ABSTRACT

Title of Dissertation:	MODULATION OF SIGNALING IN THE ANTERIOR CINGULATE CORTEX AND ITS IMPACT ON DECISION-MAKING
	Daniela Vázquez, Doctor of Philosophy, 2024
Dissertation directed by:	Dr. Matthew Roesch, Full Professor, Department of Psychology, Neuroscience and Cognitive Science Program

Attentional deficits are defining hallmarks of some of the most prevalent and disruptive neuropsychiatric disorders—including attention deficit hyperactivity disorder (ADHD) and substance abuse disorders. The anterior cingulate cortex (ACC) is a brain region that is highly implicated in shifting attention allocation towards relevant stimuli after unexpected events or outcomes occur. Importantly, increases in attention facilitate flexible learning, as attention allows you to dynamically filter relevant and necessary information during decision-making. My dissertation work seeks to identify the ACC as a novel point of intervention for the treatment of neuropsychiatric and addiction disorders by providing an in-depth perspective on its involvement in cognitive control and attentional processes.

My research explores the neural correlates of decision-making by using electrophysiology to record single unit activity while rats perform a complex reward-based decision-making task, and employing chemical, optogenetic, and epigenetic manipulations to modulate attentional correlates in the ACC. I explored the ACC's role in attention—and how it is impacted by drug use—using electrophysiology to record from ACC neurons as both cocaine-

exposed and drug-naïve rats performed a reward-guided decision-making task. Using this task, we found a dose-dependent attenuation of ACC signaling after cocaine self-administration, which was correlated with decreases in task performance and attention to the task. Rats that had self-administered large amounts of cocaine had diminished neural responsiveness to cues, which translated into reductions in behavioral measures of attention, disruptions in cognitive flexibility, and decision-making impairments. These results both supported previous findings establishing the ACC's role in attentional allocation, and revealed an intake-dependent effect of drugs on decision-making and neural encoding.

In aim 2, we wanted to be able to precisely modulate ACC activity in order to better interrogate the role of the ACC in the absence of confounding variables (e.g. cocaine use results in the dysregulation of various neural circuits), and conduct within-subject analyses. Thus, in our next experiment we used optogenetics to inactivate the ACC, and found that ACC inhibition severely impaired task engagement, as evinced by reductions in trial initiations, and trial and session completions—resulting in overall impaired session performance. In order to disambiguate whether these behavioral deficits resulted from ACC impairment dysregulating downstream action-outcome encoding, we performed chemical lesions of the ACC, and recorded neural activity from the dorsomedial striatum (DMS)—a downstream brain region that is importantly involved in goal-directed behavior—as rats performed the previously mentioned decision-making task. Again, we found that ACC lesions resulted in disrupted attention to the task, and similar behavioral deficits to the ones we observed following cocaine use. Interestingly, we found that DMS encoding was minimally impacted, reinforcing that the observed decision-making deficits stem from disruptions in attentional signaling and not dysregulations in downstream action-outcome encoding. In the aforementioned experiments, we employed an

array of techniques to dissect how disrupting ACC signaling in a variety of manners impacted task performance and engagement, so for our final experiment we sought to explore a therapeutically relevant way to potentially repair signaling disruptions that lead to the breakdown in attentional signaling. Thus, we turned to epigenetics—specifically, decreasing the expression of HDAC5, an enzyme that is involved in negatively regulating gene expression—to explore whether epigenetic changes might map onto specific alterations of neural activity and behavior. Surprisingly, we found that HDAC5 knockdown in the ACC dysregulates attentional signals that are necessary for flexible and adaptive decision-making. Together, these studies established that signaling in the functional ACC is importantly involved in attention, and that dampening these signals leads to decision-making impairments and decreased task engagement, notably characterized by significant reductions in the proportion of initiated and completed trials, and prolonged periods of inattention.

MODULATION OF ANTERIOR CINGULATE CORTEX SIGNALING AND ITS IMPACT ON DECISION-MAKING

Daniela Vázquez

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2024

Advisory Committee:
Professor Matthew R. Roesch, Chair
Professor Donald J. Bolger, Dean's Representative
Professor Xuan Li
Professor Edward M. Bernat
Professor Scott A. Juntti

Dedication

Le dedico esta disertación a mis padres—Amalia y Francisco—y a mi hermana, Alejandra—gracias por siempre creer en mí, y por apoyarme incondicionalmente en todos los sentidos de la palabra. Los amo; no soy nadie sin ustedes.

To my husband, Tyler Brown—thank you for your endless support and patience, the long coffee walks, for always re-filling my tea, for staying up and keeping me company during (most of) my late nights, for uplifting me always; I love you.

Acknowledgments

My eternal gratitude goes to Dr. Matthew Roesch—I admire you greatly, both as a scientist and as a person. You have truly been the best mentor, advocate, guide, and inspiration. Being in your lab has been the greatest honor. I will never be able to thank you enough for how much you have taught me and helped me grow.

I'm also so grateful for all of the members of the Roesch lab that I've had the pleasure of working with throughout the years—especially to Dr. Adam Brockett, Dr. Amanda Burton, Dr. Heather Pribut, Dr. Kevin Schneider, Xavier Sciarillo, and Stephen Tennyson.

Table of Contents

List of Figures

Chapter I: Introduction

Attentional impairment is a characteristic hallmark of a plethora of prevalent neuropsychiatric conditions, most notably attention deficit hyperactivity disorder (ADHD) and substance use disorders. Within the intricate neural landscape of the brain areas involved in decision-making, the anterior cingulate cortex (ACC) emerges as a pivotal region implicated in redirecting attention towards salient stimuli following unexpected events or outcomes in the service of facilitating adaptive, flexible decision-making.

The focal point of my dissertation work revolves around elucidating the role of the ACC as a potential target for intervention in the management of neuropsychiatric and addiction disorders. By offering a comprehensive examination of its involvement in cognitive control and attentional mechanisms, this research endeavors to generate a multifaceted neurophysiological and behavioral account of ACC function that provides valuable insights into novel avenues for therapeutic interventions aimed at ameliorating the attentional deficits that are associated with aberrant decision-making.

Overview of the neurocircuitry implicated in reward-guided decision-making

Decision-making is the process whereby an organism engages in goal-directed behaviors which are mediated by the formation of action-outcome associations. Optimal decision-making requires behavioral flexibility, outcome prediction, value processing, motivation, and attention. It involves an expansive network of brain regions that are responsible for learning about environmental cues, encoding expected outcomes and their associations, being motivated by valued outcomes, and updating actions to optimize behavior to obtain the best outcomes. Broadly, some of the more prominent

parts of this network (*Figure 1*) include the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), basolateral amygdala (BLA), ventral tegmental area (VTA), and the striatum. The OFC and BLA learn about valued rewards via midbrain dopaminergic (DA) modulation (phasic dopamine release from the VTA) and encode predicted outcomes, which are then transmitted to the ventral striatum (VS)—of which the nucleus accumbens (NAc) is a major component (Roesch, Calu, & Schoenbaum, 2007; Wang, 2008; Rudebeck & Murray, 2014). The NAc biases behavior towards cues predicting these outcomes, which are subsequently communicated to the dorsal regions of the striatum that are responsible for the action-selection of motor output (Wang, 2008; Floresco, 2015; Burke, Rostein, & Alvarez, 2017). Depending on the outcomes of the executed actions, the ACC plays a critical role in behavioral flexibility, monitoring changes that occur in outcome expectancies in the service of behavioral adaptation (Oliviera, McDonald, & Goodman, 2007; Sallet et al., 2007; Bryden et al., 2011). Altogether, this circuit is imperative for learning and decision-making. Because decision-making impairments are hallmarks of neuropsychiatric and substance abuse disorders, investigating the neural correlates of value-based decision-making can provide valuable insight on how to rescue these deficits.

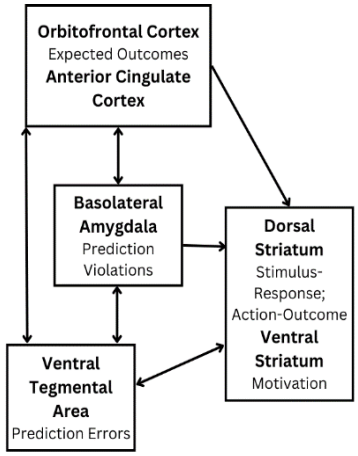

Figure 1: Circuit diagram of the key components of the decision-making network. Double-sided arrows represent a bidirectional flow of information. The ACC is highlighted in a different color, as it is the focal point of my dissertation.

Among the myriad of brain regions implicated in decision-making, the anterior cingulate cortex (ACC) emerges as a pivotal hub of cognitive control processes—including attention, feedback and error processing, and conflict monitoring (Laubach et al., 2015; Wu et al., 2017; Soltani & Izquierdo, 2019; Stolyarova et al., 2019; Schneider et al., 2020; Vázquez et al., 2020; Vázquez, Schneider, & Roesch, 2022). My graduate work has focused on the ACC, particularly honing in on its role in attentional processing during reward-guided decision-making. Studies have illustrated the key role of the ACC in shifting the allocation of attentional resources towards behaviorally relevant stimuli following outcome expectancy violations (Bryden et al., 2011; Wu et al., 2017; Schneider et al., 2020; Vázquez et al., 2020; Vázquez, Schneider, & Roesch, 2022), making this component of the corticostriatal circuit a good target for intervention following disruptions in reward-guided learning.

The role of the anterior cingulate cortex in attention

The ACC has been linked to numerous cognitive functions including conflict monitoring, arousal, surprise, feedback and error processing, perceptual decision-making, prediction errors, and attentional control (Rescorla & Wagner, 1973; D'Esposito et al., 1995; Carter et al., 1998; Botvinick, 1999; Dayan, Kakade, & Montague, 2000; Paus, 2001; Botvinick et al., 2004; Holroyd et al., 2004; Kerns et al., 2004; Weissman et al., 2004; Koob & Volkow, 2010; Bryden et al., 2011; Hayden et al., 2011; Narayanan et al., 2013; Laubach et al., 2015; Wu et al., 2017; Soltani & Izquierdo, 2019; Stolyarova et al., 2019; Schneider et al., 2020; Vázquez et al., 2020; Vázquez, Schneider, & Roesch, 2022). I have studied ACC function within the context of a reward-based behavioral paradigm (*Figure 2*—this paradigm is described, in detail, at the end of Chapter I, see Page 14—section titled "*Reward-guided decision-making task*") that allows us to

parse various critical components of decision-making—among them value encoding, reward prediction, and attentional control. This behavioral paradigm was used across all of the studies that comprise my dissertation work, and is described in further detail at the end of this chapter.

Using this task to study ACC function, we have previously found that ACC firing correlates with changes in attention proposed by the Pearce and Hall model of associative learning (Bryden et al., 2011; Roesch et al., 2012; Vázquez et al., 2020). In this model, the attention given to a cue is a product of the average unsigned prediction errors generated over trials. Unsigned prediction errors reflect the degree to which an outcome is unexpected, and result from the difference between the value of expected reward, versus the actual outcome (Pearce & Hall, 1980).

Following the model, in order for learning to occur, unsigned prediction errors should subsequently lead to increases in attention towards the cue (Pearce & Hall, 1980). Because attention is used to select, maintain, and update the relevant representations and contexts necessary for successful task completion, disruptions to attention have direct repercussions on goal-directed behaviors and flexible learning.

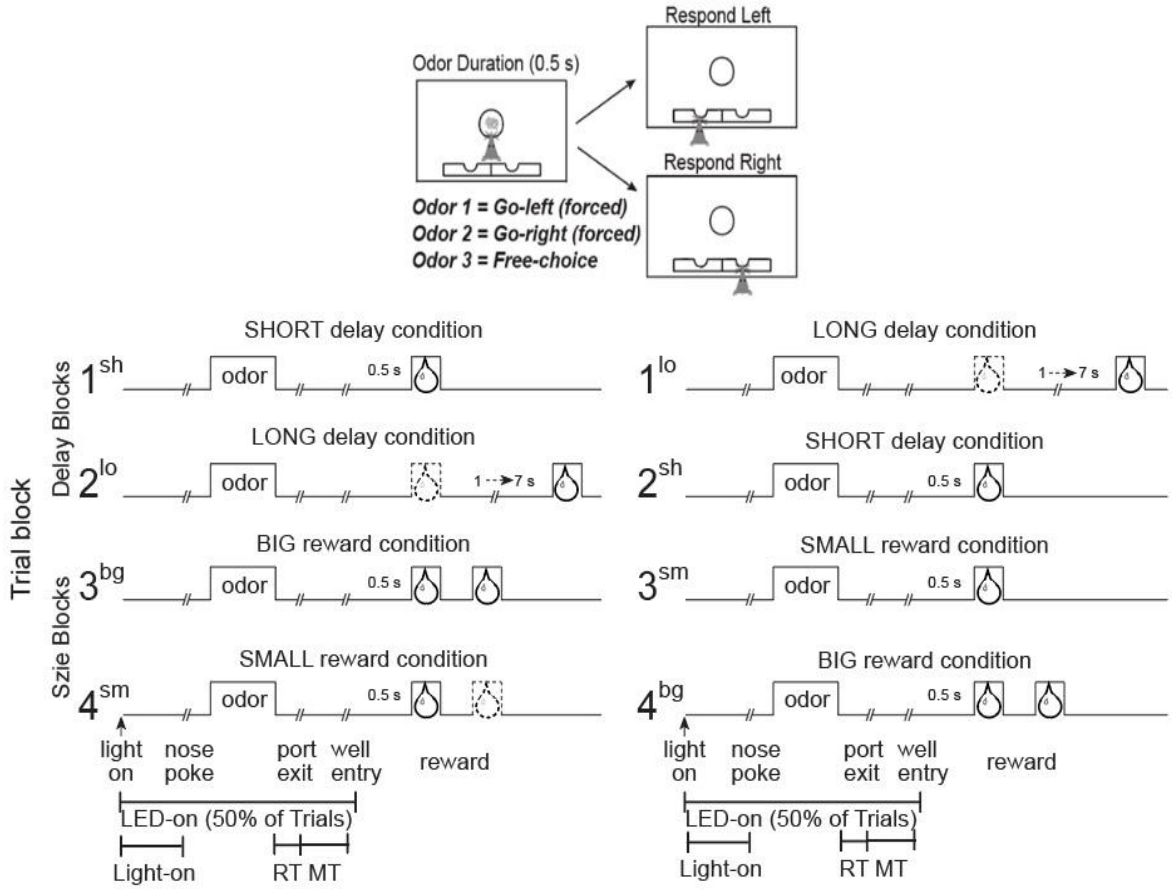

Figure 2: Task schematic—the experimental paradigm allows for independent manipulations of reward value through variations of delay (Blocks 1 and 2) and size (Blocks 3 and 4) across four sixty-trial blocks. On each trial, rats nose-poked into a central odor port to receive one of three odors, and then responded in the corresponding fluid well to receive reward. One odor signaled reward in the left well (forced-choice), another indicated reward in the right well (forced-choice), and a third odor signaled reward at either well (free choice). Forced-choice odors were counter-balanced. Lines at the bottom (e.g. light-on, RT, MT, LED-on) delineate important epochs that will be discussed and referenced throughout the text.

Of note, several human studies have also observed neural correlates of attention in the ACC. In fMRI studies manipulating the attentional load of participants, increasing attentional demands were found to augment ACC activation (Jovicich et al., 2001). In another study, individuals participated in an oddball task—a paradigm in which repetitive stimuli are presented, and are interrupted by an infrequent and

unexpected stimulus—while event-related potentials were recorded (Crottaz-Herbette & Menon, 2006). The oddball tasks used in this study involved both visual and auditory modalities; researchers found large and reliable N2 and P3 signals in the ACC after unexpected stimuli were presented (Crottaz-Herbette & Menon, 2006). N2 and P3 are well-studied event-related potentials thought to reflect focused attention (Patel & Azzam, 2005; Crottaz-Herbette & Menon, 2006; Krokhine et al., 2020). Using event-related fMRI, researchers also found that the ACC showed heightened activation during the detection of infrequent stimuli compared to frequent stimuli across both the auditory and visual oddball tasks (Crottaz-Herbette & Menon, 2006).

Our findings are also supported by human neuroimaging studies associating ADHD—the most prevalent neurodevelopmental disorder which is robustly characterized by inattentiveness (Castellanos et al., 2012; Minzenberg, 2012)—with maladaptive hypoactivation and volumetric reductions of the ACC (Bush et al., 1999; Overmeyer et al., 2001; Dickstein et al., 2006; Seidman et al., 2006; Amico et al., 2011; Bush, 2011; Banich et al., 2013; Bledsoe et al., 2013; Bayard et al., 2018; Hoogman et al., 2019; Vogt, 2019; Bayard et al., 2020; Yu et al., 2023).

In a separate study, researchers used positron emission tomography to measure resting glucose metabolism in the ACC and thus track brain activity as subjects with a previous history of drug use performed a series of neuropsychological tests that spanned several cognitive domains—and found that glucose metabolism in the ACC significantly predicted attentional performance (Goldstein et al., 2004).

Optogenetic manipulations of the ACC

While the findings in these human studies provide strong correlational evidence of an association between ACC activity and attentional engagement, methodologically they do not allow us to establish causality. Optogenetics is an experimental technique that allows researchers to activate or suppress the activity of neurons that have been genetically modified to express light-sensitive proteins known as opsins (Gradinaru, Thompson, & Deisseroth, 2004; Zhang et al., 2007; Chen et al., 2022; Emiliani et al., 2022). This method can be used to precisely modulate neural activity, allowing researchers to examine the effects of this manipulation in real-time, and interrogate the role of specific neuronal types at the cellular, circuit, and behavioral levels. Optogenetic approaches include using light-activated chloride channels, G protein-coupled receptors, and ion pumps (Zhang et al., 2007; Zhang et al., 2019; Chen et al., 2022). Specifically, in my dissertation work I used halorhodopsin (NpHR), a commonly used inhibitory light-activated chloride pump (see Chapter III for further details). NpHR pumps chloride ions into the cell in response to yellow light, thus reducing the cell's excitability and resulting in inactivation (Gradinaru, Thompson, & Deisseroth, 2004; Zhang et al., 2007; Chen et al., 2022; Emiliani et al., 2022).

Using optogenetics to dissect ACC function has been largely unexplored outside of the context of studying pain processing. Studies have demonstrated that rats with neuropathic pain exhibit hyperactive neuronal activity in the ACC (Elina et al., 2020). Additionally, research has shown that optical inhibition of the ACC can alleviate neuropathic pain in these rats (Chen et al. 2018; Moon et al. 2017; Elina et al., 2020; Moon & Park, 2022), and that inactivation of excitatory neurons in the ACC can

increase both mechanical and thermal pain thresholds (Zhuang et al. 2019; Elina et al., 2020; Moon & Park, 2022). These studies exist completely outside of the framework of studying decision-making; however, interestingly, these results can also be framed in light of attentional processing—as research has found that orienting attention helps to modulate the perception of pain (Chan et al., 2012; Sprenger et al., 2012; Villemure & Bushnell, 2012; Lier et al., 2021; Khera & Rangasamy, 2021). Attentional mechanisms have been shown to modulate pain transmission via top-down inhibition (Sprenger et al., 2012; Kerekes et al., 2021; Battison et al., 2023), suggesting that the involvement of the ACC in pain modulation that has been shown by these studies may be attributable to its role in attention, not nociception.

An overview of the dorsomedial striatum—a downstream target of the ACC

While the aforementioned studies sustain that the ACC plays an important role in attention, they do not study the impact of ACC dysfunction on other regions of the corticostriatal circuit. One such area, the DMS—a striatal subregion that plays a crucial role in action planning and goal-directed behavior (Yin, Knowlton, & Balleine, 2005; Balleine, Delgado, & Hikosaka, 2007; Thorn et al., 2010; Hart, Leung, & Balleine, 2013; Burton, Nakamura, & Roesch, 2015; Brockett & Roesch, 2021)—receives ipsilateral ACC input (Hunnicutt et al., 2016; Choi et al., 2019). However, there is limited knowledge regarding how the ACC affects downstream DMS signaling during reward-based decision-making, despite the importance of the DMS in goal-directed behavior.

Our lab has previously looked at how unilateral chemical lesions of the ACC impact downstream DMS encoding within the context of inhibitory control (Brockett et al., 2020). In

this task—known as the stop-change task—rats develop habitual responding towards a light cue that signals in which direction a rat must go in order to receive sucrose reward. On a small percentage of trials (20%), a second light cue is presented—100 ms after the initial cue—in the opposite direction of the first. On these trials, rats must suppress their prepotent responding towards the initial cue, and redirect their responding towards the second cue (Brockett et al., 2020; Brockett & Roesch, 2021). The study revealed that rats with ACC lesions had higher error rates, and a reduced number of DMS neurons that accurately encoded the correct action (Brockett et al., 2020; Brockett & Roesch, 2021). Notably, when rats made errors, DMS activity was strongly encoding the incorrect response (Brockett et al., 2020). This suggests that the functional ACC may be importantly involved in adapting directional DMS signals during trials in which rats need to update their responding (Brockett et al., 2020; Brockett & Roesch, 2021).

Other studies have shown that the DMS not only contributes to goal-directed behaviors by reflecting plans for action selection, but also by encoding prediction errors during the reception of unexpected outcomes—exhibiting phasic firing increases during receipt of unexpected reward, and decreases during unexpected reward omission (Stalnaker et al., 2010; Stalnaker et al., 2012). Further, studies have shown that lesions to the DMS promote habitual—as opposed to goal-directed—decision-making (Balleine & O'Doherty, 2010; Gremel & Costa, 2013; Burton, Nakamura, & Roesch, 2015; Peak et al., 2020; Balleine et al., 2021).

Reinforcer devaluation tests in rats that had either their dorsolateral or dorsomedial striatum lesioned confirmed that behaviors under these schedules were indeed goal- and habit-driven, respectively (Gremel & Costa, 2013). Specifically, excitotoxic lesions of the DMS impaired goal-directed behaviors, promoting habitual responding; alternatively, lesioning the

DLS resulted in an over-reliance on DMS, and increased employment of goal-directed strategies (Gremel & Costa, 2013).

Gaining a thorough understanding of the impact of the ACC on DMS encoding during goal-directed decision-making will allow us to assess whether the observed changes in behavior following ACC damage are mediated through the DMS.

Exploring the role of HDAC5 in the context of reward-guided decision-making

In our final experiment (see Chapter V), we investigated the epigenetic mechanisms underlying reward-based decision-making by using viral vectors (adeno-associated viruses encoding short hairpin RNA) to knockdown HDAC5 in the ACC, and implanting an electrode to record electrophysiological activity as rats performed the reward-based decision-making task that will be discussed in the subsequent section (see Page 14, "*Reward-guided decision-making task*").

Epigenetic modifications refer to changes in gene expression that arise from alterations in chromosomal structure, rather than by changes to the DNA sequence itself (Ravi & Kannan, 2013). Histone modification is a well-studied epigenetic mechanism that involves processes such as methylation, phosphorylation, and acetylation (Ravi & Kannan, 2013; Seto & Yoshida, 2014). Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), which add and remove acetyl groups, respectively (Seto & Yoshida, 2014). Deacetylation of histones leads to increased electrostatic affinity between histones and DNA, generally resulting in reduced gene transcription rates (Seto & Yoshida, 2014; Li et al., 2018; Malvaez et al., 2018). HDAC5 is a class IIa HDAC enzyme that shuttles between the nucleus and cytoplasm in response to intracellular signaling; it is highly expressed in the brain,

particularly in cortical regions, suggesting it may play a crucial role in cortical functions underlying learning (Yang & Gregoire, 2005; McQuown & Wood, 2011; Kim et al., 2012).

Phosphorylation of HDAC5 enables its translocation from the cytoplasm to the nucleus, where it can interact with DNA, modifying the acetylation status of histones (Greco et al., 2010). This, in turn, impacts the accessibility of chromatin to transcription factors, thus affecting the regulation of gene expression (Greco et al., 2010). Studies have shown that HDAC5 negatively regulates reward-related behaviors and may play a critical role in reward-guided learning (Taniguchi et al., 2018). Studies in rats have found decreases in nuclear phospho-HDAC5, and an accumulation of cytoplasmic phospho-HDAC5 following cocaine self-administration sessions. These findings support previous research showing that once HDAC5 is phosphorylated, it translocates from the nucleus to the cytoplasm (Host et al., 2011).

Overexpression of HDAC5 in the dorsal striatum—a brain region that plays a pivotal role in reinforcement learning—has been found to exacerbate inflexible decision-making, while inhibition of HDAC enzymes enhances long-term potentiation and memory (Levenson et al., 2004; Barrett & Wood, 2008; Morris, Karra, & Monteggia, 2010; Burns et al., 2022). Studies have shown significantly increased levels of HDAC5 expression in the frontal cortex of individuals with Alzheimer's Disease compared to age-matched controls (Anderson et al., 2015). Cocaine exposure has been shown to upregulate the nuclear localization of HDAC5 in neurons in the rodent striatum, and overexpression of nuclear-localized HDAC5 suppresses cue- and cocaine priming-induced relapse (Taniguchi et al., 2017).

There is a growing body of research that examines the impact of epigenetic modifications on behavior, studying it within the context of addiction and the reinstatement of drug abuse (Renthal et al., 2007; Renthal et al., 2009; Hui et al., 2010; Taniguchi et al., 2012; Kenny, 2014; Nestler, 2014; Li et al., 2015; Rubio et al., 2016; Taniguchi et al., 2017; Li et al., 2018; Cates et al., 2018; Li et al., 2019). Within this literature, HDAC5 was the first HDAC implicated in the negative regulation of synaptic plasticity (Kim et al., 2012). Additionally, previous studies have demonstrated that dephosphorylation of HDAC5 in the nucleus accumbens reduces the formation of reward-context associations, and suppresses the development of cocaine reward behavior (Renthal et al., 2007; Taniguchi et al, 2012).

In the context of plasticity, disrupting HDAC activity has been demonstrated to enable transient, early-phase potentiation in the hippocampus, leading to enhanced synaptic plasticity—converting short-term, subthreshold learning into long-term, transcription-dependent memory (Vecsey et al., 2007; Stefanko et al., 2009). Another study found that inhibiting HDAC activity enhanced sensory cortical plasticity (Bieszczad et al., 2015). In this study, researchers found that treatment with a class I HDAC inhibitor reorganized the primary auditory cortex in a manner that enhanced cortical representations of sound cues that were predictive of reward, suggesting that HDACs play a role in the gating of how much information is encoded during learning (Bieszczad et al., 2015).

In our lab, we previously examined the effects of HDAC5 overexpression in the dorsal striatum on decision-making, and found that HDAC5 overexpression resulted in

inflexible behavior (Pribut et al., 2021). Further, in the dorsolateral striatum—a brain region strongly implicated in habitual behavior (Lipton, Gonzales, & Citri, 2019)–we observed a reduction in the number of neurons that fired to reward, and encoding shifted towards cues that predicted more immediate reward (Pribut et al., 2021). Together, these results are suggestive of a relationship between HDAC5 overexpression in the striatum and inflexible decision-making (Pribut et al., 2021).

Another study exploring HDAC function in the dorsal striatum and its impact on behavior found that as habits were formed, HDAC3 was displaced from the promoters of genes associated with learning in the striatum (Vecsey et al., 2007; McQuown et al., 2011; Malvaez et al., 2018), and overexpression of striatal HDAC3 prevented habit formation (Malvaez et al., 2018).

In spite of these findings, the impact of HDAC5 expression modulation on decision-making in the long run remains vastly unexplored. More specifically, no work has studied how chromatin remodeling in the ACC impacts decision-making. Thus, in Chapter V we investigated the impact of HDAC5 knockdown in the ACC, in order to advance our understanding of the role of HDACs in complex decision-making.

By modulating ACC signaling and studying its subsequent impact on behavior, my dissertation work aims to contribute to our understanding of the neural correlates of attention. My dissertation work employs diverse methodologies that include behavioral analyses, in-vivo electrophysiology, optogenetic modulation of neural activity, pharmacological lesions, and virus-mediated epigenetic modifications—in the service of obtaining a more comprehensive view of the role of the ACC in complex decision-

making. The following section describes the behavioral task we used across all of these experiments in detail.

Reward-guided decision-making task

During this task, all training and recording sessions took place within an operant conditioning box—one of the walls in this operant chamber contains two houselights, below which there is a central odor port situated above two adjacent fluid wells (a left well and a right well). Odors were delivered directly into the nose port via a hemicylinder and magnetic solenoid valve positioned behind the port. Liquid sucrose (10%) was dispensed into the fluid wells solely following correct responses. Houselights would illuminate to indicate the start of a trial, which rats would initiate by nose-poking into the central odor port. The houselights would turn off at the conclusion of the trial, once the animal exited the fluid well. Entries and exits from the odor port and fluid wells were detected via photobeam breaks.

Importantly, the houselight provides a cue that a new trial is beginning, but rats self-initiate the trial once the houselight turns on—thus, the latency of nose-poke upon houselight illumination allows us to assess task engagement and attention. Once the rat nose-poked into the central odor port for 500 ms, a 500 ms odor cue was delivered.

Each trial featured one of three distinct odors (2-Octanol, Pentyl Acetate, or Carvone). One odor instructed the rat to go to the left fluid well for a reward (forced-choice), another directed the rat to choose the right fluid well for a reward (forced-choice), and a third indicated that the rat could obtain a reward from either well (free-choice). Odors were counter-balanced across rats, and their associations remained constant within-rats across sessions. Odors were presented pseudorandomly throughout the session, with the free-choice odor appearing in 7 out

of 20 trials, while the left and right odors were equally presented (each presented on 50% of forced-choice trials).

Throughout both training and recording, during the first block of each session one fluid well was randomly assigned as having a short delay (500ms) to reward, while the other had a long delay (1-7 seconds) to reward. For the second block of trials, contingencies were switched. Under the long delay conditions, the duration of the delay increased incrementally by 1 second each time the rat selected the long-delay well during free-choice odor trials, for up to a maximum of 7 seconds. Delay on forced-choice trials was yoked to delay on free-choice trials. During the final two blocks of the session, the delay preceding reward delivery remained constant (500ms), while the magnitude of the expected reward varied. Reward consisted of a 0.05 ml bolus of 10% sucrose solution. For a large reward, an additional bolus was dispensed 500ms after the first bolus. Blocks would advance once rats completed 60 correct trials, resulting in 240 trials per completed session. In sum, the task is comprised of four independent reward value manipulations (short, long, big, and small reward), each being presented in two directions (left and right), and being indicated by two stimulus types (free- and forced-choice odors).

This task consists of four blocks of sixty trials each, which allows us to independently manipulate reward value between blocks by varying the delay to (Blocks 1 and 2) or size of (Blocks 3 and 4) sucrose reward. In this task, rats must learn the contingencies between odor cues and their associated reward, and importantly must update their behavior once these contingencies are switched. There are forced-choice trials in which a rat must go to the well associated with the presented odor—regardless of reward value—and free-choice trials in which a rat may go to either well to receive a reward. Notably, delay to reward was always manipulated

during the first two blocks, and reward size was manipulated during the final two blocks to promote session completion (Bryden et al., 2011; Roesch et al., 2012; Vázquez et al., 2020).

This paradigm affords us a wealth of behavioral metrics that allow us to parse different components of complex decision-making. Optimal task performance requires rats to detect unexpected changes in reward value and update behavior accordingly to select the more favorable reward outcome on free-choice trials, while maintaining accurate responding on forced-choice trials. Thus, accuracy on forced-choice trials provides insight into the learning of stimulus-response associations, while free-choice responding allows us to assess biases towards differently valued reward. Additionally, analyzing responding on free-choice trials following block switches allows us to assess behavioral flexibility (i.e. whether they are able to update learning when contingencies switch, or whether they sustain biased responding in a maladaptive manner).

