediate rewards (i.e., short-delay trials; blue) was stronger compared to firing for delayed rewards (*Fig. 3.4H*, red). To quantify this effect, we computed the delay indices (short-long / short+long) for each neuron during the reward epoch. The distribution of delay indices from rats that self-administered cocaine were significantly shifted in the positive direction ($p < 0.001$, $\mu = 0.051$, *Wilcoxon*, *Fig. 3.4L*) and significantly different from the control distribution ($p = 0.782$, $\mu = 0.004$, *Wilcoxon*, *Fig. 3.4K*; $z = 3.081$, $p = 0.002$, *Wilcoxon*).

These results suggest that previous cocaine exposure increased neural signals for immediate rewards during short-delay trials, and also over-represented the anticipation of more immediate reward, both within long-delay trials themselves and when directly comparing immediate to delayed reward at the time of delivery.

Context-related value signals were attenuated by cocaine exposure

Upon constructing the population histograms for the analysis above (*Fig. 3.4G, H*), we unexpectedly found that neurons in insula appeared to encode block context in control rats. Specifically, firing was higher after reward delivery during size blocks (green and orange vs blue and red). Remarkably this was true even for 'small' rewards (orange) that were physically the same delay and size as reward on 'short' delay trials (blue).

To quantify this effect, we created a 'context' index comparing average activity from size and delay blocks (Size Block – Delay Block / Size Block + Delay Block) 1-2s after reward that indexed the global value differences between blocks (*Fig.3.4M, N*). This analysis epoch occurs after completion of the trial, while rats are still consuming reward and are aware of the reward that was just delivered. We found insula neurons exhibited a positive shift in distributions of activity, confirming this unexpected novel finding of higher firing after reward delivery during size compared to delay blocks at the level of single neurons ($p = 0.003$, $\mu = 0.056$; *Wilcoxon, Fig. 3.4M*). Interestingly, this effect was not observed in cocaine-exposed rats. Instead, the context index distribution was significantly shifted below zero, indicating that cells tended to fire more strongly for delay blocks compared to size blocks ($p = 0.001$, $\mu = -0.022$; *Wilcoxon, Fig. 3.4N*. Cocaine vs control: $z = -4.287$, $p < 0.001$, *Wilcoxon*).

Remarkably, we also observed that neurons that ramped up firing in anticipation of house light onset (i.e., the stimulus that signaled the start of each trial) fired more strongly during 'size' compared to 'delay' trial blocks. This finding is illustrated in *Figure 3.5A*, which plots the average firing of 69 control neurons (22%

of total). To quantify this effect, we again computed the 'context' index during the 4s prior to light onset (the period of time immediately preceding the onset of the trial; *Fig. 3.5B*). Consistent with observations in the population histogram, we found a significant positive shift in firing rate distributions, demonstrating that insula neurons tended to fire more strongly during size compared to delay blocks prior to trial onset ($p < 0.001$, $\mu = 0.062$, *Wilcoxon*).

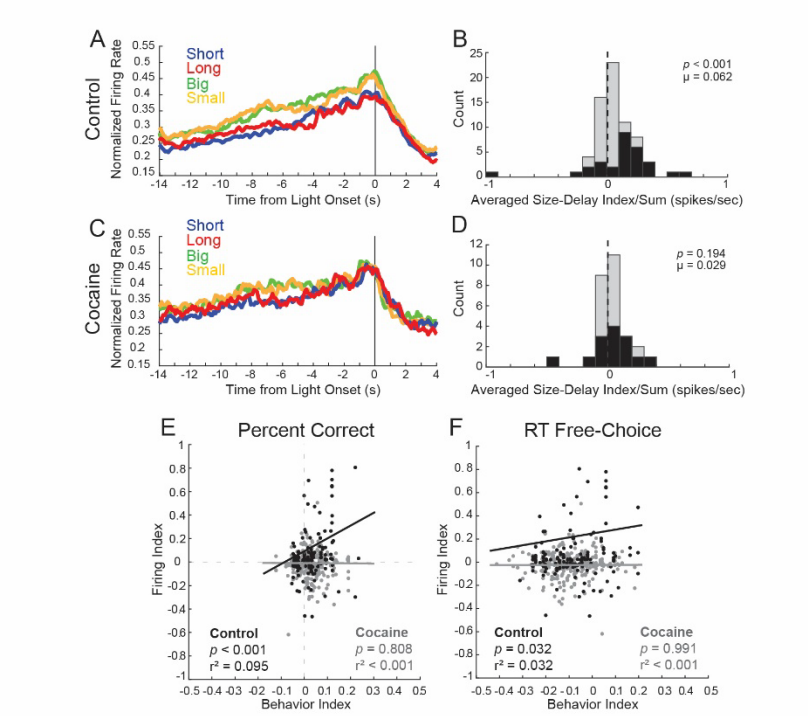

Figure 3.5: Cocaine exposure reduced context encoding and its relationships with behavior

(A) Normalized firing rates for cells from control rats that exhibited elevated activity prior to trial onset. **(B)** Distribution of context indices for controls (n = 69) reflecting changes in global value (size block – delay block/ size block + delay block) comparing average activity during size and delay blocks during the pre-trial epoch (4 s before light onset). Black bars represent counts of neurons with firing that significantly differed between trial types ($p < 0.05$, *Wilcoxon*). **(C, D)** Same as A and B for cocaine-exposed rats (n = 28). **(E, F)** Scatter plots of context index (size block – delay block/ size block + delay block) distribution data from neurons that increased activity during the reward epoch compared to ratios of size vs delay behavior for **(E)** Percent Correct, **(F)** Free-choice reaction times. Black = control; Gray = Cocaine.

Interestingly, cells that carried this signal were nearly nonexistent in rats that had self-administered cocaine (*Fig. 3.5C*). Only 7% (n = 28) of neurons exhibited such pre-trial ramping activity, which were significantly fewer cells compared to such neurons found in controls (χ^2 = 25.963, $p < 0.001$, *chi-square*). For those cells, the context index distribution was not significantly shifted ($p = 0.194$, $\mu = 0.029$, *Wilcoxon, Fig. 3.5D*), but was not significantly different compared to controls (z = -1.071, $p = 0.284$, *Wilcoxon*). Thus, we conclude that cocaine self-administration diminished contextual pre-trial value-encoding by reducing counts of responsive neurons and slightly attenuating selectivity in those that remained.

Notably, we have not observed these context signals in any other brain area that we have recorded from in rats performing this task (Brockett et al., 2018; Daniel W. Bryden, Johnson, Diao, et al., 2011; Burton, Bissonette, Lichtenberg, Kashtelyan, & Roesch, 2014; Burton et al., 2018, 2017, 2015; Burton, Kashtelyan, Bryden, & Roesch, 2014; Kashtelyan, Tobia, Burton, Bryden, & Roesch, 2012; Roesch & Bryden, 2011; Roesch et al., 2007; Roesch, Taylor, & Schoenbaum, 2006; Roesch, Bryden, Cerri, Haney, & Schoenbaum, 2012; Y. K. Takahashi, Stalnaker, Roesch, & Schoenbaum, 2017; Vázquez et al., 2019). To better understand the relationship between context-related firing and behavior, we correlated differences in firing between 'size' and 'delay' blocks to differences in percent corrects and reaction times (Size – Delay / Size + Delay).

In control rats, there was a significant positive relationship between size vs delay block accuracy and context-encoding ($p < 0.001$, $r^2 = 0.095$, *Regression*). Thus, increases in firing were correlated with better performance. Interestingly, after

cocaine exposure there was no correlation between firing and percent correct (p = 0.808, r^2 < 0.001, *Regression, Fig. 3.5E*). During free-choice trials there was also a significant, positive relationship between firing rate and reaction time in control rats (p = 0.032, r^2 = 0.032, *Regression*). This result suggests that stronger firing rates were actually related to slower reaction times during size blocks. This relationship was also disrupted by cocaine exposure (p = 0.991, r^2 < 0.001, *Regression, Fig. 3.5F*). Taken together, these novel firing patterns in insula are related to accuracy and reaction time during our Delay/Size task. These relationships are additionally disrupted by cocaine exposure.

DISCUSSION

The insula's traditional roles integrating external experiences with internal state have more recently expanded to reward-guided decision-making (Droutman, Bechara, & Read, 2015). Previous recording studies demonstrate insula activity encodes anticipation of both positive (Samuelsen et al., 2012; Samuelsen, Gardner, & Fontanini, 2013) and negative consequences (Jo & Jung, 2016), and reward consumption (Kusumoto-Yoshida et al., 2015; M. K. Wittmann et al., 2020). The insula also seems to track the subjective value of reward, reflecting how context and experience change a reward's value even when the characteristics of the reward itself has not changed. Satiety, for example, reduces insula activity for food (Livneh et al., 2017) and water (Livneh et al., 2020; Namboodiri & Stuber, 2020). Other work has shown that when a previously appetitive sucrose solution is paired with delayed (instead of immediate) access to cocaine, insula activity reflects the reduced

preference of this once favorable reward, even when the sucrose itself is unchanged (Moschak et al., 2018). Our work demonstrates signals in insula related to cues, context, and differently-valued rewards, and how chronic cocaine profoundly affects insula activity in reward-guided decision-making. First, we see enhanced signals representing reward, especially delivery-related processing of immediate reward. Second, cocaine exposure reduces signals related to cue and block context selectivity, suggesting insula plays an important part in drug-induced impairments of executive control and decision-making.

Specifically, our study found that the insula encoded predicted outcomes during presentation of reward-predictive odor cues, and fired in anticipation of reward after completion of an instrumental response. Further, we showed firing was higher for cues that predicted high-valued reward in both delay and size domains. These neurons likely contributed to subjective preferences of immediate reward and maintenance of reward representations across delays. Interestingly, these firing patterns are similar to what we have previously reported in OFC and NAc (Burton et al., 2018; Burton, Kashtelyan, et al., 2014; Y. K. Takahashi et al., 2009). Both regions are outcome-selective during cues, and fire during the anticipation and delivery of reward. However, insula's firing did not differ between rewards delivered after short and long delays in control rats. Thus, in normal insula, anticipatory reward signals were not discounted by the length of the delay that preceded it.

Interestingly, we also saw that average firing was stronger during size blocks, and correlated with changes in reaction time and accuracy. When designing our task, our goal was to completely counter-balance value manipulation and direction within

each session. We accomplished this objective by running size blocks last, countering satiation by promising rats more and faster access to reward later in the task. Indeed, rats exhibited faster reaction times and better percent correct scores during size blocks due to the increased benefit of completing trials (i.e., no long delays and potential for a larger reward). These distinct differences in behavior indicate changes in overall value between delay and size blocks, which may trigger increases in contextual firing observed in the present study. This interpretation is further supported when considering that higher firing during size blocks reflects more than just receiving a larger reward within trials, as activity was stronger before trial initiation, and after delivery of 'small' rewards that were physically identical to 'short-delay' rewards previously experienced during delay blocks (i.e., both trial types delivered 1 bolus after 500ms delay). However, presenting these size blocks last raises the question of whether or not this context encoding may be a reflection of satiety, or a recency-weighted response to blocks in the second half of the task. Directly testing this issue has proven difficult in the past, as we have found that rats are highly unmotivated to work for delayed rewards when presented with size blocks first. While beyond the scope of the current study, readdressing this question – perhaps through shorter recording sessions with reversed delay and size blocks – may add interesting insight to this data.

It may therefore be more accurate to hypothesize that these increases in activity track overall value of block contexts, and contribute to elevating motivation as satiation sets in, based on insula activity's correlations to reaction time and percent correct. Such interpretations are in line with Wittman et al. (2020), which implicates

the macaque insula in the tracking of long-term reward by demonstrating a relationship between BOLD signaling and task engagement during blocks of trials where probability of reward receipt is overall either higher or lower. Our context effects suggest such global reward signals in insula are encoded at the single-cell level, and may represent specific reward outcome in addition to reward probability. Moreover, our work supports long-held theories of insula's interoceptive functions, and demonstrates novel context-based adjustments to representations of reward value in order to adjust behavior (Droutman, Read, et al., 2015; Naqvi & Bechara, 2010).

We also determined how these correlates are disrupted after chronic cocaine self-administration. Our results replicate previous behavioral findings that preference for high-valued rewards is exaggerated in cocaine-exposed animals (Burton et al., 2018, 2017; Roesch & Bryden, 2011; Roesch et al., 2007; Vázquez et al., 2019). Interestingly, prior cocaine self-administration also shifted the balance of encoding in insula from cues and context to reward. Specifically, in rats that self-administered cocaine, fewer neurons increased firing to cues and context. Instead, significantly more neurons increased firing in anticipation of immediate reward. This activity might encourage impulsive choice by not adequately conveying the value of differently delayed and sized rewards at the time of the decision, and by more strongly representing the anticipation that reward should have been delivered sooner. Consistent with this hypothesis, not only did insula neurons fire more strongly in anticipation of reward on short-delay trials, but they also fired strongly at the start of

long-delay trials at the time when reward would have been delivered on trials with shorter delays (i.e., 500 ms after well entry).

Remarkably, insula neurons in cocaine-exposed rats also failed to increase firing at the end of recording sessions during size trial blocks. Above, we suggest this signal is important for motivating satiated animals when they encounter global increases in reward value in the context of size blocks. The explanation for its absence in cocaine-exposed rats may be that the signals (related to satiety, motivation or absolute value) necessary to drive behavior during size blocks are disrupted after cocaine exposure. Alternatively, these size-encoding signals may be absent because they are not necessary to drive motivation in cocaine-exposed rats, as seen by their faster reaction times and higher accuracy in size blocks. However, the absence of these global signals may in turn disrupt processing of long vs short-delay rewards and subsequently bias behavior for immediate rewards.

Relatedly, we found cocaine exposure disrupted relationships between context encoding and behavior- specifically, greater accuracy and slower reaction time were both associated with higher rates of context encoding. The implications of these relationships appear counterintuitive at face value, especially given that control and cocaine-exposed rats exhibited faster reaction times during size blocks. These correlations may reflect subtle increases in deliberation and attention necessary for performance as fatigue or satiety sets in, especially given the relationship between insula and anterior cingulate cortex (ACC) in governing conflict and attention (Arulpragasam, Cooper, Nuutinen, & Treadway, 2018; Cai, Ryali, Chen, Li, & Menon, 2014; Corbetta & Shulman, 2002; Damasio et al., 2000; Goulden et al., 2014;

Porter, Li, & Hillman, 2020; Seeley et al., 2007). Our lab has previously found attentional signals in ACC and behavior to be disrupted after cocaine exposure (Vázquez et al., 2019). Exploring this hypothesis may therefore be a promising area of future work. The proposed reduction of context and task structure representations in favor of reward additionally fits well with theories of drug exposure enhancing model-free behavior (Dayan & Berridge, 2011; Everitt & Robbins, 2005; Jentsch & Taylor, 1999; Lucantonio, Caprioli, & Schoenbaum, 2014; N. D. Volkow & Fowler, 2000), and could be explored further by examining insula activity in behavioral tasks where selection of immediate reward is not always the most favorable action.

Finally, we must note that insula spans a large portion of the brain and - across humans, primates, and rodents – its function varies depending on subregion (Droutman, Read, et al., 2015; Gogolla, 2017; Naqvi & Bechara, 2009). We examined a portion of anterior insula, which itself can be split into dorsal and ventral subregions. As a whole, anterior insula can be associated with cognitive function (Gogolla, 2017), which was relevant for the present study. Human and primate studies suggest this region can be further differentiated, with the ventral portion responsible for social-emotional processing, and the dorsal region responsible for cognition (Droutman, Bechara, et al., 2015). To our knowledge these same distinctions have not been found in the rodent brain, but greater understanding of insula's anatomy will be important for instructing future studies.

Thus, our findings demonstrate that cocaine exposure enhances signals related to reward delivery, while attenuating signals related to cue and context. Our novel context encoding results provide physiological evidence of the insula's role in

interoception and decision-making (Naqvi & Bechara, 2010). Moreover, disruptions of these signals and related changes in behavior due to cocaine exposure suggest an important role for insula in impaired decision-making observed after drug abuse. Further study of insula using different behavioral assays will be important for understanding how this region is involved in both optimal decision-making, and its drug-induced impairments.

CHAPTER 3B: Insula lesions reduce stimulus-driven control of behavior during odor-guided decision-making and autoshaping

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ABSTRACT

The insula has become a significant brain region in the study of both normal and impaired behavior and decision-making and has emerged as an important contributor to drug addiction. Consistent with this literature, in a previous study, we found that neural signals in rat insula encode anticipation and contextual global reward value during performance of an odor-guided delay/size choice task, and that these signals are disrupted by prior cocaine self-administration. Still, it is unknown if insula is critical for performance of this task under normal circumstances. Here, we sought to elucidate the functional role of these signals by lesioning the same region of anterior insula we previously recorded from. In addition to examining behavior during decision-making, we characterized behavior during autoshaping to further assess insula's role in behavior. We found insula damage resulted in reduced accuracy and faster reaction times, without affecting rats' choice of high-value reward, and that insula lesions reduced sign-tracking behavior. These results suggest that insula contributes to our odor-guided delay/size choice task via mechanisms that impact the control that environmental stimuli have on behavior.

INTRODUCTION

The insula has traditionally been studied as a center of interoception, integrating changes in internal state with environmental events (Craig, 2002, 2010; Damasio et al., 2000; Droutman, Read, et al., 2015). This wide-ranging but somewhat nebulous definition comes from insula's vast connectivity throughout the brain, making this region relevant for the brain's emotional, motivational, and sensorimotor control (Gogolla, 2017). Recently, a significant body of research has emerged to demonstrate an equally vast role for insula in reward-learning and decision-making (Droutman, Bechara, et al., 2015); across humans and rodents, insula has been proven necessary for cue and context-induced responding (Arguello et al., 2017; Cosme, Gutman, & LaLumiere, 2015; Ho et al., 2018; Naqvi & Bechara, 2009; Pelchat et al., 2004; R. Zhang et al., 2019), anticipation and craving (Garavan, 2010; Kusumoto-Yoshida et al., 2015; Samuelsen et al., 2013; Wang et al., 1999), and expression of goal and habit-directed behavior (Contreras et al., 2012; Kesner & Gilbert, 2007; Naqvi & Bechara, 2010). This work, while impressive, leaves the outstanding question of how insula is involved in more complex decision-making tasks that vary expected rewards across multiple dimensions, use different stimulus-response rules to guide behavior, and alter reward contingencies repeatedly within a given behavioral session.

We have begun to address this question by recording from insula during an odor-guided delay/size choice task, in which rats use odor cues to guide choice behavior between two options with shifting reward values. In this task, we manipulate value across two contexts: one in which rats choose between an immediate and

delayed reward, and one in which rats choose between large and small rewards with no delays to either option. Like many previous studies utilizing this task (Brockett et al., 2018; Burton et al., 2018, 2017; Pribut, Vázquez, Brockett, et al., 2021; Roesch et al., 2007; Vázquez et al., 2019), rats favor rewards with short as opposed to long delays, and rewards that are big as opposed to small, and learn to respond in appropriate directions to obtain more valued reward over multiple blocks of trials. While recording from insula in rats performing this task, we have recently shown that insula encodes value during reward delivery and presentation of reward-predicting cues (Pribut, Vázquez, Brockett, et al., 2021). In addition, we found that the firing of many insula neurons persisted across long delays, as rats anticipated imminent reward delivery. Finally, in that same study, we observed a novel "context" effect in which insula activity was significantly stronger during blocks when overall quantity of reward increased to compensate for end-of-session satiety. From these results, we suggested that insula's role in interoception may encourage task-engagement during odor-guided delay/size choice tasks by weighing satiety against changing reward values.

Overall, the signals we observed during task performance are consistent with the insula perturbation literature. For example, devaluation studies suggest that insula is necessary for retrieving outcome value and recalling changes in incentive value; multiple animal studies have demonstrated rats are unable to adjust responding towards a devalued option following both lesion (B. W. Balleine & Dickinson, 2000; Parkes, Bradfield, & Balleine, 2015) and inactivation methods (Parkes & Balleine, 2013; Parkes et al., 2015, 2018), suggesting a role for insula in modulating goal-

directed behavior. Given these results (i.e., insula disruption impairs goal-directed behavior), and the neural correlates above, we hypothesized that insula is also necessary for performance of our odor-guided delay/size choice task.

To test this hypothesis, here we lesioned insula in the same region we have previously recorded from (*Fig. 3.6A, D*), and examined behavior in the same odor-guided delay/size choice task (*Fig. 3.6B*). We additionally tested rats in a 10-day autoshaping procedure in different behavioral boxes (*Fig. 3.6C*) in order to determine the effects of insula lesions on goal-directed behavior. Contrary to our hypothesis, we found that lesions did not affect preference for high-value rewards and that goal-tracking during autoshaping was actually enhanced. Instead, we found that lesioned rats exhibited reduced accuracy and faster decisions during the odor-guided delay/size choice task, and reduced sign-tracking behavior during autoshaping. Overall, these results suggest insula lesions reduced stimulus-driven control of behavior, while leaving reward induced biases in behavior and free-choice unaffected.

MATERIALS AND METHODS

Subjects

16 Long-Evans rats (weight: 175-200g; 8 females, 8 males; equal sexes in control and lesion groups) were obtained from Charles River Laboratories. Subjects were held and tested at University of Maryland, College Park according to university and NIH guidelines.

Odor-guided delay/size choice task

Rats were trained on the delay-size task for one month prior to surgery. In brief, subjects needed to nose poke into a central port during house light illumination to receive one of three odor cues (odors were 2-Octanol, Pentyl Acetate, or Carvone counterbalanced across rats). These odors would instruct the rats on how to receive a liquid sucrose reward (10%) from wells located on either side of the central port (summarized in *Figure 3.6*). Two odors instructed rats to go either left or right for reward (forced), while the third indicated that rats could receive a reward from either well (free-choice). Forced odors were counterbalanced across rats, and were presented in a pseudorandom sequence with free-choice odor presented on 7 out of 20 trials. Incorrect well-selection during a forced trials resulted in no reward delivery, and 500ms time-out where the subject could not nose poke and start a new trial.

During each behavior session, reward value was independently manipulated across four blocks of 60 correct trials (*Fig. 3.6B*). During the first two blocks, one well was randomly designed to deliver reward immediately (500ms delay) while the other well delivered sucrose with delays that would gradually increase (1000-7000ms). Delay-well contingencies would then be switched at the start of the second block so that the previously higher-valued well carried the longer delay. During the final two trial blocks, delays on both wells were held constant at 500ms, and reward value was instead manipulated by size. At the start of the third block, the well that previously carried the delay condition now delivered twice as much sucrose as the previously higher valued well, which delivered a standard 0.05ml bolus of sucrose solution. Value contingencies were again switched at the fourth block. Subjects were

water-deprived to encourage motivation during the task, and their weights were recorded each week to monitor their health.

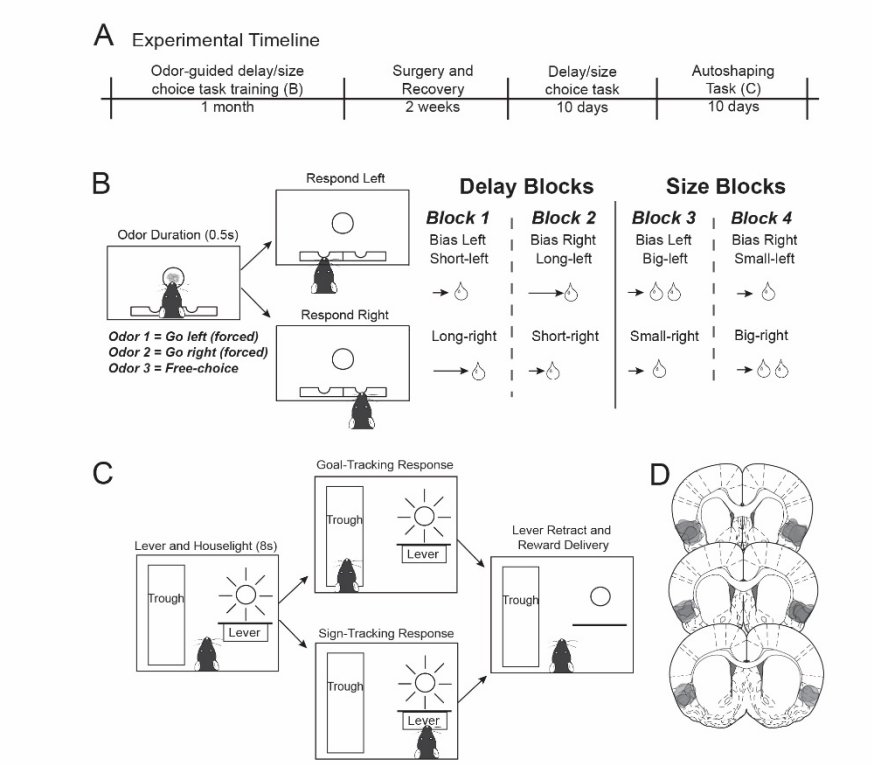

Figure 3.6: Behavioral tasks and histology
(A) Experimental timeline. **(B)** Summary schematic of the odor-guided delay/size choice task, showing the sequence of events in a single trial (left panels) and the sequence of blocks for delay and size blocks. Rats nose-poked into the central port for 0.5s to receive an odor cue for 0.5s. The odors instructed them to respond to the left or right fluid wells below, which delivered liquid sucrose rewards. Reward value was manipulated by either delivery time (ranging from 0.5 – 7s), or size of reward. Please see methods for more detail. **(C)** Summary schematic of the autoshaping task detailing the sequence of events in a single trial, and the respective classification of goal vs sign-tracking behaviors. **(D)** Lesion spread (dark areas in diagram) verified by histology displayed in templates from 2.04 mm, 1.56 mm, and 0.96 mm anterior from Bregma in Paxinos and Watson (2006).

Surgery

After training, subjects were assigned to either lesion or control. A t-test verified no significant difference in accuracy ($t(14) = 0.565$, $p = 0.581$) between the

two groups prior to surgery. Our goal was to lesion a region of insula we had previously performed single-unit recordings in (Pribut et al., 2021; electrodes placed at +1.5AP, +/-5.0ML, -5.0DV from brain surface). These coordinates placed our recordings in a more anterior region of insula, but not so far forward as to risk impinging on OFC (Chang, 2014; Gallagher, McMahan, & Schoenbaum, 1999; S. B. Ostlund & Balleine, 2007; Paxinos & Watson, 2006; Pickens et al., 2003; Saddoris, Gallagher, & Schoenbaum, 2005; Geoffrey Schoenbaum, Setlow, Saddoris, & Gallagher, 2003; Stalnaker, Franz, Singh, & Schoenbaum, 2007). Based on previous methods used in our lab (Brockett, Tennyson, DeBettencourt, Gaye, & Roesch, 2020), all rats therefore received 4 stereotactic injections, 2 per side, spaced 0.5mm in front and behind our recording AP, and at our average final recording depth to account for injection spread (1st injection: +2.0AP, +/-5.0ML, -5.77DV; 2nd injection: +1.0AP, +/-5.0ML, -5.77DV). For each injection site, a beveled 33-ga, 5µl Hamilton syringe was lowered to the target depth over the course of 5min. The bevel was always facing away from the midline of the brain. Infusions of either 0.6M ibotenic acid in saline or 0.9% saline were injected at each site at a volume of 0.2µl over 3min for an infusion rate of approximately 125nL/min. Needles were left in place for 5min to allow liquids to spread before being slowly removed. Injection site holes were then filled with sterile bonewax and the incision site was sutured.

Analysis of Odor-guided delay/size choice task

After one week of recovery from surgery, we measured behavior in the odor-guided delay/size choice task for two weeks. Behavior was recorded using four

identical Plexon Multichannel Acquisition Processor systems (Dallas, TX). Behavioral data from each session (10 days of testing from 16 rats; lesion = 4M, 4F, control = 4M, 4F) were analyzed using multi-factor ANOVAs for percent accuracy, percent choice, and reaction times on both forced- and free-choice trials. ANOVAs consisted of factors for day, group (control vs lesion), value (high vs low), block (delay vs size), and phase (first 10 vs last 10 trials). Calculations were split into first and last ten trials (phase) for each trial-type. There were 20 trials per trial-type allowing an even split into first 10 and last 10. We have previously shown that analyzing ten trials from each trial-type captures the development of learning at the start of trial blocks, and provides a large enough sample to conduct behavioral statistics (Brockett et al., 2018; Burton et al., 2017; Pribut, Vázquez, Brockett, et al., 2021; Vázquez et al., 2019).

Autoshaping

After assessing behavior in the Size-Delay task, rats were given adlib access to water and were mildly food-deprived in preparation for an autoshaping task. These procedures were conducted in modular shuttle boxes with a divider separating the box into two halves (ENV-010MD, Med Associates, St. Albans, VT). Rats were placed on one side of the box that was equipped with a stimulus light, a retractable lever, and a food trough connected to a pellet dispenser. Stimuli presentation and behavioral tracking was controlled by a computer running MED-PC IV (Med Associates). Rats were first given one day of magazine training to associate the food trough with sugar pellets. Houselights would illuminate and a 45mg sugar pellet (LabTab^{TM} AIN-76A,

Test Diet, St. Louis, MO) was delivered into the food trough. Pellets were delivered on a 90s ITI for 25 minutes.

For the next 10 days, autoshaping behavior was measured. During a single trial, a lever would randomly be inserted into the box for 8s and houselights would be illuminated. After the lever was retracted, a sugar pellet was delivered into the food trough regardless of whether or not the rat interacted with the lever. Each day's autoshaping session had 25 trials with a 90s ITI, resulting in approximately 45min sessions. Sign or goal-tracking behavior was measured by averaging interactions with the lever or the food trough over the course of the 8s waiting period.

Histology

Upon completion of recordings, subjects were euthanized via isoflurane overdose and perfused transcardially with 200ml of 0.9% saline, followed by 500ml of 4% paraformaldehyde (PFA) in order to fix brain tissue and determine the extent of the lesion. Animals were then decapitated, and brains were removed and sectioned in 40µm slices.

RESULTS

Insula lesions impaired percent accuracy on forced trials

During performance of our odor-guided delay/size choice task (*Fig. 3.6B*), rats use reward-predicting cues to guide choice behavior between two sucrose reward options, the values of which change across two manipulations: in the first two blocks, rats choose between an immediate or delayed reward; in the last two blocks, rats

choose between a large or small reward with no manipulations made to delivery time. Across blocks, rats learn which option delivers the preferred outcome (e.g. which option is the short-delay reward, or which option is the big reward), and then need to adjust behavior when these contingencies are switched after each block of 60 trials. Rats were trained on the delay-size task for approximately one month prior to surgery, during which lesion rats received bilateral injections of ibotenic acid while control subjects received injections of saline (*Fig. 3.6D*). After recovery, we measured behavior in this task for 10 days, and subsequently studied autoshaping behavior for another 10 days. Details on task structure and analysis can be found in the Experimental Procedure.

Our odor-guided delay/size choice task features two different trial-types: forced, and free-choice. Forced trials feature rules that the rat must follow in order to receive reward, otherwise no reward is delivered. Across studies (Brockett et al., 2018; Burton et al., 2017; Pribut, Vázquez, Brockett, et al., 2021; Vázquez et al., 2019) we see that rats generally perform well during these trials (~75% accuracy), but they develop biases towards the direction associated with better rewards (i.e. short or large). Specifically, rats become more accurate for high-value forced and less accurate for low-value forced trials due to their inclination to move towards fluid wells associated with better rewards while avoiding wells associated with poorer outcomes. Importantly, this behavior is learned in that differences between these differently valued rewards are not present during the first 10 trials of a block, but can be seen by the last 10 trials. In the following analyses, we show that lesioned rats exhibit many of these same behaviors, however they are worse on forced trials for

both high and low-value rewards. This result suggests lesions induced a deficit in stimulus-response processing, as opposed to altering response biases (e.g., behavior in which rats are more accurate for high-value rewards, and less accurate for low-value rewards).

We first examined accuracy during forced trials in order to measure rats' adherence to stimulus-response contingencies (i.e. which odor signals a left well response and which odor signals a right well response, and whether or not rats make the response corresponding to those signals). Similar to previous studies, we found both control (*Fig. 3.7A*, gray bars) and lesioned rats (black bars) performed the task with high accuracy. In particular, higher accuracy was observed during forced trials where rats received high-value rewards, as shown by a significant main effect of value ($F(1, 2384) = 199.83$, $p < 0.001$; as described in the methods, ANOVAs consisted of factors of day, group (control vs lesion), value (high vs low), block (delay vs size), and phase (first 10 vs last 10 trials).