Additionally, this task allows us to measure three distinct and important epochs, referred to as light-on latencies, movement, and reaction times. Light-on latencies (the time from houselight illumination until nosepoke into the odor port), are an operationalization of attention to the task. Since the houselight provides the only cue indicating the beginning of a trial, the latency at which the rat nose-pokes in response to this cue is a measure of how attentive they are. This task also allows us to measure reaction (epoch from odor offset to odor port exit) and movement times (epoch from odor port exit to well entry); although attention and motivation are difficult to disentangle, reaction and movement times appear to be proxies of motivation, as they reflect an acceleration of movement towards reward. Repeatedly across this task, we

have seen that—as rats develop behavioral biases towards fluid wells associated with higher-valued reward—reaction times become faster and slower for responses towards high- and low-valued rewards, respectively (Roesch et al., 2007; Bryden et al., 2011; Roesch & Bryden, 2011; Roesch et al., 2012; Burton et al., 2015; Burton et al., 2017; Burton et al., 2018; Chapters II-IV). Finally, movement and reaction times allow us to disambiguate whether our experimental manipulation impacted attention, and was not simply the result of impaired motor control in general (e.g. if light-on latencies are significantly different between groups, but movement and reaction times are not).

Using this task throughout all of the experiments delineated in this dissertation, we found that activity patterns within the functional ACC were strongly linked to attentional measures, and that inactivating the ACC resulted in disruptions to attention were detrimental to task performance. Furthermore, we consistently observed a decline in task engagement following ACC dysfunction, primarily characterized by significant reductions in trial initiation and prolonged periods of inattention.

CHAPTER II: Prior cocaine self-administration attenuates attention signals in the anterior cingulate cortex

Published Manuscript

Neuropsychopharmacology. 45, 833–841(2020). doi: https://doi.org/10.1038/s41386-019-0578-2

Daniela Vázquez[1,2], Heather J. Pribut[1,2], Amanda C. Burton[1,2], Stephen S. Tennyson[1] & Matthew R. Roesch[1,2]

[1]Department of Psychology, University of Maryland, College Park, MD 20742, USA

[2]Program in Neuroscience and Cognitive Science, University of Maryland, College Park, MD 20742, USA

4094 Campus Drive

University of Maryland

College Park, MD 20742

Abstract

Although maladaptive decision-making is a defining feature of drug abuse and addiction, research has not ascertained how cocaine self-administration impacts neural signals in ACC, a brain region thought to contribute to attentional control. To address this issue, rats were trained on a reward-guided decision-making task; reward value was manipulated by independently varying the size of or the delay to reward over several trial blocks. Subsequently, rats self-administered either cocaine (experimental group) or sucrose (control) during twelve consecutive days, after which they underwent a one-month withdrawal period. Upon completion of this period, rats performed the previously learned reward-guided decision-making task while we recorded from single neurons in ACC. We demonstrate that prior cocaine self-administration attenuates attention and attention-related ACC signals in an intake-dependent manner, and that changes in attention are decoupled from ACC firing. These effects likely contribute to the impaired decision-making—typified by chronic substance abuse and relapse—observed after drug use.

Introduction

Difficulties in treating addiction arise from vulnerability towards cue-induced cravings that lead to relapse and reinstatement of drug seeking behavior (Lee et al., 2006; Perry et al., 2014; Marchant et al., 2015; Burgdorf et al., 2017). Treating addiction is further complicated by drug-induced impairments of circuits that are critical for behavioral control and contribute to functions that are imperative for decision-making. Thus, addicts have the onerous task of having to overcome withdrawal symptoms and cravings without the aid of fully functioning circuits that contribute to optimal behavior.

The ACC has been implicated across a number of cognitive functions, including conflict monitoring and detection, perceptual decision-making, and attentional control (Rescorla & Wagner, 1973; D'Esposito et al., 1995; Carter et al., 1998; Botvinick, 1999; Dayan, Kakade, & Montague, 2000; Paus, 2001; Botvinick et al., 2004; Holroyd et al., 2004; Kerns et al., 2004; Weissman et al., 2004; Koob & Volkow, 2010; Bryden et al., 2011; Hayden et al., 2011; Narayanan et al., 2013; Laubach et al., 2015; Wu et al., 2017; Soltani & Izquierdo, 2019; Stolyarova et al., 2019; Schneider et al., 2020; Vázquez et al., 2020; Vázquez, Schneider, & Roesch, 2022). Across studies, the ACC contributes to behavioral adjustments that are triggered by the occurrence of unexpected events, and plays a key role in shifting the allocation of attentional resources toward behaviorally relevant stimuli when there are violations in outcome expectancies, uncertainty, or conflict between competing stimuli or behaviors.

In line with these theories, we previously reported that ACC firing correlates with Pearce and Hall-like changes in attention that occur during learning (Bryden et al., 2011). Specifically, behavioral measures of attention were correlated with increases in ACC firing on trials following unexpected outcomes (i.e., unsigned prediction errors). Furthermore, changes in ACC neural firing occurred prior to and during the processing of trial events, as rats adapted to new behavior-outcome contingencies (Bryden et al., 2011). Here, we explored whether shifts in behavior and associated neural correlates in the ACC were impacted by prior cocaine use.

To address this issue, we recorded from single neurons in the rat ACC while rats performed a two-choice reward-guided decision-making task (Bryden et al., 2011; for further details, see *Figure 2*, Chapter I). Recordings took place following twelve consecutive days of self-administration and a month-long withdrawal period, in order to assess the neural and

behavioral long-term effects of cocaine (Burton et al., 2017, Burton et al., 2018). Optimal task performance required rats to track contingency shifts, selecting high-value reward outcomes during free-choice trials, all while maintaining accurate responding during forced-choice trials. Rats that self-administered cocaine performed worse on forced-choice trials, exhibited a stronger behavioral bias towards immediate reward, and exhibited reductions in behavioral measures of attention. Here, we demonstrate that expectancy violation-induced behavioral adaptations and related ACC firing are impaired following cocaine self-administration.

Methods

Subjects

Male and female Long-Evans rats (n =18; 16M, 2F) were obtained at approximately 2-3 months of age from Charles River Laboratories, weighing in the range of 150-200g. Rats were tested at the University of Maryland (UMD), College Park in accordance with UMD and NIH guidelines. During behavioral testing, food was available ad libitum; water intake was restricted to ensure motivation for task performance.

Surgeries

All rats were trained for six weeks on the reward-guided decision-making task (*Figure 2*). Following training, all rats underwent a surgical procedure during which an intravenous catheter (Dow Corning Silastic tubing) was inserted into the right jugular vein, and a drivable chronic electrode (8 microwires; 27G cannula) was implanted into the ACC (0.2 mm anterior to bregma, ±0.5 mm lateral, 1 mm ventral to brain; Bryden et al., 2011, Burton et al., 2017). After one week of recovery, the randomly assigned experimental (n = 7; 6M, 1F) and control (n = 11; 10M, 1F) groups engaged in cocaine or sucrose self-administration for twelve consecutive days, after

which they underwent month-long withdrawal period (for further detail, see Burton et al., 2017; Burton et al., 2018). Rats lever-pressed to receive either cocaine or sucrose on a fixed ratio schedule. After each active lever press, a cue light was illuminated for 2.3 seconds—the duration of each cocaine infusion. Following each lever press, a 20 second timeout period occurred during which rats were unable to lever press for reward.

During the first six days of cocaine self-administration, each intravenous cocaine infusion was 1.0 mg/kg (maximum 30 infusions or 3 hours). The infusion dose during the final six days was reduced to 0.5 mg/kg (maximum 60 infusions or 3 hours). This procedure allowed us to assess escalations in drug-seeking behavior when doses are cut in half to maintain the desired level of drug intake. Continuous access to high cocaine doses evokes drug-taking and drug-seeking behaviors that are consistent with promoting symptoms of addiction, and has been shown to change behavior and neural signals in other brain regions (Burton et al., 2017; Burton et al., 2018). Further, individual rat variability in lever pressing parallels individual differences observed in human drug consumption. The control group followed the same protocol with the same parameters delineated above, receiving two sucrose pellets per lever press during days 1-6, and only one per active lever press for days 7-12. Recordings (Plexon) during task performance began one month after self-administration (Burton et al., 2017; Burton et al., 2018). Electrodes were advanced 40 microns daily.

Neural Analysis

Our previous work demonstrated that light-on latencies (i.e. time from houselight illumination to odor port nose poke) were significantly faster at the beginning compared to the end of trial blocks; further, ACC firing increased during early trials and was negatively

correlated to light-on latencies (Bryden et al., 2011). In this study, we replicate this analysis by examining light-on latencies and firing rates during early (first 10) and late trials (last 10) for each trial-type within a block. Other analyses break trials down into 5 trial bins to better display time course. Both light-on latencies and firing rate indices were computed to capture differences between early and late activity in each trial block (early–late/early+late). Firing rate was taken from houselight onset to completion of the response for correct trials only. Wilcoxon tests were used to measure significant shifts in the distribution of indices from zero, and to determine differences between control and cocaine-exposed groups ($p < 0.05$).

Behavioral Analysis

Behavior in the recording task was analyzed by calculating the percent of correct responses on forced-choice trials (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). Calculations were split into first and last ten trials for each trial-type. 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 and neural statistics (Burton et al., 2017; Burton et al., 2018). Free-choice reaction times were not split into early and late trials due to lower proportions of trials (e.g., fewer low-value choices late in the trial block). 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. Multi-factor analysis of variance (ANOVA; factors included group (sucrose vs. cocaine), reward value (high vs. low), value manipulation (size vs. delay), and phase of learning (early: first ten trials vs. late: last ten trials per trial type) and t-tests ($p<0.05$) were used to determine differences between the cocaine and control groups. trials per trial type).

Self-administration

All rats were trained on the reward-guided decision-making task (*Figure 2*) prior to implantation of electrodes in ACC and intravenous catheters for cocaine self-administration. Rats self-administered sucrose pellets (n=11) or cocaine (n=7) over the course of twelve days. During days 1-6 (1 mg/kg of cocaine or 2 sucrose pellets per lever press), the average number of infusions or pellet deliveries across rats out of a maximum of 30 was 18.2 (±9.2 standard deviation (s.d.)) and 29.9 (±0.4 s.d.) for cocaine and sucrose, respectively. During days 7-12 (0.5 mg/kg cocaine or 1 sucrose pellet per press), the average number infusions or pellet deliveries across rats out of a maximum of 60 was 37.9 (±16.0 s.d.) and 60 (±0 s.d.) for cocaine and sucrose, respectively.

Cocaine made rats more sensitive to reward delays, worsened task performance, and accelerated reaction times

Replicating previous results, rats exhibited bias toward higher value rewards during delay blocks (Burton et al., 2017; Burton et al., 2018). In an ANOVA with percent choice as the dependent variable, there was a significant main effect of reward value (*Figure 3.1a;* $F(1,2658) = 2781.3$, $p < 0.01$), as both control and cocaine rats preferred high-value reward (short; large). There was a significant interaction between group,

value, and block phase ($F(1,2658) = 415.0$, $p < 0.001$), with cocaine rats choosing high-value reward significantly more often than controls in the last ten free-choice trials during delay manipulations (*Figure 3.1a;* $t_{(1197)} = 2.08$, $p < 0.05$; $d = 0.26$). Furthermore, cocaine rats were significantly faster at responding on all free-choice trial-types compared to controls (*Figure 3.1c;* ANOVA; main effect of group ($F(1,2630)=6.55$, $p < 0.05$; $d = 1.3$). Although cocaine made rats more sensitive to delay manipulations, cocaine and control rats chose large over small reward at similar rates (*Figure 3.1a*; $t_{(1197)} = 0.19$, $p = 0.85$; $d = 0.06$).

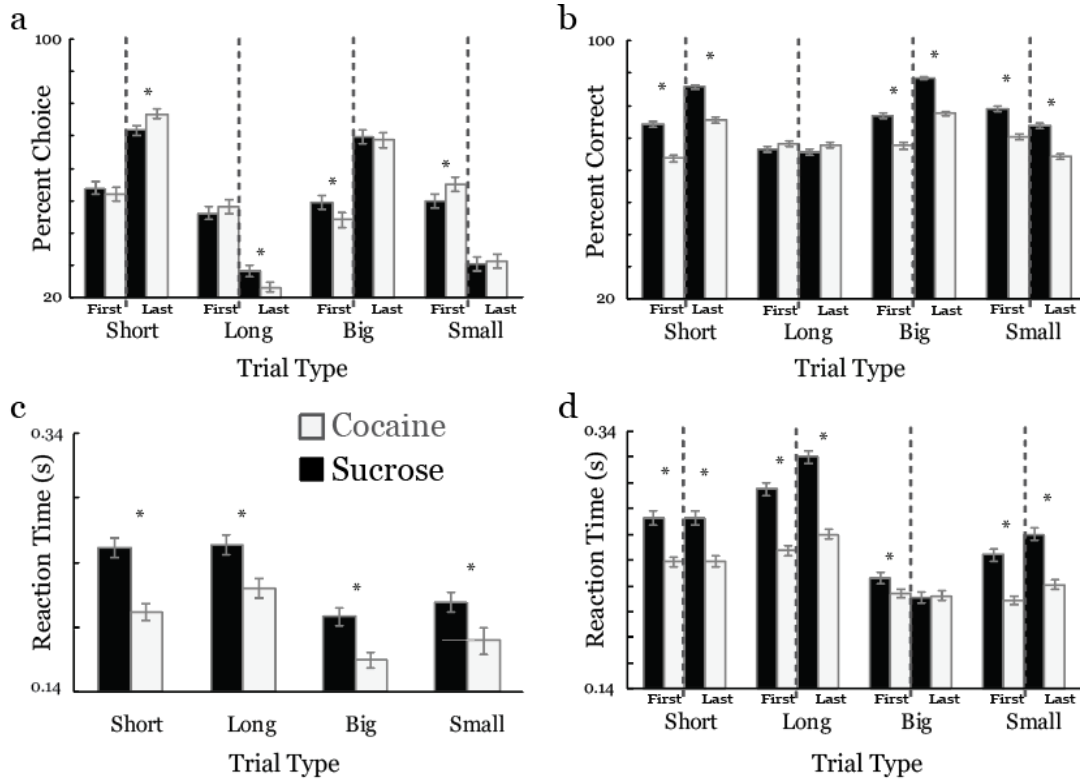

Figure 3.1: a) Percent choice on free-choice trials in each value manipulation over the first ten and last ten trials of each block. b) Percent correct on forced-choice trials in the same manner as a. c) Reaction time (odor port exit minus odor offset) on all free-choice trials for each value manipulation. d) Reaction time (odor port exit minus odor offset) on forced-choice trials. For these analyses, behavior was analyzed by session (controls, black bars; cocaine, gray bars). Error bars indicate SEM. Asterisks (*) indicate significance ($p < 0.05$) in multi-factor ANOVA and/or post-hoc t-tests. For the cocaine group, n = 7; control group, n = 11.

In ANOVAs with percent correct and reaction time on forced-choice trials as the dependent variables,

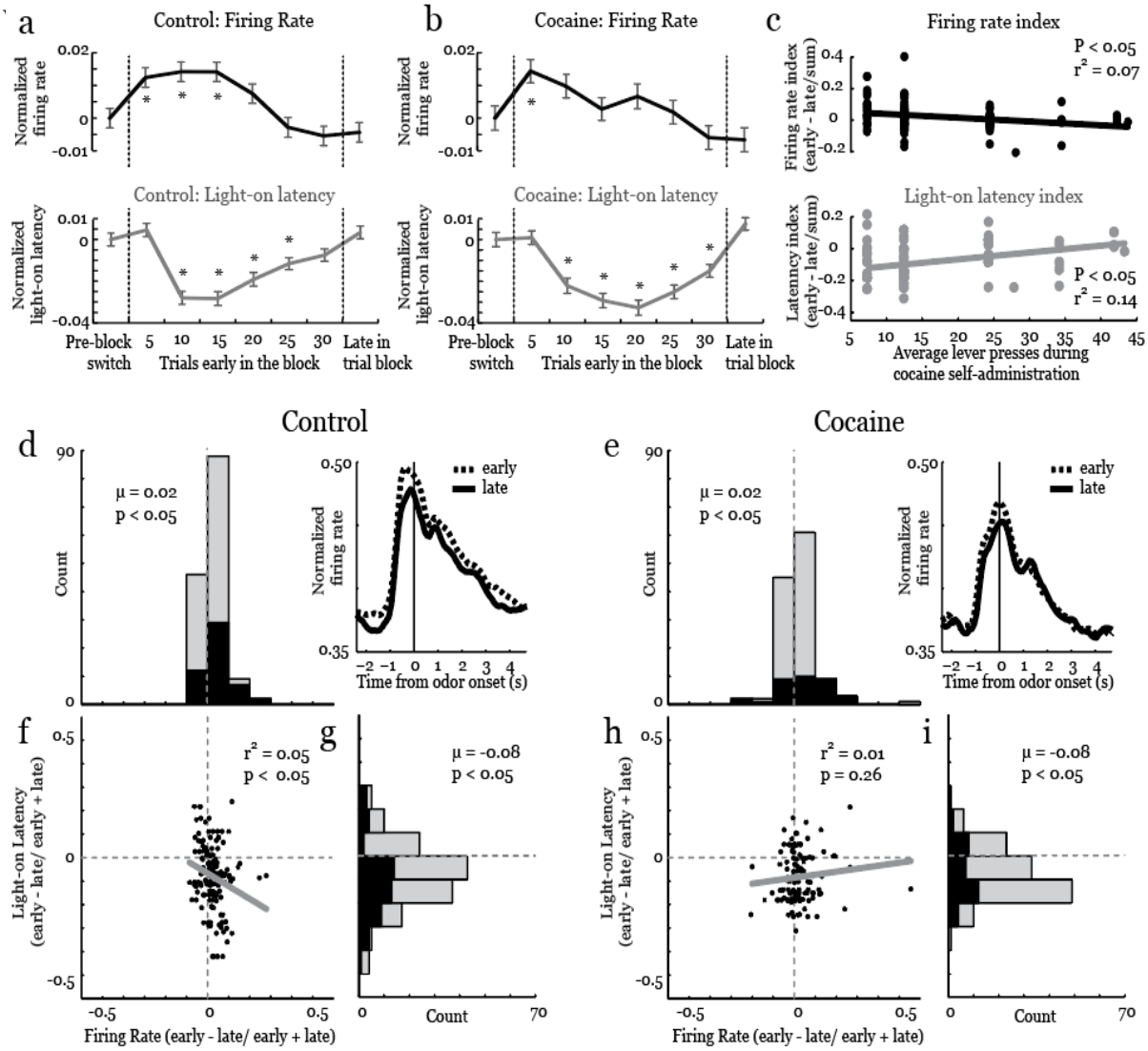

Figure 3.2a-b, Normalized firing rates (black; epoch = houselights on to well entry) and light-on latencies (gray; latency to enter odor port upon houselight illumination) for the average of the last 5 trials in a block before a switch in contingencies ("pre-block switch"), the trials post-switch in 5 trials bins, and the average of the last 5 trials in the current trial block ("late in trial block") for control (a; n = 145 cells; 11 rats) and cocaine-exposed (b; n = 123 cells; 7 rats) rats. Firing rates and light-on latencies are normalized to the max values within each session, averaged, and then subtracted from "Pre-block switch" trials so that comparisons can be made across groups. c, Correlation between firing-rate indices (black) and total number of infusions during cocaine self-administration and between light-on indices (gray) and total number of infusions during cocaine self-administration. d-e, Distributions of firing-rate indices for controls (d) and cocaine-exposed (e) rats. Insets to the right of each panel illustrate normalized average firing during early (dashed) and late (solid) trials averaged over all trial-types. Firing is aligned odor onset. Nose poke into the odor port occurred 500 ms prior to odor presentation and was triggered by illumination of the houselights. Port exit occurred roughly 750 ms after odor onset (500 ms odor presentation + ~250 ms reaction time). f, i) Correlation between firing rate indices (d,e) and light-on latency indices (g,i) for control (f) and cocaine (i) rats. Black bars in G and I represent sessions where light-on latencies significantly differed between early and late trials (ttest; p < 0.05).

reaction time: $F(1,2658) = 16.84$, $p < 0.01$) and an interaction of value and phase (percent correct: $F(1,2658) = 144.99$, $p < 0.01$). This indicates that both control and cocaine rats were significantly better and faster on high-value forced-choice trials in the late phase of each block. Importantly, there was also a main effect of group in the ANOVAs on forced-choice behavioral measures (percent correct: $F(1,2658)=185.80$, $p < 0.001$; forced-choice reaction time: $F(1,2658)=112.54$, $p < 0.001$), with cocaine rats being significantly faster and worse on forced-choice trials compared to controls (*Figures 3.1b&d*). Notably, the majority of these behavioral measures were correlated with the number of cocaine infusions and the differential drug-seeking following dosage reduction. We found that both were negatively correlated with percent correct on forced-choice trials (infusions versus percent correct: $p < 0.001$, $r^2 = 0.08$; difference between weeks 1 and 2 versus percent correct: $p < 0.001$, $r^2 = 0.09$) and were positively correlated with a response bias towards more immediate reward on free choice trials (infusions versus percent correct: $p < 0.05$, $r^2 = 0.030$; difference between week 1 and 2 versus percent correct: $p < 0.05$, $r^2 = 0.034$). Lastly, there was a significant negative correlation between reaction times on forced-choice trials and increases in cocaine seeking during week two (difference between weeks 1 and 2 versus percent correct: $p < 0.05$, $r^2 = 0.037$).

We conclude that previous cocaine self-administration had a long-term impact on behavior—overall, cocaine rats exhibited stronger response biases toward more immediate reward on free-choice trials, and were significantly faster and worse on forced-choice trials. These results are consistent with previous work demonstrating that cocaine self-administration makes rats more impulsive during delay tasks (Saddoris et al., 2016; Takahashi et al., 2019).

Cocaine self-administration attenuates attentional signals in a dose-dependent manner

Previously, we have shown that attention and firing in the ACC is elevated during early trials when rats are updating action-outcome contingencies (Bryden et al., 2011). One measure of attention is the latency at which rats respond to external stimuli that signal the initiation of behavioral trials. Here, we examine how quickly rats nose poke into the central port upon illumination of house lights—referred to as light-on latency. Latency to approach the odor port precedes any knowledge of the upcoming reward; thus, this measure cannot reflect the nature or evaluation of the reward to be received at the end of the trial. Further, this measure cannot reflect a reduction in motivation over the course of the session, because latencies are significantly shorter on early trials following a block shift, irrespective of the amount of preceding trials (Rescorla & Wagner, 1972). Faster light-on latencies are thought to reflect accelerated processing of trial events (e.g. cues, responses) as rats increase their reception of unexpected shifts in reward contingencies. Light-on latencies might also reflect an investigatory reflex, or a reengagement of instrumental task performance (i.e., increased cognitive control). A similar phenomenon has been described during recovery from habituation following shifts in learned contingencies, mirroring theoretical changes in Pearce and Hall models of attention. Consistent with previous reports, light-on latencies became faster several trials after the block transition before returning to pre-block switch levels (5 pre-block switch trial bin versus second bin of 5 trials post-switch (*Figure 3.2a;* ttest; control: $t_{(144)} = 4.80$; $p < 0.0001$; cocaine: $t_{(122)} = 3.64$; $p < 0.0001$)).

To determine the impact that block transitions had on neural firing, we examined neurons that increased firing from house light onset (i.e., trial start) to well entry (i.e., completion of the behavioral response) averaged across all rewarded trials and trial-types. Neurons that exhibited

general increases in firing during the trial were previously reported to increase following violations in reward expectancies (Bryden et al., 2011). Overall, 145 (20%) and 123 (21%) of ACC neurons increased firing during the 'trial epoch' compared to baseline (1 second preceding light onset; Wilcoxon; $p< 0.05$) in control and cocaine exposed rats, respectively. For both groups, the proportion of neurons that increased firing during the trial epoch was significantly higher than expected from chance alone ($p < 0.05$), and the frequency of counts relative to the total sample did not significantly differ between groups ($\chi^2 = 0.26$; $p = 0.61$).

As previously reported, we found that ACC activity was stronger at the beginning compared to the end of each trial block. As with light-on latencies, firing rate changes in ACC were significantly different early in trial blocks (*Figure 3.2a ,b*). However, unlike changes in light-on latencies, firing rate changes were significantly different within the first block of five trials (*Figure 3.2a, b*; 5 pre-block switch trial bin versus first bin of 5 trials post-switch; ttest; control: $t_{(144)} = 2.32$; $p = 0.02$; cocaine: $t_{(122)} = 2.41$; $p = 0.02$). Thus, changes in firing preceded changes in light-on latencies.

To further quantify changes from the beginning and end of trial blocks, we computed an index that captured differences between early (first 10 trials) and late (last 10 trials) trials for each trial-type for each neuron in both control and cocaine groups (index=early-late/early+late). For light-on latencies we observed significant shifts below zero for both groups (indicating faster latencies early in the trial blocks), with no difference between them (*Figures 3.2g&i;* Wilcoxons: control ($n = 145$; $\mu = -0.08$; $p < 0.0001$; cocaine: $n = 123$; $\mu = -0.08$; $p < 0.0001$; control versus cocaine: $z = 0.72$; $p = 0.47$). Likewise, for distributions of firing rate indices, we found significant shifts above zero for both groups—indicative of higher firing at the beginning of trial

blocks—with no significant difference between groups (*Figures 3.2d&e;* Wilcoxons: control: n = 145; μ = 0.23; $p < 0.0001$; cocaine: n = 123; μ = 0.22; $p < 0.0001$; control versus cocaine: z = 1.04; $p = 0.30$).

At the single neuron level, firing of 50 (34%) and 24 (20%) of neurons were significantly modulated during first compared to the last 10 trials in control and cocaine-exposed rats, respectively (*t* test; $p < 0.05$). This difference between groups approached significance ($\chi^2 = 3.76$; $p = 0.053$). Of these neurons, the firing rates of 38 and 12 neurons from control animals were significantly higher and lower during the first 10 trials compared to the last 10 trials (*t* test; $p < 0.05$). For the cocaine group, firing of 22 and 12 neurons exhibited significantly higher and lower firing during early compared to late trials, respectively (*t* test; $p < 0.05$). Only for controls did the counts of neurons that exhibited significantly higher firing during early trials significantly outnumber those showing significantly lower firing (control: $\chi^2 = 13.41$; $p < 0.05$; cocaine: $\chi^2 = 1.44$; $p = 0.09$); however, the frequency of neurons did not differ significantly between groups ($\chi^2 = 0.77$; $p = 0.38$).

In summary, for both groups of rats we found that early increases in ACC firing preceded faster light-on latencies—suggesting that the two processes were related, and that ACC might be contributing to changes in attention that occur following unexpected shifts in reward contingencies. Consistent with our previous work, we show that ACC firing and light-on latencies are correlated in control rats (Bryden et al., 2011). In control rats, the two indices were negatively correlated *(Figure 3.2f;* $p = 0.009$, $r^2 = 0.05$), suggesting that during sessions in which ACC firing rates were higher, rats were more attentive. Interestingly, in cocaine rats, the correlation was positive but not significant (*Figure 3.2h;* $p = 0.26$*;* $r^2 = 0.01$), and was

significantly different than controls (Fisher r-to-z transformation; $z = 2.64$; $p = 0.008$), suggesting that attentional control was decoupled from firing in ACC after cocaine exposure.

Next, we explored whether these changes were impacted by the amount of cocaine rats self-administered as measured by the total amount of cocaine infused, and the degree to which rats escalated drug seeking from week 1 to week 2 during self-administration. Remarkably, we found a positive correlation between light-on latencies and both measures of self-administration (*Figure 3.2c*; infusions versus light-on latency indices ($p < 0.0001$; $r^2 = 0.20$; difference between weeks versus light-on latency indices: $p = 0.001$; $r^2 = 0.11$). The opposite relationship was

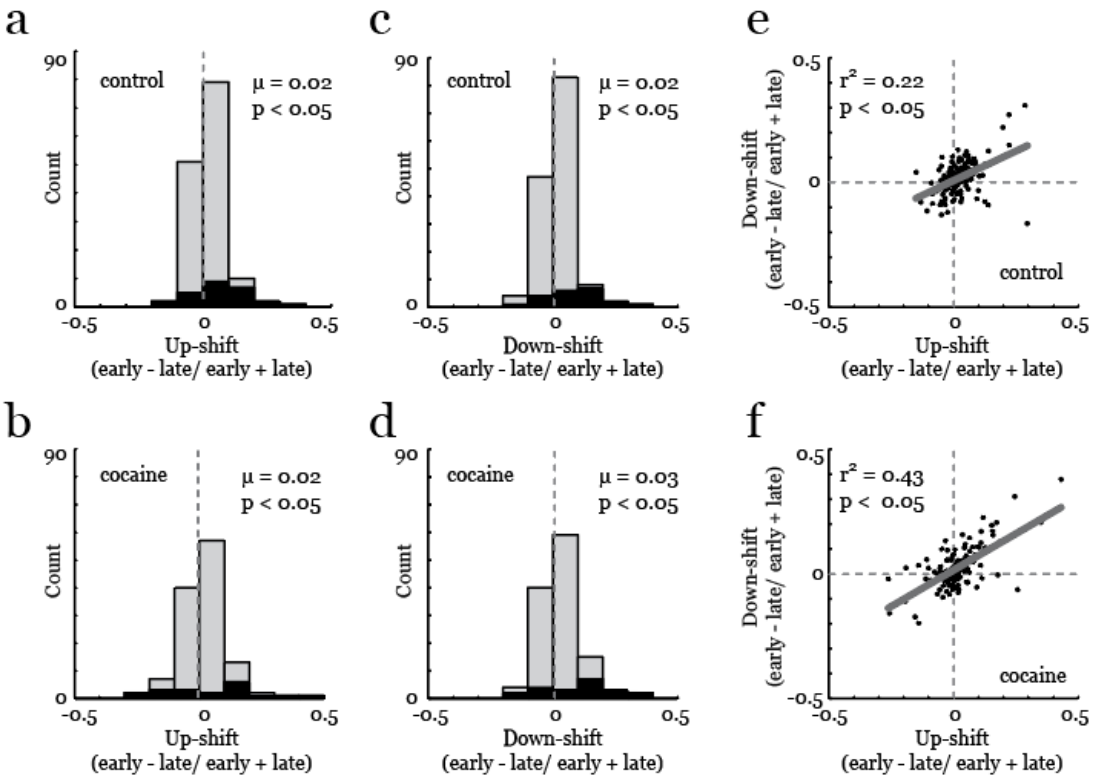

Figure 3.3: Firing-rate indices for upshifts (i.e., shift to short delay or large reward); a, b and downshifts (i.e., shift to long delay or small reward); c, d were computed by subtracting late trials (last ten trials) from early trials (first ten trials) for each shift type and dividing by the sum (early − late/early + late) for control (a, c; n = 145; 11 rats) and cocaine-exposed (b, d; n = 123; 7 rats). Black bars represent cells whose firing was significantly different for early compared with late trials (t test; p < 0.05). e, f Correlation between up- and downshift firing-rate distributions for controls (e) and cocaine-exposed (f) rats.

observed between firing rate indices and both measures of self-administration (*Figure 3.2c;* i.e., negative correlation; infusions versus firing rate indices: $p = 0.002$*;* $r^2 = 0.08$; difference between weeks versus firing rate indices: $p = 0.0009$*;* $r^2 = 0.08$). Thus, rats that had self-administered more cocaine exhibited reduced attention and attention-related firing early in trials blocks.