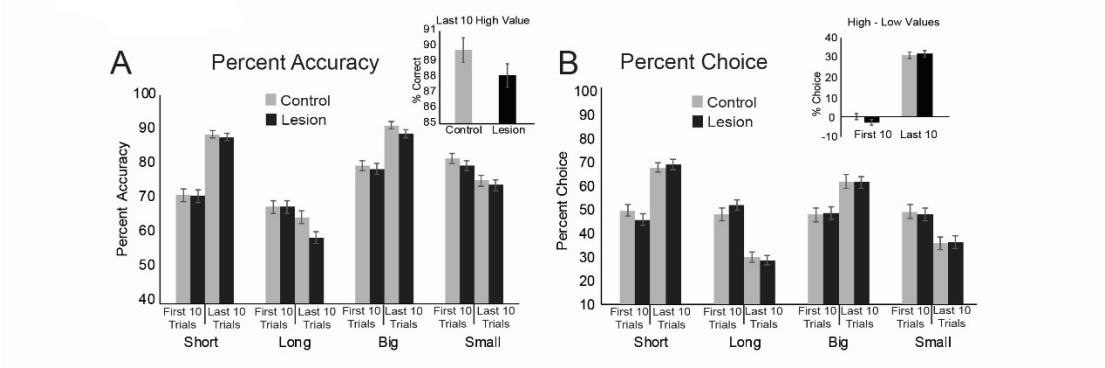

Figure 3.7: Lesions reduced accuracy while sparing reward preference
Control rats are represented by light bars and lesion rats represented by black bars. All error bars indicate SEM. **(A)** Percent accuracy on all forced trials for each value manipulation, during the first and last 10 trials of each block. Inset: Average percent accuracy for high-value rewards in the last 10 trials. **(B)** Percent choice on all free-choice trials, during the first and last 10 trials of each block. Inset: Response bias (percent choice high – low value rewards, during the first and last 10 trials).

Because reward contingencies are switched every 60 trials, we also measured accuracy at the beginning and end of every block to confirm that rats are learning the new contingencies and adapting their performance. We found a main effect of phase ($F(1,2384) = 27.64$, $p < 0.001$), demonstrating that all rats were more accurate in the last 10 trials of a block compared to the first 10 trials. Examining *Fig. 3.7A*, this improvement in performance was only seen in high-value rewards; rats were less accurate for long and small rewards compared to short and big rewards by the last 10 trials in a block (long and small rewards). An interaction between value and phase ($F(1,2384) = 168.69$, $p < 0.001$) supported this observation, with post-hoc t-tests showing no difference between accuracy for high and low-value rewards during the first 10 trials (t-test, $t(1198) = 0.726$, $p = 0.468$), but greater accuracy for high-value rewards by the last 10 trials of a block ($t(1198) = 20.489$, $p < 0.001$). Finally, similar to previous studies, we found a main effect of block ($F(1,2384) = 134.7$, $p < 0.001$) and a value and block interaction ($F(1,2384) = 26.49$, $p < 0.001$) demonstrating higher accuracy in size blocks.

These findings demonstrate that rats from both groups performed better and worse on high and low value forced trials, respectively, and that these biases developed over the course of learning within each block of trials. Thus, rats understood the task, performing forced trials well, but their biases influenced their performance as we have described previously (Burton et al., 2018, 2017; Pribut, Vázquez, Brockett, et al., 2021; Vázquez et al., 2019). We also found that lesioned rats performed slightly, but significantly, worse on both high and low value forced trials, as demonstrated by a main effect of group ($F(1, 2384) = 5.08$, $p = 0.024$; *Fig*

3.7A, inset). Further, there were no significant interactions between group and block ($F(1,2384) > 0.001$, $p = 0.987$), group and phase ($F(1,2384) = 1.27$, $p = 0.260$), or group and value ($F(1,2384) = 0.680$, $p = 0.409$) demonstrating that lesioned rats' behavior did not differ in respect to the type of block performed (delay vs size), the phase of learning (early vs late), or the value of the reward (high vs low value).

Finally, we examined group differences on each day of testing to determine if lesions were producing transient effects on percent accuracy on forced trials due to compensatory processes after insula damage. An ANOVA with factors of day and group showed a main effect of day ($F(9,1180) = 2.43$, $p = 0.010$) and no interaction between group and day ($F(9,1180) = 0.54$, $p = 0.848$), indicating that both lesion and control rats improved in accuracy over the course of testing at similar rates.

Lesions did not impact choice of high value reward on free-choice trials

Recall from the odor-guided delay/size choice task structure that rats also have free-choice trials, in which they receive a reward regardless of the well they select. Importantly, value contingencies remain in place so that free-choice trials can directly test how much a rat prefers one reward over another. Although the relationship between direction and value can be inferred through changes in accuracy on forced trials as described above (i.e., better at high value forced; worse at low value forced choice), free-choice trials allow for a more direct measure of response bias that reflects the development of knowledge pertaining to response direction-value contingencies. Across many studies we have observed that rats prefer and learn to select high-value rewards more than low-value rewards during these free-choice

trials (Brockett et al., 2018; Burton et al., 2017; Pribut, Vázquez, Brockett, et al., 2021; Vázquez et al., 2019). Here also we found our rats generally selected high value rewards 60-70% of free-choice trials, while low-value rewards were selected around 30-40% of free-choice trials (*Fig. 3.7B*, gray bars). A main effect of value further demonstrated this preference across control and lesioned rats, in that all rats chose high-value rewards more frequently than low-value rewards ($F(1,2384) = 149.11$, $p < 0.001$). Similar to forced trials, we also found reward selection during free-choice trials changed from the beginning to the end of a block to reflect the rats' learning of new reward contingencies. A significant interaction between value and phase ($F(1,2384) = 175.47$, $p < 0.001$) indicated that selection of high-value rewards was no different from low-value rewards at the start of a block ($t(1198) = -0.724$, $p = 0.4690$. However, high-value reward selection increased by the last 10 trials ($t(1198) = 18.489$, $p < 0.001$). Although there was a significant interaction between value and block ($F(1, 2384) = 5.11$, $p = 0.024$), preference for high value rewards was significantly greater across both delay ($t(1198) = -10.723$, $p < 0.001$) and size blocks ($t(1198) = -6.326$, $p < 0.001$). We did not observe any significant main effects of phase ($F(1,2384) = 0.04$, $p = 0.851$) or block ($F(1,2384) = 0.04$, $p = 0.851$), nor did we observe significant interactions between these two factors ($F(1,2384) = 0.04$, $p = 0.851$). These results indicate that the behavior of the rats in this study was similar to behaviors observed in previous studies, with rats selecting higher value reward by the end of both delay and size blocks.

Surprisingly, we found that lesioned rats' selection of rewards did not significantly differ from control rats (*Fig. 3.7B*, black bars); there were no significant

group main or interaction effects (main effect: $F(1,2384) < 0.001$, $p = 0.970$; group x phase and group x block interactions: $F(1,2384) < 0.001$, $p = 0.970$; group x value interaction: $F(1,2384) = 0.21$, $p = 0.646$). Together, these results show that both control and lesioned rats significantly choose high over low-value rewards at similar rates.

One possibility for a lack of a lesion effect on free-choice trials is that impairment may have been observed early but not later in testing. To address this issue, we examined percent choice of high-value rewards across each day of testing. Using an ANOVA with factors for group, phase, and day, we found a significant main effect of phase ($F(1,1160) = 86.69$, $p < 0.001$), showing that both control and lesioned rats selected high-value rewards significantly more by the last 10 trials of a block. We found no significant main effects of group ($F(1,1160) = 0.12$, $p = 0.727$) or day ($F(9,1160) = 0.23$, $p = 0.99$), nor did we find any significant interactions (GroupxPhase: $F(1,1160) = 0.37$, $p = 0.45$; GroupxDay: $F(9,1160) = 0.49$, $p = 0.884$; PhasexDay: $F(9,1160) = 0.6$, $p = 0.795$). Taken together, these results show that lesions to insula did not affect free-choice preference for high-value rewards.

Insula lesions made rats respond more quickly

In addition to performance, we can also examine reaction time, or the time it takes for rats to leave the odor port after odor onset. Much like accuracy during forced trials, we see with forced reaction time that biases towards higher reward-value affect the speed at which rats make their decisions. As rats develop their biases towards fluid wells associated with better reward, rats become faster to respond for

high-value wells and are slower when they need to work for a reward of lesser value. This behavior also is learned and adaptable, developing over the course of a block and changing as contingencies switch. In the following analyses, we show similar trends as presented in our forced percent accuracy data, in that lesions produced general changes to reaction time without altering specific biases for high or low-value reward.

During forced trials we found rats were generally faster for high- versus low-value rewards (*Fig 3.8A*, $F(1,2384) = 25.49$, $p < 0.001$). Although there was no significant main effect of phase ($F(1,2384) = 129.82$, $p = 0.360$), we found a significant interaction between phase and value ($F(1,2384) = 6.35$, $p = 0.012$), indicating that, although there was no difference at the start of a block ($t(1198) = -1.782$, $p = 0.075$), rats became faster in their decision-making by the end of a block for high-valued rewards ($t(1198) = -5.020$, $p < 0.001$). A main effect of block also indicated that rats were faster during size blocks ($F(1,2384) = 39.58$, $p < 0.001$). Lastly, our lesioned rats were significantly faster than our control rats (*Fig. 3.8A*, black bars), as evidenced by a significant main effect of group ($F(1,2384) = 129.82$, $p < 0.001$). As there were no other significant interactions (with the closest to significant interaction being $F(1,2384) = 1.07$, $p = 0.301$), lesions seemed to produce a general speeding effect across forced trials of the odor-guided delay/size choice task.

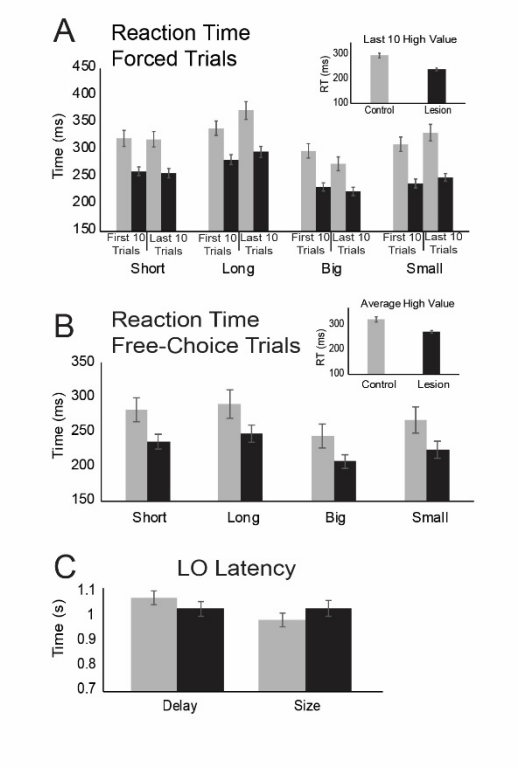

Figure 3.8: Lesions reduced reaction time and attentional differences between delay and size blocks
(A) Reaction time (odor offset to odor port exit) in milliseconds for correct forced trials, during the first and last 10 trials of each block. Inset: Average reaction time in milliseconds for high-value rewards in the last 10 trials. **(B)** Reaction time in milliseconds across all free-choice trials. Inset: Average reaction time in milliseconds for high-value rewards. **(C)** Average light-on latency (houselight illumination to odor port entry) in seconds, averaged across delay and size blocks.

We next examined forced reaction time across the 10 days of testing to determine whether these differences in speed were transient effects. We found a main effect of day ($F(9,1180) = 3.51$, $p < 0.001$) and an interaction between group and day ($F(9,1180) = 3.11$, $p = 0.001$). Post hoc t-tests for days 1 and 10 demonstrated lesioned rats were significantly faster on the first day of testing ($t(120) = -2.371$, $p = 0.019$), but there was no significant difference between controls and lesions on day 10 ($t(117) = -1.433$, $p = 0.155$). Together, these results indicate that rats were faster to respond to odor stimuli, but effects were transient, possibly reflecting post-lesion experience induced compensatory mechanisms.

We observed similar speeding effects when looking at reaction time for free-choice trials (*Fig. 3.8B*). Main effects of value ($F(1,1142) = 3.99$, $p = 0.046$) and block ($F(1,1142) = 13.71$, $p < 0.001$) demonstrated that all rats exhibited faster reaction times for high-value rewards and during size blocks of the task. A main

effect of group additionally showed that lesioned rats were faster than controls ($F(1,1142) = 30.51$, $p < 0.001$). A lack of significant interactions again demonstrated that lesions produced an overall effect on speed ($F(1,1142) \leq 0.43$, $p \geq 0.510$). Analyzing this effect by day again showed a main effect of group ($F(1,1130) = 33.92$, $p < 0.001$), as well as a main effect of day ($F(9,1130) = 7.05$, $p < 0.001$) and a significant interaction between these factors ($F(9,1130) = 3.69$, $p < 0.001$). A post hoc t-test of the first day revealed that lesioned rats trended toward being faster, ($t(89) = -1.824$, $p = 0.072$), and that there was no significant difference between these groups on day 10 ($t(114) = -1.181$, $p = 0.240$).

Finally, we measured the latency at which rats entered the odor port after they are cued to start a trial by houselight illumination (i.e. light-on latency; *Fig. 3.8C*). This measurement has been used in past studies to examine attention and task engagement, as light-on latencies are thought to reflect the processing of trial events and cognitive control needed for task performance. Because rats do not have any knowledge of the upcoming reward as they are initiating a trial, we took an average LO latency across all trials of each block type (delay and size) and performed an ANOVA of group and block. In past studies we have generally found faster LO latencies in size blocks of the task, perhaps indicative of increased attentional processes for blocks with overall greater amounts of reward in spite of fatigue and satiety that develops as rats complete a session (Vázquez et al., 2019). Here we found a significant main effect of block ($F(1, 1196) = 5.66$, $p = 0.018$), indicating that light-on latencies were overall faster for size blocks (*Fig. 3.8E*). Interestingly, although there was no significant effect of group ($F(1,1196) = 0.02$, $p = 0.901$), we observed a

significant interaction between group and block ($F(1,1196) = 6.06$, $p = 0.014$). Post-hoc t-tests indicated that, while controls were significantly faster in size compared to delay blocks ($t(590) = -3.558$, $p < 0.001$), there was no difference between blocks in the lesioned group ($t(606) = -0.056$, $p = 0.955$). This finding suggests the speed at which lesioned rats initiated trials was not modulated by the block context that they were in. This result also demonstrates that lesioned rats do not exhibit a general motor speeding as they responded to the house-light by initiating the trial via nose poke at the same speed as controls.

To examine whether the difference between size and delay blocks changed over the course of the 10 days of testing, we took the difference between size and delay blocks (e.g. size – delay) and performed an ANOVA with factors for group and day. we found a significant main effect of group ($F(1,580) = 10.27$, $p = 0.001$), however we did not find a significant main effect of day ($F(9,580) = 1.01$, $p = 0.433$) nor did we find a significant interaction between group and day ($F(9,580) = 0.91$, $p = 0.520$). These results suggest that the group differences we observed in LO latency remained consistent throughout the 10 days of odor-guided decision-making testing.

Insula lesions reduced sign-tracking

Altogether, insula lesions seemed to produce changes in behavior during the odor-guided delay/size choice task including, significantly faster reaction times, reduced accuracy, and reduced differences in which rats initiate trials dependent on block context.