Signals related to both up- and down-shifts in value were impacted by cocaine

In a final neural analysis, we investigated whether observed drug-induced changes in firing rates were more or less pronounced after up- versus down-shifts in value. Recall that block shifts were only cued by rats experiencing unexpected increases (i.e., larger or more immediate reward: up-shifts) or decreases (i.e., smaller or delayed reward: down-shifts) in reward value. One tenant of the Pearce and Hall model of attention is that signals increase following both types of value shifts. Indeed, we have shown that firing rate changes in ACC exhibit unsigned increases in activity to both up- and down-shifts in value (D'Esposito et al., 1995; Bryden et al., 2011). Here, we replicate this effect in ACC for both control and cocaine-exposed rats. Firing rate indices (early-late/early+late) were significantly shifted in the positive direction for both up- and down-shifts in value (*Figures 3.3a-d;* Wilcoxons: *control*: up-shift: $\mu = 0.02$; $p < 0.0001$; down-shift: $\mu = 0.02$; $p = 0.002$; *cocaine*: up-shift: $\mu = 0.02$; $p = 0.006$; down-shift: $\mu = 0.03$; $p = 0.001$). Further, up- and down-shift indices were positively correlated with each other, demonstrating that single neurons exhibited similar increases in firing following either an up- or down-shift in value (*Figures 3.3e-f*; control: $p < 0.0001$; $r^2 = 0.22$; cocaine: $p < 0.0001$*;* $r^2 = 0.43$). Lastly, both up- and down-shift distributions were negatively correlated with the cocaine infusions (up-shifts: $p = 0.01$*;* $r^2 = 0.05$; down-shifts: $p = 0.003$*;* $r^2 = 0.07$) and increases in drug-

seeking during week 2 during self-administration (up-shifts: $p = 0.007$; $r^2 = 0.06$; down-shifts: $p = 0.0007$; $r^2 = 0.10$).

Discussion

In summary, we show that neural signals related to attentional control in the ACC are attenuated following cocaine exposure, and that neural firing and behavioral changes were correlated with the degree to which rats self-administered cocaine. Altered attentional control observed after cocaine exposure potentially impacts decisions that require increased neural processing in the face of competing information, uncertainty, or expectancy violations, likely contributing to impaired decision-making that leads to drug use and relapse.

Here, we have used light-on latencies as a measure for when and how strongly attentional control processes are engaged. During performance of our task, faster light-on latencies are thought to reflect accelerated processing of trial events (e.g. cues, responses) after unexpected shifts in reward contingencies—task reengagement when habitually learned block structures must be updated following unexpected shifts in reward value—mirroring theoretical changes in Pearce and Hall models of attention. Consistent with this observation, the percent errors that occurred early in trials blocks were correlated with changes in light-on latencies. We also think that the functional ACC contributes to maintaining attention to cues as block adjustments are occurring, allowing rats to follow forced-choice rules while updating action-outcome contingencies during free-choice trials. This idea fits with the finding that when attention was low, rats tended to perform worse on forced choice trials.

Overall, neural and behavioral changes observed after cocaine self-administration were maladaptive. Cocaine rats were more impulsive in that they exhibited a behavioral bias towards

more immediate over delayed reward, and performed significantly worse on forced-choice trials. As a result, cocaine-exposed rats acquired less reward overall—due to the non-delivery of reward on incorrect forced-choice trials, and because short delay trials were normalized so that over-selection of more immediate reward did not result in greater reward over multiple trials.

Although it is difficult to know the exact homolog of the human ACC, rat ACC connectivity does overlap with that of the primate ACC (Bryden et al., 2018), and is optimally suited to mediate behavioral control via its monosynaptic projections to the dorsal striatum (Gabbott et al., 2005; Mailly et al., 2013), subthalamic nucleus (Mailly et al., 2013), prefrontal cortex (Hoover & Vertes, 2007), and locus coeruleus (Maurice et al., 1998). Further, our recordings are consistent with signals reported in the primate ACC (Hayden et al., 2011). Our findings also align with research in humans studying cocaine-induced impairments of attention and cognitive control (Pace-Schott et al., 2008; Balodis et al., 2016; van Son et al., 2016; Almeida et al., 2017). Thus, our work likely translates well to the human condition, providing additional evidence that elucidates the long-term impact of drug use on behavior and in the brain—at the resolution of single neurons—while controlling for confounding variables that might impact the findings of research in humans (e.g., life history, polysubstance abuse).

Studies have already found that neural signals in nucleus accumbens core (NAc), dorsolateral striatum (DLS) and ventral tegmental area (VTA) dopamine (DA) neurons are abnormal following cocaine self-administration (Saddoris et al., 2016). Cocaine disrupts the NAc's ability to encode reward predictions and expectancies during delays preceding reward delivery (Burton et al., 2017). It also impairs signed prediction error signals generated by VTA DA neurons, and alters DLS encoding, in line with elevated response biases on free-choice trials

(Burton et al., 2017, Brockett et al., 2018). Our findings add a new dimension to the growing number of problems that arise after drug use, paralleling observed drug-induced changes to the striatal and dopamine circuits that give rise to reward predictions and signed prediction errors.

We suspect that enhancing ACC firing in cocaine-exposed rats could repair many of these deficits—resulting in the redirection of neural resources to relevant task events following expectancy violations, subsequently leading to better prediction and error encoding in both NAc and VTA DA neurons, and stronger cognitive control over the DLS.

Chapter III: Optogenetic inhibition of the rat anterior cingulate cortex impairs the ability to initiate and stay on task

Published Manuscript

The Journal of Neuroscience, In Press

Authors: Daniela Vázquez[1,2], Sean R. Maulhardt[1], Thomas A. Stalnaker[3], Alec Solway[1,2], Caroline J. Charpentier[1,2] & Matthew R. Roesch[1,2]

[1]Department of Psychology, University of Maryland, College Park, MD 20742, USA

[2]Program in Neuroscience and Cognitive Science, University of Maryland, College Park, MD 20742, USA

[3]Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD, USA.

Abstract

Substance abuse studies have given rise to a lot of seminal findings in the field of neuroscience; however, the plasticity of the brain—and its consequent ability to engage compensatory mechanisms during learning—poses fundamental challenges to these types of studies. Our prior research has identified neural correlates of attention in the functional ACC, and demonstrated that disruptions in ACC signaling following cocaine self-administration impair decision-making. Whether the observed attentional impairments are solely due to these attenuated ACC signals, or occur as a result of the global circuit disruptions that arise following substance abuse, remains to be studied. Thus, in this study we used optogenetics to transiently inhibit ACC activity while rats performed a reward-guided two-choice task previously used in our research. ACC inhibition had a profound impact on behavior that extended beyond deficits in attention during learning when expected outcomes were uncertain. We found that ACC inactivation slowed and reduced the number of trials rats initiated, and impaired both their accuracy and their ability to complete sessions. Together, these results suggest that in addition to attention-related functions, the ACC contributes to the ability to initiate trials and generally stay on task.

Introduction

By increasing attention, the anterior cingulate cortex facilitates flexible learning, allowing for the online filtering of relevant and necessary information. We previously have seen that—in our task—ACC firing at the start of trials, from trial onset until completion of the behavioral response, was correlated with changes in attention during flexible learning of response-outcome

contingencies on trials following reward prediction errors (Bryden et al., 2011). In another study, we found that disruption of ACC signaling after cocaine self-administration results in dose-dependent decision-making impairments that arise from attenuations in attention during behavioral tasks (Vázquez et al., 2020). However, these experiments did not allow us to study the ACC's role in the absence of compensatory mechanisms that might be engaged following cocaine self-administration, nor did they allow us to conduct within-subject modulation of ACC activity. To address these limitations, we used optogenetics to transiently inactivate the ACC in a spatiotemporally precise manner while rats performed the two-choice task (*Figure 2*) that we have previously used to study reward-guided decision-making (Bryden et al., 2011; Vázquez et al., 2020).

We found that ACC inactivation had a profound impact on the rats' basic ability to initiate and complete trials—with rats being slower to initiate trials, and initiating and completing fewer trials—which resulted in reduced session completion and obtaining less reward overall. ACC inactivation also impaired free-choice performance for contingencies associated with learning in the first block of trials (i.e., original discrimination). Inactivation did not impact overall movement time or reduce time spent consuming reward, suggesting that the observed behavioral impairments during inhibition days were not a result of motor or motivational deficits. Together, these findings suggest that optogenetic inhibition not only impairs attention, but it also drastically impedes rats' ability to initiate and sustain task performance.

Methods

Male Long-Evans rats (n =10) were obtained at approximately 2-3 months of age from Charles River Laboratories, weighing in the range of 150-200g. Rats were tested at

the University of Maryland (UMD), College Park in accordance with UMD and NIH guidelines. During behavioral testing, food was available ad libitum; water intake was restricted to ensure motivation for task performance. They were trained on the aforementioned decision-making task (refer to *Figure 2* and the in-depth description of the task on *pages 14-17)* for a month, and then underwent surgery.

Surgery

Following training, all rats underwent a surgical procedure to receive virus injections and fiber optic implants. We bilaterally injected halorhodopsin (AAV-CaMKIIa-eNPHR3.0-EYFP from the UNC Vector Core; 0.6μL per hemisphere) and implanted optical fibers into the rat ACC (coordinates: 0.2 mm anterior to bregma, ±0.5 mm lateral, and 1.0 mm ventral to brain). The virus we used has been reliably established in optogenetics literature (Zhang et al., 2007; Gradinaru, Thompson, & Deisseroth, 2008; Zhao et al., 2008; Fenno, Yizhar, & Deisseroth et al., 2017)—across both in-vivo and in-vitro experiments—as an inhibitory opsin, and one that specifically inhibits pyramidal cells as a result of its CamKII promoter (Han & Boyden, 2007; Zhang et al., 2007; Arrenberg et al., 2009; Calu et al., 2013; Stefanik et al., 2013; Kang et al., 2015; Chang et al., 2016; Gardner et al., 2017; Gholami & Sayyah, 2018; Hart et al., 2020). Moreover, patch-clamp experiments using the same halorhodopsin virus we used have shown that yellow light illumination results in clear inhibition of excitatory postsynaptic currents, with no rebound excitation (de Lima et al., 2022).

Following a week of recovery after surgery, rats performed the previously described decision-making task. Every two days, we alternated between using a blue (control days) or yellow (inhibition days) LED to optogenetically inactivate the ACC while rats performed the

aforementioned task (*Figure 2*). Throughout the task, LED delivery randomly occurred on 50% of trials—and lasted from the onset of houselights until completion of the behavioral response (e.g. "LED-on" period—the epoch from houselight-on to well entry—see *Figure 2*). During sessions, the LED light was shielded so as not to be a distracting stimulus—electrical tape was wrapped around the sleeves connecting the optical fibers to the ferrules. Regardless, since our control was a blue LED light—if the LEDs were distracting or reinforcing in some way, then that would be controlled for as well (Wikenheiser et al., 2017); many studies—both in vivo and in vitro—which do optical excitation and inhibition in the same cells—use both halorhodopsin and channelrhodopsin; because of the difference in excitation wavelengths, these two opsins can be co-expressed (e.g. only yellow light will activate halorhodopsin—blue light will have no impact) (Gradinaru et al., 2007; Han & Boyden, 2007; Zhang et al., 2007; Kang et al., 2015; Sharpe et al., 2017; Chang et al., 2018). Further—as we will describe below—inhibition also impacted trials in which the LED was not illuminated, demonstrating that the behavioral changes observed during inhibition sessions cannot be a product of the physical presence of LED illumination. We used PlexBright LED Optogenetics Modules controlled by PlexBright Dual LED Commutators to deliver light (blue: 465 nm, yellow: 590 nm) via Plexon's 200 μm core optical fibers.

Behavioral analysis

Behavior for each session was analyzed by calculating the total number of reward trials, percentage of initiated trials (i.e., how often rats responded to houselights), total number of incomplete trials (i.e., rats did not maintain hold centrally or respond to one of the two wells), percentage of correct responding on forced-choice trials (i.e., the number of trials the animal correctlsy responded to the side corresponding to the directional odor), the percentage of trials

rats chose a high-valued condition (i.e., short delay, large reward) on free-choice trials, reaction times (odor offset to odor port exit; *Figure 2*: RT), movement times (port exit to well entry *Figure 2*: MT), time spent in the fluid well after reward onset, and light-on latencies (latency to enter odor port upon illumination of houselights; *Figure 2*: LO Latency). Behavioral analyses were computed for each individual session (separated by control and inhibition days), and then averaged across sessions within each group. Calculations for completed sessions were split into the first and last ten trials of each block. We have previously shown that analyzing the first ten trials from each trial type captures learning that occurs at the start of trial blocks, and provides a statistically sufficient sample size (Bryden et al., 2011; Burton et al., 2017; Burton et al., 2018; Vázquez et al., 2020). Behavioral analyses were computed for each individual session (separated by control and inhibition days), and then averaged across sessions within each group. Multifactor analysis of variance (ANOVA) statistics with relevant factors were conducted on the above-mentioned behavioral measures. Factors included experimental manipulation (inhibition vs. control session), LED (on vs. off), phase (early vs. late in learning), block-type (delay vs. size), discrimination (original vs. learning), and value (high vs. low). Post-hoc ttests corrected for multiple comparisons were used to explore significant interaction terms in the ANOVAs.

Results

Inhibition of the anterior cingulate cortex reduced the number of trials and sessions completed

Rats performed the task for 92 inhibition sessions and 89 control sessions. As previously mentioned, LED illumination occurred on 50% of trials, lasting from houselight-on to well entry (*Figure 2*)—as this is the epoch during which we saw firing related to initiation of trials and attention during recordings in our previous studies (Bryden et al., 2011; Vázquez et al., 2020).

Further, it is during that epoch that we have observed changes in firing due to chronic cocaine self-administration (Vázquez et al., 2020). With *Figure 4.1*, and in the subsequent section, we will demonstrate that ACC inhibition had a profound impact on the rats' basic ability to initiate and complete trials. We will first show, by conducting our initial analyses across all behavioral sessions–including those that were incomplete (i.e., sessions in which rats did not complete all four blocks in the allotted two hours per session)–that general measures of task performance that were common across blocks (i.e., percentage of initiated and completed trials, latencies to initiate trials, the number of rewarded trials per session, and forced-choice accuracy) were impaired not only on LED-on trials, but also LED-off trials. That is, when we included LED (on vs. off) as one of the factors in the ANOVA, we found no significant main or interaction effects with the LED being on vs. off. For the percentage of initiated trials, light on latencies, percentage of completed trials and number of rewards obtained, we found main effects of session-type (inhibition vs. control sessions; $F(1,339) > 14.4$, $p < 0.0002$), but no significant main or interaction effects ($F(1,339)$ 1.52, $p > 0.22$) with LEDs being on vs. off. To summarize these findings, rats were impaired on both LED-on and LED-off trials during inhibition sessions compared to control sessions. Due to finding no main or interaction effects with LED on vs. off across measures, we collapse across these trial-types in the analysis below.

Figure 4.1A illustrates the percentage of trials that rats initiated, averaged across sessions (bars) and for each individual rat (dots) during inhibition (right) and control (left) sessions. An initiated trial is defined by a nose-poke into the odor port upon illumination of the houselights (*Figure 2*; houselight on). If the rat did not initiate a trial within 5s, then the houselights turned

off for a 2s time interval. On average, during inhibition of the ACC, there were significantly fewer trials initiated in response to the houselights (*Figure 4.1A*; $t_{(173)} = 3.63$, $p = 0.0004$).

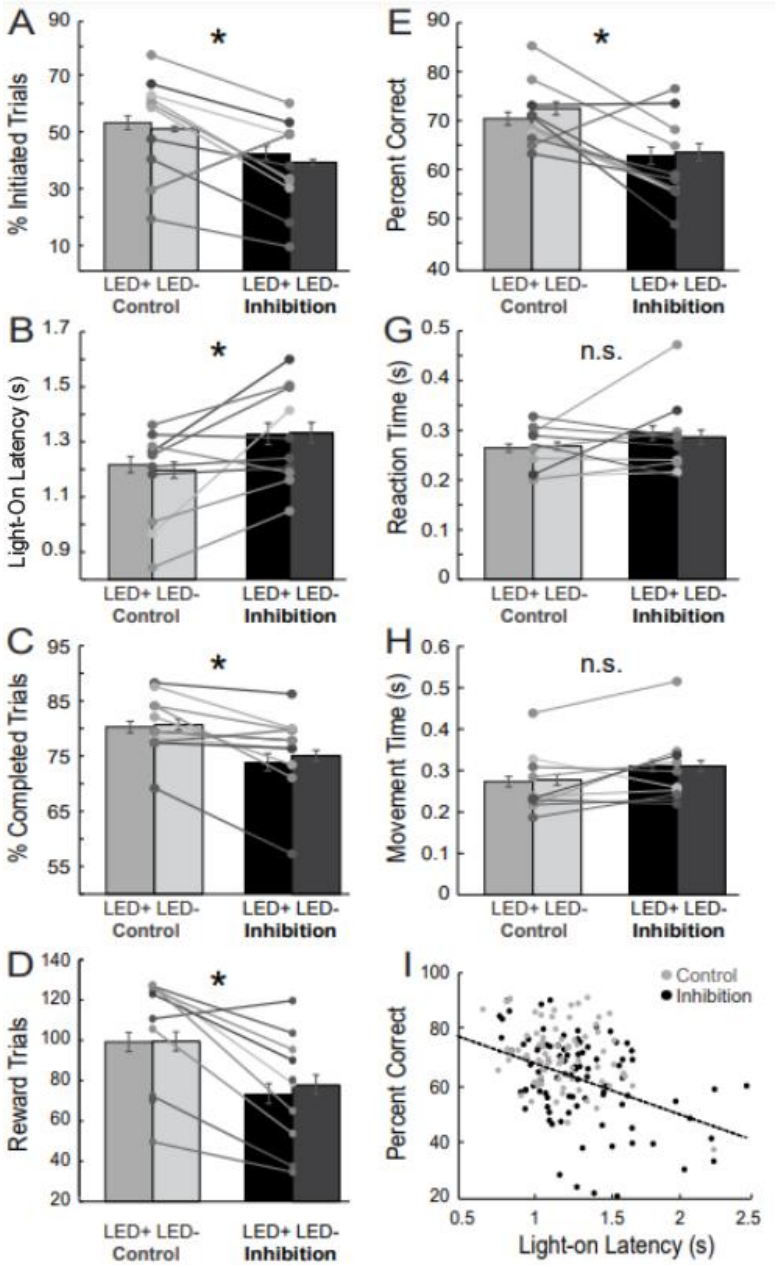

Figure 4.1: Behavioral analyses across all sessions, comparing inhibition to control days. Bar graphs are further broken down into LED-on (labeled "LED+") vs. LED-off ("LED-") trials. A) Percentage of times that rats initiated trials. B) Average light-on latency denotes the latency to enter odor port upon house-light illumination (which indicates the beginning of a new trial). C) Average percentage of trials rats completed each session, where we define completed trials as trials in which rats executed the entire behavioral sequence to completion—maintaining their nose-poke in the central odor port for one second, and then responding to one of the two fluid wells (regardless of whether correctly or incorrectly) within the allotted three seconds for trial completion. D) Average number of rewarded trials. E) Percentage correct forced-choice trials. F) Percentage of times rats chose high-value reward on free-choice trials F) Average reaction times (odor offset to odor port exit). G) Average movement times (port exit to well entry). H) Correlation between percent correct and light-on latencies on inhibition (black) and control (gray) days. For all graphs, lines indicate the averages of individual rats across each condition (control and inhibition, n=10); bar graphs represent the average across all sessions within each condition. Asterisks indicate significance ($p < 0.05$); ANOVAs and post-hoc t tests ($p < 0.05$) were used to determine differences between inhibition and control days.

Not only did rats initiate trials less frequently, but they were also slower to respond to the houselights when they did initiate trials (i.e., nose poking upon houselight illumination). *Figure 4.1B* illustrates the latency at which rats responded to the houselights on initiated trials (i.e., epoch between houselight onset and rat entry into the central odor port within the 5s of houselight illumination; *Figure 2*: LO latency) during inhibition (right) and control (left) sessions. In our previous research, we have found that this measure serves as a proxy for how strongly attentional processes are being engaged (Bryden et al., 2011; Roesch et al., 2012; Vázquez et al., 2020). On average, during inhibition sessions latencies were significantly longer, indicating that rats were slower to initiate trials when the ACC was inhibited ($t_{(173)} = 2.80$, $p = 0.006$).

So far, we have shown that rats are slow to initiate trials, and did so less frequently on inhibition days; additionally, ACC inhibition significantly diminished trial completion. *Figure 4.1C* plots the percentage of completed trials – once initiated – during inhibition (right) and control (left) sessions. Here, we define completed trials as trials in which rats executed the entire behavioral sequence to completion—maintaining their nose-poke in the central odor port for 1s, and then responding to one of the two fluid wells (regardless of whether correctly or incorrectly) within the allotted 3s for trial completion (*Figure 2*: nosepoke to well-entry). During inhibition sessions, there were significantly more failures to complete trials ($t_{(173)} = 2.87$, $p = 0.005$). As a result, during inhibition sessions, rats received fewer rewards (*Figure 4.1D*; $t_{(168)} = 3.46$, $p < 0.0001$) and finished fewer sessions (inhibition = 36 (45%); control = 58 (65%); $\chi 2 = 7.10$, $p < 0.01$).

Additionally, ACC inhibition had a deleterious impact on forced-choice trial accuracy. Recall that during forced-choice trials, rats must adhere to the learned stimulus-response contingency regardless of the reward value that it produced. *Figure 4.1E* plots the average percent correct over all forced-choice trials during inhibition and control sessions. On inhibition days, rats performed significantly worse (*Figure 4.1E*; $t_{(168)} = 4.23$, $p < 0.0001$).

Inhibition did not impact motivation, motor control, nor the ability to perform the task, but it impacted attention

To ensure that the observed effects of ACC inhibition reducing task involvement and impacting performance was not due to a generalized impact on motivation to perform the task or motor control, we analyzed the time it took the rat to move across the different trial epochs during inhibition and control sessions averaged over trial-types. Reaction time is a measure the amount of time rats took to exit the odor port upon odor offset (*Figure 2*: RT), and movement times measured the epoch from odor port exit to well entry (*Figure 2*: MT). Their reaction and movement times on inhibition days were not significantly different from their reaction and movement times on control days (*Figure 4.2F-G;* reaction times: $t_{(168)} = 1.31$, $p = 0.19$; movement times: $t_{(168)} = 0.95$, $p = 0.34$), demonstrating that ACC inhibition was not impacting motivation to perform the task (e.g., their latency to approach the well for reward was indistinguishable on control vs. inhibition days) or motor control itself (e.g. their movement times were not impacted by inhibition).

Lastly, our previous study found that behavioral measures of attention (the aforementioned "light-on latencies") were associated with better forced-choice performance across rats (Vázquez et al., 2020). Thus, here too we assessed whether there was a correlation

between light-on latencies and forced-choice performance; we found that faster latencies were associated with better forced-choice performance, with no differences between manipulation (*Figure 4.2H*; control: $p < 0.01$; $r = -0.31$; inhibition: $p < 0.0001$; $r = -0.41$; Fisher r-to-z transformation: $z = 0.74$; $p = 0.46$)—replicating our previous findings that heightened attention is conducive to better performance.

In summary, ACC inhibition diminished and slowed the ability of rats to initiate and complete trials; it also impaired their accuracy on the task, as evinced by diminished forced-choice accuracy during inhibition sessions. Importantly, reaction and movement times averaged over all trial-types and choices were unaffected by ACC inhibition, suggesting that the changes in behavior are not related to impaired motor control or decreased motivation.

ACC inhibition disrupted free-choice selection

Following these findings, we sought to break down behavior by learning phase and reward value manipulations. To do so, we then proceeded to analyze completed sessions only (i.e., sessions in which all four blocks were completed; inhibition = 36; control = 58).

During performance of the reward-guided decision-making task described previously (*Figure 2*), we independently manipulate reward value across two dimensions; during the first two blocks, rats choose between an immediate or delayed reward, and during the last two blocks, rats choose between a large or small reward. During the first block of trials, optimal responding requires that rats track which response direction yields the short delay, and select that direction on free-choice trials (i.e. original association). After sixty trials, the location of the short delay switches to the opposite side. Thus, in order to respond optimally, rats must detect errors in reward prediction and update their free-choice behavior accordingly (i.e., reversal of originally

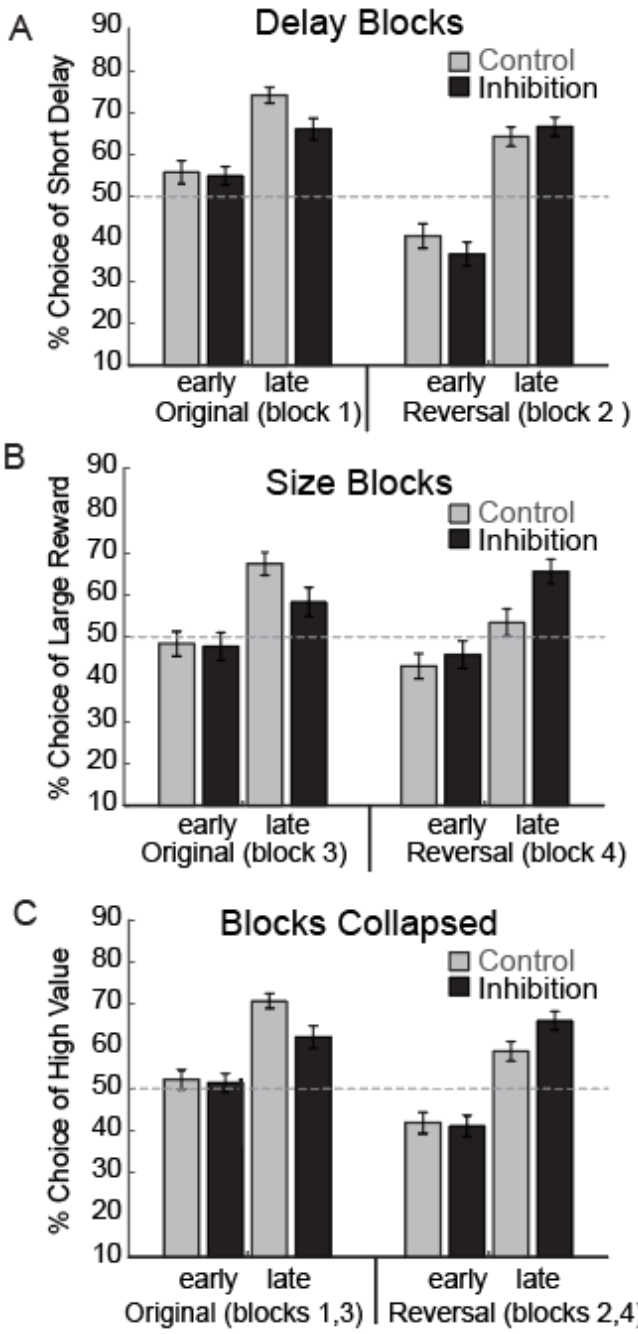

Figure 4.2: Breakdown of free choice behavior on completed trials. A) Percentage of times rats chose short-delay reward on free-choice trials during completed sessions, broken down by learning phase (the first ten trials in a block, e.g. "early" vs. the last ten trials in a block, e.g. "late in the block once contingencies have been learned) and side-value contingencies. We refer to the side-value contingencies defined in the first block of trials (which are repeated in the third block of trials) as the "original" learned associations (e.g. blocks 1&3). In the second block of trials, these contingencies were interchanged (Block 2). As this is a reversal of the originally learned contingencies, we refer to the side-value contingencies defined in the second block of trials (which are repeated in the fourth block of trials) as the "reversal" of the originally learned associations (e.g. blocks 2&4) B) Percentage of times rats chose large reward on free-choice trials during completed sessions, broken down by learning phase (early in session vs. late in session once contingencies have been learned) and contingencies (originally learned contingencies vs. contingency reversal). C) Percentage of times rats chose high-value reward (i.e. collapsing A&B) during completed sessions, broken down by learning phase (early in session vs. late in session once contingencies have been learned) and contingencies (originally learned contingencies vs. contingency reversal).

learned contingencies). During the third block of trials, the direction that produced the long delay now produces a large reward, while the reward on the other side remains the same size. As a result, rats must switch their selection bias back in the original direction in order to obtain high-value reward more often. Finally, in the fourth block, side-value contingencies switch for the last time.

We have previously shown that by the end of each trial block, rats learn to bias their behavior on free-choice trials towards high-value rewards, and perform worse during reversals—reflecting the difficulty of overriding the originally learned associations (Bryden et al., 2011; Vázquez et al., 2020). Here too, we see that during both inhibition and control sessions, rats selected high value rewards more often during the last ten free-choice trials within a block (i.e., late) compared to the first ten free-choice trials in the block (i.e., early). We found a main effect of phase ($F(1,736) = 73.2$, $p < 0.0001$) in a four factor ANOVA with manipulation (control vs. inhibition), block type (delay vs. size), reversal (original vs. reversal) and phase (early vs. late in learning) as factors. This is illustrated in Figure 4.2, which plots the percentage of times rats chose high value reward on free-choice trials across each of the four trial blocks in the order that they were run (i.e., blocks 1-4). Interestingly, during blocks of trials where delay was manipulated (delay blocks; *Figure 4.2A*), rats chose short-delay less on inhibition days (black bars) compared to control days by the end of block one (i.e., 'original') but not block two (i.e., 'reversal'). We observed similar effects during blocks of trials where size was manipulated ("original" size blocks; *Figure 4.2B*); however, when the size contingencies reversed, selection of the large reward was higher during inhibition. Thus, as expected, during control sessions, discrimination performance was better than reversals; however, during reversals, the opposite tended to be true. These results are supported by a significant interaction between manipulation, phase and reversal in the ANOVA ($F(1,736) = 4.56$, $p = 0.0331$), but no significant main effect ($F(1,736) = 2.50$, $p = 0.1144$) or interaction effects with block-type ($F(1,736)$'s < 1.59, p's > 0.2095).

Since there were no significant effects of block-type, we then collapsed data across delay and size blocks (*Figure 4.2C*) to further illustrate that during inhibition days, rats selected high-value reward significantly less by the end of trial blocks during the original discrimination (ttest; $t_{(186)} = 2.2346$, $p = 0.0266$), but this effect disappeared following contingency reversals (ttest; $t_{(186)} = 1.87$, $p = 0.0716$).

Forced-choice behavior was not significantly impacted during sessions that were fully completed

During forced-choice trials, rats had to respond in the cued direction in order to receive reward (i.e. correct trial); if rats responded in the incorrect well, no reward was delivered (i.e., error trial). As a reminder—when analyzing across all sessions, we found that inhibition disrupted forced-choice behavior (*Figure 4.2E*). However, in order to be able to analyze our data by learning phase and reward value manipulations, we then conducted analyses across only completed sessions (inhibition = 36; control = 58). We found that—when solely analyzing sessions that rats were able to complete—there were no significant differences in performance on forced-choice trials. As previously reported, we found that rats tended to be more and less accurate on high- and low-value forced choice trials, respectively (main effect of value: $F(1,1472) = 77.1$, $p < 0.0001$; *Figures 4.3A&B*; solid vs. dashed). This presumably reflects their perseverance towards the fluid well associated with the more favorable reward, further demonstrating their awareness of block contingencies. As with free-choice behavior, these contingency differences are learned within blocks, and the value preferences of the rat are thus established by the end of the blocks (interaction effect between phase and value: $F(1, 1472) = 81.79$, $p < 0.0001$). Also noteworthy is that rats performed better in the context of size blocks—presumably because they are more motivated by obtaining larger rewards and having no delays

(main effect of block-type: $F(1, 1472) = 30.89$, $p < 0.0001$). Thus—similar to our findings in previous studies—we demonstrate that by the end of blocks, rats performed high-value forced-choice trials with higher accuracy, and perform better during size blocks (Bryden et al., 2011; Chapter II). Remarkably, there was no significant main effect of inhibition ($F(1,1472) = 0.81$, $p = 0.37$), nor interactions between manipulation and value ($F(1,1472) = 1.38$, $p = 0.24$), phase ($F(1,1472) = 0.40$, $p = 0.53$) or block-type ($F(1,1472) = 2.91$, $p = 0.09$). Thus, when considering only completed sessions, accuracy on forced-choice trials was not impacted by inhibition. This suggests that the observed effects on accuracy across all sessions (*Figure 4.2E*) were driven by session completion—a

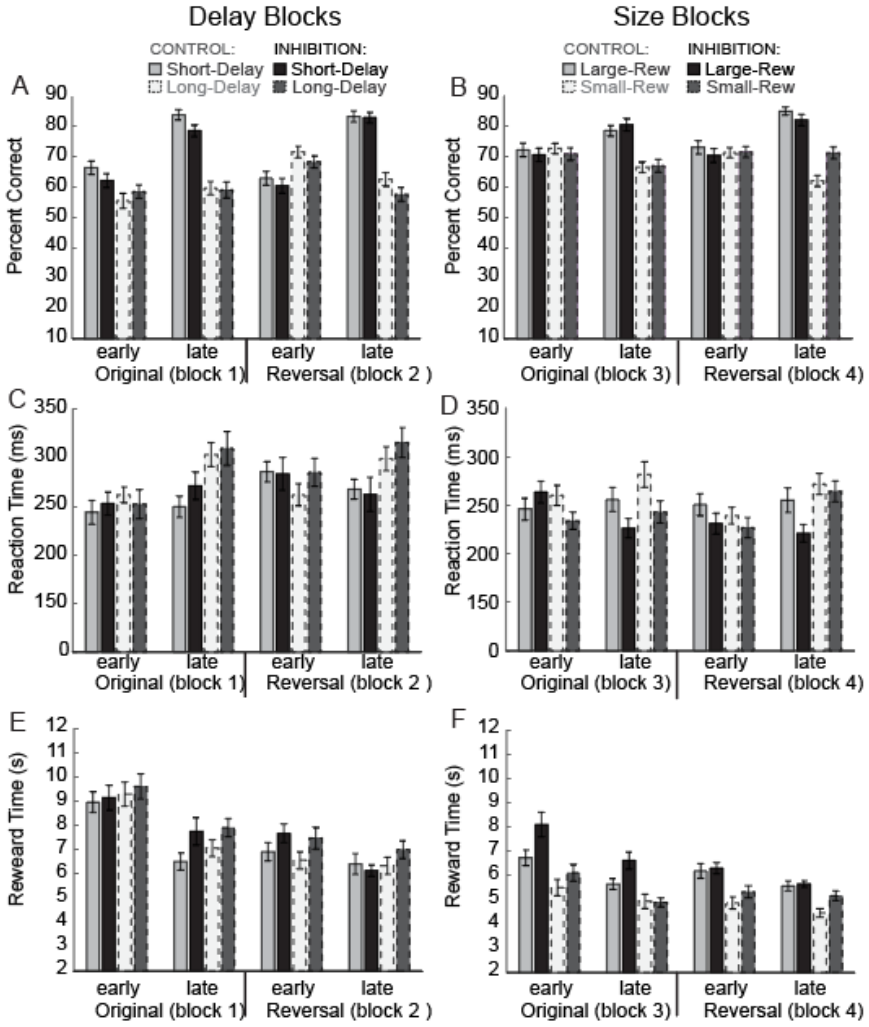

Figure 4.3: Behavioral analyses across completed sessions, with behavior during delay blocks on the left column of graphs, and size blocks on the right column. Analyses are broken down by learning phase (early in session vs. late in session once contingencies have been learned) and contingencies (originally learned contingencies vs. contingency reversal). A&B) Average percent correct on forced-choice trials during completed sessions. C&D) Average reaction times during completed sessions. E&F) Average time spent consuming reward during completed sessions.

finding which is also consistent with *Figure 4.2H,* wherein higher heightened attention is conducive to higher accuracy.

ACC inhibition did not reduce the motivational modulation of reaction time

Similar to accuracy on forced-choice trials, reaction times (odor offset to odor port exit) can reflect motivational biases towards more favorable reward (*Figure 4.3C&D*). That is, as rats develop behavioral biases towards fluid wells that are associated with higher-valued reward, reaction times become faster and slower for responses into high- and low-value wells, respectively (main effect of phase ($F(1,1472) = 4.91$, $p = 0.0269$) and value ($F(1,1472) = 6.05$, $p = 0.014$), and these factors interact ($F(1,1472) = 10.22$, $p = 0.0014$)). Further, rats become faster overall during size blocks compared to delay blocks. Interestingly, rats were even faster on "small" reward trials, which are identical to "short" delay trials (i.e. one sucrose bolus, no delay), suggesting that size blocks are generally more motivating (main effect of block-type: $F(1,1472) = 19.52$, $p < 0.0001$). Notably, the task design is constructed in this way to promote completion of all four trial blocks.

As above for percent correct, we analyzed completed sessions only and found that inactivation of the ACC did not globally impact reaction times—as evidenced by no main effect of inhibition ($F(1,1472) = 0.84$, $p = 0.3591$)—nor did it impact the effect that reward value had on reaction times (no interaction between manipulation and value ($F(1,1472) > 0.0001$, $p = 0.9509$). The only significant factor that included manipulation (control vs. inhibition) was the interaction between inhibition and block-type ($F(1,1472) = 4.54$, $p = 0.0332$), which can be explained by overall faster reaction times during size compared to delay blocks. With that said, post-hoc comparisons conducted for individual trial types produced no significant effects (ttests

comparing control vs. inhibition for early and late phases, and short, long, large and small trial-types; $t_{(92)}$'s > 1.46, p's > 0.1466) suggesting that the interaction emerged from both a slowing and acceleration of reaction times in delay and size blocks, respectively.

ACC inhibition did not reduce time spent consuming reward

Finally, it might be argued that ACC inactivation decreases the desirability or motivational value associated with reward. If true, then rats might spend less time consuming reward after delivery, especially late in sessions. To address this issue, we plotted time spent in the well after reward delivery (i.e., reward delivery onset to well exit) for all four blocks in the order that they were performed (*Figure 4.3E&F*). As expected, the time spent in the well after the onset of reward delivery was longer for large compared to small reward (*Figure 4.3F*; solid vs. dashed). It is no surprise that it took rats longer to drink two drops of sucrose compared to one. However, and more interestingly, rats spent the most time in the well following reward delivery early in the first block of trials—when they first initiated the task, and thus were most thirsty (*Figure 4.3E*; 'Original'); this declined over the course of the four trial blocks (main effect of phase ($F(1,1472) = 33.47$, $p < 0.0001$) and block-type ($F(1,1472) = 98.91.54$, $p < 0.0001$)). Importantly, decreases in time spent in the well across the four trial blocks were not more prominent during ACC inhibition, as evidenced by no significant interactions between inhibition and phase ($F(1,1472) = 0.03$, $p = 0.8569$) or block-type ($F(1,1472) = 0.02$, $p = 0.8936$). Instead, we found a main effect of inhibition ($F(1,1472) = 9.64$, $p = 0.0019$), demonstrating that rats tended to spend more time in the well post-reward delivery when the ACC was inhibited. We speculate that this result reflects the rat's drive to consume as much

reward as possible given their difficulties in initiating and completing trials; regardless, at the very least it shows that ACC inhibition did not reduce the desirability of the reward.

Discussion

We have previously published two studies highlighting the role of the ACC in shifting the allocation of attentional resources towards behaviorally relevant stimuli following outcome expectancy violations, and how disruption of these attentional signals via cocaine self-administration leads to impairments in decision-making and cognitive flexibility (Bryden et al., 2011; Vázquez et al., 2020). In these studies, we showed that ACC firing was correlated with behavioral shifts in attention following unsigned prediction errors—as rats updated previously learned behavior-outcome contingencies. Additionally, we found that chronic cocaine self-administration attenuates attention-related ACC signals, which correlated with a variety of task impairments **(**Chapter II). From these results, we had hypothesized that ACC signaling must give rise to specialized functions related to attention and cognitive control that promote optimal reward-guided decision-making when contingencies change. Here, we tested this prediction by transiently inactivating the ACC using optogenetics. We found that ACC inactivation had a profound impact on the rats' basic abilities to initiate and complete trials, which resulted in fewer rewards and completed sessions overall. Importantly, reaction times, movement times, and reward consumption averaged across trial-types and choices were not impaired, suggesting that these deficits in the rats' ability to initiate, complete, and attend to the task were not due to motor control or motivational deficits.

Our main hypothesis going into this study was that the functional ACC was necessary for allocating attention to reward-predicting cues so that value contingencies can be learned,

consistent with studies that have implicated the ACC in reward-processing, conflict monitoring, error detection and the allocation of attention (Aarts et al., 2008; Barch, 2001; Botvinick et al., 2004; Braver, 2001; Carter et al., 1998; Hayden et al., 2011; Holroyd et al., 2004; Hyman et al., 2013; Kerns et al., 2004; Laubach et al., 2015; Newman et al., 2015; Soltani & Izquierdo, 2019; Totah et al., 2009; Weissman, 2004; Wu et al., 2017; Yeung et al., 2004). In our study, we found that ACC inactivation severely impeded continuous task performance—as demonstrated by reductions in trial initiations, as well as in diminished trial and session completions; in turn, this severely impaired task accuracy. Further, light-on latency—the measure of attention to the task we have used throughout our studies—was significantly slower on inhibition days; consistent with our previous research, performance on the task was correlated with this behavioral proxy of attention to the task (Chapter II; Bryden et al., 2011).

When analyzing across only completed sessions, the only deficits that we observed were on free-choice trials during the original learning, potentially reflecting weaker response-outcomes associations, which might stem from changes in attention (Krajbich et al., 2010, 2012; Nunez et al., 2017; Retzler et al., 2020).

Overall, the ACC seems to be critically involved in promoting the initiation and completion of trials and behavioral sessions, which might reflect general deficits in attention or motivation, or willingness to engage in a cognitively demanding task. Motivation and attention are sometimes difficult to disambiguate. Deficits in either could explain the observed decreased rates of trial initiation and completion. However, neither explain why there were inhibition sessions which rats were able to complete, and how during these completed sessions they were still able to reverse contingencies, respond appropriately on forced-choice trials, and show

stronger motivation on high value compared to low value reward trials (i.e., rats were better and faster on high value forced choice trials). Further, if anything, during completed inhibition sessions rats were faster during size blocks relative to delay blocks, and actually spent more time in the fluid well consuming reward compared to control sessions. Thus, there were sessions during which rats were able to attend to (i.e., respond to cues, reverse contingencies) and process motivational cues (i.e., better/faster for high value) during ACC inhibition. Although it is difficult to determine whether the observed deficits reflect general decreases in attention, it is clear that rats seem to be less engaged with the task during inhibition sessions.

Inhibition of the ACC resulted in deficits that were present throughout the entire session during both LED+ and LED- trials (i.e., did not differentially impact behavior on a trial-by-trial basis, but rather had a global impact on task engagement). Thus, it appears that inhibiting ACC on only 50% of trials is sufficient to place rats in a generally less engaged state. Studies using halorhodopsin have shown that it can enable prolonged hyperpolarization and suppress action potentials on a time-scale of minutes, with slow recovery from inactivation (Mahn et al., 2018; Zhang et al., 2019), which could also explain why behavioral effects were present during both LED on and off trials.

The lack of engagement throughout the task might reflect the inability to allocate cognitive resources to complete trials, but might also specifically reflect the inability to exert effort to complete a task that is cognitively demanding. It has been shown that rats with ACC lesions are less able to exert effort to obtain large reward (Holec et al., 2014; Hosking et al., 2014; Walton et al., 2003). For example, studies have found that rats with ACC lesions exhibit a decreased inclination to scale a physical barrier in order to acquire a large reward, opting instead

for a small reward that requires minimal effort to acquire (Holec et al., 2014; Walton et al., 2003); further, in separate case studies involving human subjects with bilateral ACC lesions, researchers found that these individuals exhibited motivational and attentional impairments, as well as akinetic mutism (Barris & Schuman, 1953; Laplane et al., 1981). Although our task does not require physical exertion, it is a cognitively demanding task in that rats must follow basic trial structure (i.e., respond when houselights are on, maintain hold, respond to one of the wells) and learn, track, and update response-outcome associations—which vary across two reward dimensions throughout four sixty-trial blocks—all while following forced-choice rules. Perhaps the ACC is particularly important when rats need to sustain attention in a task that requires considerable cognitive effort or complexity.

In summary, we found that ACC inhibition reduced trial initiation and both session and trial completion, and deleteriously impacted task performance without impacting reaction or movement times. Finally, choice selection during inhibition sessions was biased towards the direction associated with the originally learned response-outcome association. Overall, behavior on inhibition days was suboptimal, in that fewer trials were completed and better rewards were selected less often. While these results may reflect ACC's role in attention, we speculate that it reflects that the ACC is necessary to initiate and stay on task, especially during cognitively demanding tasks. Importantly and consistently, one of the hallmarks of ADHD —which has been associated with ACC hypofunction (Bush et al., 2005; Ernst et al., 2003; Konrad et al., 2006; Pliszka et al., 2006; Rubia et al., 1999; Zilverstand et al., 2017) and structural abnormalities (Makris et al., 2008, 2010; Qiu et al., 2011; Seidman et al., 2006; Vogt, 2019; Yu et al., 2023)–is

difficulty with task initiation and sustained engagement (Dieckhaus et al., 2021; Lauth et al., 2006; Mills et al., 2018).

Chapter IV: Anterior cingulate cortex lesions impair multiple facets of task engagement during reward-guided decision-making that are not mediated by deficits in downstream dorsomedial striatum firing

Daniela Vázquez[1,2], Norma Peña-Flores[1,2], Sean R. Maulhardt[1], Alec Solway[1,2], Caroline J. Charpentier[1,2] & Matthew R. Roesch[1,2]

[1]Department of Psychology, University of Maryland, College Park, MD 20742, USA

[2]Program in Neuroscience and Cognitive Science, University of Maryland, College Park, MD 20742, USA

Abstract

Previously, we found that cocaine self-administration attenuates attentional control processes and associated ACC firing; similarly, optogenetic inactivation of the ACC disrupts task engagement. However, these studies did not address whether these impairments impacted downstream action-outcome encoding, which is necessary for goal-directed behavior. Research suggests that the DMS is an important locus for the encoding of action-outcome representations that are imperatively implicated in goal-directed behavior and decision-making (Walton et al., 2003; Yin et al., 2005; Burton et al., 2015; Haber, 2016).

The ACC sends projections to the DMS, constituting part of the corticostriatal circuit—a circuit which has been extensively implicated in the development of goal-directed behaviors (Walton et al., 2003; Walton et al., 2009; Haber, 2016; Smith et al., 2021). However, there is little understanding of how DMS signaling during reward-guided decision-making might be impacted by disruptions in ACC function. In this study, we aimed to elucidate the role of the ACC in the modulation of downstream response-outcome encoding and action planning in the dorsomedial striatum.

To address this issue, rats were trained on a reward-guided decision-making task as described above; reward value was manipulated by independently varying the size of or the delay to reward over several trial blocks. Subsequently, we unilaterally injected either ibotenic acid (experimental group) or saline (control group) into the rat ACC, and rats performed the previously learned reward-guided decision-making task while we recorded from single neurons in DMS. We found that ACC lesions impacted several facets of task performance—including decreasing the initiation and completion of trials, slowing reaction times, and resulting in

suboptimal and inaccurate action selection. Reductions in movement times towards the end of behavioral sessions further suggested attenuations in motivation, which paralleled reductions in directional action selection signals in the DMS that were observed later in recording sessions. Surprisingly, however, beyond altered action signals late in sessions—neural correlates in the DMS were largely unaffected, even though behavior was disrupted at multiple levels. We conclude that ACC lesions result in overall deficits in task engagement that impact multiple facets of task performance during our reward-guided decision-making task, which—beyond impacting motivated action signals—arise from dysregulated attentional signals in the ACC and are not driven by disruptions to downstream action-outcome encoding.

Introduction

Cognitive control is necessary for complex goal-directed thought and adaptive decision-making, and deficits in cognitive control are hallmarks of neuropsychiatric and substance abuse disorders. As described above, the ACC plays an important role across a plethora of cognitive functions (Rescorla & Wagner, 1973; D'Esposito et al., 1995; Carter et al., 1998; Botvinick et al., 1999; Dayan, Kakade, & Montague, 2000; Paus, 2001; van Veen & Carter, 2002; Botvinick, Cohen, & Carter, 2004; Holroyd et al., 2004; Kerns et al., 2004; Weissman et al., 2004; Koob & Volkow, 2010; Bryden et al., 2011; Hayden et al., 2011; Narayanan et al., 2013; Laubach et al., 2015; Bryden et al., 2019; Soltani & Izquierdo, 2019; Stolyrova et al., 2019; Brockett et al., 2020; Schneider et al., 2020; Vázquez et al., 2020; Brockett & Roesch, 2021; Vázquez, Schneider, & Roesch, 2022). Previous research has identified a putative circuit between the ACC and DMS, wherein the ACC provides ipsilateral input to the DMS (Hunnicutt et al., 2016; Choi et al., 2019). However, in spite of the extensive literature implicating the DMS in action planning

and action-outcome encoding—characterizing this striatal subregion as primordially important for goal-directed decision-making—there is currently limited understanding of how the ACC impacts downstream DMS signaling during reward-guided decision-making.

In order to address whether more biologically relevant disruptions to ACC firing would replicate our optogenetic inactivation findings—as well as assess how these disruptions might impact downstream action-outcome encoding—we performed chemical lesions of the ACC, and recorded neural activity from the dorsomedial striatum DMS. Optogenetic inhibition might have resulted in incomplete network perturbation—allowing for a fraction of unaffected ACC neurons to sustain certain functions, but not others. Further, task-related neural correlates in the ACC have been observed in trial epochs outside of the window during which we inactivated the ACC. Specifically, it is known that the ACC also fires in response to rewards, and during errors of commission and omission (Totah et al., 2009; Bryden et al., 2011; Shen et al., 2015); thus, more complete lesions might uncover other functions that the ACC is critical for. With that said, it has also been argued that chronic perturbation induced by lesions, unlike optogenetic inhibition, might allow for compensatory mechanisms that mask the effects of inactivation (Lee et al., 2020; Murphy & Corbett, 2009; Whishaw, 2000).

Here, we address these issues by chemically lesioning the ACC and subsequently recording neural firing in the dorsomedial striatum (DMS) as rats perform the same task we used in our aforementioned studies (for a more comprehensive breakdown of the behavioral paradigm, see *Figure 2*)—a task consisting of four sixty-trial blocks, during which rats choose between differently delayed or sized rewards. It is known that firing in the DMS encodes predicted outcomes and action selection (Walton et al., 2003; Yin et al., 2005; Burton et al., 2015; Haber,

2016)—thus, in addition to obtaining behavioral readouts related to these functions, recording from the DMS provides an additional measure that allows us to assess the downstream impact of ACC signaling disruptions. Further, we can determine whether observed changes in behavior following ACC damage are mediated through the DMS.

We found that—much like we previously observed during optogenetic inhibition (Chapter III)—ACC lesions impaired task engagement (i.e., rats were slower to initiate trials and completed fewer trials overall) and choice selection on free-choice trials. However, rats were also impaired across other facets of the task—including reaction times, reversal learning, and accuracy on forced-choice trials. Further, rats with lesions appeared less motivated towards the end of recording sessions, as evidenced by slower movement speeds. Consistent with this behavior, we observed reduced directional encoding in the DMS at the end of recording sessions during manipulations of reward size. Surprisingly, we did not find widespread effects of ACC lesions on DMS firing, suggesting that the robust behavioral impairments we observed were not mediated through disruptions in DMS action-outcome encoding, but rather driven by the reductions in task engagement. We conclude that ACC lesions impact multiple facets of task performance that are only partially mediated through the DMS; these results support our previous findings that ACC signaling disruptions result in task engagement reductions that are necessary for optimal decision-making to occur.

Methods

Subjects

Male and female Long–Evans rats (control: n = 6; 5M males, 1F; lesion: n = 6; 5M, 1F) were obtained at ~2–3 months of age from Charles River Laboratories, weighing in the range of

150–200 g. Rats were tested at the University of Maryland (UMD), College Park in accordance with UMD and NIH guidelines. During behavioral testing, food was available ad libitum; water intake was restricted to ensure motivation for task performance.

Surgery

We unilaterally injected ibotenic acid—an excitotoxic, non-selective glutamate receptor agonist commonly used in inactivation studies (Winn, Tarbuck, & Dunnett, 1984; Jarrard, 1989; Morris et al., 1990; Newman & McGaughy, 2011; Newman, Creer, & McGaughy, 2014; Martínez-Torres, 2021)—into the ACC of the experimental group (n = 6); control rats (n = 6) received saline injections. Rats received two unilateral stereotactic injections spaced 1mm a part targeting the ACC (Injection 1: AP: +0.2 mm; ML: ±0.5 mm; DV: -2.2 mm; Injection 2: AP: +1.2 mm; ML: ±0.5 mm; DV: -2.2 mm). Coordinates were chosen based on previous optogenetic and recording studies targeting the same area (Bryden et al., 2011; Chapters II-III). For each injection site, a beveled 33 gage 5 µl Neuros Syringe (Hamilton) was lowered slowly over the course of five minutes to its final depth, with the bevel of the needle positioned away from the midline of the brain (Brockett et al., 2020). Rats received unilateral infusions of either 0.2 µl of 0.6M ibotenic acid in saline (experimental group), or 0.2 µl of 0.9% saline (controls) per site over the course of 3 minutes (approximately 125 nl/ minute). Needles were left in place for 5 minutes before being slowly withdrawn—over the course of an additional 5 minutes—in order to minimize the risk of tissue damage and backflow. Holes were loosely filled with sterile bonewax prior to beginning electrode implantation.

Unilateral electrode implantation procedures were carried out after stereotaxic injection, and electrodes were implanted ipsilaterally to the hemisphere that was targeted by injection.

Hemispheres were counterbalanced across groups, and the methods for implantation have been previously described in detail (Bryden et al., 2012; Brockett et al., 2022). Rats were chronically implanted with a drivable bundle of ten 25 µm diameter FeNiCr wires (Stablohm 675, California Fine Wire, Grover Beach, CA) into either the left or right hemisphere of the dorsal medial striatum (DMS) using the following coordinates relative to bregma (AP: -0.4 mm; ML: ±2.4 mm; DV: -3.5 mm). Coordinates were chosen based on our previous results investigating the role of DMS in rats performing the aforementioned reward-guided decision-making task (Burton et al., 2017; Burton et al., 2018). Immediately prior to implantation, wires were freshly cut with surgical scissors to extend ~1 mm beyond the cannula, and were electroplated with platinum (H2Cl6Pt) to an impedance of ~300kOhms. Cephalexin (15 mg/kg p.o.) was administered twice daily for seven days following surgery in order to prevent infection.

Histology

Following the completion of testing, rats were overdosed on isoflurane and transcardially perfused with 4% paraformaldehyde (PFA). Following perfusion, the electrode assembly was removed from the skull, and the brain was extracted. Brains were post-fixed for 48 hours in 4% PFA, before being moved to a 30% sucrose solution for cryoprotection. Following cryoprotection brains were blocked, flash frozen in alcohol, and sectioned on a freezing microtome. 40 µm coronal sections were cut throughout the extent of ACC and DMS. Sections were collected, mounted to positively charged Superfrost slides, and underwent Nissl staining. Slides were viewed under a light microscope, and the extent of the lesion and presence or absence of electrode tracks were verified and cross-referenced with score sheets demarcating

electrode assembly advancement. Lesion and electrode tracks were traced onto coordinate matched printouts of stereotaxic space.

Behavioral Analysis

Behavior was analyzed by calculating the percent of correct responses on forced-choice trials (when the animal responded to the well signaled by the directional odor cue), the percentage of times rats chose a particular valued (short, long, big, small) condition during free-choice trials, reaction times for both free- and forced-choice trials (time from odor offset to odor port exit), movement times for both free- and forced-choice trials (odor port exit to fluid well entry), and light-on latency times collapsed across size and delay blocks (from houselight illumination—indicating start of trial—to odor port nose-poke). Light-on latency was calculated this way because it occurs during an epoch which precedes odor presentation, so it cannot be split by reward value. Calculations on forced-choice trials were split into the first and last ten trials of each trial type within each block. Our previous work has demonstrated that this analysis allows us to capture learning as it occurs at the beginning of trial blocks, and provides a large sample to conduct neural and behavioral statistics. Free-choice movement and reaction times were not split into early and late trials due to a lower amount of trials that could be analyzed (e.g. a fewer amount of low-value choices made later in trial blocks). Behavioral analyses were conducted for individual sessions (split by control and lesion groups), and then averaged across sessions within each group. Multifactor analysis of variance (ANOVA) factors included treatment group (control vs. lesion), reward value (high vs. low), value manipulation (size vs. delay), and block phase (first vs. last ten trials per trial type within a block). We used *t*-tests ($p < 0.05$) to determine differences between the control and lesion group sessions.

Neural Analysis

The procedures for conducting single-unit recordings have been described previously (Bryden et al., 2011; Burton et al., 2018; Vázquez et al., 2020). Wires were screened daily for activity; if no activity was detected, rats were removed from the testing box, and the electrode assembly was advanced 40-80 µm. If activity was detected, the session occurred and the electrode assembly was advanced 40-80 µm at the end of testing. Neural activity was recorded using four identical Plexon Multichannel Acquisition Processor systems (Plexon Inc). Signals from electrode wires were amplified 20x by an op-amp headstage located on the electrode array. Immediately outside the testing chamber, signals were passed through a differential pre-amplifier (Plexon Inc) where single units 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. For neural analyses, single units were sorted using template matching (Plexon, Offline Sorter), and exported to NeuroExplorer and Matlab. Increasing and decreasing cells were identified as cells whose activity significantly increased or decreased, respectively, during the epoch from odor cue onset to well entry (trial epoch) relative to their baseline firing rate (1s preceding odor onset, Wilcoxon; $p < 0.05$). Wilcoxons (rank-sum; $p < 0.05$) were used to measure significant shifts in the distribution of indices from zero, and to determine differences between control and lesion groups. Chi-squares ($p < 0.05$) were performed on counts of neurons in each group to determine whether there were any significant differences between groups.

Results

ACC lesions impaired multiple facets of task performance

Overall, lesioned rats took significantly longer to complete sessions ($t_{(372)} = 5.64$, $p < 0.00001$; control average time per session: 1.80 hours, lesioned: 2.08 hours), and were significantly less engaged in the task—initiating trials with less frequency than controls (*Figure 5.1A*; $t_{(372)} = 4.94$, $p < 0.00001$) and with slower latencies (*Figure 5.1B*; $t_{(372)} = 3.22$, $p < 0.001$). Further, across all trial-types, lesioned rats performed significantly worse on forced-choice trials compared to controls ($t_{(372)} = 4.02$, $p < 0.0001$). Due to these factors, lesioned rats had to carry out significantly more trials than control rats on average (controls: $\mu = 275.7$ trials, lesion: $\mu = 284.8$ trials) in order to complete a session ($t_{(370)} = 1.97$, $p < 0.01$).

As previously reported (Roesch, Taylor, & Schoenbaum, 2006; Roesch et al., 2009; Roesch & Bryden, 2011; Bryden et al., 2011; Vázquez et al., 2020), both groups chose short-delay over long-delay, and large-reward over small reward on free choice trials; however, rats with ACC lesions selected high-value rewards less often than controls. Difference scores calculating the percentage of free-choice trials where rats chose high-valued vs low-valued rewards showed that control rats chose high-valued rewards more often than lesion rats across

both block types (*Figure 5.1C*; short delays: $t_{(372)} = 2.28$, $p < 0.05$; big rewards: $t_{(372)} = 2.00$, $p < 0.05$). On average, control rats chose the high-value reward 6.39% more often than lesion rats.

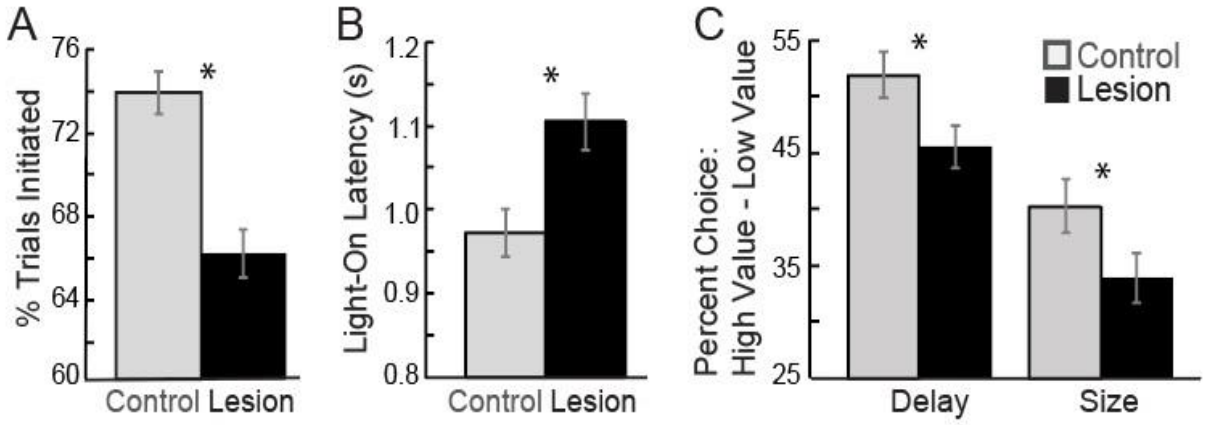

Figure 5.1: Behavioral analyses across sessions. Lesioned rats were slow to initiate trials, initiated fewer trials and chose high value reward less often. a) Percentage of trials initiated by lesion and control rats. In order to initiate a trial, rats had to nose poke into the odor port for approximately 500 ms once the house lights came on. If rats did not nose poke, the trial would time out. b) Average light-on latency denotes the latency to enter odor port upon house-light illumination (which indicates the beginning of a new trial). c) Percentage of times rats chose high-value reward on free-choice trials, split by block types.