Next we examined insula's role in autoshaping, a task that has traditionally been used to determine goal or sign-tracking respectively, an animal's attraction to a reward or its associated stimuli (Brown & Jenkins, 1968; Chudasama & Robbins, 2003; Fitzpatrick et al., 2013; Flagel, Akil, & Robinson, 2009; Flagel, Watson, Robinson, & Akil, 2007; Lee et al., 2018; Lovic, Saunders, Yager, & Robinson, 2011; Morrison, Bamkole, & Nicola, 2015; Tunstall & Kearns, 2015). Goal-tracking suggests the animal is focused on the outcome of receiving reward and is considered a more flexible, adaptable behavior. In contrast, sign-tracking suggests an animal is prone to attributing incentive salience to these reward-related stimuli and subsequently may exhibit habitual, inflexible behavior to obtain a reward (Morrison et al., 2015; Nasser et al., 2015). It is therefore suggested that these behaviors are respectively driven by actions and their associated outcomes (A-O associations), or by stimuli and their associated responses (S-R). Successful performance in the odor-guided decision-making task requires both forms of associative learning because rats must form odor-response associations for forced trials (S-R), and remember the location of high-value rewards during free-choice trials (A-O). To further study insula's role in associative learning we quantified trough entries and lever-presses during presentation of the conditioned stimulus in the 10-day autoshaping task. Similar to the odor-guided delay/size choice task, we analyzed each behavior across each day of testing using an ANOVA with factors of group and day. Examining trough entries during lever extension, we found a main effect of group, with higher rates of trough entries in lesioned rats compared to controls ($F(,1,140) = 9.74$, $p = 0.002$; *Fig. 3.9A*). For lever-pressing behavior, we found a significant main effect of

group (F(1,140) = 4.15, p = 0.043; *Fig. 3.9B*), indicating that lesioned rats exhibited significantly lower rates of lever-pressing compared to controls. Lastly, there was no significant main effect of day (F(9,140) = 1.24, p = 0.278), nor was there a significant interaction (F(9,140) = 0.54, p = 0.843) for trough entries and or lever pressing (Day: F(9,140) = 1.11, p = 0.359; interaction: F(9,140) = 1.31, p = 0.239)

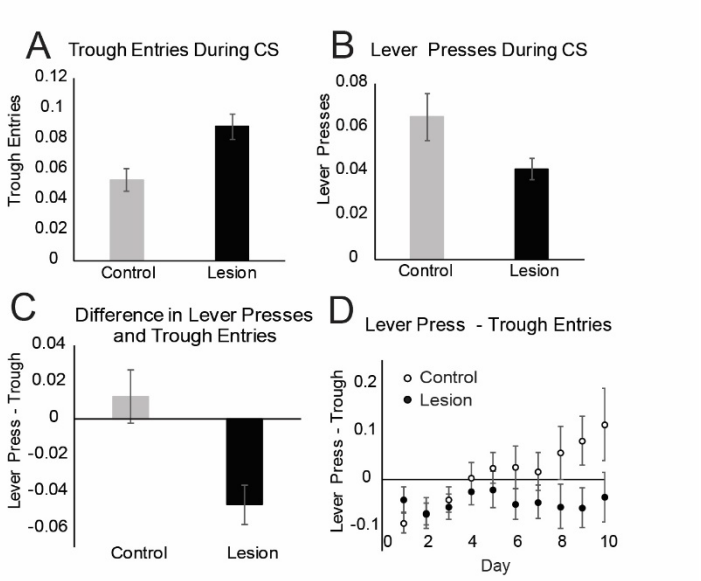

Figure 3.9: Lesions reduced sign-tracking and increased goal-tracking during autoshaping
Control rats are represented by light bars and open circles. Lesion rats are represented by black bars and black circles. Error bars represent SEM. **(A)** Trough entries during the 8s lever presentation for control and lesion rats. **(B)** Lever presses during the 8s lever presentation for control and lesion rats. **(C)** Total Lever – Trough difference for control and lesion rats. **(D)** Lever – Trough difference across each day of autoshaping for control and lesion rats.

As a final measure of goal and sign tracking behaviors, we computed the difference between these behaviors (lever presses - trough) and compared them across control and lesion groups (*Fig. 3.9C*). A positive and negative difference score would indicate sign-tracking and goal-tracking behavior, respectively. As predicted from *Figures 3.9A* and *B*, control and lesion difference scores were positive and negative, respectively, and significantly different from each other as indicated by a significant

main effect of group in the ANOVA ($F(1,140) = 10.83$, $p = 0.001$). There was no significant main effect of day ($F(9,140) = 1.54$, $p = 0.139$; *Fig. 3.9D*), nor a significant interaction with day ($F(9,140) = 1.26$, $p = 0.265$). Together, these results suggest that rats with insula lesions exhibited overall stronger goal-tracking behavior compared to controls.

Behaviors during autoshaping and odor-guided delay/size choice tasks were not correlated

Because insula lesions reduced accuracy in the odor-guided delay/size choice task and sign-tracking in the autoshaping task, our last set of analyses were designed to ask whether sign-tracking tendencies were related to rats' ability to follow S-R rules during forced trials. We took the lever-trough difference in autoshaping performance, and correlated this result to behavioral measures from the odor-guided delay/size choice task using regression analyses. We found no significant relationship between accuracy during forced trials in the odor-guided delay/size choice task and lever-trough difference in the autoshaping task (control: $p = 0.135$, $r^2 = 0.331$; lesion: $p = 0.417$, $r^2 = 0.112$; *Fig. 3.10A*, black dots). There was also no significant relationship between lever-trough difference and reaction time during forced trials (control: $p = 0.101$, $r^2 = 0.385$; lesion: $p = 0.592$, $r^2 = 0.051$; *Fig. 3.10B*).

Our next set of analyses examined the relationship between free-choice behavior in the odor-guided delay/size choice task and autoshaping behaviors. Here we took the average of high-value reward selection during the last 10 free-choice trials for each rat, as at this point reward-contingencies of the task would be well-

established (*Fig. 3.10C-D*). We observed no significant relationship between high-value reward choice and lever-trough differences (control: $p = 0.521$, $r^2 = 0.072$; lesion: $p = 0.882$, $r^2 = 0.004$; *Fig. 3.10C*). We additionally found no significant relationships between free-choice reaction time and lever-trough difference (control: $p = 0.151$, $r^2 = 0.310$; lesion: $p = 0.619$, $r^2 = 0.044$; *Fig. 3.10D*). Together, these results suggest that, although lesions altered sign-tracking behavior, these changes in S-R processing were not related to behavioral changes observed during the odor-guided delay/size choice task in a simple and straightforward way.

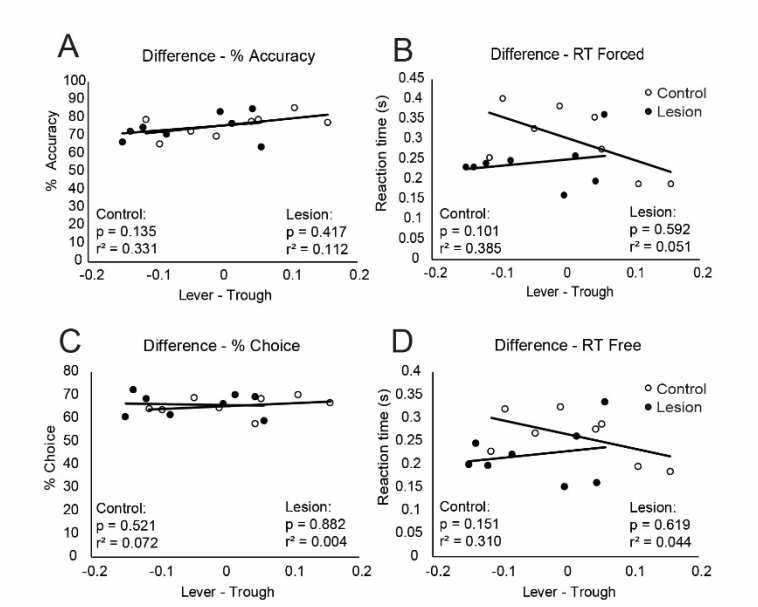

Figure 3.10: Odor-guided delay/size choice task performance was not correlated with autoshaping Control rats are represented by open circles, while lesion rats are represented by black circles. **(A – D)** Scatter plots comparing Lever – Trough difference during autoshaping with **(A)** percent accuracy, **(B)** reaction time during forced choice trials, **(C)** percent choice, and **(D)** reaction time during free choice trials of the odor-guided delay/size choice task.

DISCUSSION

Here we show that damage to the insula resulted in reduced accuracy, faster reaction times, and differences in light on latencies between blocks of our odor-guided delay/size choice task. Insula damage additionally disrupted sign-tracking behavior, while leaving goal-tracking intact and evaluative decision-making intact.

Surprisingly, however, the deficits observed in the two tasks – autoshaping and reward-guided decision-making – were not correlated with each other.

Our autoshaping task was used to assess the potential role of insula in forming S-R and A-O associations needed to perform our decision-making task. Autoshaping has traditionally been used to assess an animal's tendencies towards flexible or inflexible behavior by classifying them as goal or sign-trackers, with sign-tracking in particular being considered a predictor for habit-formation and impulsivity that potentially heightens the risk for future drug abuse or poor decision-making behavior (Lovic et al., 2011; Morrison et al., 2015; Saddoris et al., 2016; Tomie et al., 2008). Our findings suggest lesioning the insula reduced the incentive salience of the lever during autoshaping and reduced the processing of odor stimuli during the decision-making task, while not affecting reward preference or choice selection. Further, lesioned rats were faster to leave the odor port, suggesting that they were timing their response as opposed to fully processing the odors and their meaning. From these results, insula seems necessary for the maintenance of sign-tracking behavior and potential habit-formation, as well as processing sensory stimuli while performing a more complex behavioral task.

Previous studies in insula have largely shown anterior regions to be necessary for retrieving reward-memories and updating reward contingencies, while posterior regions are responsible for acquisition of reward responses (Contreras et al., 2012, 2007; Forget et al., 2010; Kesner & Gilbert, 2007; Parkes et al., 2018; Pelloux et al., 2013; Pushparaj & Le Foll, 2015). Other studies have also demonstrated that anterior insula is involved in devaluation (B. W. Balleine & Dickinson, 2000; Parkes et al.,

2015; Pelloux et al., 2013) and taste aversion (Cubero, Thiele, & Bernstein, 1999; Ferreira, Miranda, De La Cruz, Rodríguez-Ortiz, & Bermúdez-Rattoni, 2005; Kayyal et al., 2019; Miranda & McGaugh, 2004). Recent work from the Calu lab has also found that inactivating the pathway between basolateral amygdala and anterior insula reduced food cup contacts and increased the latency to approach the food cup in rats that had previously been identified as goal-trackers (Nasser, Lafferty, Lesser, Bacharach, & Calu, 2018).

However, when comparing our results to this body of work it is important to consider that the nomenclature surrounding insula's subregions can vary from study to study, and oftentimes can cross over into other categorizations of insula's anatomy and functions. When developing our first recording study in insula, we discovered that anterior insula manipulations frequently affected OFC due to these regions' close proximity (Chang, 2014; Gallagher et al., 1999; McDannald, Saddoris, Gallagher, & Holland, 2005; S. B. Ostlund & Balleine, 2007; Pickens et al., 2003; Saddoris et al., 2005; Stalnaker et al., 2007). However, we were interested in studying this subregion for its potential role in our decision-making task. To address these conflicting issues, we sought a part of insula that would be further rostral while having no chance of impinging on OFC.

While we avoided such accidental overlap, our coordinates do contribute to some of this ambiguity of which subregion we are studying. For example, our coordinates are within the boundary of gustatory cortex, which has been found to encode taste perception (Mukherjee, Wachutka, & Katz, 2019), task-related information for attaining food rewards (Vincis et al., 2020), and signals related to

anticipation (Gardner & Fontanini, 2014; Samuelsen et al., 2012). Our lesions may have also crossed into regions of mid-insula, which has in fact been found to mediate odor cue-guided reinstatement for cocaine (Di Pietro, Black, & Kantak, 2006). The current study highlights the need for more careful dissection of insula subregions. Performing these procedures on another region within insula may further establish distinct subregional functions. However, even if we are outside typical borders of anterior insula, it is suggested information within the entirety of insula is consolidated in a caudal-to-rostral pathway (Centanni, Janes, Haggerty, Atwood, & Hopf, 2021). Signals from more posterior parts of insula – including those observed in the current study – may therefore contribute to the cognitive functions of anterior insula (Castro & Berridge, 2017; Centanni et al., 2021; Méndez-Ruette et al., 2019).

Our lesions in the present study may have also produced independent compensatory processes within the brain that occur over time and/or with experience. In this regard, emerging evidence has shown that experience can strongly influence the expression of goal or habit-directed behavior. For example, the presentation of multiple options can conserve goal-directed behavior even after overtraining (Colwill & Rescorla, 1985; Holland, 2004; Kosaki & Dickinson, 2010). Here, our rats had been previously trained in a complex task with two choices, and flexible reward contingencies before they were tested in a much simpler autoshaping task. This prior experience may have influenced plasticity of insula and its subregions, as well as the rats' behavioral strategies, and in turn affected how strongly stimuli influence behavior. These results suggest previous literature using single-outcome instrumental

tasks should be examined alongside complex tasks to fully study behavior and their related neural processes.

Our findings may additionally inform studies of insula's role in drug addiction. While recording from the insula in this same decision-making task, we have previously demonstrated a novel "context encoding" signal in which we observed overall higher neural firing during size blocks compared to delay. This activity was correlated to higher accuracy and reaction times during these blocks, and such relationships were also found to be disrupted by prior cocaine exposure (Pribut, Vázquez, Brockett, et al., 2021). From these analyses, we suggested that this context encoding conveyed the overall higher value of size blocks compared to delay, and was important for maintaining motivation despite fatigue and satiation that inevitably sets in by the time size blocks are presented. The reduction in context-encoding observed after cocaine exposure may in turn disrupt such processing and produce cocaine-induced biases in reward selection.

The present study potentially elucidates some of the mechanisms by which insula produced these behavioral changes. Here we observed that damaging the region we recorded from resulted in reduced accuracy and faster reaction times. We additionally observed disruptions in attention between delay and size blocks. Recall that our control rats exhibited faster light-on latencies during size blocks compared to delay, but there was no significant difference in performance from our lesioned rats. Importantly, we still observed block effects in our percent accuracy and reaction time measures. These subtle changes may be due to compensation from other regions of insula and its connecting circuitry, and differences in recording from an intact insula

within a cocaine-exposed rat. However, we believe the current results highlight alterations in attention and salience of stimuli that may also occur after exposure to drugs of abuse and contribute to impairment of behavior. As previously mentioned, future study could further dissociate the contributions of insula subregions to this behavior by comparing our findings here to manipulations of more anterior and posterior insula. We could additionally explore the potential of sex differences in behavior, a question we were not able to adequately explore in the current study.

Taken together, our results suggest insula is necessary for mediating how quickly and accurately stimulus guided decisions are made and the control that stimuli have over behavior. Interestingly, at least in the context of these two tasks, rats can still process reward predictions and adjust choice behavior based on the value of expected outcomes, as well as goal-tracking during autoshaping. The nuances of insula's role in stimulus guided behavior, choice behavior and autoshaping, and the interactions they have with complex experiences and drug addiction, all provide important avenues for future study.

CHAPTER 4: Prior cocaine self-administration impacts sensitivity to delays to reward after reversal but not the ability to delay gratification during diminishing returns with reset

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ABSTRACT

Previous exposure to drugs of abuse produces impairments in studies of reversal learning, delay discounting, and response inhibition tasks. While these studies contribute to the understanding of normal decision-making and how it is impaired by drugs of abuse, they do not fully capture how decision-making impacts the ability to delay gratification for greater long-term benefit. To address this issue, we used a diminishing returns task to study decision-making behavior from rats that had previously self-administered cocaine. This task is designed to test the ability of the rat to choose to delay gratification in the short term to obtain more reward over the course of the entire behavioral session. Anecdotally, this is similar to an addict choosing to take the time to go to rehab to improve their life in the long run. In this task, rats were presented with two choices. One choice had a fixed amount of time delay needed to obtain reward (FD), while the other choice had a progressive delay (PD) that started at 0s and progressively increased by 1s each time the PD option was selected. During the "reset" variation of the task, rats could choose the FD option to reset the time delay associated with the PD option, while in the "no reset" variation, the PD delay continued to increase regardless of FD selection. Consistent with previous results, we found that prior cocaine exposure reduced rats' overall preference for the PD option in post-reversal no reset sessions, suggesting that cocaine exposure made rats more sensitive to the increasing delay of the PD option. Surprisingly, however, we found that rats that had self-administered cocaine adapted behavior during reset sessions by delaying gratification to obtain more reward in the long run similar to control rats. That is, both groups selected the FD lever with a

longer delay to reset the PD delay back to zero prior to the equality point, thus achieving more reward over the course of the session. Although these findings do not support our hypotheses, they still support previous work demonstrating general deficits in reversal learning after previous drug use. Given these results, studying neural signals related to diminishing returns behavior and using progressive ratio variations of the diminishing returns task may lead to a deeper understanding of these behavioral findings.

INTRODUCTION

Existing research has proven addiction negatively affects executive functioning, self-control, and impulsivity, which can often be observed through impairments in reversal learning, delay discounting, and response inhibition tasks (Brockett et al., 2018). Subjects previously exposed to drugs of abuse consistently show decreased ability to adjust their responses to reversed reward-contingencies, as shown by humans (Ersche, Roiser, Robbins, & Sahakian, 2008; Moreno-Lõpez et al., 2015) and rats that have previously been exposed to cocaine (Calu et al., 2007; Geoffrey Schoenbaum et al., 2004). Studies have also found that impairments in delay discounting tasks, in which rats (Mendez et al., 2010; M. R. Mitchell et al., 2014) and humans previously exposed to cocaine will more steeply discount the value of a delayed reward in favor of a smaller reward that arrives sooner (Camchong et al., 2011; Kirby & Petry, 2004). Lastly, studies show response inhibition impairments in human subjects exposed to cocaine (Albein-Urios, Martinez-González, Lozano, Clark, & Verdejo-García, 2012; Fillmore & Rush, 2002), as well as rats exposed to cocaine (Paine & Olmstead, 2004).

This collection of work has contributed immensely to researchers' understanding of both normal and impaired decision-making by drugs of abuse, especially when used in conjunction with neurophysiology techniques that give us a better understanding of how these behaviors are correlated to neural activity. However, these existing studies do not capture all aspects of decision-making, specifically the ability to select a less favorable option for greater long-term benefit. Examples of such optimal decision-making can be seen in both humans and animals.