During forced-choice trials, rats had to respond in the cued direction in order to receive reward (i.e. correct trial); if the rats responded in the opposite direction, no reward was delivered (i.e., error trial). Although rats performed well on forced-choice trials, their performance and reaction times (RT; odor offset to odor port exit) were swayed by the location of the more favorable reward (i.e. short or large). That is, rats tended to perform better and worse on high- and low-value forced choice trials, respectively. Consistent with previous reports, we found that both groups performed better and were faster on high- compared to low-value forced-choice trials (*Figure 5.2A* and *C*; Main effect of value for percent correct: $F(1,5968) = 284.23$, $p < 0.0001$; interaction between reward value and phase for percent correct: $F(1,5968) = 153.25$, $p < 0.0001$; interaction between group and block for percent correct: $F(1,5968) = 4.97$, $p < 0.05$;

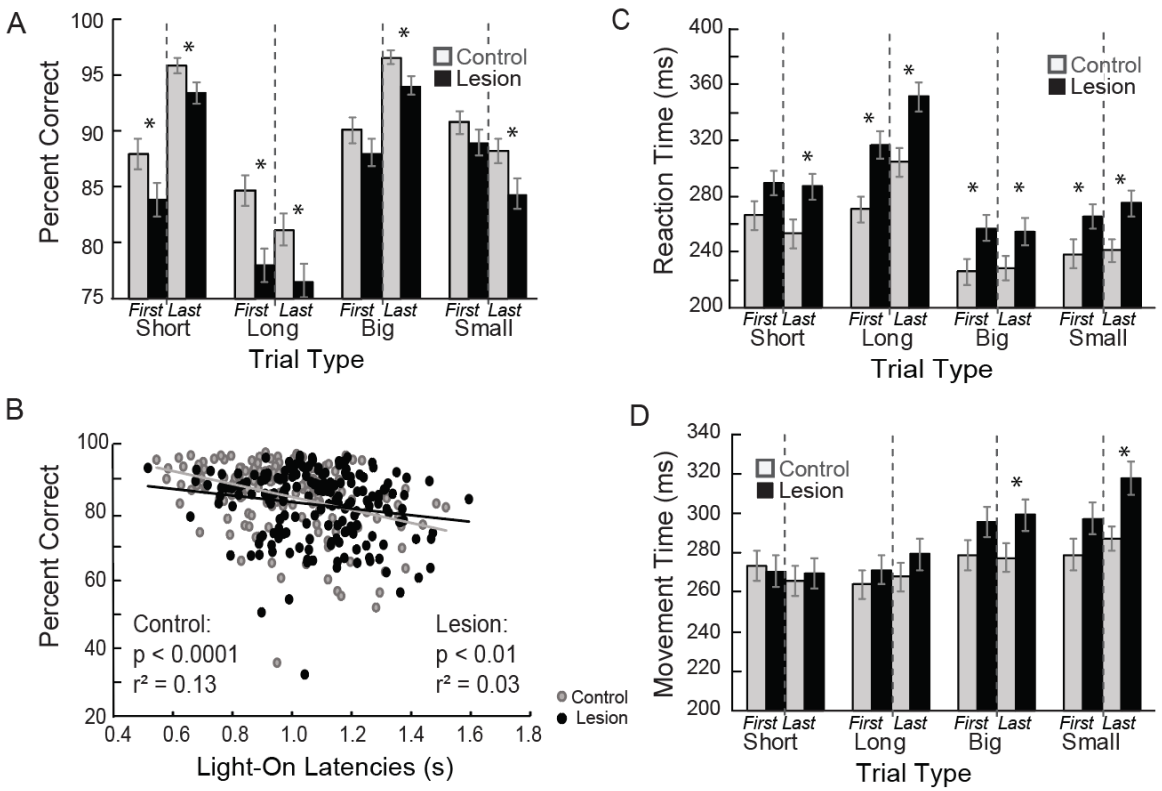

Figure 5.2: Forced-choice trials analyses by trial type across sessions. Lesion rats were slower and less accurate. a) Percentage of times rats accurately responded during different trial types. b) Correlation between light-on latencies and percent correct. c) Reaction time (odor port exit – odor offset) on forced-choice trials for each value manipulation. d) Movement times (time from odor port exit to fluid well entry) on forced-choice trials for each value manipulation.

main effect of value for reaction time: $F(1,5968) = 49.2$, $p < 0.0001$; interaction between reward value and phase for RT: $F(1,5968) = 11.21$, $p < 0.001$). However, we also found main effects of group for both accuracy and reaction time, indicating that lesion rats performed significantly worse and were slower over all trial-types (Main effect of group for percent correct: $F(1,5968) = 67.68$, $p < 0.0001$; main effect of group for RT: $F(1,5968) = 86.56$, $p < 0.0001$).

Our previous research has revealed consistent correlations between behavioral measures of attention—specifically the aforementioned "light-on latencies"—and enhanced forced-choice performance across rats (Vázquez et al., 2020; Vázquez et al., in press). Here, we replicated that effect–finding that faster latencies were correlated to higher forced-choice trial accuracy, with no

significant differences between groups (*Figure 5.2B*; control: $r^2 = 0.13$, $p < 0.0001$; lesion: $r^2 = 0.03$, $p < 0.01$; Fisher r-to-z transformation: $z = 1.90$; $p = 0.06$). Overall, this suggests that decreases in attention might contribute to worse performance.

Lastly, we found that lesions made rats slower to move from the odor port to the fluid well (MT = movement time). Notably, both groups were slower during size-blocks compared to delay-blocks, however rats with ACC lesions were significantly slower than controls in size blocks (*Figure 5.2C*). Consistent with this observation, we found a significant main effect of group ($F(1,5968) = 22.5$, $p < 0.0001$; main effect of block: $F(1,5968) = 56.57$, $p < 0.0001$; and interaction effects of group and block: $F(1,5968) = 9.02$, $p < 0.005$), suggesting that rats with ACC lesions were less motivated to perform the task later in sessions.

Overall, ACC lesions had a deleterious impact on behavior. General measures of attention and task engagement were diminished, as well as the ability to select the better reward on free-choice trials in delay and size trial blocks. Lesioned rats also displayed significant deficits in task performance on force-choice trials (i.e., worse and slower), and decreases in motivation toward the end of trial blocks, consistent with longer session times and fewer trials initiated.

ACC lesions attenuated directional tuning in the DMS during large reward trials

Single neurons in the DMS have been shown to increase or decrease firing during task performance (Burton et al., 2014, 2015; Stalnaker, 2010). Consistent with these reports, we found that 15.7% (n=235) and 16.9% (n=208) of neurons in control and lesion groups increased firing, and that 44.8% (n=669) and 42.6% (n=527) decreased firing, respectively (Wilcoxon's; p

< 0.05). ACC lesions did not impact the frequency of increasing and decreasing cells (Increasing: $\chi^2 = 0.35$, $p = 0.55$; Decreasing: $\chi^2 = 0.47$, $p = 0.49$).

Figure 5.3 illustrates average firing over all increasing (*Figure 5.3A-D*) and decreasing (*Figure 5.3E-H*) cells. Previous reports have shown that neurons in the DMS tend to fire selectively for specific outcomes and response directions; thus, to make this plot we sorted trials based on preferred trial-type according to which condition produced the maximal firing response (Burton et al., 2014, 2015; Stalnaker, 2010). The remaining trials were categorized relative to the preferred trial type—depending on whether the response and outcome occurred in the same or opposite directions, and whether the outcome was the same or opposite value for both manipulations (delay and size). For example, if a neuron fired maximally for large-reward trials to the right, then large reward and right became the preferred outcome and direction, small and left became the non-preferred outcome and direction, and short and long became outcomes of the same and opposite value (e.g. high and low value), respectively. Thus, in *Figure 5.3*, trials are sorted based on which reward outcome produced maximal firing (*blue lines: "preferred")* and its counterpart in the same value manipulation (*red: "nonpreferred"),* as well as firing to rewards of the same (green) or opposite (yellow) value, for both preferred and nonpreferred response directions. The purpose of presenting average population firing in this way is twofold. First, we can appreciate that the overall pattern of firing is similar between controls and lesions—and that firing is selective, firing more strongly for one trial-type and response direction. Secondly, these qualitative plots with error bars can give us a feel for the strength and timing of firing selectivity across both populations. Overall, it appeared that the temporal pattern was similar across groups, but selectivity appeared attenuated after lesions for increasing cells.

To explore this difference, we quantified directional selectivity for each trial type (short, long, big and small), by computing the differences in firing rates for responses made into and away from the response field (i.e., directional index = (preferred – nonpreferred)/ (preferred + nonpreferred)) during the trial epoch (odor onset to fluid well entry). Here, we defined a

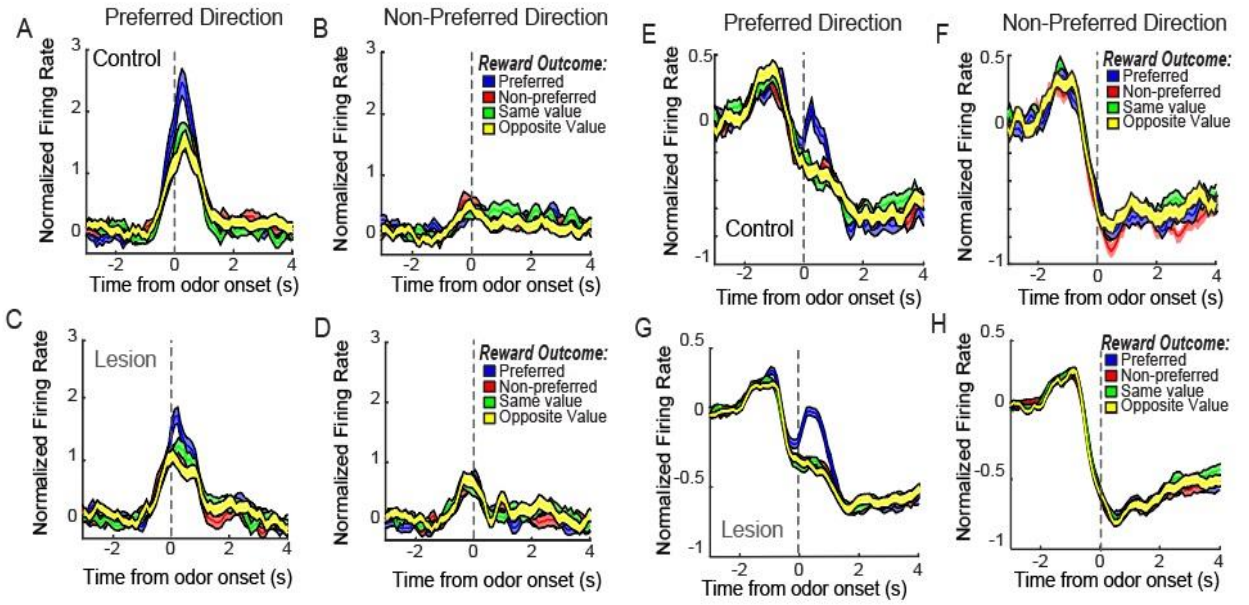

Figure 5.3: Population histograms. Activity of DMS neurons that increased (a-b) control: n = 235; c-d) lesion: n = 208) and decreased (e-f) control: n = 669; g-h) lesion: n = 527) firing in their preferred (left column) and non-preferred (right column) directions. Trials were sorted based on which reward outcome and response direction produced maximal firing (blue lines: "preferred"), firing to non-preferred reward (red), firing to rewards of the same value (yellow; e.g. in the case of preferential firing to short delay rewards, subsequent firing to large reward), and firing to rewards of the opposite value (green; e.g. in the case of preferential firing to short delay rewards, subsequent firing to small reward). Ribbons = SEM.

neuron's preferred direction as the direction that elicited the strongest firing collapsed across all trial-types, as a non-biased estimate of preferred direction. We then examined response direction separately for each reward type to determine whether the directional signal is stronger for trials where rats exerted more effort (i.e., high valued rewards), and if encoding differed between groups for each trial-type (i.e., short, long, big and small). In addition, at the single neuron level, we performed within-session comparisons to determine whether activity differed significantly

between directions (Wilcoxon; $p < 0.05$; black bars in distributions). Note, all analyses were performed at the end of trial blocks (last 10 trials for each trial-type) after learning had occurred (*Figure 5.6*). By assigning direction in this way, all distributions are shifted in the preferred direction—the critical question is whether or not directional index distributions are different between groups.

Significant differences were only observed on large-reward trials for the population of increasing cells. The distributions of directional indices computed on large-reward trials was significantly different between controls and lesions (*Figure 5.4;* $z = 2.01$; $p < 0.05$). Though this difference was present at the population level, the frequency of significant neurons only trended in that direction (control: 113 (48%)—black bars to the left of the distribution vs 2 (1%) cells to the right of the distribution; lesion: 90 (43%) vs 7 (3%); $\chi^2 = 2.65$; $p = 0.10$)—in other words, the overall attenuation in firing resulted from a shift in population-level firing, and was not driven by counts of significant single units.

Although the strength of the population directional signal was attenuated in rats with ACC lesions during performance of large-reward trials, the strength of the directional signal was unaffected for the other three trial-types: for short-delay trials, the distribution of directional indices was not significantly different between controls and lesions (*Figure 5.4;* $z = -1.34$; $p = 0.18$), and the frequency of significant neurons did not differ between groups (control: 102 cells—black bars to the left of the distribution vs. 10 cells to the right of the distribution; lesion:

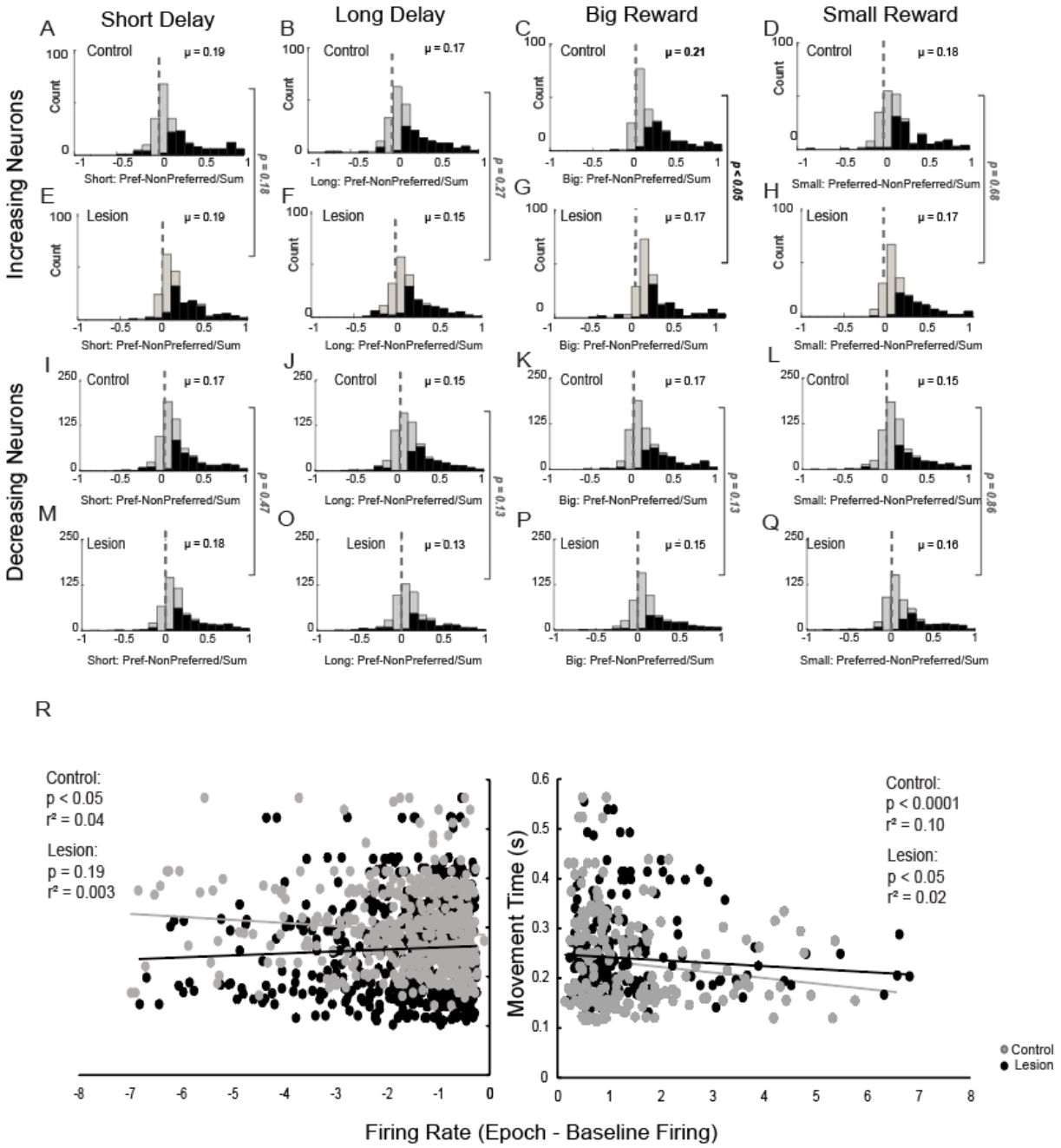

Figure 5.4: Lesions weakened directional signals during big reward trials as evidence by examining differences in firing for responses made into and away from the response field (preferred – nonpreferred)/(preferred + nonpreferred) for each trial type (short, long, large and small) for control (increasing cells: a-d; decreasing cells: i-l) and lesion (increasing cells: e-h; decreasing cells: m-q) rats. Black bars indicate individual neurons whose activity was significantly modulated by direction during the response epoch (Wilcoxon, $p < 0.05$). r) Correlation between firing rate and average movement times across sessions.

106 vs 5; $\chi^2 = 1.11$; $p = 0.29$). For long-delay trials, the distribution of directional indices was not

significantly different between controls and lesions (*Figure 5.4; z* = 1.10; *p* = 0.27), and the frequency of significant neurons did not differ between groups (control: 103--black bars to the left of the distribution vs 8; lesion: 90 vs 12; χ^2 = 0.82; *p* = 0.37). For small-reward trials, the distribution of directional indices was not significantly different between controls and lesions (*Figure 5.4; z* = 0.42; *p* = 0.68), and the frequency of significant neurons did not differ between groups (control: 106—black bars to the left of the distribution vs 7 cells to the right of the distribution; lesion: 88 vs 1; χ^2 = 2.16; *p = 0.14*).

As we did for increasing-type cells, we next quantified the strength of the directional signal for responses made into and away from the response field for each trial-type (short, long, large and small) for decreasing-type cells. We found no significant differences between groups for any trial-type. For short-delay trials, the distribution of directional indices was not significantly different between controls and lesions (*Figure 5.4; z* = -0.73; *p* = 0.47), and the frequency of significant neurons did not differ between groups (control: 269 cells—black bars to the left of the distribution vs. 25 cells to the right of the distribution; lesion: 217 vs 12; χ^2 = 1.62; *p* = 0.20). For long-delay trials, the distribution of directional indices was not significantly different between controls and lesions (*Figure 5.4; z* = 1.53; *p* = 0.13), and the frequency of significant neurons did not differ between groups (control: 245 vs 28; lesion: 170 vs 33; χ^2 = 3.23; *p* = 0.07). For large-reward trials, the distribution of directional indices was not significantly different between controls and lesions (*Figure 5.4; z* = 1.52; *p* = 0.13), and the frequency of significant neurons did not differ between groups (control: 263 vs. 11; lesion: 184 vs 13; χ^2 = 1.09; *p = 0.30*). For small-reward trials, the distribution of directional indices was not significantly different between controls and lesions (*Figure 5.4; z* = 0.17; *p* = 0.86), and the

frequency of significant neurons did not differ between groups *(*control: 235 vs. 15; lesion: 179 vs 15; $\chi^2 = 0.28$; $p = 0.60$).

Combined, these results suggest that firing decreases late in recording sessions might be related to the decreases in motivation evidenced by the slower movement times. To confirm this relationship, we plotted movement time against firing rate changes in increasing cells during the response epoch (*Figure 5.4R*). We found a significant negative correlation for both groups, and the correlations were significantly different from each other (*Figure 5.4R*; control: $p < 0.0001$; $r^2 = 0.10$; lesion: $p < 0.05$; $r^2 = 0.02$; Fisher r-to-z transformation: $z = 2.00$; $p < 0.05$). In decreasing-type cells, there was a significant correlation in control—but not lesion rats—(*Figure 5.4R*; control: $p < 0.05$; $r^2 = 0.04$; lesion: $p = 0.19$; $r^2 = 0.003$; Fisher r-to-z transformation: $z = 2.66$; $p < 0.01$). These findings indicate that slower movement times were indeed correlated with lower firing rates in increasing-type cells, suggesting that reduced signaling during size blocks is related to decreases in motivation.

Autoshaping—Behavioral deficits do not reflect disruptions to motivation or motor control

After our initial experiment was conducted, we trained (one day of magazine training) the remaining rats (controls: $n = 3$; lesions: $n = 4$) to perform a classic autoshaping task for a week (30 trials per session). During each trial of this task, a retractable lever was presented (conditioned stimulus) for a duration of 8 seconds, after which a sucrose pellet (unconditioned stimulus) was immediately delivered into an adjacent food cup. We analyzed average response bias, calculated as (Food Cup Entries − Lever Presses) / (Food Cup Entries + Lever Presses)—where a higher number reflects increased goal-tracking, whereby behavior is directed towards the reward, as opposed to towards the stimulus associated with reward. We found that control

rats exhibited higher goal-tracking (independent samples *t*-test; $t_{(55)} = 2.25, p < 0.05$). Control rats entered the food cup during the conditioned stimulus significantly more times than lesions ($t_{(55)} = 2.83, p < 0.05$), but there was no significant difference between how many lever presses were made ($t_{(55)} = -0.72, p = 0.48$), suggesting that the differences in goal-tracking were driven by food cup entries. This emphasizes that there were no differences in either group's interaction with the conditioned stimulus. Further, lesioned rats exhibited higher locomotion compared to controls ($t_{(55)} = -2.15, p < 0.05$), and they did not attribute high incentive salience towards the US; control rats were better goal-trackers. Lesioned rats engaged the CS lever about as frequently as control rats, in spite of the fact that the sucrose pellet would appear independently of their engagement with the lever. This also suggests that ACC lesions did not impact how much effort rats were willing to expend—in a simple task where they received reward independently of performing an action, they still engaged the CS. Consequently, we can conclude that the vigorous behavioral deficits observed in the decision-making task were not a reflection of rats experiencing motor or motivational deficits following lesions. Rather, these findings seem to suggest that the behavioral deficits we saw on the decision-making task—a task much more involved, requiring frequent updating of reward-outcome contingencies—are tied to the complexity of the decision-making.

Discussion

We have previously published two studies highlighting the role of the anterior cingulate cortex in shifting the allocation of attentional resources towards behaviorally relevant stimuli following outcome expectancy violations, and how disruption of these attentional signals leads to impairments in decision-making and cognitive flexibility (Bryden et al., 2011; Vázquez et al., 2020). We found increases in ACC firing that were correlated with behavioral shifts in attention

following unsigned prediction errors (i.e. unexpected trial outcomes)—as rats had to update previously learned behavior-outcome contingencies. These findings uncovering attention for learning signals in the ACC were replicated in our paper examining the effects of cocaine exposure on ACC signaling (Vázquez et al., 2020). Additionally, we found that cocaine intake induced graded modulatory effects in ACC signaling, resulting in maladaptive cognitive flexibility disruptions and decision-making impairments (Vázquez et al., 2020). From these results we had hypothesized that normal ACC signaling gives rise to increases in attention and cognitive control that promote flexible reward-guided decision-making by informing downstream neural signals in regions such as the DMS. We observed global changes in behavior that likely reflect gross changes in task engagement—possibly encompassing functions related to cognitive effort, motivation, or attention. These results suggest that the significant behavioral impairments observed following ACC dysfunction do not arise as a result of simple disruptions of downstream action-outcome encoding when attention is necessary for learning (i.e., during block transitions), but rather are related to a breakdown in functions necessary to engage in a complex reward-guided decision-making task.

One possibility is that ACC lesions bias behavior in a way that minimizes the effort an organism is willing to exert to obtain reward. Consistent with this notion, rats also exhibited less frequent food cup entries during autoshaping; however, rats engaged with the lever at the same rate as control rats—making this explanation unlikely.

It might also be argued that lesioned rats exhibited some sort of gross motor deficit; however, during delay blocks—which always occurred first during recording sessions—there were no significant differences in movement times between control and lesioned rats.

Another possibility is that ACC lesions affected attentional processes. Attentional allocation is used to select, maintain, and update the relevant representations and contexts necessary for successful task completion; consequently, disruptions to attention have direct repercussions on goal-directed behaviors and flexible learning. Supporting this hypothesis, light-on latencies ($F(1,5968) = 113.91$, $p < 0.0001$)—operationalizing attention to the task—and reaction times were significantly slower across all trial types in lesioned rats (forced-choice: $F(1,5968) = 86.56$, $p < 0.0001$; free-choice: $F(1,2786) = 10.36$, $p < 0.001$), and movement times were significantly slowed towards the end of the task ($F(1,2786) = 6.93$, $p < 0.01$). Importantly, there were block effects—so that lesioned rats were significantly slower towards the end of sessions, after having performed many trials—again seeming to suggest that ACC impairment impacts the ability to continuously attend to task demands. These changes further suggest general decreases in task engagement that may include reduced motivation, attention or willingness to exert cognitive effort late in sessions.

Overall, these findings are also consistent with the notion that the ACC is necessary to engage model-based strategies and exhibit cognitive control (Akam et al., 2021). Model-based strategies implicate using learned environmental models to inform subsequent predictions (Doody, Van Swieten, & Manohar, 2022). One study found that the ACC plays an important role in model-based action selection by representing task space information, as well as whether changes in task space components match predictions made (Akam et al., 2021). The complicated task used in our experiment requires rats to regularly update multiple changing action-outcome representations; thus, the inability of lesioned rats to exhibit flexible decision-making might be a consequence of disruptions to model-based learning.

The reduction in directional signals observed here are also consist with our previous work examining DMS activity after ACC lesions in rats performing a stop-change task—a paradigm which asseses cognitive control processes, as it requires the abrupt repression of learned automatic responses. In this task, rats must respond to a directional cue light that directs them to a fluid well in order to receive reward (GO trials). On a different kind of trial (STOP-change trials; 20% of trials), the GO cue is again presented, but is subsequently followed (0-100 ms after first cue light) by a cue light on the opposite direction of the initial cue signal. A correct response on a STOP-change trial would require the rat to redirect its movement towards the side indicated by the second cue light, thus requiring a suppression of their established automatic response to the first cue. It is important to note that in this stop-change task, GO trials are significantly easier than STOP-change trials. In this study, we found that lesioned rats exhibited reduced accuracy and were slower to shift responses on trials that required the suppression of a prepotent response in lesioned rats (STOP-change trials)—but that ACC lesions did not impact performance on GO trials (Brockett et al., 2020). Additionally, DMS firing in lesioned rats did not reflect response direction on STOP-change trials (Brockett et al., 2020). When rats made errors, DMS firing represented incorrect, unresolved responses (Brockett et al., 2020). On STOP-change trials, the DMS of lesioned rats fired significantly more strongly for incorrect responses that were made in the direction signaled by the initial cue light (Brockett et al., 2020). In the stop-change task there were no group differences between the number of total trials performed or the latency to initiate trials (Brockett et al., 2020). This suggests that rats in both groups exhibited similar levels of motivation and effort, and that ACC is most important for sustaining engagement during complex tasks that implicate higher cognitive demands—such as the one discussed in this paper.

Previous research has identified a putative circuit between the anterior cingulate cortex and the DMS, and extensive studies have shown that goal-directed decision-making is driven by DMS encoding (Yin et al., 2005; Stalnaker et al., 2010; Thorn et al., 2010; Stalnaker et al., 2012). In this study, we sought to examine how ACC lesions impacted behavior and DMS encoding of response-outcome associations and directional encoding. The robust impact of ACC lesions highlights an important relationship between ACC activity, attentional modulation, and task performance—findings that are replicated here and exemplified by worse performance across all trial types and significantly slowed light-on latencies, reaction, and movement times of lesioned rats.

Here, we show that ACC lesions had deleterious effects across all behavioral measures. Specifically, rats with ACC lesions performed significantly worse across all trial-types, chose high-valued rewards less often, and exhibited slower light-on latencies, reaction times and movement times. Notably, these overarching deficits in task performance are unique to ACC lesions, as they have not been observed after perturbations of orbitofrontal cortex, nucleus accumbens core, dorsolateral striatum, or basolateral amygdala in rats performing the same task (Burton et al., 2014, 2015, 2018; Roesch et al., 2006; Roesch & Bryden, 2011). Further, the effects we see here are more profound than those observed with optogenetic inactivation of the ACC during performance of the task. As with lesions, transient ACC inactivation from houselights on until termination of the response on 50% of trials impaired latencies to initiate trials, frequency of trial initiation and completion, accuracy, and choice performance (Chapter III). Unlike optogenetic inhibition, ACC lesions also impaired reaction and movement times.

Further, as evinced by the results of our autoshaping experiment immediately following the reward-based decision-making task, these behavioral deficits were not due to aberrant deficits in lever pressing or locomotion. Notably, lesioned rats locomoted more, which might reflect their reduced engagement with the task (i.e. they were roaming around the chamber instead of interacting with the food cup and lever). Indeed reflective of this, and consistent with findings in our optogenetic inactivation study, lesioned rats took significantly longer to complete recording sessions ($t_{(372)} = -5.64$, $p < 0.00001$), and were significantly less engaged in the task—initiating trials with less frequency than controls ($t_{(372)} = -4.94$, $p < 0.00001$).

In our opinion, the simplest explanation is that—much as we observed during optogenetic inhibition of the ACC (Vázquez et al., in press)—rats are less engaged after ACC lesions and as a result, performed poorly on all other facets of task performance. In our previous research, we have repeatedly discovered a negative correlation between a measurable proxy of attention–specifically, the aforementioned "light-on latencies"—and improved performance on forced-choice trials (Chapters II-III). Our findings have consistently revealed that faster latencies are linked to enhanced forced-choice performance, irrespective of the experimental manipulation. Our current finding replicates our earlier results, underscoring that heightened attention correlates with enhanced task performance (Vazquez et al., 2020; Vazquez et al., in press). Importantly, these deficits did not reflect any sort of gross motor abnormalities as none were observed, and movement times were unaffected during delay blocks. Differences in movement times were only observed during size blocks—which always occurred towards the end of sessions—reflecting the rats' level of satiation/motivation rather than their ability to move.

Paralleling these changes in behavior, it was only during size blocks where we observed deficits in DMS encoding, in that directional tuning was attenuated relative to controls on large reward trials. Note that the task is designed so that size blocks always occur at the end of recording sessions in order to promote session completion (Bryden et al., 2011; Roesch et al., 2012; Vázquez et al., 2020; Vázquez et al., in press). This task design was successful in that rats in both groups exhibited faster reaction times and better performance in size blocks compared to delay blocks; however, lesion rats did complete fewer trials, and exhibited significantly slower movement speeds during size blocks. Combined, these results suggest that the ACC might be critical for maintaining task engagement or sustaining motivation later during sessions.

Consistent with this interpretation, inactivation of the ACC impairs the ability of rats to exert effort to obtain more valued reward. For example, studies have shown that ACC disruptions reduce the amount of physical effort a rat is willing to exert in order to obtain reward (Hart et al., 2020; Holec et al., 2014; Porter et al., 2019). Although our task does not require physical effort, it is cognitively demanding in that rats must follow basic trial structure (i.e., respond when houselights are on, maintain hold, respond to one of the wells), learn and update response-outcome associations across multiple dimensions, and follow forced-choice rules. Perhaps the ACC is most necessary when rats need to engage in a task that requires considerable cognitive or physical effort (Barris & Schuman, 1953; Holec et al., 2014; Walton et al., 2003; Laplane et al., 1981).

Another interpretation of the neural effects of ACC lesions being isolated to size blocks is that the ACC does not encode information pertaining to delay to reward. Previous work using this same task has shown that activity in the ACC reflects expectations in reward size, but there

was no preferential tuning for shorter delays to reward (Bryden et al., 2011). Further, the previously mentioned study that demonstrated the ACC's involvement in effort also showed that the ACC was not critically involved in overcoming delays to large rewards (Holec et al., 2014). Thus, the ACC might not be as critical for delay processing. However, we did observe clear behavioral deficits during delay blocks, suggesting that the ACC is involved to some degree. We speculate that the ACC does contribute to delay valuation, but modulates behavior through other downstream brain regions such as the nucleus accumbens core (NAc). The NAc is strongly associated with reward expectation, and is implicated in delay discounting and intertemporal choice (Cardinal & Cheung, 2005; Acheson et al., 2006; Galtress & Kirkpatrick, 2010; Joutsa et al., 2015; Wu et al., 2018). Future work should record from the NAc following ACC perturbations to determine whether reward expectancy and prediction signals are disrupted. It is also possible that behavioral changes in value encoding might stem from disruption of the ACC's contribution to attention-related functions (Bryden et al., 2011; Vázquez et al., 2020). Further, it's important to note that many other cortical and thalamic afferents innervate the striatum; studies mapping striatal inputs have found that throughout the striatum, the dorsomedial subdivision receives heterogeneous and multimodal cortical inputs (Collins & Saunders, 2020). Thus, modulation of directional signals during delay blocks are likely informed by inputs other than the ACC. Indeed, recent work has shown that prefrontal and thalamic populations of neurons can be differentiated by their projection targets to downstream regions, and that these populations play distinctive roles in cognitive control (de Kloet et al., 2021; Brockett et al., 2020, 2022). Consequently, the unilateral nature of the lesions in our study—compounded by the

heterogeneity of DMS inputs—may also contribute to why the impact of ACC lesions on DMS encoding was not robust.