Animals foraging for food will sometimes need to forego the benefit of a known food source to explore for other resources, delaying immediate gratification to ensure future security should their current food source be depleted. Similarly, a human may pursue higher education and halt other aspects of their life for greater opportunity and financial security in the future.

In the lab, this behavior can be examined using diminishing returns tasks (Hayden, 2016). In such tasks, the value of a reward constantly changes based on a subject's choice: consecutive selection of one of the two possible options results in a gradual decrease of that option's value because either the work needed to obtain reward or the delay to reward delivery progressively increases, while the other option which has a fixed amount of work or time needed to obtain reward across the entire experiment. Importantly, this fixed option is initially set to be less rewarding than the progressive option. In this situation, optimal behavior would be shown by initially selecting the progressive option, and then switching to the fixed option when the costs of each option became equivalent.

However, in a variation of this task, known as a "reset" condition, selecting the fixed option enables the cost associated with the progressive option to be reverted back to its original value. Referring back to the foraging example, this task variation would be equivalent to an animal traveling to a slightly more distant food source so that its closer source could be replenished. Interestingly, computational analyses of the diminishing returns task have demonstrated that it is most beneficial to reset reward prior to the two options reaching equivalence in work or time (Hayden, 2016). Such computations demonstrate the analogous nature of diminishing returns with

reset to situations requiring delayed gratification: in order to maximize future rewards, subjects must forego the benefit of less work/time from the progressive option, and select the fixed option for overall greater rates of reward receipt. Indeed, subjects have shown time and time again that they are able to distinguish between these two schedules and maximize the reward they receive by "resetting" before the progressive option is equal to the fixed option, across humans (Hackenberg & Axtell, 1993; Jacobs & Hackenberg, 1996), pigeons (Hackenberg & Hineline, 1992), and chimpanzees (Hodos & Trumbule, 1967).

In addition to this work, we have recently shown that rats are also capable of performing this task and appropriately adjusting their behavior to the two schedules of reinforcement (Schuweiler, Rao, Pribut, & Roesch, 2021), opening new possibilities for studying this behavior in combination with techniques and principles used in behavioral neuroscience. To this end, we can also study how behavior in this task may be disrupted by previous exposure to drugs of abuse, and whether such disruptions are in line with previous observations from other decision-making tasks. Such work could potentially provide insight into long-term decision-making deficits often seen in humans with addiction, as these individuals are often unable to forego the immediate benefit of drug use to seek and successfully complete treatment for improved quality of life.

To address this question, here we asked whether prior cocaine-exposure would impact the demonstrated ability of rats to maximize reward via delayed gratification. We hypothesized rats that had previously self-administered cocaine would "reset" reward at a later rate compared to control rats, demonstrating deficits in

delaying gratification and reducing their maximum net reward rate. Although our results reinforced findings that previous drug use impairs reversal learning, we intriguingly found no effects on reset rate. Our findings therefore affirm that drugs of abuse produce long-lasting impairments of these basic elements of reward-learning, but their role in more complex decision-making requires further exploration and development of appropriate tasks.

MATERIALS AND METHODS

Subjects

Twenty-two Long-Evans rats (equal males and females; weight, 175g – 225g) were purchased from Charles River Laboratories. Rats were housed and tested at the University of Maryland, following National Institute of Health (NIH) guidelines and the Institutional Care and Use Committee (IACUC) at the University of Maryland, College Park. The rats were single-housed in a temperature and humidity-controlled vivarium, set on a diurnal light cycle (12 h light/dark). Water was available *ad libitum*. Prior to starting behavioral training, rats were given approximately 12-15 g of chow and 2.5 g of sucrose pellets (45mg; LabDiet, St. Louis, MO) to begin food deprivation and prevent neophobia for sucrose. Throughout diminishing returns training and testing subjects were given 12-15g of chow a day, resulting in a minimum body weight of 85% of their initial pre-experimental weight. After the completion of the experiment, the subjects were euthanized via isoflurane overdose.

Behavioral apparatus

The behavioral procedures of this experiment were conducted in two modular shuttle boxes, with a divider placed in the center of the box (ENV-010MD, Med Associates, St. Albans, VT). Both shuttle boxes were stored inside of a cabinet that attenuates sound and light to reduce distractions for subjects during behavioral procedures. The right end walls of the shuttle boxes were equipped with a central nose port, and pellet troughs connected to pellet dispensers (Med Associates ENV-200R2M-6, and ENV-203M-45), while the left end walls were equipped with a house light (ENV-221M).

Shaping

To perform the diminishing returns task, all rats underwent 5 weeks of training to become accustomed to waiting for reward and to learn the different schedules of reinforcement on the two troughs (See *Fig. 4.1A* timeline). Training sessions were 1hr, Monday-Friday. In week 1, rats were shaped to nosepoke into the central port by being rewarded sugar pellets delivered to either trough for each port entry. The time required to nosepoke was initially set at 1 centisecond, and increased by 1 centisecond-intervals after at least 10 successful trials until the rats were able to hold their nose in the port for 1s. For this week, rewards from both troughs were delivered immediately.

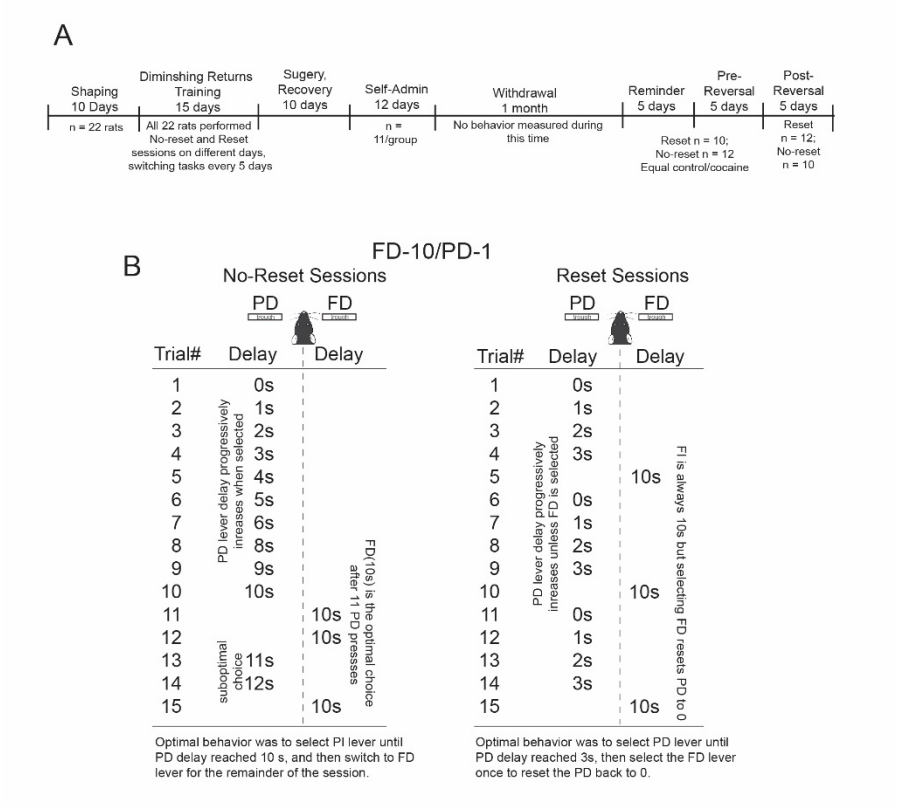

Figure 4.1: Experimental timeline and task overview
(A) Experimental timeline. Rats were counterbalanced between control and cocaine groups, sex, and the experience of reset or no-reset sessions first. **(B)** In the diminishing returns task, rats choose between two troughs. One delivers reward after a fixed delay (FD), while the other delivers reward on a progressive delay (PD) schedule where the delay increases by 1s with each entry into the trough. In the reset condition (right), the rat is able to reset the PD delay back to zero by entering the FD trough. In the no-reset condition (left), the PD delay continues to increase regardless of FD choice. In the reset condition, rats should switch between the FD and PD options prior to the PD delay reaching equality with the FD trough in order to maximize the amount of rewards they earn in a session.

In week 2, rats were next trained to wait for progressively higher delays from the trough. After holding their nose in the central port for 1s, rats could then go to either trough for reward. Reward was delivered immediately on the first trial, and then gradually increased by increments that were increased each day. On the first day, progressive delays increased by increments of 0.1s. Delays increments then increased to 0.25s on the second day, 0.5s on the third, and 1s increments on the final day of PD training.

Once rats were trained to nosepoke and wait for long delays of at least 30s, they began training in both reset and no-reset variations of the diminishing returns task. Rats were counterbalanced across reset and no-reset conditions, with conditions being switched each week. For example, rats would go through three weeks of training that consisted of one week reset, second week no-reset, and third week reset. The switching conditions allowed the rats to learn the diminishing returns task and adapt behavior across the two variations of the task. PD and FD troughs assignments were counterbalanced across rats, and trough assignments were also switched alongside task variations to prevent formation of side biases.

Diminishing returns task

To perform the diminishing returns task (summarized in *Fig. 4.1B*), rats followed visual light cues and chose between two troughs that would deliver reward on different temporal schedules. The central nose port first illuminated to signal that the rat could begin a trial. After nosepoking for 1s, lights within both troughs illuminated and the rat could select one by nosepoking into the trough. One trough delivered a pellet at a fixed-interval (FD) delay of 10s, while the other delivered a pellet immediately on the first selection and progressively increased (PD) its delay by 1s each time it was selected.

Behavior that maximizes the reward earned is different for the two conditions of reset and no-reset. In order to maximize rewards during the reset condition, the average delay on the progressive delay (PD) trough should be lower than the delay on the fixed delay (FD) trough; in other words, rats should nosepoke into the FD trough

to reset the PD trough before the delay on the PD trough reaches 10s, equivalent to the fixed delay on the FD trough. As seen in the right side of *Fig. 4.1B*, rats should ideally select the PD option 4 consecutive trials, and then make a single response in the FD trough. On the other hand, maximizing rewards during the no-reset condition requires rats to nosepoke into the PD trough until the delay increases to 10 s, then switch over to the FD trough for the remainder of the trial. Here, optimal behavior would be demonstrated as selecting the PD option for 10 consecutive trials, and then permanently selecting the FD trough for the rest of the session (*Fig. 4.1B*, left).

Surgery

All subjects were implanted with catheters in the jugular vein for self-administration. Catheters were made from Silastic tubing (0.02 x 0.037 in; Dow Corning, Midland, MI) with a modified G 5-up cannula (Plastics One, Roanoke, VA). The cannula traveled through the fascia layer over the shoulder and was cemented on top of the skull. Rats were given Rimadyl (pain reliever, 0.1 mg/kg, s.c.) before and 24h after surgery to minimize post-surgical discomfort. They additionally received post-surgical antibiotics of Cephalexin (orally), and catheters were flushed with 0.9% saline + Gentamicin (0.1 mg/ml) each day for one week. We then split rats into cocaine (male = 6, female = 5) and control groups (male = 5, female = 6) based on catheter patency and switching performance, ensuring that there was no significant difference between the average switching rate during reset sessions ($t(20) = -0.589$, $p = 0.563$).

Cocaine self-administration

After recovery from surgery, rats underwent a 12d self-administration protocol in separate Med Associates operant chambers. These boxes were equipped with a single trough, a retractable lever (ENV 112CM), and a green stimulus light on one side of the box. To further prevent generalization across the two different operant chamber contexts, self-administration chambers were additionally equipped with different flooring (ENV-005A), and the walls of the box had horizontal strips of tape placed on the outside.

The self-administration protocol involved subjects in the cocaine group receiving an infusion of cocaine with each lever press, whereas control rats pressed for sugar pellets. The cocaine dosages were calculated by weight, with separate doses for male and female rats to account for sex differences. For the first 6 days of self-administration, rats received either 1mg/kg infusion of cocaine or two sucrose pellets per lever press, with a maximum of 30 presses or for a 3h time limit. For the last 6 days of self-administration, the cocaine dosage was halved to 0.5mg/kg, and only 1 sucrose pellet was delivered for each press, with a maximum of 60 lever presses. This procedure of halving the dose allowed us to examine increases in drug-seeking behavior to maintain the desired level of drug intake.

Importantly, the 5-week training for the diminishing returns task took place before cocaine self-administration, and no diminishing returns testing occurred during self-administration or within the 1-month withdrawal period that followed. The purpose of this timeline was to examine how cocaine exposure produces long-term

impairments in decision-making behavior, similar to our previous studies (Brockett et al., 2018; Burton et al., 2018, 2017; Vázquez et al., 2019).

Experimental design and behavioral analyses

After the 12 days of cocaine self-administration and 4 weeks of withdrawal, testing took place over 3 weeks, for 1 hour each Monday through Friday. The first 5 days of testing served as the reminder days, where rats were reminded of the diminishing returns task after surgery, self-administration, and withdrawal. The second 5 days of testing served as a baseline pre-reversal period. During these first two weeks, rats were tested on the last variation of the task they experienced during training. The final 5 days, during the third week of testing, served as the post-reversal period, where the task variation (reset or no-reset) was switched and the PD trough and FD trough assignments were reversed.

To analyze the data, we computed separately for reset and no-reset sessions (1) the average choice on the PD trough, (2) the average delay of the PD trough for all nosepokes within each session, and (3) the average delay on the PD trough prior to selection of the FD trough. For these behavioral measures, we performed a series of ANOVAs across sessions. First, we examined if there were any differences between groups during the first 5 days of reminder training by performing ANOVAs separately for reset and no-reset with group (cocaine vs control) and session day (1-5) as factors. Second, we examined pre- and post-reversal test days in one large ANOVA with group (cocaine vs control), session day (1-5), session type (reset vs no-rest), and reversal (pre vs post) as factors. Third, to follow up on interaction terms, we

performed post-hoc t-tests and ANOVAs for reset and no-reset session factors that included group (cocaine vs control), session day (1-5), and reversal (pre and post).

RESULTS

During the first 6 days of self-administration, rats received 1 mg/kg infusions of cocaine per lever press. This dose was halved for the next 6 days to examine increases in drug-taking behavior in order to maintain a desired level of drug-intake. We observed that cocaine-exposed rats did increase drug-seeking from the first to second half of self-administration, although this difference was not significant ($t(20) = -2.048$, $p = 0.054$; gray line; *Fig. 4.2*). We have shown previously that similar rates of self-administration alter reward-guided decision-making and neural firing in anterior cingulate cortex, insula, dorsal striatum and nucleus accumbens (Brockett et al., 2018; Burton et al., 2018, 2017; Pribut, Vázquez, Brockett, et al., 2021; Vázquez et al., 2019). Control rats exhibited the maximum number of responses each session, across the first and second half of self-administration (black line; *Fig. 4.2*).

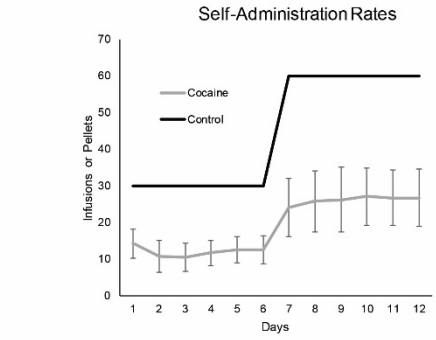

Figure 4.2: Self-administration data
Average lever-press rates for control (black) and cocaine-exposed (gray) rats for each day of self-administration. Bars on the cocaine-exposed group represent SEM.

Below we will examine how cocaine self-administration impacted performance during reset and no-reset variations of the task. If rats recognize the difference between the two sessions-types, reset and no-reset, then they should select

the PD trough at different rates. During no-reset sessions, rats should choose the PD trough until the delay reaches the equality point (i.e., 10 seconds) and then switch to the FD trough for the remainder of the session, thus during the no-rest sessions rats should choose PD lever far more often than the FD lever, and optimally the delay on the PD lever should not exceed 10s.

During reset sessions, selection of the FD trough resets the PD trough back to zero. If rats do not recognize this, then they would also select the PD trough until they experienced a PD delay of 10, and then switch to FD trough. However, if they understand the reset contingencies, then they would not wait until the delay became 10 s, but instead would switch several trials before when the delay was 4 seconds. Thus, rats performing reset sessions should choose the PD lever far more often than the FD lever and average delays on the PD should significantly low during reset compared to no-reset sessions. We have previously found that rats, like most species (Hackenberg & Axtell, 1993; Hackenberg & Hineline, 1992; Hodos & Trumbule, 1967; Jacobs & Hackenberg, 1996), reset prior to the equality point, at near optimal levels.