One study found long-term synaptic potentiation in the DMS early in training, so that DMS lesions only have a deleterious impact during the initial acquisition of goal-directed behavior (Yin et al., 2009). Because our training sessions occur prior to recording sessions, we are unable to explore this finding.

Additionally, a recent study has found that neuronal populations in prefrontal regions can be disambiguated by their projection targets to downstream regions, and that these populations play distinctive roles in cognitive control (de Kloet et al., 2021). Of note, targeted thalamic subdomains are important for guiding goal-directed behavior through the maintenance of task representations, making this a region of interest for future studies (de Kloet et al., 2021). Other research has found that cortical motivational signal inputs to the striatum are sparse (Balleine, Delgado, & Hikosaka, 2007), reaffirming that we should look towards other downstream regions important for task performance—such as the thalamus, basolateral amygdala, ventral striatum, ventral tegmental area, orbitofrontal cortex, and insula.

Finally, a plethora of studies suggest that the DMS is an important locus for the encoding of action-outcome representations that are imperatively implicated in goal-directed behavior and decision-making (Walton et al., 2003; Yin et al., 2005; Burton et al., 2015; Haber, 2016). However, the impact of ACC lesions on DMS firing was only evident in the attenuated directional signals during large reward trials across the population. Furthermore, the impact of ACC lesions on DMS neural encoding was limited to size blocks. These findings suggest that the observed behavioral impairments cannot be solely attributed to disruptions in downstream DMS

encoding. This suggests that the reason we do not see global disruptions to DMS encoding is because the observed robust behavioral impairments were not a result of a breakdown in downstream action-outcome encoding, but rather tied to reductions in task engagement we have previously observed following ACC perturbation (Chapters II-III).

Chapter V: Histone Deacetylase 5 knockdown in the anterior cingulate cortex decouples attentional signals and disrupts reward-guided decision-making

Daniela Vázquez[1,2], Xavier A. Sciarillo[1], Kiana J. Yeganeh, Clarissa Xia, Xuan Li[1,2], Matthew R. Roesch[1,2]

[1]Department of Psychology, University of Maryland, College Park, MD 20742, USA

[2]Program in Neuroscience and Cognitive Science, University of Maryland, College Park, MD 20742, USA

Abstract

In previous studies, we have found that disruption of anterior cingulate cortex (ACC) signaling—via manipulations ranging from chemical lesions to optogenetic inhibition—results in attenuated attention during performance of a decision-making task. How these attentional disruptions may be repaired—using therapeutically relevant and biologically feasible treatment—in the service of restoring decision-making impairments remains to be explored. One interesting molecular target for modulating cognition and neural activity is histone deacetylase 5 (HDAC5), an epigenetic enzyme involved in regulating gene expression. Studies have found that heightened HDAC expression contributes to memory impairments and cognitive decline (Dos Santos Sant' Anna, 2013; Taniguchi et al., 2017), and that expression of nucleus-localized HDAC5 in the nucleus accumbens reduces neuron excitability (Anderson et al., 2023). Taking these findings into account, we sought to explore whether chromatin remodeling resulting from HDAC5 knockdown might enhance attentional signals that are necessary for flexible decision-making. To this aim, we trained rats on a reward-guided decision-making task consisting of four sixty-trial blocks; reward value was manipulated by independently varying the delay to (Blocks 1 and 2) or size of reward (Blocks 3 and 4). Subsequently, we either knocked down (scAAV1-CMV-shHDAC5-GFP; n=8), or did not manipulate (control virus, scAAV1-CMV-shLuc-GFP; n=7) HDAC5 expression, and recorded ACC activity while rats performed the aforementioned task. We found that HDAC5 knockdown lead to decoupled attentional signaling, significantly worse performance across all trial-types, and reduced task engagement; thus, counter to our hypothesis, HDAC5 knockdown in the ACC did not improve performance.

Introduction

Dysregulated gene transcription is a hallmark of many neuropsychiatric disorders, and this dysregulation can—in part—be attributed to the aberrant activity of epigenetic enzymes (Duman & Newton, 2007; Tsankova et al., 2007; Stuffrein-Roberts et al., 2008; Karsli-Ceppioglu, 2016; Nestler et al., 2016; Tseng et al., 2020; Grezenko et al., 2023), making these enzymes an important target for therapeutic intervention. Among these epigenetic enzymes are histone deacetylases (HDACs); deacetylation of histones leads to increased electrostatic affinity between histones and DNA, generally resulting in reduced gene transcription rates *(*Seto & Yoshida, 2014; Li et al., 2018; Malvaez et al., 2018*).* HDAC5 is a class II HDAC enzyme that shuttles between the nucleus and cytoplasm in response to intracellular signaling; it is highly expressed in the brain—particularly in cortical regions—indicating that it may play a crucial role in cortical functions underlying learning (Yang & Gregoire, 2005; McQuown & Wood, 2011; Kim et al., 2012). There have been studies showing that—in the nucleus accumbens (NAc)—HDAC5 expression negatively modulates cocaine seeking (Renthal et al., 2007); similarly, viral overexpression of nuclear-localized accumbal HDAC5 decreases cue-induced reinstatement of drug seeking (Taniguchi et al., 2012; 2017), suggesting that HDAC5 plays a significant role in reward-associated learning and memory.

Although there is increasing research looking at epigenetic regulation of gene expression, and how it contributes to drug abuse and the reinstatement of addiction (Nestler, 2014; Li et al., 2015; Rubio et al., 2016; Taniguchi et al., 2017; Li et al., 2018; Cates et al., 2018; Li et al., 2019), there is limited research on whether histone modification may exert a macro impact neural signaling and behavior. More specifically, no work has explored how chromatin remodeling in the anterior cingulate cortex (ACC) impacts decision-making.

HDAC5 overexpression in the dorsal striatum—a brain region that plays a pivotal role in reinforcement learning—has been found to exacerbate inflexible decision-making, while inhibition of HDAC enzymes in hippocampal regions enhances long-term potentiation and memory (Levenson et al., 2004; Barrett & Wood, 2008; Morris, Karra, & Monteggia, 2010; Burns et al., 2022). While these studies did not look at cortical regions, studies in humans have found significantly increased levels of HDAC5 expression in the frontal cortex of individuals with Alzheimer's Disease compared to age-matched controls (Anderson et al., 2015). Here, we wanted to study how the downregulation of HDAC5 might influence neural activity at the single cell level, and whether this might translate to changes in behavior.

Only a few studies have investigated the effects of HDAC manipulation on reward-based learning outside of the context of drug-seeking. Previously, our research demonstrated that overexpression of HDAC5 in the dorsal striatum resulted in quicker and less adaptable behavior (Pribut et al., 2021). Conversely, another study examining the influence of HDAC3 on habitual behavior found that inhibiting HDAC3 function in either the dorsolateral or dorsomedial striatum promoted habit formation, whereas enhancing HDAC3 function via viral-mediated overexpression prevented habit formation. Taken together, these findings indicate that HDAC3 negatively regulates habit formation within the dorsal striatum (Malvaez et al., 2018).

In our previous research, we have also discovered an imperative role of the ACC in attention and task engagement (Chapters II-IV). Attentional reductions and inflexible decision-making are hallmarks of age-related cognitive impairment (Kim & Giovanello, 2011); relatedly, aging has been shown to increase HDAC activity in the rat

hippocampus (Dos Santos Sant'Anna, 2013). Other studies have shown that the administration of HDAC inhibitors can improve age-related memory deficits in rodents (Reolon et al., 2011). Together, these findings seem to suggest that heightened HDAC expression contributes to the cognitive decline that is characteristic of aging, and that inhibiting or reducing HDAC activity might help repair some of these deficits.

Very recent work has also shown that the expression of nucleus-localized HDAC5 in the nucleus accumbens reduced intrinsic neuron excitability in both D1 and D2-class MSNs (Anderson et al., 2023). This same study found that HDAC5 overexpression in the nucleus accumbens dampened context-associated heroin-seeking behavior (Anderson et al., 2023). These findings—coupled with the findings that HDAC5 overexpression is associated with the negative regulation of reward-related behaviors in the context of cocaine seeking (Taniguchi et al., 2017)—make HDAC5 an interesting target for the study of how modulation of histone deacetylase expression may impact decision-making and neural firing in the ACC.

Thus—in order to identify a more biologically feasible and therapeutically relevant mechanism by which to potentially repair disrupted attentional signaling and, subsequently, decision-making—we sought to explore how HDAC5 knockdown might impact neural firing in the ACC and behavior. We found that HDAC5 knockdown lead to decoupled attentional signaling, significantly worse performance across all trial-types, and reduced task engagement. Our current findings implicate dysregulated HDAC5 expression in abnormal decision-making.

Methods

Rats were trained on the aforementioned decision-making paradigm (*Figure 2*) for one month until they reached criterion responding (>70% on forced-choice trials). Following

training, they underwent surgery, after which they had a week-long recovery period. Groups were counterbalanced based on task performance. After recovery, we recorded neural data while they performed the same task they had been previously trained on. At the end of each recording session, each rat's electrode was advanced ~40 μm. Following three months of recording neural data, rats were perfused and the location of recording and injection sites were histologically confirmed.

Surgery

Twelve Sprague-Dawley rats were obtained at 175-200 g from Charles River Laboratories. Before surgery, rats were trained on the aforementioned reward-based decision-making task (*Figure 2).*

Rats (control: n=8; HDAC5 underexpressed: n=7) received bilateral injections (0.75 μl/hemisphere) of either scAAV1-CMV-shHDAC5-GFP (experimental) or scAAV1-CMV-shLuc-GFP (control) in their ACC (0.2 mm anterior to bregma, ±0.5 mm lateral, 1 mm ventral to brain). Comprehensive plasmid maps available on request. The in-vivo confirmation of HDAC5 knockdown was previously illustrated by Li et al. (Li et al., 2018). Virus delivery occurred at a rate of 0.375 μl/min via Hamilton syringes (32 gauge). Following each injection, we left the injection needle in place for an additional minute to allow for full virus diffusion.

Following viral injection, we implanted unilateral drivable electrodes (0.2 mm anterior to bregma, ±0.5 mm lateral, 1 mm ventral to brain; hemispheres counterbalanced) for single-unit recordings. Coordinates were chosen based on previous optogenetic and recording studies targeting the same area (Bryden et al., 2011; Vázquez et al., 2020; Vázquez et al., in press).

Single-Unit Recordings

Single-unit recording procedures followed those outlined in previous publications (Bryden et al., 2011; Vázquez et al., 2020; Vázquez et al., in press). Neural recordings were collected using four identical Plexon Multichannel Acquisition Processor systems. Initially, signals from the electrode wires underwent 20x amplification via an op-amp headstage situated on the electrode array. Single-unit signals were subsequently amplified 50x and filtered within the range of 150-9000 Hz by a differential preamplifier (Plexon, PBX2/16sp-r-G50/16fp-G50). These single-unit signals were then directed to the Multichannel Acquisition Processor box, where they were further filtered at 250-8000 Hz, digitized at 40 kHz, and amplified at 1-32x.

Histology

Rats were deeply anesthetized with isoflurane and transcardially perfused using 500 mL of 0.01 M phosphate buffered saline (PBS). Brain tissue was fixed for one hour in 500 mL of 4% paraformaldehyde (PFA), and was subsequently 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 imaging, 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 using Fluormount G (Electron Microscopy Sciences). We acquired fluorescent images of the ACC using a Zeiss AXIO Imager M2 microscope.

Behavioral Analyses

Behavioral analyses were conducted on individual sessions, and subsequently averaged across all sessions within each group. The analyses spanned several parameters, including percentage of initiated trials, percentage of correct responding during forced-choice trials (i.e.,

trials where the animals correctly responded to the well corresponding to the directional odor cue), percentage of trials where rats selected a high-valued condition (i.e., short delay, large reward) during free-choice trials, reaction times (epoch from odor offset to odor port exit), percentage of trials rats initiated (nosepoke upon houselight illumination), movement times (epoch from port exit to well entry), light-on latencies (time taken to enter the odor port upon the illumination of houselights), and correlations between light-on latencies and correct responding.

For analyses conducted solely across completed sessions (all four sixty-trial blocks), calculations were split into the first ten trials (early in trial block) and the last ten trials (late in trial block) in order to assess within-block learning. Prior studies have demonstrated that analyzing the initial ten trials of each trial type captures learning that occurs at the onset of trial blocks, and ensures a sufficiently large sample size for analysis (Bryden et al., 2011; Burton et al., 2018; Roesch et al., 2006; Vázquez et al., 2020; Vázquez et al., in press). Multifactor analysis of variance (ANOVA) was performed on behavioral measures, incorporating relevant factors such as group (underexpressed vs. control), phase (early vs. late in learning), block-type (delay vs. size), and value (high vs. low). Post-hoc t-tests, adjusted for multiple comparisons, were employed to explore significant interaction terms identified in the ANOVA.

Neural Analyses

For neural analyses, single units were sorted using template matching (Plexon, Offline Sorter), and exported to NeuroExplorer and Matlab. Increasing and decreasing cells were identified as cells whose activity significantly increased or decreased, respectively, during the epoch from odor cue onset to well entry (trial epoch) relative to their baseline firing rate (1s preceding odor onset, Wilcoxon; $p < 0.05$). 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., 2017, 2018; Vázquez et al., 2020; Pribut et al., 2021) to capture firing related to the anticipation and delivery of reward. 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 light-on latencies collected during each recording session. Wilcoxons (rank-sum; $p < 0.05$) were used to measure significant shifts in the distribution of indices from zero, and to determine differences between control and underexpressed groups. Chi-squares ($p < 0.05$) were performed on counts of neurons in each group to determine whether there were any significant differences between groups.

Results

HDAC knockdown reduced task accuracy and impacted some facets of task engagement

Overall, HDAC5 knockdown reduced task accuracy (*Figure 6.1A*; $t_{(273)} = 3.48$, $p < 0.00001$), and decreased the amount of times rats chose high-value reward on free-choice trials (*Figure 6.1B*; $t_{(273)} = -2.58$, $p < 0.01$). Further, knockdown rats initiated trials

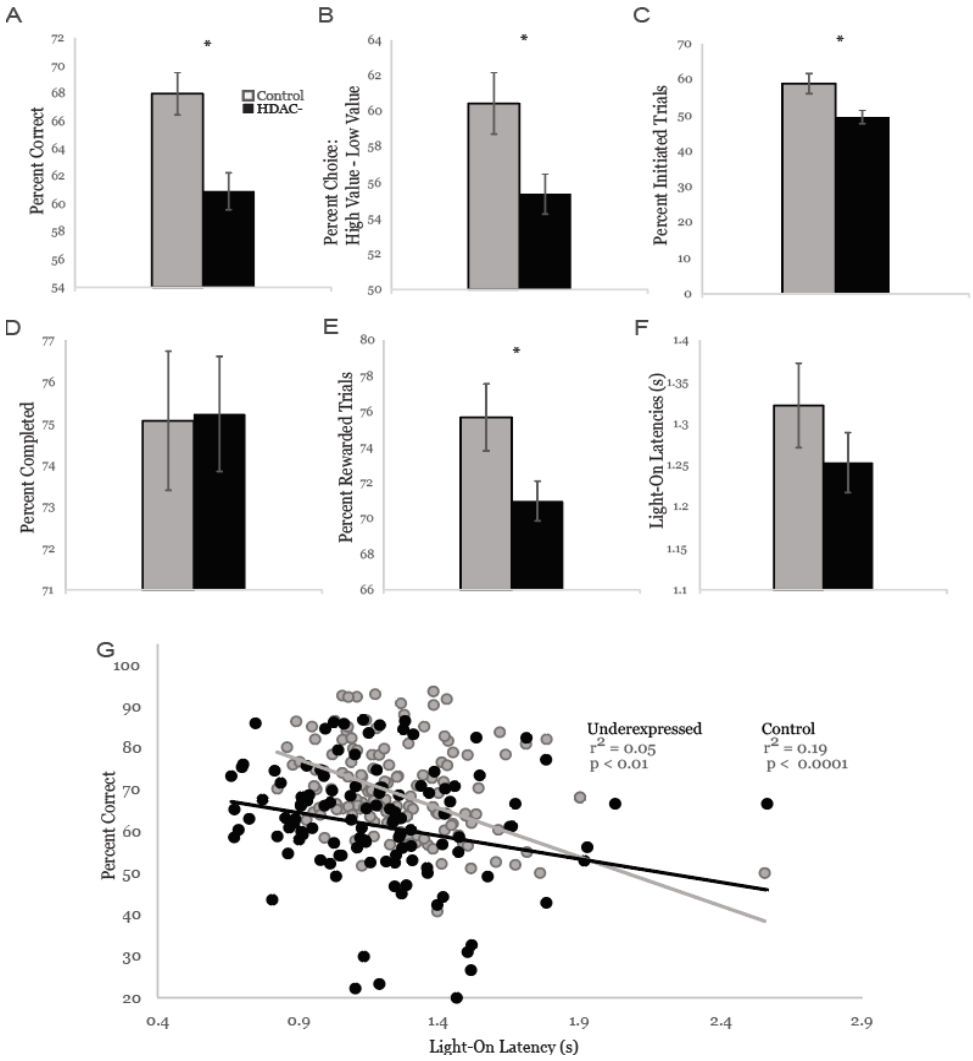

Figure 6.1: Behavioral analyses across all sessions. HDAC5 knockdown worsened performance and diminished engagement. A) Percentage correct forced-choice trials. B) Percentage of times rats chose high-value reward on free-choice trials C) Percentage of trials rats initiated (defined as rats nose-poking into the central odor port until odor presentation upon illumination of the houselights). D) Percentage of completed sessions (i.e. sessions in which rats completed all 240 trials (four sixty-trial blocks). E) Percentage of rewarded trials. F) Average light-on latency denotes the latency to enter odor port upon house-light illumination (which indicates the beginning of a new trial). G) Correlation between light-on latencies and percent correct.

less frequently (*Figure 6.1C*; $t_{(273)} = 2.93$, $p < 0.005$); however, once they initiated a trial, they completed it at a rate similar to controls (*Figure 6.1D*; $t_{(273)} = 0.08$, $p = 0.94$). Due to their decreased accuracy, they were rewarded significantly less (*Figure 6.1E*; $t_{(273)} = 2.24$, $p < 0.05$).

Light-on latencies were not significantly different between the two groups (*Figure 6.1F*; $t_{(273)} = 1.13$, $p = 0.26$).

Our previous research has uncovered a consistent relationship between behavioral measures of attention—denominated "light-on latencies"—and task accuracy (Chapters II-IV). Here, we have once again replicated this phenomenon, demonstrating that quicker latencies were linked to higher accuracy on forced-choice trials, and the correlations were significantly different from each other (see *Figure 6.1G*; control: $r^2 = 0.19$, $p < 0.0001$; underexpressed: $r^2 = 0.05$, $p < 0.01$; Fisher's r-to-z transformation: $z = -2.02$; $p < 0.05$). In summary, these findings suggest that reductions in attentional focus may contribute to diminished task performance.

During forced-choice trials, rats had to respond in the cued direction in order to receive reward (i.e. correct trial); if the rats responded in the opposite direction, no reward was delivered (i.e., error trial). As we have shown in previous studies, behavior was biased towards the location of the more favorable reward (i.e. short delay or large reward size; Pribut et al., 2021). That is, rats tended to perform better and worse on high- and low-value forced choice trials, respectively. Thus, we found that both groups performed better on high- compared to low-value forced-choice trials (*Figure 6.2A*; Main effect of group for percent correct: $F(1, 5983) = 21.52$, $p < 0.0001$; phase: $F(1,5983) = 10.99$, $p < 0.0001$; value: $F(1,5983) = 29.59$, $p < 0.0001$; and an interaction between group, phase, and reward value: $F(1,5983) = 21.08$, $p < 0.0001$; interaction between group and block for percent correct: $F(1,5983) = 4.97$, $p < 0.05$.) Rats were also swayed towards high reward value on free-choice trials towards the end of trial blocks (*Figure 6.2B*; interaction between reward value and phase for percent choice: $F(1,5983) = 226.18$, $p < 0.0001$). There

were no significant differences in movement (*Figure 6.2C*; $F(1,5983) = 0.14$, $p = 0.71$) or reaction times (*Figure 6.2D*; $F(1,5983) = 0.14$, $p = 0.71$).

Attentional control was decoupled from ACC firing in HDAC5 knockdown rats

In order to quantify neural (firing rate) and behavioral (light-on latencies) changes occurring

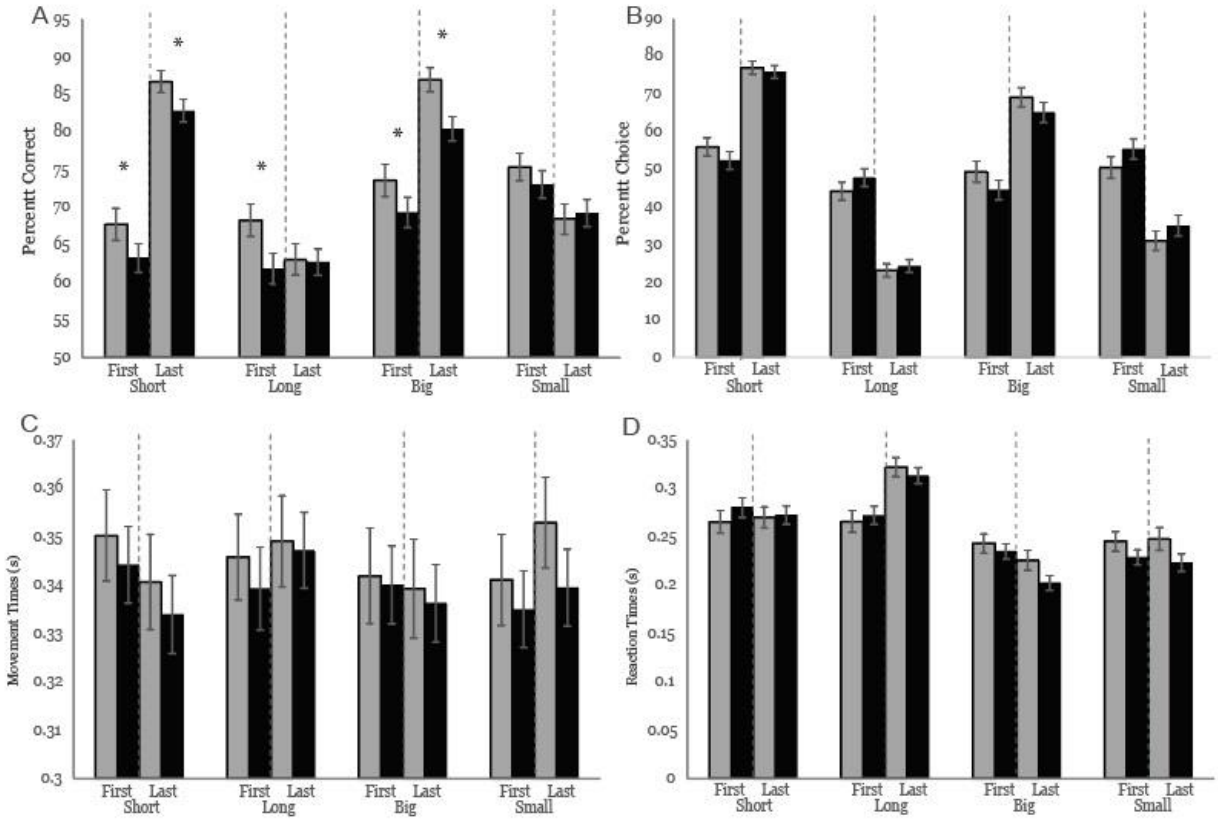

Figure 6.2: HDAC5 diminished task accuracy across completed sessions. Analyses were broken down by learning phase (early in session, when contingencies first switch vs. late in session, once contingencies have been learned). A) Percentage of times rats accurately responded during completed sessions. B) Percentage of times rats chose each valued reward during completed sessions. C) Movement times (time from odor port exit to fluid well entry) on forced-choice trials for each value manipulation during completed sessions. D) Reaction times (time from odor offset to odor port exit) on forced-choice trials for each value manipulation during completed sessions.

at the beginning of trial blocks (when learning occurred), we computed an index that captured differences in activity between early (first ten trials) and late (last ten trials) trials for each neuron in both control and knockdown groups (*Figure 6.3*; index = early − late/early + late). For

distributions of firing-rate indices, we found significant shifts above zero for controls (*Figure 6.3A;* Control: $\mu = 0.01$; $p < 0.0001$)—indicative of higher firing at the beginning of trial blocks. However, we did not see this effect in knockdown rats (*Figure 6.3B;* Knockdown: $\mu = 0.002$; $p = 0.11$); the distributions did not significantly differ between groups (control vs. knockdown: $z = 1.55$; $p = 0.12$). For light-on latency indices, we found significant shifts for control (*Figure 6.3D;* Control: $\mu = 0.02$; $p < 0.01$) but not knockdown (*Figure 6.3E;* Knockdown: $\mu = -0.01$; $p = 0.16$) rats; further, the distributions were significantly different from each other (control vs. knockdown: $z = 3.09$; $p < 0.05$). We then explored the correlation between firing rate and light-on latencies. In line with our previous findings, we demonstrate a correlation between ACC firing rates and light-on latencies in control rats (*Figure 6.3C*). Specifically, in control rats, these two measures were negatively correlated (*Figure 6.3C*, $p < 0.0005$, $r^2 = 0.07$), indicating that sessions characterized by elevated ACC firing rates corresponded to heightened levels of attention. Interestingly, in HDAC5 knockdown rats, this relationship disappeared (*Figure 6.3E*, $p = 0.78$; $r^2 = 0.0004$), and—notably—significantly differed from the control group (determined via Fisher r-to-z transformation; $z = 2.78$; $p < 0.005$). This suggests that there is a disassociation between attentional control and ACC firing in HDAC5 knockdown rats, similar to what we previously saw with cocaine rats (see Chapter II).

At the single-neuron level, the firing of 25 (14%) and 19 (9.5%) neurons was significantly modulated during the first compared with the last ten trials in control and HDAC5 underexpressed rats, respectively (*Figure 6.3A*, B; black bars; t test; $p < 0.05$). In controls, 18 and

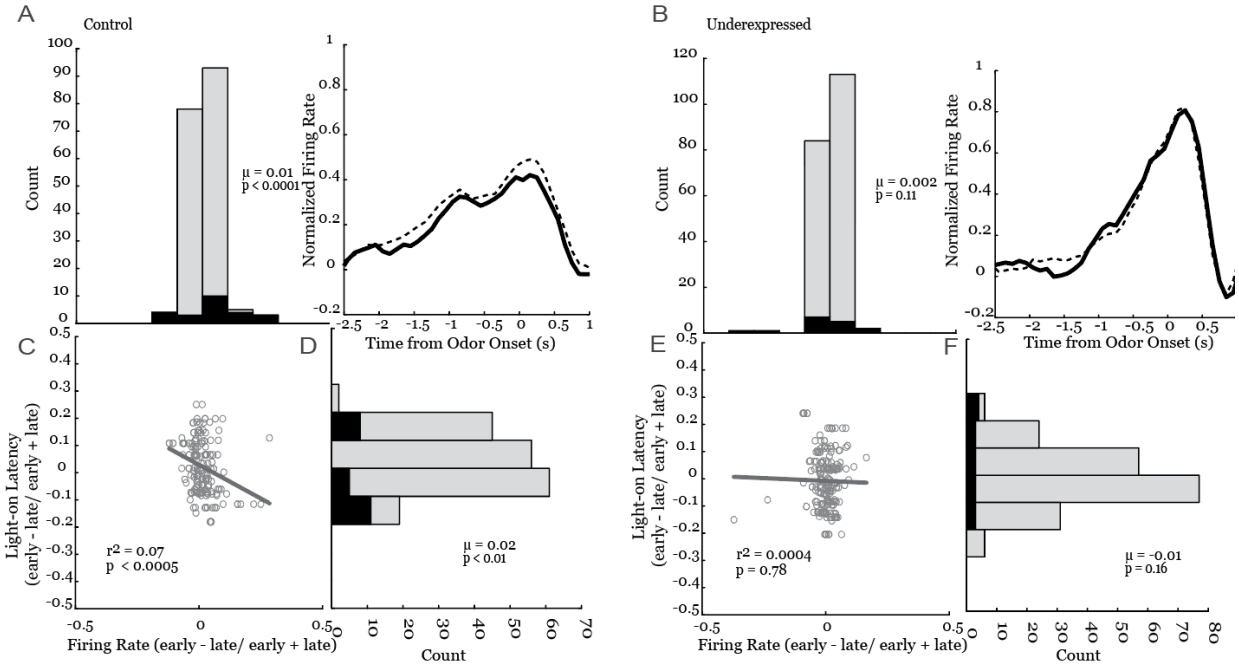

Figure 6.3: HDAC5 knockdown decoupled attention signals. A, B) Distributions of firing-rate indices for control (A) and HDAC5 knockdown (B) rats. Black bars represent cells with firing that significantly differed for early compared to late trials (t test; $p < 0.05$). Insets to the right of each panel illustrate z-score normalized average firing during early (dashed line) and late (solid) trials averaged over all trial types. Firing is aligned odor onset. Nose poke into the odor port occurred 500 ms prior to odor presentation and was triggered by illumination of the houselights. Port exit occurred roughly 750 ms after odor onset (500 ms of odor presentation +~250 ms of reaction time). C) The correlation between firing-rate indices (A) and light-on latency indices (D) for control rats. E) The correlation between firing-rate indices (B) and light-on latency indices (F) for knockdown rats. Black bars in D and F represent sessions where light-on latencies significantly differed between early and late trials (t test; $p < 0.05$). Wilcoxon tests were used to measure significant shifts in the distribution of indices from zero, and to determine differences between control and knockdown groups ($p < 0.05$).

7 neurons significantly increased or decreased their firing during the beginning compared to the end of trial blocks (*Figure 6.3A*, black bars; t test; $p < 0.05$). In the knockdown group, 10 and 9 neurons significantly increased or decreased their firing during early compared to late trials,

respectively (*Figure 6.3B*, black bars; t test; $p < 0.05$). The frequency of neurons did not significantly differ between the two groups ($\chi 2 = 1.01$; $p = 0.31$).