In the following sections we demonstrate that prior cocaine self-administration produced no significant differences in behavior during the 5 reminder days. We additionally demonstrate that both groups are able to adapt to the different task variations – i.e. switching prior to the equality point during reset sessions and switching later during no-reset sessions – regardless of previous cocaine experience. However, we also show rats that previously self-administered cocaine exhibit greater

sensitivity to delays after the reversal, by showing lower PD trough preference in no-reset sessions post-reversal.

Cocaine and control rats stabilize over reminder days at similar rates

After cocaine self-administration and withdrawal rats were tested for 5 days to stabilize performance after not performing the tasks for 7 weeks. The 5 days of reminder training is plotted in *Fig. 4.3*. Here, we plotted the percentage of PD choices (*Fig. 4.3A*), the delay on the average PD trough over the entire session (*Fig. 4.3B*), and the average delay on the PD trough prior to a selection of the FI trough (*Fig. 4.3C*). For each of these measures we performed ANOVAs with group (cocaine vs control) and session day (1-5) as factors separately for reset and no-reset sessions to determine if performance changed during reminder days and were different between cocaine and control groups.

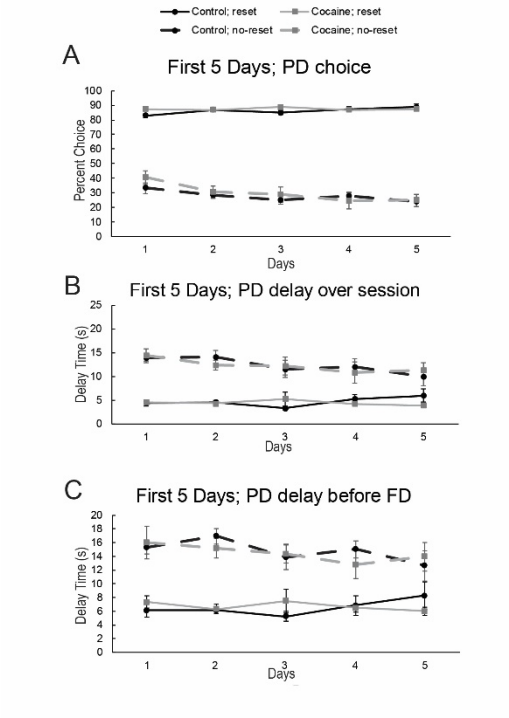

Figure 4.3: Cocaine-exposed and control rats' performance on reminder days of training
Data recorded for 5 days after the month-long withdrawal period. **(A)** Overall percent choice of the PD trough for control and cocaine-exposed rats, for both reset and no-reset variations of the diminishing returns task. **(B)** Average delay on the PD trough over the entire session for control and cocaine-exposed rats, for both reset and no-reset variations of the diminishing returns task. **(C)** Average delay on the PD trough prior to switching to the FD trough for control and cocaine-exposed rats, for both reset and no-reset variations of the diminishing returns task.

For reset sessions, we observed no significant main effect of day ($F(4,40) = 1.04$, $p = 0.397$) or group ($F(1,40) = 2.1$, $p =$

0.155), indicating that both control (black, solid line) and cocaine-exposed rats (grey, solid line) showed similar selection of the PD trough across all 5 days of reminder training. There was additionally no significant main effect of group for PD trough choice during no-reset sessions ($F(1,50) = 0.21$, $p = 0.647$), indicating similar performance across control (black, dashed line) and cocaine-exposed rats (grey, dashed line). However, we found a significant main effect of day ($F(4,50) = 3.28$, $p = 0.018$). This finding suggested all rats selected the PD trough less over the course of the reminder days, although post-hoc t-tests found no significant difference between the days 1 and 5 ($t(42) = 0.617$, $p = 0.540$). This test was performed across both cocaine and control groups, as there was no significant interaction between group and day ($F(4,50) = 0.64$, $p = 0.639$). Thus, rats' PD choice was largely established early in reminder training, and was similar across control and cocaine-exposed treatment groups.

PD choice can be indicative of overall preference, but it does not inform us of the number of consecutive selections a rat is making. We therefore examined the average PD delay across the entire session, for both reset and no-reset sessions (*Fig. 4.3B*). For this measure we observed no significant main effects of group (reset: $F(1,40) = 0.25$, $p = 0.617$; no-reset: $F(1,50) < 0.001$, $p = 0.960$), indicating both control and cocaine exposed rats had a similar consecutive selection of the PD trough for both variations of the diminishing returns task. There was also no significant main effect of day for either task variation (reset: $F(4,40) = 0.24$, $p = 0.911$; no-reset: $F(4,50) = 1.58$, $p = 0.195$), indicating no differences in selection rates across the 5

reminder days. There was no significant interaction between these factors, for either reset ($F(4,40) = 1.77$, $p = 0.153$) or no-reset session ($F(4,50) = 0.38$, $p = 0.819$).

Finally, we examined the average PD delay prior to FI selection, as a measure of switching behavior (*Fig. 4.3C*). Here, we observed no significant main effects of group (reset: $F(1,40) = 0.09$, $p = 0.768$; no-reset: $F(1,50) = 0.08$, $p = 0.785$) or day (reset: $F(4,40) = 0.2$, $p = 0.939$; no-reset: $F(4,50) = 0.95$, $p = 0.445$). We also found no significant interaction between these factors (reset: $F(4,40) = 1.14$, $p = 0.353$; no-reset: $F(4,50) = 0.42$, $p = 0.794$).

Thus analyses of the overall percentage of PD choice, average delay on the PD trough, and the average PD delay prior to switching to the FD trough for both reset and no-reset sessions largely reveal that all rats showed stable performance on the diminishing returns task across the 5 reminder days and the performance did not significantly differ between cocaine and control groups. Performance during the reminder days were also unaffected by prior cocaine exposure.

Cocaine and control rats both reset prior to equality point

After behavior had stabilized during the reminder sessions we then examined behavior for an additional 5 days (i.e., pre-reversal) prior to reversing contingences during the 5 days. To determine if prior cocaine self-administration impacted performance over pre- and post-reversal days, we performed ANOVAs with group (cocaine vs control), session-day (1-5), session-type (reset vs no-reset) and reversal (pre vs post) as factors. These analyses were performed for our three behavioral measures of PD choice (*Fig. 4.4A* and *4.5A*), average PD delay for the entire session (*Fig. 4.4B* and *4.5B*), and average PD delay prior to an FI switch (*Fig. 4.4C* and *4.5C*)

across both reset and no-reset sessions, and across the 5 days pre (*Fig. 4.4*) and post reversal (*Fig. 4.5*). Importantly, we found main effects of session-type, demonstrating that rats chose the PD trough more often during reset compared to no-reset. Surprisingly, and inconsistent with our hypothesis, we did not find a session-type by group interaction, suggesting that there was no difference in the way the two groups performed the task. Instead, we only found a group by reversal interaction where cocaine exposed rats were more sensitive to delay, but only after the reversal. Below we describe the statistics in detail.

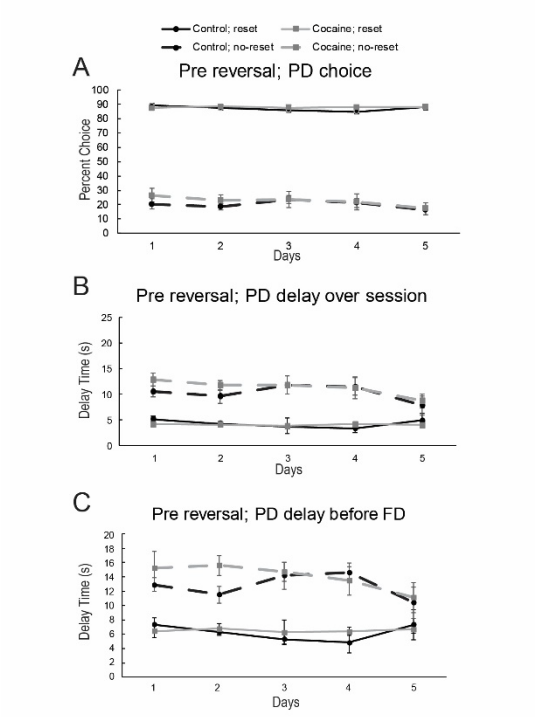

Figure 4.4: Cocaine-exposed and control rats' performance on pre-reversal days of training
Data recorded for 5 days before the reversal. **(A)** Overall percent choice of the PD trough for control and cocaine-exposed rats, for both reset and no-reset variations of the diminishing returns task. **(B)** Average delay on the PD trough over the entire session for control and cocaine-exposed rats, for both reset and no-reset variations of the diminishing returns task. **(C)** Average delay on the PD trough prior to switching to the FD trough for control and cocaine-exposed rats, for both reset and no-reset variations of the diminishing returns task.

PD choice: As in our reminder day analyses, we first examined overall PD choice behavior in the two task variations (*Fig. 4.4A* and *4.5A*). We found no significant main effect of group ($F(1,170) = 0.51$, $p = 0.475$), suggesting similar performance across control and cocaine-exposed rats for both variations of the task. We also found no significant main effect of day

(F(4,170) = 0.76, p = 0.552), indicating choice behavior among all rats did not fluctuate over the 5 days of either pre (*Fig. 4.4A*) or post reversal (*Fig. 4.5A*).

However, a significant main effect of reset (F(1,170) = 2778.5, $p < 0.001$), showed all rats, regardless of cocaine exposure, selected the PD trough significantly more often in reset (solid lines) compared to no-reset conditions (dashed lines). A significant interaction between the effects of reset and day (F(4,170) = 3.05, p = 0.019) demonstrated that, across all days of testing, rats selected the PD trough significantly more in the reset condition (t-tests for all 5 days significant, with least significant test on day 1: t(42) =12.021, $p < 0.001$). Finally, we found a significant interaction between group and reversal condition (F(1,170) = 4.58, p = 0.034), suggesting group performance only diverged after the reversal. This interaction will be explored below when examining reset and no-reset ANOVAs independently. All other interactions were not significant (most significant interaction: F(4,170) = 2.25, p = 0.066). Thus, all rats largely selected the PD trough more often during the reset variation of the diminishing returns task, and switch contingencies appropriately after the reversal.

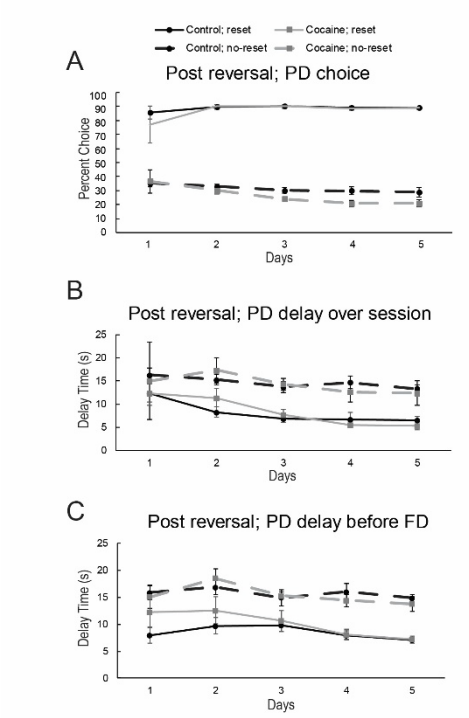

Figure 4.5: Cocaine-exposed and control rats' performance on post-reversal days of training Data recorded for 5 days after the reversal. **(A)** Overall percent choice of the PD trough for control and cocaine-exposed rats, for both reset and no-reset variations of the diminishing returns task. **(B)** Average delay on the PD trough over the entire session for control and cocaine-exposed rats, for both reset and no-reset variations of the diminishing returns task. **(C)** Average delay on the PD trough prior to switching to the FD trough for control and cocaine-exposed rats, for both reset and no-reset variations of the diminishing returns task.

PD session delay: When examining the PD delay for the whole session (*Fig. 4.4B* and *4.5B*) we found no significant main effect of group ($F(1,170) = 0.2$, $p = 0.656$), nor any significant interactions with group (all non-significant interactions greater than $F(4,170) = 3.14$, $p = 0.016$), suggesting prior cocaine exposure did not affect how often a rat switched between the PD and FD troughs. However, we found a significant main effect of reset ($F(1,170) = 222.49$, $p < 0.001$), demonstrating all rats had a lower PD delay in reset sessions (solid lines) compared to no-reset sessions (dashed lines). We additionally found a significant main effect of reversal ($F(1,170) = 85.2$, $p < 0.001$), in which the PD delay was significantly higher post-reversal (*Fig. 4.5B*). A significant main effect of day ($F(4,170) = 6.64$, $p = 0.001$) also demonstrated that total PD delay length decreased from day 1-5. A significant interaction between reversal and day ($F(4,170) = 3.14$, $p = 0.016$) revealed that the session PD delay was generally significantly higher post-reversal (days 1: $t(42) = -3.715$, $p = 0.001$; day 2: $t(42) = -3.664$, $p = 0.001$; day 5: $t(32) = -2.195$, $p = 0.036$), except for two sessions (day 3: $t(42) = -1.725$, $p = 0.09$; day 4: $t(42) = -1.183$, $p = 0.244$). All other interactions were not significant (closest to significance: $F(4,170) = 1.53$, $p = 0.196$). Thus, rats generally had lower session PD delays during the reset conditions of the task, although delays increased after the reversal and persisted throughout all 5 post-reversal days.

PD delay prior to reset: Finally, we examined the average PD delay prior to a switch (*Fig. 4.4C* and *4.5C*). We found no significant main effect of group ($F(1,170)$

= 2.59, $p = 0.110$), indicating cocaine experience had no effect on switching behavior. We also found no significant interactions (closest to significance $F(1,170) \leq 2.58$, $p \geq 0.110$). Similar to session delay, we did observe a significant main effect of reversal ($F(1,170) = 32.03$, $p < 0.001$), which indicated significantly higher PD delays post-reversal (*Fig. 4.5C*) compared to pre-reversal (*Fig. 4.4C*). We also found a significant main effect of reset, in which rats switched from PD to FD trough significantly later in no-reset sessions (dashed lines) compared to reset ($F(1,170) = 210.77$, $p < 0.001$; solid lines). Finally, a significant main effect of day showed all rats switched sooner by the 5th day of testing.

Conclusion: Taken together, all rats largely adapted their decision-making across the two tasks by selecting the PD trough more often and switching between the two troughs at an earlier rate during reset sessions. We surprisingly found previous cocaine-exposure had little effect on behavior, except in slightly altering overall PD choice preference post-reversal.

Cocaine self-administration reduces selection of PD trough during no-reset sessions post reversal

To explore the interaction between group and reversal, we performed separate ANOVAs independently for each session-type with group (cocaine vs control), day (1-5) and reversal (pre and post) as factors. Consistent with the previous analysis, we found no main or interaction effects of group during reset sessions, suggesting that cocaine did not impact the ability to reset and delay gratification in this task. During no-reset sessions, also consistent with the previous analysis, we found no main effect of group or interaction of group with session-day or session-type, suggesting that

overall cocaine did not impact performance on no-reset trials. However, we did find a significant group by reversal interaction demonstrating that cocaine-exposed rats began to select the PD trough less often than controls several days after the reversal, suggesting these rats were more sensitive to delays compared to controls. Statistic details are presented below.

PD choice: For PD choice selection (*Fig. 4.4A* and *4.5A*) we found for reset sessions (solid lines) no significant main effects of group ($F(1,87) = 0.8$, $p = 0.773$), reversal ($F(1,87) = 0.05$, $p = 0.817$, or day ($F(4,87) = 0.86$, $p = 0.491$). We additionally found no significant interactions (closest to significance: $F(4,87) = 1.24$, $p = 0.299$). During no-reset sessions (dashed lines), we also observed no significant main effect of group ($F(1,83) = 0.54$, $p = 0.466$). However, we found significant main effects of reversal ($F(1,83) = 21.65$, $p < 0.001$) and day ($F(4,83) = 2.96$, $p = 0.024$), indicating that all rats had higher preference for the PD trough after the reversal (*Fig. 4.5A*), but showed lower preference across the 5 days of behavior for both pre- and post-reversal. These contradictory effects could be explained by a significant interaction between group and reversal ($F(1,83) = 5.04$, $p = 0.028$). Post-hoc t-tests indicated that, prior to reversal, control and cocaine-exposed rats showed a similar preference for the PD trough ($t(51) = 1.137$, $p = 0.261$). However, after the reversal, cocaine-exposed rats (grey, dashed line) had significantly lower preference for the PD trough compared to control rats (black, dashed line) ($t(48) = -2.021$, $p = 0.049$). These analyses were performed across all days of training, due to no significant interaction with day. All other interactions were not significant (closest to significance: F(4,83)

≤ 1.34, $p \geq 0.262$). Thus, cocaine-exposure reduced rats' overall preference for the PD trough, but only in no-reset sessions post-reversal.