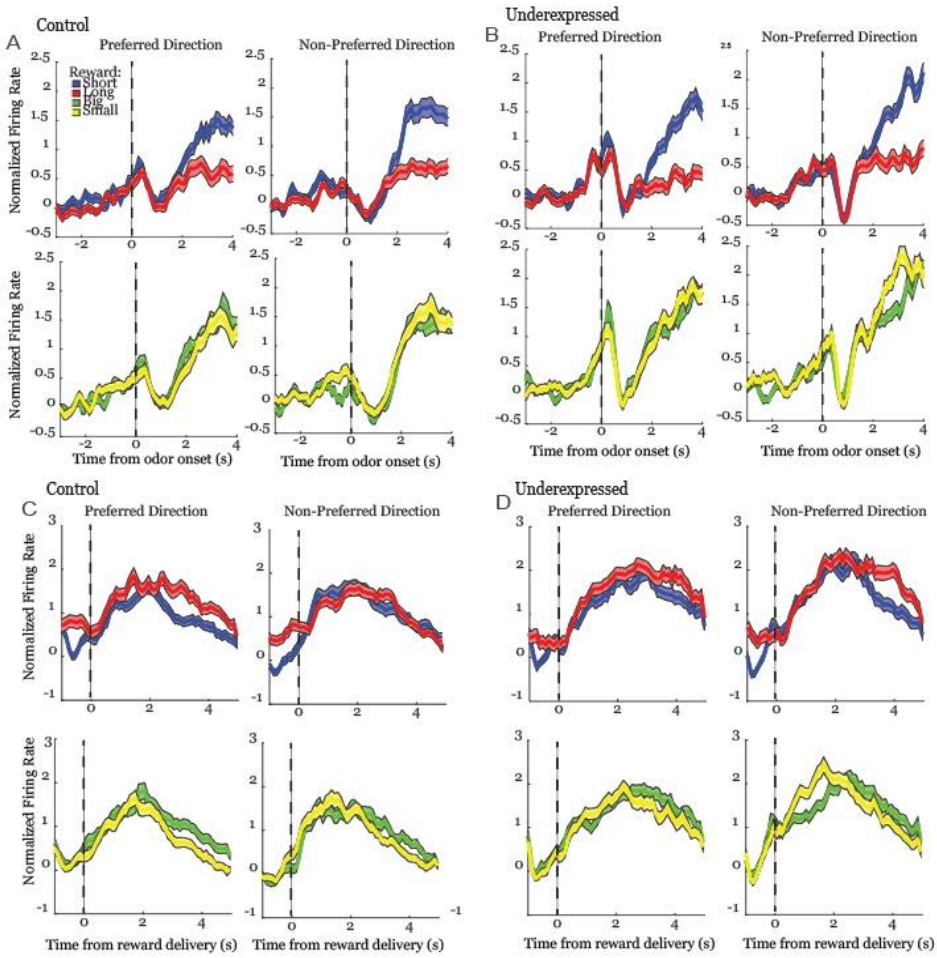

Figure 6.4: HDAC5 knockdown dysregulated firing during odor sampling. A) Z-score normalized average firing over all control cells that increased firing during odor sampling aligned to odor onset. We sorted neurons according by firing into their preferred (i.e. towards the neuron's response field; left column) and non-preferred directions (i.e. away from the neuron's response field; right column), and based on the direction and outcome (short, long, big, small reward) that elicited the highest firing rate. B) Same as (A), but for HDAC5 knockdown rats. C) Z-score normalized average firing over all control cells that increased firing during the reward epoch aligned to reward delivery. D) Same as (C), but for HDAC5 knockdown. Ribbons = SEM.

Figures 6.4 A and *B* illustrate z-score normalized firing–aligned to odor onset–for neurons that increased firing in control (*A*) and HDAC5 underexpressed (*B*) rats. However, there were no differences in baseline activity between these two populations of cells ($t_{(75)} = -0.963$, $p = 0.338$, *unpaired t-test*). *Figure 6.4 C* and *D* depict z-score normalized average neural firing aligned to reward delivery for both control (*C*) and HDAC5 underexpressed (*D*)

rats. In order to quantify this reward selectivity, we then plotted the normalized difference between firing to short and long reward (*Figure 6.5, A&B*; delay index = short – long /short + long) for each neuron in their preferred (*A*) and non-preferred (*B*) directions for control (*Figure 6.5*; top row) and knockdown (*Figure 6.5*; bottom row) rats. Gray bars reflect the distribution of indices across the entire population of neurons, while black bars represent neurons that either

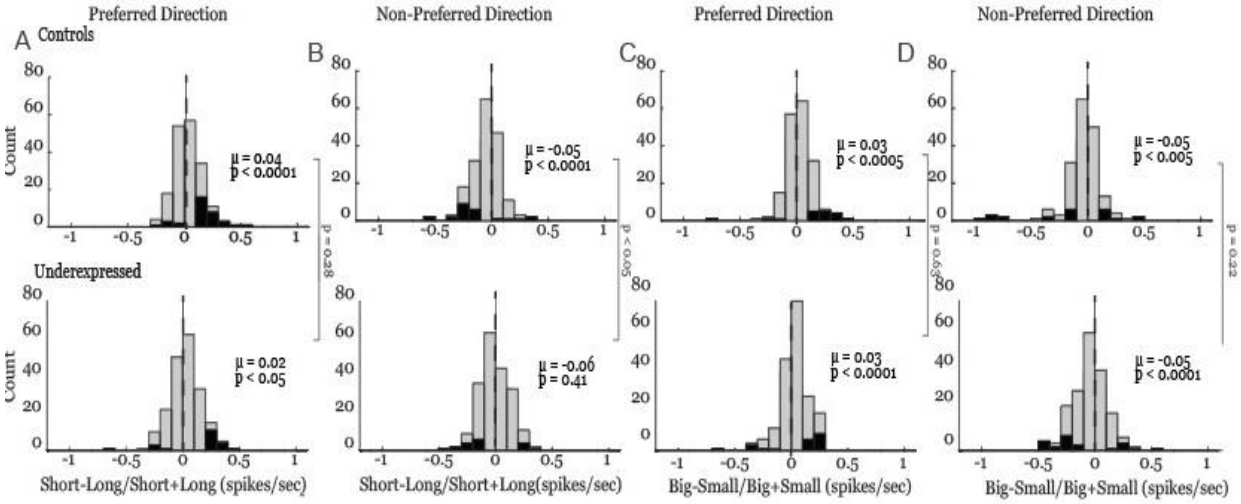

Figure 6.5: HDAC5 knockdown weakened delay indices in the non-preferred direction. A) Distribution of delay indices computed for neuron during the odor epoch (short-long/short+long) for control (top row) and knockdown (bottom row) rats. Gray bars reflect the distribution of indices across the entire population of neurons, while black bars represent neurons that exhibit a 'preference' for short- or long-delay trials, firing significantly more (to the right of the dashed line) or significantly less (left of the dashed line) for cues that predicted short delayed reward in their preferred (A) and non-preferred (B) directions (Wilcoxon, $p < 0.05$). C) Same as (A), but for small and big reward outcomes D) Same as (B), but for small and big reward.

significantly increased (*Figure 6.6, A&B*; right of dashed line) or significantly decreased (*Figure 6.5, A&B*; left of dashed line) their firing for cues that predicted short delayed reward relative to long delays. We found that distributions of delay indices were only significantly different between groups in that the neural firing of HDAC5 knockdown rats was not significantly modulated in the non-preferred direction (control: preferred direction: $\mu = 0.04$, $p < 0.0001$; non-preferred: $\mu = -0.05$, $p < 0.0001$; knockdown: preferred direction: $\mu = 0.02$, $p < 0.05$; non-

preferred: μ = -0.06, $p = 0.41$; $z = 2.01$; $p < 0.05$). The distributions of reward indices computed on delay trials in the non-preferred direction was significantly different between controls and knockdown rats (*Figure 6.5;* $z = 2.97$; $p < 0.05$). Though this difference was present at the population level, there was no difference between groups in the counts of significant neurons that significantly altered their firing (control: 20 (11%)—black bars to the left of the distribution vs 5 (3%) cells to the right of the distribution; knockdown: 13 (4%) vs 5 (2%); $\chi^2 = 0.05$; $p = 0.82$)—in other words, the overall attenuation in firing resulted from a shift in population-level firing, and was not also present in the counts of significant single units.

Finally, we quantified reward selectivity in the same way for size blocks, (*Figure 6.5, C&D*; size index = big – small / big + small) for each neuron in their preferred (*C*) and non-preferred (*D*) directions for control (*Figure 6.5*; top row) and knockdown (*Figure 6.5*; bottom row) rats. There were no significant differences in firing between the two groups (control: preferred direction: μ = 0.03, $p < 0.0005$; non-preferred: μ = -0.05, $p < 0.005$; knockdown: preferred direction: μ = -0.03, $p < 0.0001$; non-preferred: μ = -0.05, $p = 0.0001$).

Discussion

There is a growing body of research that examines the epigenetic regulation of gene expression, studying it principally within the context of addiction and the reinstatement of drug abuse (Renthal et al., 2007; Renthal et al., 2009; Hui et al., 2010; Taniguchi et al., 2012; Kenny, 2014; Nestler, 2014; Li et al., 2015; Rubio et al., 2016; Taniguchi et al., 2017; Li et al., 2018; Cates et al., 2018; Li et al., 2019). Prior research has shown that nucleus-localized HDAC5 in the nucleus accumbens negatively regulates the formation of reward-cue associations in the context of drug-seeking behaviors (Renthal et al., 2007; Taniguchi et al, 2012).

Disrupting HDAC activity has been demonstrated to enable transient, early-phase potentiation in the hippocampus, leading to enhanced synaptic plasticity—converting short-term, subthreshold learning into long-term, transcription-dependent memory (Vecsey et al., 2007; Stefanko et al., 2009). Epigenetic events impact a cascade of molecular events; the study of how these cascades alter the encoding properties of neurons, and subsequently lead to macro changes in behavior, is in its nascent stages. In this study, we wanted to explore whether epigenetic changes map onto specific alterations of neural activity and behavior—more specifically, how knocking down HDAC5 might alter attentional signaling in the ACC.

Similarly to what we observed during optogenetic inhibition of the ACC (Vázquez et al., in press) when analyzing across all sessions—both complete and incomplete—HDAC underexpression reduced task accuracy, selection of high-value rewards, and reduced task engagement. However, when looking solely at sessions during which rats were able to complete (4 blocks, 240 trials total), accuracy impairments remained, but differences in choice selection and movement and reaction times disappeared. This aligns with our previous findings in studies in which we chemically and optogenetically inactivated the ACC, wherein the robust behavioral impairments we observed seemed to be driven by global reductions in task engagement (Chapters II-IV). Supporting this, we replicated previous findings showing that heightened attention (operationalized as "light-on latencies") were correlated with better task performance. We also replicated our previous research that showed heightened attentional signals in the ACC when contingencies switch and learning has to be updated (Bryden et al., 2011; Vázquez et al., 2020). In the past, we have seen that these heightened firing patterns in the ACC are associated

with attentional adjustments that are important for learning and correlated with enhanced performance (Bryden et al., 2011; Vázquez et al., 2020). However, these changes in firing were not present in the HDAC5 knockdown rats.

One major limitation of our current study was that due to the size of the electrode array, and the very dorsal nature of the ACC, we were unable to collect tissue samples that would allow us to probe the transcriptional profile of ACC neurons after HDAC5 knockdown using methods such as qPCR. For reasons to do with methodological feasibility, the limited amount of studies that have both used electrophysiology and explored the transcriptional impact of HDAC5 knockdown have studied these properties in different cohorts of animals, and using ex-vivo patch clamp recordings (Anderson et al., 2023). Research has shown that significant changes in the biophysical properties of neurons are induced post-mortem, and that in vivo and ex vivo recordings show significant differences in both the strength and frequency of cell response dynamics (Rossmanna et al., 2014; Opitz et al., 2017; Márquez Loza et al., 2021; Pandeya et al., 2021). Thus, while patch-clamp recording studies provide very important insights about the electrophysiological properties of the neuron—as well as its alteration following HDAC manipulations—the focal point of our research is seeing how these neurons behave in a consortium and within the landscape of other brain regions, providing translatable signals that help explore how epigenetic manipulations alter event-specific responding and real-time, complex behavior.

Phosphorylation of HDAC5 triggers its translocation from the nucleus to the cytoplasm, effectively isolating it from target histones. The reality is that because HDAC5 is phosphorylated in an activity-dependent manner, it is one of the more interesting enzymes to hone in on within

the context of learning and plasticity; however, this simultaneously makes it a significantly more complicated target to study. Recently, studies have found that HDAC5 can modulate overall neural activity—specifically, that HDAC5 overexpression in the NAc reduces the intrinsic excitability of both D1 and D2 MSNs (Anderson et al., 2023), and HDAC5 overexpression in the dorsal striatum increases activity for cues that predict immediate reward, and diminishes reward encoding (Pribut et al., 2021). Although our present study found that ACC signals were decoupled from attention in HDAC5 knockdown rats—similarly to what we observed following cocaine exposure (see Chapter II)—we did not find that knocking down HDAC5 in the ACC significantly altered baseline or task-related firing. This could partially be due to region-specific differences; for example, one study found that HDAC5 overexpression in the dorsal striatum enhances incubation of meth seeking after abstinence, while knockdown decreases this incubation (Li et al., 2018), which contrasts with findings showing that HDAC5 overexpression in the nucleus accumbens negatively regulates drug-seeking (Renthal et al., 2007; Taniguchi et al., 2012).

While beyond the scope of our current study, another important consideration is that the brain might engage in compensatory modulation of other HDACs in response to HDAC5 knockdown (Li et al., 2018), and these HDACs may have differential impacts on behavior. For example, overexpression of HDAC5 in the dorsal striatum promoted inflexible behavior—specifically, rats formed biased, inflexible responding that was contingent upon the initial action-outcome associations learned (Pribut et al., 2021). However, studies manipulating that activity of HDAC3—a class I HDAC that is the most highly expressed and is primarily localized in the nucleus (Broide et al., 2007; McQuown et al., 2011; Thomas, 2014)—in the dorsal striatum

found that inhibiting its function accelerated habitual responding, whereas overexpressing it decreased habit formation (Malvaez et al., 2018).

Bringing in an additional level of complexity, the molecular cascades that are modulated by HDAC manipulations are dependent on a plethora of variables. For example, Npas4—an activity-dependent brain-specific transcription factor that regulates experience-dependent learning and memory (Fu et al., 2020)—has been shown to be negatively regulated by HDAC5 (Di Ciano et al., 2001; Taniguchi et al., 2017), but in other studies where HDAC5 is overexpressed, no differences in Npas4 expression are observed (Li et al., 2018). These contrasting findings could be attributable to factors such as differences in the activity of the impacted brain region, the type of drug being administered, the learning periods being studied (i.e. acquisition vs. performance), and the behavioral paradigms (e.g. conditioned place preference vs. cue-induced reinstatement of drug seeking) (Li et al., 2018).

In human studies there also seem to be indications of sub-region specific effects. Studies examining HDAC expression in patients with schizophrenia—a neuropsychiatric disorder characterized by chronic cognitive deficits—have shown that, compared to controls, patients with schizophrenia showed lower HDAC expression in the dorsomedial prefrontal cortex and orbitofrontal gyrus, but higher HDAC expression in cerebral white matter, pons, and cerebellum (Gilbert et al., 2019), suggesting that the activity and impact of these enzymes might be sub-region specific.

A variety of neurodevelopmental and psychiatric disorders are associated with dysregulated cell excitability, synaptic transmission, and alterations in functional connectivity (Egger et al., 2004; Feng et al., 2007). Research indicates that an imbalance in excitatory and

inhibitory (E/I) activity within fronto-striatal circuitry contributes to the attentional impairments that are characteristic of attention-deficit/hyperactivity disorder (Naaijen et al., 2017; Mamiya et al., 2022). While beyond the scope of our current study, altering HDAC5 expression leads to a cascade of modifications in gene expression, impacting diverse target proteins that are important for the homeostatic maintenance of E/I—such as Npas4. Together, these findings suggest that irregularities in epigenetic processes governing gene expression—through either up- or down-regulation of these enzymes—can have deleterious effects on cognition.

Chapter VI: General Discussion

My dissertation work probed the role of the ACC in complex decision-making by employing a diverse array of methodologies—including in-vivo electrophysiology, optogenetic inactivation of neural activity, pharmacological lesions, and virus-mediated epigenetic modifications—in order to obtain a holistic understanding of the ACC's role in attention. Our findings are supported by a plethora of human studies that have repeatedly shown persistent hypoactivation in the ACCs of patients with attention deficit disorders during attention-related tasks (Banich et al., 2013; Bledsoe et al., 2013; Bayard et al., 2018; Hoogman et al., 2019; Vogt, 2019; Bayard et al., 2020; Yu et al., 2023).

Summary of Results

We first explored the ACC's role in attention—and how it is impacted by drug use—using electrophysiology to record from ACC neurons as both cocaine-exposed and drug-naïve rats performed a reward-guided decision-making task. Using this task, we found a dose-dependent attenuation of ACC signaling after cocaine self-administration, which was correlated with decreases in task performance and attention to the task. Rats that had self-administered large amounts of cocaine had diminished neural responsiveness to cues, which translated into reductions in behavioral measures of attention, disruptions in cognitive flexibility, and impaired decision-making. These results both supported previous findings establishing the ACC's role in attentional allocation, and revealed an intake-dependent effect of drugs on decision-making and neural encoding.

Rats that had self-administered cocaine displayed heightened impulsivity, demonstrating a preference for immediate rewards over delayed ones, and exhibiting notably poorer performance on forced-choice tasks. Consequently, these cocaine-exposed rats accrued fewer reward overall. This was attributed to the absence of rewards on incorrect forced-choice trials and the normalization of short-delay trials, which prevented an accumulation of greater rewards despite the tendency to perseverate and select immediate options repeatedly across multiple trials.

Additionally, we proposed that the ACC plays a role in sustaining attention to cues during block adjustments, enabling rats to adhere to forced-choice rules while updating action-outcome contingencies in free-choice trials. This notion aligns with our observation that rats tended to perform poorly on forced-choice trials when attention levels were diminished.

Our results align with studies in humans investigating cognitive control impairments that are induced by cocaine use (Pace-Schott et al., 2008; Balodis et al., 2016; van Son et al., 2016; Almeida et al., 2017), reinforcing the translational validity of our findings, and offering further evidence of drug-induced alterations in brain function and behavior at the level of individual neurons. Importantly, our study controls for confounding variables that could affect research in humans, such as life history and polysubstance abuse, enhancing the validity of our findings.

The dose-dependent effects we found were particularly intriguing, but due to the correlational nature of these findings we could not disambiguate whether more impulsive rats self-administered more cocaine, or whether having self-administered higher quantities of cocaine made them subsequently more impulsive. Further, we could not disambiguate whether the

behavioral disruptions arose from ACC signaling disruptions, or were partially attributable to the global impact of drug use throughout the brain.

Thus, in our subsequent experiment we wanted to be able to precisely modulate ACC activity in order to better interrogate the role of the ACC in the absence of confounding variables (e.g. cocaine use results in the dysregulation of various neural circuits), and conduct within-subject analyses. Subsequently, we used optogenetics to transiently inactivate the ACC. Our results unveiled that inhibiting the ACC significantly hindered the rats' fundamental abilities to both initiate and complete trials, leading to a decrease in both obtained reward and completed sessions. Notably, reaction times, movement times, and reward consumption remained unimpaired across different trial types and choices, indicating that the deficits observed in trial initiation, completion, and task engagement were not related to issues with motor control or motivation.

Our primary hypothesis for this study was that the functional ACC played a crucial role in directing attention towards cues predicting rewards, thereby facilitating both the learning and updating of value contingencies. This aligns with previous studies implicating the ACC in various aspects of reward processing, conflict monitoring, error detection, and attention allocation (Aarts et al., 2008; Barch, 2001; Botvinick et al., 2004; Braver, 2001; Carter et al., 1998; Hayden et al., 2011; Holroyd et al., 2004; Hyman et al., 2013; Kerns et al., 2004; Laubach et al., 2015; Newman et al., 2015; Soltani & Izquierdo, 2019; Totah et al., 2009; Weissman, 2004; Wu et al., 2017; Yeung et al., 2004). Our findings demonstrated that ACC inactivation severely disrupted the rats' ability to sustain task performance, evident by the reductions in trial initiations, and trial and session completions—resulting in overall impaired session performance.

Moreover, their latency to respond to the trial initiation cue—much as we observed in our cocaine study—was significantly longer on inhibition days, and task performance was negatively correlated with this behavioral proxy of attention to the task. The reductions in accuracy could potentially be attributable to attenuated response-outcome associations, which have been shown to stem from alterations in attention (Krajbich et al., 2010, 2012; Nunez et al., 2017; Retzler et al., 2020).

The observed lack of engagement throughout the task on inhibition days could be related to an inability to sustain attention in a task that is highly cognitively demanding. Research has demonstrated that rats with ACC lesions are less inclined to surmount physical barriers in order to access higher-valued reward, instead opting for an easily obtained, smaller reward that requires minimal effort (Walton et al., 2003; Rudebeck et al., 2006; Holec et al., 2014). Similarly, investigations involving human subjects with bilateral ACC lesions have revealed motivational and attentional deficits, alongside akinetic mutism—a disorder characterized by a state of profound apathy and a paucity of goal-directed behaviors (Barris & Schuman, 1953; Laplane et al., 1981).

While our task does not involve physical exertion, it is highly cognitively demanding, requiring rats to adhere to a basic trial structure (e.g., responding to houselights, maintaining the hold in the odor port, choosing a well) and to learn, track, and revise response-outcome associations, which vary across two reward dimensions across four sixty-trial blocks—all while adhering to forced-choice rules. It's conceivable that the ACC plays a particularly crucial role when rats are tasked with sustaining attention during activities that demand significant cognitive effort or complexity.

However, we found that—while inactivation disrupted attention in a way that impaired task performance—we did not observe decision-making impairments on sessions which rats were able to complete. Thus, it appears that when task engagement is conserved, rats are able to perform the task well—suggesting that action-outcome encoding is preserved.

In order to confirm this, we chemically lesioned the ACC, and recorded neural activity from the dorsomedial striatum (DMS)—a downstream brain region that is importantly involved in goal-directed behavior—as rats performed the previously mentioned decision-making task in order to explore whether ACC signaling disruptions impacted downstream action-outcome encoding.

ACC projections to the DMS are an important component of the corticostriatal circuit—a critical circuit for motivated behavior—with ample evidence demonstrating that DMS encoding drives goal-directed decision-making (Yin et al., 2005; Stalnaker et al., 2010; Thorn et al., 2010; Stalnaker et al., 2012). Our findings revealed detrimental effects of ACC lesions across various behavioral measures. Specifically, rats with lesions exhibited significantly inferior performance across all trial types, showed reduced preference for high-valued rewards, and demonstrated slower light-on latency, reaction, and movement times.

In spite of these significant behavioral impairments, the impact of ACC lesions on DMS encoding was small—evident primarily in the attenuated directional signals during large reward trials across the population. These results suggest that the pronounced behavioral deficits following ACC dysfunction do not stem from disruptions to downstream action-outcome encoding, but rather relate to a breakdown in functions necessary for engaging in a complex

reward-guided decision-making task when attention is required for learning (e.g., during block transitions).

One plausible explanation is that ACC lesions bias behavior in a manner that minimizes the effort exerted by an organism to obtain a reward. Supporting this notion, rats with lesions exhibited fewer food cup entries during autoshaping; however, they interacted with the lever at a comparable rate to control rats, making this explanation less probable (see Chapter IV). It could also be argued that lesioned rats displayed gross motor deficits; however, during delay blocks—which always occurred first during recording sessions—there were no notable differences in movement times between control and lesioned rats. These significant reductions in movement times towards the end of sessions, suggest that ACC dysfunction impacts the capacity to sustain attention.

Moreover, the results of our autoshaping experiment immediately following the reward-based decision-making task suggest that these behavioral deficits were not attributable to aberrant deficits in lever pressing or locomotion. Notably, lesioned rats engaged in more locomotion, which may reflect their reduced task engagement (i.e., they roamed around the chamber instead of interacting with the food cup and lever). In line with this interpretation, studies in humans with ADHD have identified high locomotor, non-task-related activity as a core presentation (Stray et al., 2013; Gawrilow et al., 2014; Garcia Murillo et al., 2015).

Indeed, consistent with findings from our optogenetic inactivation study, lesioned rats took significantly longer to complete recording sessions and were markedly less engaged in the task, initiating trials less frequently than controls. Additionally, akin to observations in previous studies (Chapters II and III), when examining both complete and incomplete sessions,

differences in movement and reaction times—but not in light-on latencies—were no longer apparent.

We found that ACC lesions resulted in disrupted attention to the task, and similar behavioral deficits to the ones we observed following cocaine use. Interestingly, we found that DMS encoding was minimally impacted—reinforcing the idea that the ACC is critical for task engagement, but not for informing downstream action-outcome representations. The disruptions to DMS encoding during delay blocks may also suggest that the valuation of this variable relies more heavily on ACC output to the nucleus accumbens, a region strongly implicated in reward expectation and central to delay discounting and intertemporal choice tasks (Cardinal & Cheung, 2005; Acheson et al., 2006; Galtress & Kirkpatrick, 2010; Joutsa et al., 2015; Wu et al., 2018).

In the aforementioned experiments, we employed an array of techniques to dissect how disrupting ACC signaling in a variety of manners impacted task performance and engagement, so for our final experiment we sought to explore a therapeutically relevant way to potentially repair signaling disruptions that lead to the breakdown in attentional signaling. Thus, we turned to epigenetics—specifically, decreasing the expression of HDAC5, an enzyme that is involved in regulating gene expression—explore whether epigenetic changes might map onto specific alterations of neural activity and behavior.

When analyzing across all sessions—both complete and incomplete—HDAC5 knockdown led to diminished task accuracy, decreased selection of high-value rewards, and reduced task engagement. However, focusing solely on sessions in which rats completed the entire task (e.g. all four blocks), accuracy impairments persisted, while differences in choice selection, movement, and reaction times vanished. This mirrors our earlier findings, where the

pronounced behavioral deficits appeared to stem from overall reductions in task engagement and not when rats were able to sustain engagement.

Additionally, we replicated earlier findings showing that attentional signals are enhanced following shifts in contingencies and updates to learning (Bryden et al., 2011; Vázquez et al., 2020). Historically, we have also noted that heightened ACC firing follows contingency changes—when learning needs to be updated—and are linked to improved performance (Bryden et al., 2011; Vázquez et al., 2020). However, these alterations in firing were not seen in the HDAC5 knockdown rats.

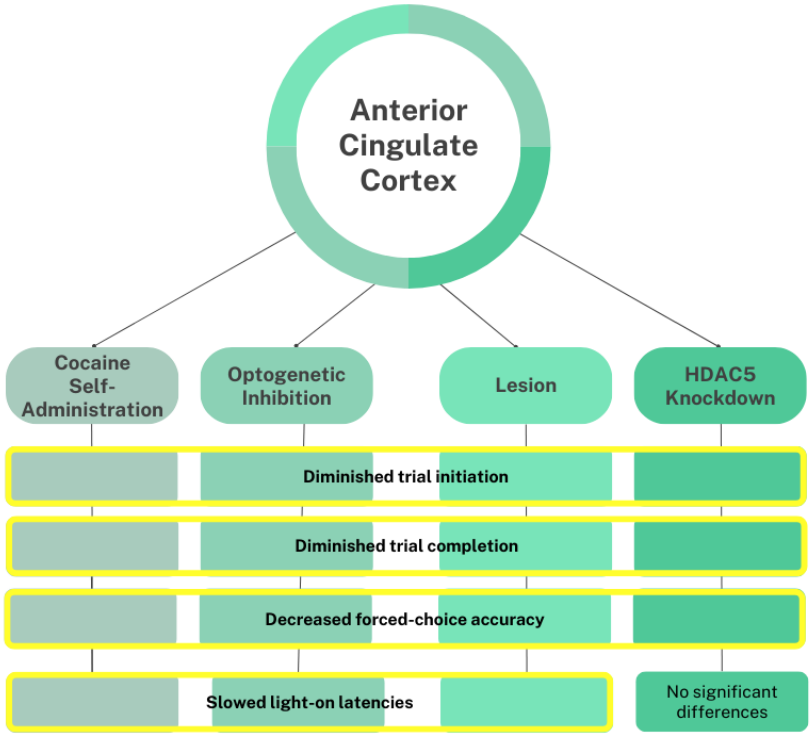

Figure 7.1: Activity patterns within the functional ACC were closely associated with measures of attention, and across the board there are correlations between these behavioral measures of attention and task accuracy. Additionally, there was a persistent decline in task engagement following ACC dysfunction, characterized by notable reductions in the proportion of initiated and completed trials and prolonged periods of inattention (measured by light-on latencies), except following HDAC5 knockdown. Yellow highlight denotes overlapping findings; bold font indicates findings were consistent across all four studies.

Across all of the studies we conducted, light-on latencies served as a behavioral proxy that allowed us to gauge the timing and intensity of attentional control processes. Faster light-on latencies during task performance are believed to indicate accelerated processing of trial events (such as cues and responses) following unexpected shifts in reward contingencies. This

acceleration mirrors theoretical shifts proposed in Pearce and Hall models of attention, particularly in terms of task reengagement being necessary for updating learned block structures in response to changes in reward value (Pearce & Hall, 1980). Accordingly, throughout all of our presently discussed studies we found correlations between attenuated attention and diminished task accuracy.

Thus, we found that signaling in the functional ACC correlates with behavioral measures of attention, and that attenuation of these signals results in disruptions to decision-making behaviors, primarily evinced by decreased accuracy and sub-

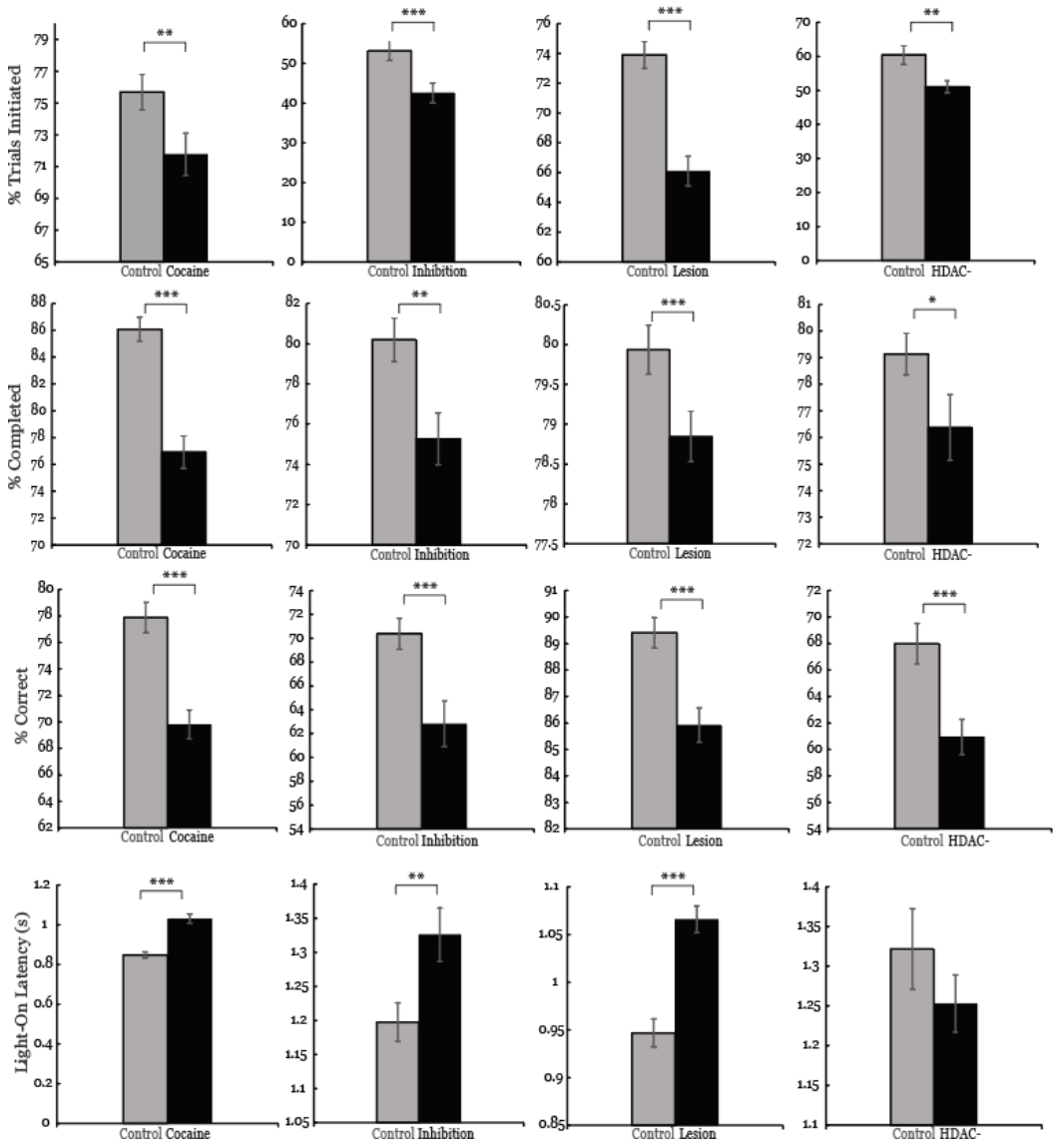

Figure 7.2: A brief overview of some of the major findings that existed across all manipulations; these analyses occurred across all sessions. This figure is a graphical depiction of the summary of results presented in Figure 7.1. Across all four manipulations of the ACC, we found decreased accuracy on task performance. With the exception of the HDAC- study, we found longer light-on latencies—suggestive of decreased task engagement (e.g. they were slow to initiate trials). Along those same lines, ACC manipulations decreased trial initiation and trial completion across the board. Finally, there were no significant consistencies across movement and reaction times—a finding highlighted here to show that light-on latencies are distinctly impacted by disruptions to ACC signaling. These results make sense with regards to the impacts on task engagement. Although motivation is difficult—if not impossible—to fully disentangle from attention to the task, their indistinguishable movement and reaction times suggest that these findings are not attributable to decreased motivation to obtain a reward. Although movement times were impacted in the lesioned rats, there were other factors suggesting that this was not due to decreased motivation (see Chapter IV discussion).

optimal free-choice responding. Across the board, we also found a consistent breakdown in task engagement upon ACC dysregulation—specifically, significant decreases of the percentage of trials rats initiated and completed, and longer periods of inattention (operationalized by light-on latencies), although HDAC5 knockdown did not impact these latencies. *Figure 7.1* presents a qualitative summary of findings, and *Figure 7.2* serves as a graphical depiction of these findings.