PD sessions delay: Similarly, when examining the overall average PD delay during reset sessions we also found no significant main effect of group ($F(1,87) < 0.001$. $p = 0.961$). We found significant main effects of reversal ($F(1,87) = 54.84$, $p < 0.001$), in which the average delay over session significantly increased post-reversal. We also found a main effect of day, in which the average delay decreased from the first to last day of testing ($F(4,87) = 5.78$, $p < 0.001$). A significant interaction between reversal and day ($F(4,87) = 3.94$, $p = 0.006$) further explained these findings by demonstrating that, while post-reversal (*Fig. 4.5B*) delays were significantly longer than pre-reversal (*Fig. 4.4B*) delays on days 1-4 (day 1: $t(20) = -4.389$, $p < 0.001$; day 2: $t(20) = -4.295$, $p < 0.001$; day 3: $t(20) = -4.290$, $p < 0.001$; day 4: $t(20) = -2.705$, $p = 0.014$), post-reversal delays decreased over time and were no different from pre-reversal by the 5^{th} day of testing ($t(17) = -1.489$, $p = 0.155$). All other interactions were not significant (closest to significance: $F(1,87) = 0.27$, $p = 0.605$). In no-reset sessions, we found only a significant main effect of reversal, in which the average PD delay was significantly longer post-reversal compared to pre-reversal (dashed lines; $F(1,83) = 33.12$, $p < 0.001$). There were no group ($F(1,83) = 0.3$, $p = 0.587$) or day ($F(4,83) = 2.35$, $p = 0.060$) main effects, and all interactions were not significant (closest to significance: $F(1,83) \leq 1.14$, $p \geq 0.289$). Average delays over session therefore were not significantly different between control and cocaine-exposed rats. The only observable change came from all rats in general selecting the

PD trough for more consecutive trials post-reversal, an effect that was especially transient during reset sessions.

PD delay prior to reset: Finally, we examined the average delay before a switch (*Fig. 4.4C* and *4.5C*), for both reset and no-reset sessions. In both reset and no-reset sessions, we found no significant main effects of either group (reset: $F(1,87) = 2.33$, $p = 0.131$; no-reset: $F(1,83) = 0.59$, $p = 0.443$) or day (reset: $F(4,87) = 1.58$, $p = 0.187$; no-reset: $F(4,83) = 1.89$, $p = 0.121$). We additionally observed no significant interactions in either task variation (interaction closest to significance for reset: $F(4,87) = 1.53$, $p = 0.201$; and no-reset: $F(1,83) = 1.39$, $p = 0.241$). In both reset and no-reset sessions, we only observed a significant main effect of reversal (reset: $F(1,87) = 22.3$, $p < 0.001$; no-reset: $F(1,83) = 11.03$, $p = 0.001$), both of which demonstrated that rats switched after significantly longer delays following the reversal. These rates of switching were not affected by prior cocaine exposure.

Conclusion: In sum, we found that rats performed accordingly to the different task parameters by showing overall greater preference for the PD trough and switching between the PD and FD troughs at earlier rates during reset sessions. Switching rates and overall PD trough delays generally had longer delays immediately after a reversal, for both cocaine-exposed and control rats, which rats typically adjusted over the 5 post-reversal days. Finally, we found that cocaine exposure reduced preference for the PD trough, but this effect was only seen post reversal and actual switching behavior was largely unaffected.

DISCUSSION

The negative effects of substance use on executive function, self-control, and impulsivity has been extensively demonstrated through impairments in reversal learning, delay discounting, and response inhibition tasks (Brockett et al., 2018). While beneficial, none of these tasks capture long-term consequences of addiction on optimal decision-making skills, such as the ability to select a less favorable option in the short-term for a greater reward in the long-term. To address this question, we studied behavior in the diminishing returns task in rats that had previously been exposed to cocaine.

In the diminishing returns task, the rats are presented with two choices. One choice has a fixed delay to reward delivery (FD), while the other choice first delivers reward immediately and progressively increases the delay each time that choice is selected (PD). The FD option is initially less rewarding, but choosing this option once during the reset condition will allow the increasing cost associated with the progressive option to be reset back to the initial cost. In the current study, we had hypothesized that rats with prior cocaine experience would show impaired optimal behavior, as shown by earlier resetting rates compared to control rats. We replicated previous findings that rats are capable of adapting their behavior between reset and no-reset variations of the task by switching between FD and PD options more frequently and at faster rates during reset sessions compared to no-reset sessions. However, and to our surprise, we found that prior cocaine exposure had minimal effects on behavior; our analyses showed that cocaine exposure actually reduced overall preference for the PD trough, suggesting these animals had higher sensitivity

to the increasing delays compared to control rats. This increased sensitivity to delays in reward was only found during post-reversal sessions.

A potential cause for the lack of cocaine's behavioral effects during pre-reversal may come from rats being highly trained on the task before self-administration. It is possible that the rats were trained to a point where the task had become habitual, despite its complexity, so that task performance before reversal remained unaffected by cocaine. However, once contingencies reversed and rats had to re-engage with the task, the effects of prior cocaine exposure became evident. These reversal-learning deficits are in fact similar to results we have observed using another reward-guided decision-making task (Brockett et al., 2018; Burton et al., 2018, 2017; Stalnaker et al., 2006; Y. Takahashi et al., 2007; Vázquez et al., 2019), and support literature demonstrating the impact of drugs of abuse on flexible decision-making (Lucantonio, Stalnaker, Shaham, Niv, & Schoenbaum, 2012; Mendez et al., 2010) in spite of our initial hypotheses being unsupported.

Further understanding of these behavioral findings may also come from studying neural signals related to diminishing returns behavior. We have shown across several studies that previous cocaine exposure produces changes in neural signals, which can be correlated to performance in a task where rats must choose between differently sized and delayed rewards. For example, Vázquez et al. (2019) found that cocaine exposure leads to attenuated neural signals related to attentional control in the anterior cingulate cortex. Cocaine exposure also disrupts insula's cue-encoding and contextual signals related to task accuracy and deliberation (Pribut, Vázquez, Brockett, et al., 2021). Finally, cocaine reduces the degree and flexibility of

neural firing rates in the ventral striatum while over-representing associations and actions related to high-value reward in dorsolateral striatum. These alterations in neural firing are related to inflexible behavior that is skewed towards high-value reward, regardless of its availability (Burton et al., 2018, 2017). While we do not know how neural signals in these brain regions correlate to performance on our diminishing returns task, it would be worth exploring to better understand the neural mechanism behind the behavioral patterns seen in cocaine-exposed rats of the present study, as lack of behavioral change is not definitive proof of lack of neural change.

Finally, it may be worth examining how previous drug experience alters behavior in other tasks based on principles of diminishing returns. Studies utilizing progressive ratios have investigated how increased response effort decreases the value of the reward, instead of altering the time of reward delivery (Wanchisen, Tatham, & Hineline, 1988). Others have examined foraging behavior, and are studying neural correlates within these tasks related to task engagement and temporal delay's effect on reward value (Kane et al., 2019, 2017). Both tasks could be utilized to examine whether the correlates we have observed are also present in these other forms of decision-making. Taken together, the present study adds to the growing body of literature by demonstrating cocaine-induced deficits in reversal-learning can be found in other behaviors. Moreover, our work highlights the need for complex behavioral assays for the study of both normal and impaired decision-making behavior.

CHAPTER 5: GENERAL DISCUSSION

Effective decision-making is a crucial part of everyday life, but is also relevant for the study of drug addiction. Addiction neuroscience has made great strides in understanding how motivational and reward-learning systems are dysregulated to drive the cycle of drug craving, consumption, and withdrawal. However, the extensive circuitry involved in decision-making is also disrupted by this condition. As a result, individuals suffering from addiction have long-lasting impairments in cognitive control even if they have abstained from drug use for long periods of time.

Previous work has found that, in normal decision-making, OFC and ABL form expectations that are constantly compared to and updated by prediction errors generated in the NAc and VTA (D. W. Bryden & Roesch, 2015; Daniel W. Bryden, Johnson, Tobia, et al., 2011; Burton et al., 2018; Burton, Kashtelyan, et al., 2014; Kashtelyan et al., 2012; Keiflin & Janak, 2015; Morrison et al., 2011; Geoffrey Schoenbaum et al., 2011; Y. K. Takahashi et al., 2017, 2009; Wilson et al., 2014). Attention is redirected as needed by ACC (Daniel W. Bryden, Johnson, Tobia, et al., 2011; Hayden et al., 2011), and the automaticity of such behavior is modulated by the DLS and DMS (Corbit & Janak, 2010; Gremel & Costa, 2013; McNamee et al., 2015; K. S. Smith & Graybiel, 2013; Stalnaker et al., 2012). After exposure to drugs of abuse, value representations in NAc are disrupted to prevent effective outcome prediction (Burton et al., 2018), downstream action-outcome policies in DLS become biased towards high-valued options (Burton et al., 2017), and attentional functions of ACC are reduced to prevent behavior from being updated (Vázquez et al., 2019).

These changes in circuitry are thought to be related to higher rates of impulsive, inflexible behavior that is skewed towards immediate benefit- behaviors which have been extensively measured across species in various behavioral assays of impulsivity and cognitive control. This collection of work has greatly contributed to understanding the insidious nature of addiction and how it can continue to have such a strong hold on behavior even when drug use has stopped.

My dissertation sought to expand on this work in two informative ways: first, by elucidating mechanisms that interact with known parts of circuitry; and second, by uncovering new regions that contribute to value-assessment and reward-guided decision-making. My work additionally examined these issues in the context of addiction, finding potential mechanistic commonalities between drug-induced impairments of cognitive control and uncovering new neural correlates that are disrupted by previous drug abuse.

Summary of Results

The first objective of my research sought to explore how epigenetics influence reward-guided decision-making circuitry and behavior. HDACs, epigenetic enzymes that suppress gene transcription, have been well-studied for their role in learning and memory (McQuown et al., 2011; Vogel-Ciernia & Wood, 2012), habit formation (Malvaez et al., 2018), and psychiatric conditions like addiction (Botia, Legastelois, Alaux-Cantin, & Naassila, 2012; Chen et al., 2016; Renthal et al., 2007). In particular, rodent studies of HDAC5 have found activity of this enzyme within dorsal striatum plays an important role in both the hedonic experience of drugs, and in models of drug relapse (X. Li et al., 2018; X. Li, Rubio, et al., 2015). Given the dorsal

striatum's role in governing goal and habit-directed learning (Gremel & Costa, 2013), it was possible HDAC5 would also play a role in DLS encoding of action-outcome policies necessary for complex decision-making, as had previously been observed in the Roesch lab (Burton et al., 2017). Thus, my first project examined whether dorsal striatal HDAC5 could play a role in reward-guided decision-making and previously observed firing patterns within DLS (Chapter 2).

To explore this question, I used viral techniques to overexpress HDAC5 in dorsal striatal neurons, and measured single-unit activity within DLS. This combination of techniques allowed us to explore how molecular mechanisms influence the activity of single neurons and their related behaviors. At the behavioral level, we observed rats with HDAC5 overexpression exhibited significantly faster reaction times (i.e. movement from the odor port to the fluid well; essentially measuring decision-making speed) compared to controls. We additionally found that rats with HDAC5 overexpression showed strong preference for high-value rewards, even very early in a block of trials. However, rats with HDAC5 overexpression showed a decrease in their selection of high-value rewards in the second block of trials, suggesting these rats were unable to adapt their responding when a block ended and contingencies switched. These results suggest that HDAC5 overexpression resulted in faster, inflexible behavior that was based on strong preferences for high-value reward in the first block, but could not be adapted for changing contingencies in the second block.

In Chapter 2 I additionally demonstrated that HDAC5 overexpression impaired DLS encoding of cues and reward, likely contributing to the inflexible

behavior observed in the task. In control rats, we found neurons that showed responsiveness to odor cues and to reward. After HDAC5 overexpression, there were significantly larger populations of neurons that showed increases in activity for odor cues. Within this population, we also observed stronger representations of odors that predicted high-value reward- especially rewards delivered after a short delay. These neurons also demonstrated strong signals related to movement towards these rewards. We additionally observed fewer reward-responsive DLS neurons from our HDAC5 rats compared to controls. Taken together, HDAC5 overexpression resulted in faster, inflexible behavior that could not be adapted when reward contingencies changed. This behavior was related to greater encoding of cues that predicted immediate reward, and reduced encoding of anticipation and reward delivery.

These results suggest that epigenetics can play an important role in decision-making and its neural correlates. Intriguingly, the behavioral and neural changes observed in this study also paralleled previous findings on long-term impairments of decision-making observed after prior cocaine exposure. In Burton et al. (2017), we found action-outcome encoding within DLS, represented by signals tying odor cues to associated outcomes and reflecting the outcome the rat chose from trial to trial. After cocaine exposure, DLS activity reflected reward preference regardless of what reward was available and what was signaled by the presented odor cue. These signals would be present even before odor presentation, and were suggested to reflect the strong behavioral biases cocaine-exposed rats exhibited for high-value reward. HDAC5 may play a role in these drug-induced aberrations of action-outcome encoding by enhancing DLS's representation of cues predicting high-value reward, while

dampening signals encoding reward receipt and in turn reducing this region's ability to update such associations when contingencies change.

Our findings in this study and the Roesch lab's previous research are additionally compelling when viewed in the context of the Li lab's HDAC5 work. Previous studies suggest a bidirectional relationship between HDAC5 in DS and the expression of methamphetamine relapse, finding higher concentrations of HDAC5 during relapse tests and additionally that overexpressing HDAC5 in DS leads to greater rates of methamphetamine-seeking after a period of forced abstinence (X. Li et al., 2018; X. Li, Rubio, et al., 2015).

Taken together, the findings from this project illustrate important molecular mechanisms involved in previously observed alterations of decision-making behavior and associated neural activity. This work additionally suggests that HDAC5's potential as a common mechanism behind both drug relapse, and drug-induced impairments of cognitive control.

The second objective of my research was to uncover novel regions involved in decision-making circuitry. Striatal regions have been well-studied for their role in associative learning and in the formation of goals and habits. However, there are many cortical regions that have not been fully explored, despite what is known of these areas' involvement in higher cognitive functioning. One region that quickly gained interest in this respect was the insula, which is considered the seat of consciousness and is a complex site where the integration of vast neural signaling occurs (Gogolla, 2017; Livneh & Andermann, 2021). Moreover, in spite of growing evidence that this region is involved in the experience of addiction (Naqvi et al.,

2014, 2007; Sahani, Hurd, & Bachi, 2022), there have been few studies to date recording from insula to determine how signaling within this area of the brain is altered by prior exposure to drugs of abuse. In Chapter 3, I was therefore interested in recording from insula in drug-naïve and cocaine-experienced rats, and studying the functional relevance of insula using lesioning techniques. This collection of work gave me the opportunity to expand on decision-making circuitry while also directly studying the long-term influence of drug exposure on decision-making behavior and its neural correlates.

At the behavioral level, I found that all rats were able to differentiate between high and low-value rewards across both delay and size manipulations. However, similar to previous studies (Brockett et al., 2018; Burton et al., 2018, 2017; Vázquez et al., 2019), rats with prior cocaine exposure demonstrated a bias for high-value rewards and exhibited overall faster reaction times compared to control rats.

These behavioral changes occurred alongside changes in insula activity. Within control rats, insula neurons showed activity encoding odor cues, and their associations with specific reward outcomes. Insula additionally had neurons that responded to reward, and either maintained activity over the course of a delay or failed to represent reward across a delay period. Finally, I observed a novel "context" signal within the insula that seemed to encode the blocked structure of the task; insula exhibited stronger firing during size manipulations compared to delay, regardless of whether the trial resulted in the delivery of high or low-value reward. Such signals seemed to be involved in task deliberation. After exposure to cocaine, neural firing within the insula was profoundly changed so that cue and context signaling was

attenuated. There were additionally alterations in reward-cell populations so that "expectancy" signals (i.e. firing that was not maintained during a delay period, but represented reward when it was expected to be delivered after short delays) were more strongly represented.

From these findings we hypothesized that insula may reinforce many functions within the circuit by encoding cue expectancy signals and representing reward during long delays. We also suggested the context signals within insula modulate rats' deliberation and accuracy as they progress through the task. Importantly, size blocks allow for overall greater amounts of reward due to the larger boli delivered and the lack of delay to reward, but they are presented towards the end of the task when animals are beginning to experience satiety and fatigue. The context signal may therefore help rats persist through the task by weighing the overall greater value of size blocks against decreasing motivation.

To test this hypothesis, I lesioned the recording site in a new cohort of rats, and measured behavior in the decision-making task. I found lesioned rats were less capable of following odor cues during forced-choice trials, as seen by reductions in accuracy, although preference for high-value rewards was not altered. Rats with insula lesions additionally exhibited faster reaction times, similar to rats that self-administered cocaine in the recording study. Finally, I observed an interesting change in light-on latency, or the time it takes to start a trial after houselight illumination, between delay and size blocks. This measurement has been used in previous studies to examine attention (Vázquez et al., 2019), and typically rats will exhibit faster light-on latencies during size blocks in spite of growing fatigue and satiety. While this

finding was replicated in control rats, lesioned rats showed no difference in attention between delay and size blocks.

Remaining Questions

Taken together, prior exposure to drugs of abuse produces long-lasting changes in cognitive control that have been previously characterized in our lab by faster decision-making and behavior that is skewed towards high-value reward. These behavioral changes are accompanied by widespread impairments of neural activity. The projects within this dissertation suggest the insula is yet another region within this disrupted network, specifically via its inability to modulate relationships between cues and their appropriate responses, and the disruption of global value signals needed to weigh motivation and attention against internal state changes. Moreover, epigenetic factors like HDAC5 may play a role in how such neural changes occur. Here, we demonstrated HDAC5's role in disrupting activity within DLS, but its influence likely spreads to many other regions within the decision-making circuit. This and other epigenetic mechanisms may additionally provide a physiological link between addiction's acute effects of craving, and its more insidious impairments in executive functioning. In light of these results, there are several questions to consider for future study.

HDAC5's role within DS

One key limitation of my first project was our overexpression of HDAC5 in the entire DS. As a result, it is unclear whether these behavioral findings were driven by the neural changes observed in DLS alone, or whether there were also changes in

DMS involved. In the Li lab's previous study, these same methods were required to demonstrate HDAC5's role in methamphetamine relapse; overexpression in either DMS or DLS alone were not sufficient to produce behavioral change (X. Li et al., 2018). The exact causes of these results, both here and in the Li lab's previous work, may involve distinct mechanisms of HDAC5 action within different regions of DS and the type of HDAC used in this study.

Within DS, the medial and lateral regions have long been respectively parceled into modulating associative goal-directed learning and sensorimotor habitual responding (Barnes et al., 2005; Jog et al., 1999; Regier, Amemiya, & Redish, 2015; K. S. Smith & Graybiel, 2013; Thorn et al., 2010). Such relationships have been replicated in rodents (Corbit & Janak, 2010; Yin et al., 2004; Yin, Knowlton, et al., 2005; Yin, Ostlund, et al., 2005), primates (Miyachi et al., 2002, 1997), and humans (Liljeholm et al., 2011; McNamee et al., 2015; Tricomi et al., 2009). Based on these findings, it therefore seems counterintuitive that the same manipulation across both DMS and DLS would produce a similar result instead of effectively cancelling each other's functional changes. However, DMS and DLS have distinct cellular compositions with differing functions, which in turn may produce distinct results of HDAC5 manipulation even when applied across the entire DS. In both DMS and DLS, medium spiny neurons (MSNs) project to the basal ganglia to transmit information about goals and habits. Striatonigral (dMSNs) D1R-neurons directly project to the basal ganglia, while striatopallidal (iMSNs) D2R-neurons indirectly modulate basal ganglia activity by inhibiting its upstream regions (Peak, Chieng, Hart, & Balleine, 2020). Research manipulating these projections have found distinct

functions of each cell type depending on whether they originate from DMS or DLS. For example, goal-directed learning has been linked to greater dMSN and reduced iMSN activity within DMS (Shan, Ge, Christie, & Balleine, 2014), while in DLS the execution of habit seems to be rely on finely coordinated activity between the two-dMSNs may promote the continued performance of previously learned behavior to promote habit, while iMSNs inhibit competing behavior (Cui et al., 2013; Tecuapetla, Jin, Lima, & Costa, 2016). Given this information, it is possible that the results from the present study are due to differential effects of HDAC5 on these distinct cell lines within DS.

Further compounding these ambiguities is the fact that HDAC5 is just one of many HDACs that can all influence behavior and neural activity by acting on different molecular targets and promoter regions within a cell's DNA (Malvaez et al., 2018). The Wassum group has a collection of work studying the role of HDAC3, a prominent class I HDAC that is concentrated in the nucleus of a cell (Broide et al., 2007; McQuown et al., 2011). In many respects their findings are contradictory to ours, with HDAC3 inhibition accelerating habit formation and its action being significant in successful goal-directed learning and behavior regardless of whether such manipulations take place in DLS or DMS (Malvaez et al., 2018). While we manipulated HDAC5 in the current study, it is unclear how other HDACs may have been working within DS, or if our manipulations produced downstream effects that interacted with other HDAC activity.

What is insula's role in the broader decision-making circuit?

An additional question for future study involves exploring how exactly insula fits into the broader decision-making circuitry. In the present work I studied a small facet of a large, highly intra and inter-connected brain region with numerous functions. While there are abundant ways insula can be explored in further studies, there are a few key questions that arise.

As previously mentioned, the insula can be roughly split into three anatomical categories of anterior, mid, and posterior insula. In the present work, I explored anterior insula due to its high connectivity to frontal and midbrain regions- findings which have been replicated across humans (Menon & Uddin, 2010; Nomi, Schettini, Broce, Dick, & Uddin, 2018; Wiech et al., 2010) and rodents (Gehrlach et al., 2019, 2020; Ongur & Price, 2000; Tsai et al., 2020). Such connectivity has implicated this part of insula in higher cognitive functions, and these hypotheses have been largely supported by experimental work in rodents (Di Pietro, Black, Green-Jordan, Eichenbaum, & Kantak, 2004; Kesner & Gilbert, 2007; Nasser et al., 2018; Nerad, Ramírez-Amaya, Ormsby, & Bermúdez-Rattoni, 1996; Ragozzino & Kesner, 1998).

The present findings further support this body of work, but they also highlight the need for additional study of anterior insula's connections to other frontal areas of the brain. For example, we observed signals within anterior insula that suggest some basic functions in expectancy signaling- signals that have also been observed in our previous studies of OFC (D. W. Bryden & Roesch, 2015; Burton, Kashtelyan, et al., 2014). Insula has significant bidirectional connectivity with OFC, made possible by how physically close these structures are to each other (Gogolla, 2017; Naqvi et al.,

2014). In fact, a number of studies have found lesions of anterior insula typically can result in damage to OFC due to its close proximity (Chang, 2014; Gallagher et al., 1999; McDannald et al., 2005; S. B. Ostlund & Balleine, 2007; Pickens et al., 2003; Saddoris et al., 2005; Stalnaker et al., 2007). Given this connectivity it is unsurprising that there is overlap in signals, and it may be possible that insula informs outcome expectancy in OFC.

Anterior insula is also well-connected to ACC (Gasquoine, 2014; Grodin, Cortes, Spagnolo, & Momenan, 2017; Menon & Uddin, 2010). These regions have long been thought of as important parts of a "salience network" that helps an organism detect and respond to sudden changes in the environment. Several hypotheses are used to describe this relationship, with insula and ACC integrating salient sensory information with emotional and bodily states (Seeley et al., 2007), and insula generating interoceptive prediction errors that aid ACC in redirecting attention when necessary (Droutman, Bechara, et al., 2015; Paulus, Rogalsky, Simmons, Feinstein, & Stein, 2003). A recent study in rats also found LFP activity between ACC and insula that seemed to track the amount of effort needed for a physical task, and changes in this activity were correlated to when the rat decided to quit the task (Porter et al., 2020). Examining the findings of the present study, it is possible that similar activity may be observed in cognitive tasks. If insula is additionally maintaining some sort of greater task structure, it may also be possible that some coordinating activity with ACC is needed to signal when such changes occur.

Additionally, anterior insula is also vastly connected to its middle and posterior regions. Connectivity studies suggest information travels in a caudal-to-

rostral direction, but also highlights distinct functions and activity patterns in each region (Centanni et al., 2021). For example, studies mapping the connectivity of rat insula have found opposing trends in activity between anterior and posterior regions, which may suggest coordination between them (Castro & Berridge, 2017; Méndez-Ruette et al., 2019), while the few studies specifically examining the middle region suggest this part of insula may operate somewhat independently of the anterior and posterior areas (Centanni et al., 2018; Luchsinger et al., 2021). Recordings from different subregions of insula will be necessary to further elucidate how signals are both generated and coordinated with insula to contribute to complex behavior.

Diminishing returns as a model of optimal decision-making

In my two main projects, I utilized a well-established task that examines basic elemental cogs of learning, including value assessment, anticipation, and prediction errors. But from these findings alone, it is unclear how the disrupted signals in this task can then translate to disruptions in optimal decision-making behavior. Specifically, while the reward-guided decision-making task allows for in-depth study of value assessment and its neural encoding, it does not allow for the study of impulsive choice because there is no greater benefit to waiting for a delayed reward. Without a stable behavioral model of optimal decision-making, it is also impossible to study the neural correlates of this behavior and how this behavior is disrupted in psychiatric conditions.

The final project of my dissertation was meant to begin addressing this question by studying behavior in a diminishing returns paradigm. The Roesch lab has previously established that rats - along with pigeons (Wanchisen et al., 1988),

primates (Hodos & Trumbule, 1967), and humans (Hackenberg & Axtell, 1993) – are capable of forgoing immediate benefit for long-term gain by selecting a delayed reward in order to receive reward faster in subsequent trials (Schuweiler et al., 2021). In the present study, I asked whether rats that had previously self-administered cocaine were less capable of performing this behavior. Specifically, I hypothesized these rats would exhibit higher delays on the PD trough compared to control rats, reflecting their inability to select the FD trough in order to reset the PD trough's delay. Surprisingly, I found that prior cocaine exposure did not affect resetting rates, and cocaine-exposed rats overall selected the PD trough less than control rats only after a reversal test.

The results from this work do not necessarily refute what we have previously studied- in fact, the deficits we observe post-reversal mirror our results with the reward-guided decision-making task found in previous studies conducted by the Roesch lab (Brockett et al., 2018; Burton et al., 2018, 2017; Stalnaker et al., 2006; Y. Takahashi et al., 2007; Vázquez et al., 2019). To this point, much like the reward-guided decision-making task, the rats were well-trained in the Diminishing Returns task prior to cocaine exposure. It is therefore possible that the extensive training they received prevented cocaine's effects on decision-making behavior until the reversal, when rats needed to engage attentional mechanisms and adjust responding to new task parameters.

Furthermore, even if behavior is not remarkably changed, it is still possible that neural activity is altered. Rats previously exposed to cocaine may need to expend greater amounts of effort to show the same behavior as control subjects, and such

increases in effort may be reflected in neural recordings. Such a finding may provide clarity to the reversal effect seen in the present study: perhaps neural activity reflected greater effort to work against poorer decision-making skills, and such alterations in neural firing were enough to rescue behavior until task demands were raised by the reversal.

Finally, it is important to consider other tasks that also examine optimal decision-making. Here, we utilized a task that pitted differing temporal schedules against each other. A task that relies on progressive ratios may yield different results and would perhaps capture elements of foraging more accurately than a delay-based task, as animals need to expend energy and effort in the wild to retrieve food and water as opposed to waiting for a set amount of time. Studies of patch foraging tasks in both humans (Constantino & Daw, 2015) and rodents (Kane et al., 2019, 2017) have also begun uncovering the computational and neural processes behind this task, finding brain regions related to task disengagement (Kane et al., 2017) and cross-species methods that relate to principles of temporal discounting (Constantino & Daw, 2015; Kane et al., 2019). Utilizing other tasks based on diminishing returns may therefore reflect principles of optimal decision-making in ways not observed in the present study while also tying more complex behaviors to foundational elements of cognitive control.

Future Directions

Revisiting the broader decision-making circuit (*Figure 6*), OFC forms outcome predictions that are either met or violated when an animal performs an

action. The results and comparison to OFC's predictions are compared in ABL, and conflict is resolved within ACC while motivation is adjusted by NAc. Together, this information adjusts prediction error signaling within VTA and action-outcome policies are adjusted accordingly in the DMS and DLS. After cocaine exposure, disrupted value signaling within NAc alters prediction error formation and drives DS activity to promote behavioral biases, and such biases are not as easily corrected due to reduced functionality of ACC.

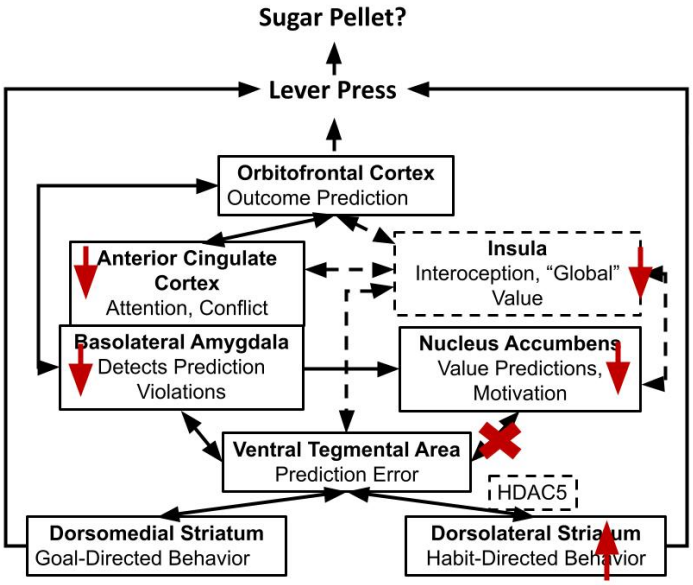

Figure 6: Revised circuit incorporating epigenetic and anatomical studies within this dissertation. Dashed lines indicate areas for future study.

From the work presented in this dissertation, HDAC5 within DS likely contributes to the faster reaction times of such automatic behavior. Enhanced HDAC5 expression after drugs of abuse increases activity for cues that predict immediate reward and movement towards such reward, while diminishing reward signaling itself (Pribut, Vázquez, Wei, et al., 2021). These changes in decision-making speed are

likely further reinforced by altered signaling within the insula. Immediate rewards are more highly represented in this region through greater expectancy signaling and reduced signaling of global value and task structure (Pribut, Vázquez, Brockett, et al., 2021). The reduction of these signals likely reduces the ability to utilize stimulus-response associations to guide behavior, resulting in faster responding while keeping preferences for high-value reward – especially preference for immediate reward – intact (Pribut et al., 2022).

The projects within this dissertation greatly contribute to our understanding of the neural mechanisms behind decision-making, and how lasting changes can be brought out in both the brain and behavior after exposure to drugs of abuse. However, these projects just scratch the surface of several intriguing lines of future study. First, HDAC5's role within DS could be further elucidated by discovering how this enzyme is influencing activity within DMS. Performing this same experiment while recording from DMS could determine whether HDAC5 exerts differential effects on subregions of DS. From there, additional studies could determine if HDAC5 acts on specific cell types within DS, which may explain why this enzyme needs to be manipulated throughout DS to exert effects on behavior (X. Li et al., 2018). Future studies could also more directly probe HDAC5's common role in relapse and drug-induced impairment of decision-making by combining incubation of craving procedures with our reward-guided decision-making task. Based off of the Li lab's previous work, it would be intriguing to discover if there was any relationship among HDAC5 concentrations, relapse behavior, and the severity of drug-induced impairment to decision-making. This relationship could be further explored by determining if

HDAC5 knockdown could prevent relapse behavior and additionally rescue decision-making behavior.

Further study of the insula provides equally vast opportunities for additional lines of work. Recordings from other regions of insula may provide insight into their distinct functions and the ways in which information is integrated within insula as a whole. For example, the more cognitive functions of anterior insula may rely on more basic interoceptive processing from posterior regions of insula. Recording studies may find prediction error-type signaling that contributes to insula's roles in anticipation and expectation. Connectivity studies also provide considerable avenues for additional work. Insula is highly interconnected with OFC, and may contribute to the formation and alteration of outcome predictions. Such findings would also support the commonalities we found in signaling within insula and OFC (D. W. Bryden & Roesch, 2015; Burton, Kashtelyan, et al., 2014; Pribut, Vázquez, Brockett, et al., 2021). While beyond the scope of the current project, insula is also vastly connected to ACC as a part of a "salience network", with its interoceptive detections being used by ACC to respond to conflict (Koob & Volkow, 2016; Wiech et al., 2010). Insula's anticipatory signaling and its encoding of global value may help ACC make adjustments in attention on both a trial-by-trial and long-term basis.

Taken together, this dissertation expands on our understanding of decision-making circuitry by introducing how molecular mechanisms contribute to cellular change. Moreover, it expands on how cortical regions encode value on multiple scales, and how the disruption of such activity contributes to long-lasting impairments of decision-making behavior observed after exposure to drugs of abuse. Continued

study in both of these topics in the context of complex behavioral assays will be key to having a richer understanding of decision-making's neural processes, and how deficits in these processes can be addressed in those struggling with substance abuse.

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