Future Directions

Exploring excitatory/inhibitory balance

Studies suggest that the dysregulation of excitatory and inhibitory activity (E/I) seems to contribute to the pathogenesis, as well as the inattention and impulsivity symptoms, of neuropsychiatric disorders such as ADHD (Naaijen et al., 2017; Shepard et al., 2019; Kim et al., 2021; Liu et al., 2021; Mamiya et al., 2022). Importantly, the manipulations presented in this dissertation principally impacted pyramidal neurons—excitatory cells. Future work exploring how manipulating activity in interneurons that inhibit excitatory cells—such as parvalbumin- and somatostatin- positive interneurons (Nahar et al., 2021)—modulates attention might be beneficial to perform a more thorough interrogation of ACC circuitry.

Related to E/I imbalance, future studies should look into overexpressing Npas4—a target gene of HDAC5—in order to assess its impact on the ACC's activity and modulation of attention. Npas4 is a transcription factor that is expressed solely in neurons, and is very importantly implicated in both excitatory and inhibitory synaptogenesis in the service of promoting homeostasis (Lin et al., 2008; Coutellier et al., 2012; Spiegel et al., 2014; Shepard et al., 2019; Fu et al., 2020; Kim et al., 2021). Our epigenetic manipulation centered around knocking down HDAC5—which negatively regulates Npas4 expression (Di Ciano et al., 2001;

Taniguchi et al., 2017; Zhu et al., 2019; Rein et al., 2022). Naturistically, in order to negatively regulate transcriptional processes, class IIa HDACs need to be localized to the nucleus (Clocchiatti et al., 2011); because HDAC5's nucleocytoplasmic shuttling is stimulus-dependent—and thus, its ability to repress transcription is activity-dependent—this enzyme is simultaneously both a critical and challenging target to study in the context of learning. Honing in on the modulation of one of its main target genes—Npas4—may help us precisely identify molecular contributors to the genetic basis of attention disorders such as ADHD.

HDAC5 following drug use

The study of HDAC5's impact on reward learning has largely been restricted to the context of drug-seeking. Researchers studying the link between cocaine and HDAC5 expression have noted that repeated cocaine administration causes CaMKII-dependent phosphorylation of HDAC5, which results in its nuclear export and loss of function as a transcriptional repressor (Renthal et al., 2007; Taniguchi et al., 2012). Within an hour, however, cAMP-mediated activation of protein phosphatase dephosphorylates HDAC5, and it is subsequently imported back into the nucleus (Renthal et al., 2007; Taniguchi et al., 2012). While these studies do not examine the long-term impact of drug abuse on deacetylase activity, nor the effects following—but outside of—the context of immediate drug use, our findings in Chapter II suggest that the impact of drug abuse on executive function extends far beyond the acute effects of drug use. Future research should explore whether the chronic impact of cocaine abuse on decision-making is associated with aberrant deacetylase activity or expression.

Probing the circuit optogenetically

Another interesting avenue of research will be employing anatomically precise and cell-specific optogenetic manipulation to probe which network connections are necessary for attention. We would use a retrograde label, such as glycoprotein-deleted rabies virus, to target presynaptic inputs with halorhodopsin (Kim et al., 2017). Among regions of interest to inject the retrograde virus would be the nucleus accumbens, for its extensive role in reward processing and motivation, and the insula—for its involvement in the salience network.

Complementary behavioral assays

Consistently throughout our studies, we were able to replicate that the ACC plays a critical role in task engagement. As evinced by the lack of behavioral differences on completed sessions across the optogenetic study, coupled with the lack of robust disruptions to action-outcome encoding during the lesion experiment, it appears that ACC inactivation did not have a behavioral impact on the ability of rats to acquire action-outcome associations—findings that are supported by inactivation studies showing that the ACC is not necessary for the acquisition of conditioned behaviors (Cardinal et al., 2003; Schweimer et al., 2005; Aly-Mahmoud et al., 2017; Halbout et al., 2022; Stawicka et al., 2022). Instead, in Chapters III and IV we saw that ACC inactivation diminished attention, which impacted task engagement and—subsequently—accuracy during sessions which they were unable to complete. The structure of our behavioral paradigm—while allowing us to parse out various facets of complex decision-making—does not allow us to conduct normalized analysis on unfinished sessions—which are the sessions in which ACC inactivation was deleterious to task execution. Thus, future research should study the effects of optogenetic inactivation of the ACC during less complex tasks—such as the 5-choice

serial reaction time task (5CSRTT), which is a behavioral assay commonly used to study attention (Asinof et al., 2014).

In this task, rats are placed in an operant chamber that contains a panel with five openings. A trial consists of a brief cue light illuminating one of the chambers, at which point the rat must nosepoke into the opening in order to receive reward (Robbins, 2002; Bari et al., 2008; Asinof et al., 2014; Remmelink et al., 2017). This paradigm allows you to assess attention at its most basic level, as well as impulsive behavior or inhibitory control. Premature responding during the inter-trial interval, for example, is a measure of prepotent and impulsive behavior. Unfortunately, there is a trade-off—this task is significantly less cognitively demanding, and thus would not allow us to parse the impacts of inattention on higher-order decision-making flexibility (e.g. tracking contingency switches), which attention is particularly important for.

In sum, our work at present identifies the ACC as an important hub for targeted therapeutic interventions aimed at ameliorating the attentional impairments that are hallmarks of some of the most prevalent neuropsychiatric disorders that ail modern society. A continued interrogation of the ACC's role in attention at both the systems- and cellular-level will provide insight into how to repair these deficits.

REFERENCES

Acheson A, Farrar AM, Patak M, Hausknecht KA, Kieres AK, Choi S, de Wit H, Richards JB. Nucleus accumbens lesions decrease sensitivity to rapid changes in the delay to reinforcement. Behav Brain Res. 2006 Oct 16;173(2):217-28. doi: 10.1016/j.bbr.2006.06.024. Epub 2006 Aug 1. PMID: 16884790; PMCID: PMC4083481.

Akam T, Rodrigues-Vaz I, Marcelo I, Zhang X, Pereira M, Oliveira RF, Dayan P, Costa RM. The Anterior Cingulate Cortex Predicts Future States to Mediate Model-Based Action Selection. Neuron. 2021 Jan 6;109(1):149-163.e7. doi: 10.1016/j.neuron.2020.10.013. Epub 2020 Nov 4. PMID: 33152266; PMCID: PMC7837117.

Almeida PP, de Araujo Filho GM, Malta SM, Laranjeira RR, Marques ACRP, Bressan RA, Lacerda ALT. Attention and memory deficits in crack-cocaine users persist over four weeks of abstinence. J Subst Abuse Treat. 2017 Oct;81:73-78. doi: 10.1016/j.jsat.2017.08.002. Epub 2017 Aug 5. PMID: 28847458.

Aly-Mahmoud M, Carlier P, Salam SA, Houari Selmani M, Moftah MZ, Esclapez M, Boussaoud D. Role of Anterior Cingulate Cortex in Instrumental Learning: Blockade of Dopamine D1 Receptors Suppresses Overt but Not Covert Learning. Front Behav Neurosci. 2017 May 15;11:82. doi: 10.3389/fnbeh.2017.00082. PMID: 28555096; PMCID: PMC5430040.

Amico F, Stauber J, Koutsouleris N, Frodl T. Anterior cingulate cortex gray matter abnormalities in adults with attention deficit hyperactivity disorder: a voxel-based morphometry study. Psychiatry Res. 2011 Jan 30;191(1):31-5. doi: 10.1016/j.pscychresns.2010.08.011. Epub 2010 Dec 3. PMID: 21129938.

Anderson KW, Chen J, Wang M, Mast N, Pikuleva IA, Turko IV. Quantification of histone deacetylase isoforms in human frontal cortex, human retina, and mouse brain. PLoS One. 2015 May 11;10(5):e0126592. doi: 10.1371/journal.pone.0126592. PMID: 25962138; PMCID: PMC4427357.

Anderson EM, Tsvetkov E, Galante A, DeVries D, McCue LM, Wood D, Barry S, Berto S, Lavin A, Taniguchi M, Cowan CW. (2023) Epigenetic function during heroin self-administration controls future relapse-associated behavior in a cell type-specific manner. *Proceedings of the National Academy of Sciences*. doi.org/10.1073/pnas.2210953120.

Asinof SK, Paine TA. The 5-choice serial reaction time task: a task of attention and impulse control for rodents. J Vis Exp. 2014 Aug 10;(90):e51574. doi: 10.3791/51574. PMID: 25146934; PMCID: PMC6592595.

Balleine BW, Delgado MR, Hikosaka O. The role of the dorsal striatum in reward and decision-making. J Neurosci. 2007 Aug 1;27(31):8161-5. doi: 10.1523/JNEUROSCI.1554-07.2007. PMID: 17670959; PMCID: PMC6673072.

Balleine BW, O'Doherty JP. Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.* 2010;35:48–69.

Balleine, B.W., Peak, J., Matamales, M., Bertran-Gonzalez, J., and Hart, G. (2021). The dorsomedial striatum: an optimal cellular environment for encoding and updating goal-directed learning. Curr. Opin. Behav. Sci. 41, 38–44.

Balodis IM, Kober H, Worhunsky PD, Stevens MC, Pearlson GD, Carroll KM, Potenza MN. Neurofunctional Reward Processing Changes in Cocaine Dependence During Recovery. Neuropsychopharmacology. 2016 Jul;41(8):2112-21. doi: 10.1038/npp.2016.11. Epub 2016 Jan 21. PMID: 26792441; PMCID: PMC4908642.

Banich MT, Burgess GC, Depue BE, Ruzic L, Bidwell LC, Hitt-Laustsen S, Du YP, Willcutt EG. The neural basis of sustained and transient attentional control in young adults with ADHD. Neuropsychologia. 2009 Dec;47(14):3095-104. doi: 10.1016/j.neuropsychologia.2009.07.005. Epub 2009 Jul 18. PMID: 19619566; PMCID: PMC3703501.

Bari A, Dalley JW, Robbins TW. The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. Nat Protoc. 2008;3(5):759-67. doi: 10.1038/nprot.2008.41. PMID: 18451784.

Barrett RM, Wood MA. Beyond transcription factors: the role of chromatin modifying enzymes in regulating transcription required for memory. *Learn Mem.* 2008;15:460–467.

Battison EAJ, Brown PCM, Holley AL, Wilson AC. Associations between Chronic Pain and Attention-Deficit Hyperactivity Disorder (ADHD) in Youth: A Scoping Review. Children (Basel). 2023 Jan 11;10(1):142. doi: 10.3390/children10010142. PMID: 36670692; PMCID: PMC9857366.

Bayard, F., Nymberg Thunell, C., Abé, C. *et al.* Distinct brain structure and behavior related to ADHD and conduct disorder traits. *Mol Psychiatry* **25**, 3020–3033 (2020). https://doi.org/10.1038/s41380-018-0202-6

Bieszczad KM, Bechay K, Rusche JR, Jacques V, Kudugunti S, Miao W, Weinberger NM, McGaugh JL, Wood MA. Histone Deacetylase Inhibition via RGFP966 Releases the Brakes on Sensory Cortical Plasticity and the Specificity of Memory Formation. J Neurosci. 2015 Sep 23;35(38):13124-32. doi: 10.1523/JNEUROSCI.0914-15.2015. PMID: 26400942; PMCID: PMC4579377.

Bledsoe JC, Semrud-Clikeman M, Pliszka SR. Anterior cingulate cortex and symptom severity in attention-deficit/hyperactivity disorder. J Abnorm Psychol. 2013 May;122(2):558-65. doi: 10.1037/a0032390. PMID: 23713508.

Botvinick MM, Nystrom LE, Fissell K, Carter CS, Cohen JD. Conflict monitoring versus selection-for-action in anterior cingulate cortex. Nature. 1999;402:179–81.

Botvinick MM, Cohen JD, Carter CS. Conflict monitoring and anterior cingulate cortex: an update. Trends Cogn Sci. 2004;8:539–46.

Brockett AT, Pribut HJ, Vázquez D, Roesch MR. The impact of drugs of abuse on executive function: characterizing long-term changes in neural correlates following chronic drug exposure and withdrawal in rats. Learn Mem. 2018 Aug 16;25(9):461-473. doi: 10.1101/lm.047001.117. PMID: 30115768; PMCID: PMC6097763.

Brockett AT, Roesch MR. Anterior cingulate cortex and adaptive control of brain and behavior. Int Rev Neurobiol. 2021;158:283-309. doi: 10.1016/bs.irn.2020.11.013. Epub 2020 Dec 15. PMID: 33785148.

Brockett AT, Tennyson SS, deBettencourt CA, Gaye F, Roesch MR. Anterior cingulate cortex is necessary for adaptation of action plans. Proc Natl Acad Sci U S A. 2020 Mar 17;117(11):6196-6204. doi: 10.1073/pnas.1919303117. Epub 2020 Mar 4. PMID: 32132213; PMCID: PMC7084129.

Bryden, D. W., Johnson, E. E., Tobia, S. C., Kashtelyan, V., & Roesch, M. R. (2011). Attention for learning signals in anterior cingulate cortex. *The Journal of Neuroscience, 31*(50), 18266–18274. https://doi.org/10.1523/JNEUROSCI.4715-11.2011

Bryden DW, Brockett AT, Blume E, Heatley K, Zhao A, Roesch MR. Single Neurons in Anterior Cingulate Cortex Signal the Need to Change Action During Performance of a Stop-change Task that Induces Response Competition. Cereb Cortex. 2019 Mar 1;29(3):1020-1031. doi: 10.1093/cercor/bhy008. PMID: 29415274; PMCID: PMC6373672.

Burgdorf CE, Schierberl KC, Lee AS, Fischer DK, Van Kempen TA, Mudragel V, Huganir RL, Milner TA, Glass MJ, Rajadhyaksha AM. Extinction of Contextual Cocaine Memories Requires $Ca_v1.2$ within D1R-Expressing Cells and Recruits Hippocampal $Ca_v1.2$-Dependent Signaling Mechanisms. J Neurosci. 2017 Dec 6;37(49):11894-11911. doi: 10.1523/JNEUROSCI.2397-17.2017. Epub 2017 Oct 31. PMID: 29089442; PMCID: PMC5719973.

Burke DA, Rotstein HG, Alvarez VA. Striatal Local Circuitry: A New Framework for Lateral Inhibition. Neuron. 2017 Oct 11;96(2):267-284. doi: 10.1016/j.neuron.2017.09.019. PMID: 29024654; PMCID: PMC5649445.

Burns AM, Farinelli-Scharly M, Hugues-Ascery S, Sanchez-Mut JV, Santoni G, Gräff J. The HDAC inhibitor CI-994 acts as a molecular memory aid by facilitating synaptic and intracellular communication after learning. Proc Natl Acad Sci U S A. 2022; 119: 1-12.

Burton AC, Nakamura K, Roesch MR. From ventral-medial to dorsal-lateral striatum: neural correlates of reward-guided decision-making. Neurobiol Learn Mem. 2015 Jan;117:51-9. doi: 10.1016/j.nlm.2014.05.003. Epub 2014 May 21. PMID: 24858182; PMCID: PMC4240773.

Burton AC, Bissonette GB, Zhao AC, Patel PK, Roesch MR. Prior Cocaine Self-Administration Increases Response-Outcome Encoding That Is Divorced from Actions Selected in Dorsal Lateral Striatum. J Neurosci. 2017 Aug 9;37(32):7737-7747. doi: 10.1523/JNEUROSCI.0897-17.2017. Epub 2017 Jul 10. PMID: 28694335; PMCID: PMC5551065.

Burton AC, Bissonette GB, Zhao AC, Patel PK, Roesch MR. Prior Cocaine Self-Administration Increases Response-Outcome Encoding That Is Divorced from Actions Selected in Dorsal Lateral Striatum. J Neurosci. 2017 Aug 9;37(32):7737-7747. doi: 10.1523/JNEUROSCI.0897-17.2017. Epub 2017 Jul 10. PMID: 28694335; PMCID: PMC5551065.

Burton AC, Bissonette GB, Vazquez D, Blume EM, Donnelly M, Heatley KC, Hinduja A, Roesch MR. Previous cocaine self-administration disrupts reward expectancy encoding in ventral striatum. Neuropsychopharmacology. 2018 Nov;43(12):2350-2360. doi: 10.1038/s41386-018-0058-0. Epub 2018 Apr 10. PMID: 29728645; PMCID: PMC6180050.

Bush G, Frazier JA, Rauch SL, Seidman LJ, Whalen PJ, Jenike MA, Rosen BR, Biederman J. Anterior cingulate cortex dysfunction in attention-deficit/hyperactivity disorder revealed by fMRI and the Counting Stroop. Biol Psychiatry. 1999 Jun 15;45(12):1542-52. doi: 10.1016/s0006-3223(99)00083-9. PMID: 10376114.

Bush G. Cingulate, frontal, and parietal cortical dysfunction in attention-deficit/hyperactivity disorder. Biol Psychiatry. 2011 Jun 15;69(12):1160-7. doi: 10.1016/j.biopsych.2011.01.022. Epub 2011 Apr 13. PMID: 21489409; PMCID: PMC3109164.

Cardinal RN, Parkinson JA, Marbini HD, Toner AJ, Bussey TJ, Robbins TW, Everitt BJ. Role of the anterior cingulate cortex in the control over behavior by Pavlovian conditioned stimuli in rats. Behav Neurosci. 2003 Jun;117(3):566-87. doi: 10.1037/0735-7044.117.3.566. PMID: 12802885.Cardinal RN, Cheung TH. Nucleus accumbens core lesions retard instrumental learning and performance with delayed reinforcement in the rat. BMC Neurosci. 2005 Feb 3;6:9. doi: 10.1186/1471-2202-6-9. PMID: 15691387; PMCID: PMC549214.

Carter CS, Braver TS, Barch DM, Botvinick MM, Noll D, Cohen JD. Anterior cingulate cortex, error detection, and the online monitoring of performance. Science. 1998;280:747–9.

Castellanos FX, Proal E. Large-scale brain systems in ADHD: beyond the prefrontal-striatal model. Trends Cogn Sci. 2012 Jan;16(1):17-26. doi: 10.1016/j.tics.2011.11.007. Epub 2011 Dec 12. PMID: 22169776; PMCID: PMC3272832.

Cates HM, Li X, Purushothaman I, Kennedy PJ, Shen L, Shaham Y, Nestler EJ. Genome-wide transcriptional profiling of central amygdala and orbitofrontal cortex during incubation of methamphetamine craving. Neuropsychopharmacology. 2018 Nov;43(12):2426-2434. doi: 10.1038/s41386-018-0158-x. Epub 2018 Jul 20. PMID: 30072726; PMCID: PMC6180053.

Chan SC, Chan CC, Kwan AS, Ting KH, Chui TY. Orienting attention modulates pain perception: an ERP study. PLoS One. 2012;7(6):e40215. doi: 10.1371/journal.pone.0040215. Epub 2012 Jun 29. PMID: 22768257; PMCID: PMC3387012.

Chen T, Taniguchi W, Chen QY, Tozaki-Saitoh H, Song Q, Liu RH, Koga K, Matsuda T, Kaito-Sugimura Y, Wang J, Li ZH, Lu YC, Inoue K, Tsuda M, Li YQ, Nakatsuka T, Zhuo M. Top-down descending facilitation of spinal sensory excitatory transmission from the anterior cingulate cortex. Nat Commun. 2018 May 14;9(1):1886. doi: 10.1038/s41467-018-04309-2. PMID: 29760484; PMCID: PMC5951839.

Chen W, Li C, Liang W, Li Y, Zou Z, Xie Y, Liao Y, Yu L, Lin Q, Huang M, Li Z, Zhu X. The Roles of Optogenetics and Technology in Neurobiology: A Review. Front Aging Neurosci. 2022 Apr 19;14:867863. doi: 10.3389/fnagi.2022.867863. PMID: 35517048; PMCID: PMC9063564.

Choi K, Holly EN, Davatolhagh MF, Beier KT, Fuccillo MV, Integrated anatomical and physiological mapping of striatal afferent projections, Eur J Neurosci. 49 (2019) 623–636. https://doi.org/10.1111/ejn.13829.

Clocchiatti A, Florean C, Brancolini C. Class IIa HDACs: from important roles in differentiation to possible implications in tumourigenesis. J Cell Mol Med. 2011 Sep;15(9):1833-46. doi: 10.1111/j.1582-4934.2011.01321.x. PMID: 21435179; PMCID: PMC3918040.

Collins AL, Saunders BT. Heterogeneity in striatal dopamine circuits: Form and function in dynamic reward seeking. J Neurosci Res. 2020 Jun;98(6):1046-1069. doi: 10.1002/jnr.24587. Epub 2020 Feb 13. PMID: 32056298; PMCID: PMC7183907.

Cooper S, Robison AJ, Mazei-Robison MS. Reward Circuitry in Addiction. Neurotherapeutics. 2017 Jul;14(3):687-697. doi: 10.1007/s13311-017-0525-z. PMID: 28324454; PMCID: PMC5509624.

Crottaz-Herbette S, Menon V. Where and when the anterior cingulate cortex modulates attentional response: combined fMRI and ERP evidence. J Cogn Neurosci. 2006 May;18(5):766-80. doi: 10.1162/jocn.2006.18.5.766. PMID: 16768376.

Dayan P, Kakade S, Montague PR. Learning and selective attention. Nat Neurosci. 2000;3:1218

de Kloet SF, Bruinsma B, Terra H, Heistek TS, Passchier EMJ, van den Berg AR, Luchicchi A, Min R, Pattij T, Mansvelder HD. Bi-directional regulation of cognitive control by distinct prefrontal cortical output neurons to thalamus and striatum. Nat Commun. 2021 Mar 31;12(1):1994. doi: 10.1038/s41467-021-22260-7. PMID: 33790281; PMCID: PMC8012364.

de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J. 2003 Mar 15;370(Pt 3):737-49. doi: 10.1042/BJ20021321. PMID: 12429021; PMCID: PMC1223209.

D'Esposito M, Detre JA, Alsop DC, Shin RK, Atlas S, Grossman M. The neural basis of the central executive system of working memory. Nature. 1995;378:279–81.

Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ. Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. J Neurosci. 2001 Dec 1;21(23):9471-7. doi: 10.1523/JNEUROSCI.21-23-09471.2001. PMID: 11717381; PMCID: PMC6763894.

Dickstein SG, Bannon K, Castellanos FX, Milham MP. The neural correlates of attention deficit hyperactivity disorder: an ALE meta-analysis. J Child Psychol Psychiatry. 2006 Oct;47(10):1051-62. doi: 10.1111/j.1469-7610.2006.01671.x. PMID: 17073984.

Doody, M, Van Swieten, MMH, & Manohar, SG. Model-based learning retrospectively updates model-free values. *Sci Rep* **12**, 2358 (2022). https://doi.org/10.1038/s41598-022-05567-3

Dos Santos Sant' Anna G, Rostirola Elsner V, Moysés F, Reck Cechinel L, Agustini Lovatel G, Rodrigues Siqueira I. Histone deacetylase activity is altered in brain areas from aged rats. Neurosci Lett. 2013 Nov 27;556:152-4. doi: 10.1016/j.neulet.2013.10.016. Epub 2013 Oct 16. PMID: 24140005.

Elina KC, Moon HC, Islam J, Kim HK, Park YS. The Effect of Optogenetic Inhibition of the Anterior Cingulate Cortex in Neuropathic Pain Following Sciatic Nerve Injury. J Mol Neurosci. 2021 Mar;71(3):638-650. doi: 10.1007/s12031-020-01685-7. Epub 2020 Aug 18. PMID: 32808249.

Emiliani, V., Entcheva, E., Hedrich, R. *et al.* Optogenetics for light control of biological systems. *Nat Rev Methods Primers* **2**, 55 (2022). https://doi.org/10.1038/s43586-022-00136-4

Floresco SB. The nucleus accumbens: an interface between cognition, emotion, and action. Annu Rev Psychol. 2015 Jan 3;66:25-52. doi: 10.1146/annurev-psych-010213-115159. Epub 2014 Sep 17. PMID: 25251489.

Frodl T, Skokauskas N. Meta-analysis of structural MRI studies in children and adults with attention deficit hyperactivity disorder indicates treatment effects. *Acta Psychiatr Scand.* 2012;125:114–126. doi: 10.1111/j.1600-0447.2011.01786.x.

Fu J, Guo O, Zhen Z, Zhen J. Essential Functions of the Transcription Factor Npas4 in Neural Circuit Development, Plasticity, and Diseases. Front Neurosci. 2020 Dec 1;14:603373. doi: 10.3389/fnins.2020.603373. PMID: 33335473; PMCID: PMC7736240.

Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby SJ. Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol. 2005;492:145–77.

Galtress T, Kirkpatrick K. The role of the nucleus accumbens core in impulsive choice, timing, and reward processing. Behav Neurosci. 2010 Feb;124(1):26-43. doi: 10.1037/a0018464. PMID: 20141278; PMCID: PMC3909469.

García Murillo L, Cortese S, Anderson D, Di Martino A, Castellanos FX. Locomotor activity measures in the diagnosis of attention deficit hyperactivity disorder: Meta-analyses and new findings. J Neurosci Methods. 2015 Aug 30;252:14-26. doi: 10.1016/j.jneumeth.2015.03.001. Epub 2015 Mar 11. PMID: 25770940; PMCID: PMC4522351.

Gawrilow C, Kühnhausen J, Schmid J, Stadler G. Hyperactivity and Motoric Activity in ADHD: Characterization, Assessment, and Intervention. Front Psychiatry. 2014 Nov 28;5:171. doi: 10.3389/fpsyt.2014.00171. PMID: 25506329; PMCID: PMC4246670.

Goldstein RZ, Leskovjan AC, Hoff AL, Hitzemann R, Bashan F, Khalsa SS, Wang GJ, Fowler JS, Volkow ND. Severity of neuropsychological impairment in cocaine and alcohol addiction: association with metabolism in the prefrontal cortex. Neuropsychologia. 2004;42(11):1447-58. doi: 10.1016/j.neuropsychologia.2004.04.002. PMID: 15246283.

Gradinaru V, Thompson KR, Deisseroth K. eNpHR: a Natronomonas halorhodopsin enhanced for optogenetic applications. Brain Cell Biol. 2008 Aug;36(1-4):129-39. doi: 10.1007/s11068-008-9027-6. Epub 2008 Aug 2. PMID: 18677566; PMCID: PMC2588488.

Gremel, C., Costa, R. Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. *Nat Commun* **4**, 2264 (2013). https://doi.org/10.1038/ncomms3264

Greco TM, Yu F, Guise AJ, Cristea IM. Nuclear import of histone deacetylase 5 by requisite nuclear localization signal phosphorylation. Mol Cell Proteomics. 2011 Feb;10(2):M110.004317. doi: 10.1074/mcp.M110.004317. Epub 2010 Nov 16. PMID: 21081666; PMCID: PMC3033682.

Grimm O, van Rooij D, Tshagharyan A, Yildiz D, Leonards J, Elgohary A, Buitelaar J, Reif A. Effects of comorbid disorders on reward processing and connectivity in adults with ADHD. Transl Psychiatry. 2021 Dec 16;11(1):636. doi: 10.1038/s41398-021-01758-0. PMID: 34911950; PMCID: PMC8674233.

Haber SN. Corticostriatal circuitry. Dialogues Clin Neurosci. 2016 Mar;18(1):7-21. doi: 10.31887/DCNS.2016.18.1/shaber. PMID: 27069376; PMCID: PMC4826773.

Halbout B, Hutson C, Wassum KM, Ostlund SB. Dorsomedial prefrontal cortex activation disrupts Pavlovian incentive motivation. Front Behav Neurosci. 2022 Oct 13;16:999320. doi: 10.3389/fnbeh.2022.999320. PMID: 36311857; PMCID: PMC9608868.

Harstad E, Levy S; Committee on Substance Abuse. Attention-deficit/hyperactivity disorder and substance abuse. Pediatrics. 2014 Jul;134(1):e293-301. doi: 10.1542/peds.2014-0992. PMID: 24982106.

Hart G, Leung BK, Balleine BW. Dorsal and ventral streams: the distinct role of striatal subregions in the acquisition and performance of goal-directed actions. Neurobiol Learn Mem. 2014 Feb;108:104-18. doi: 10.1016/j.nlm.2013.11.003. Epub 2013 Nov 11. PMID: 24231424; PMCID: PMC4661143.

Hayden BY, Heilbronner SR, Pearson JM, Platt ML. Surprise signals in anterior cingulate cortex: neuronal encoding of unsigned reward prediction errors driving adjustment in behavior. J Neurosci. 2011;31:4178–87.

Holroyd, CB, Nieuwenhuis, S, Mars, RB, & Coles, MGH. Anterior cingulate cortex, selection for action, and error processing. In Posner MI, editor. Cognitive neuroscience of attention. New York, NY, US: Guilford Press; 2004. 219–231.

Hoogman M, Muetzel R, Guimaraes JP, Shumskaya E, Mennes M, Zwiers MP, Jahanshad N, Sudre G, Wolfers T, Earl EA, Soliva Vila JC, Vives-Gilabert Y, Khadka S, Novotny SE, Hartman CA, Heslenfeld DJ, et al., Brain Imaging of the Cortex in ADHD: A Coordinated Analysis of Large-Scale Clinical and Population-Based Samples. Am J Psychiatry. 2019 Jul 1;176(7):531-542. doi: 10.1176/appi.ajp.2019.18091033. Epub 2019 Apr 24. PMID: 31014101; PMCID: PMC6879185.

Hoover WB, Vertes RP. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct. 2007;212:149–79.

Host L, Dietrich JB, Carouge D, Aunis D, Zwiller J. Cocaine self-administration alters the expression of chromatin-remodelling proteins; modulation by histone deacetylase inhibition. J Psychopharmacol. 2011 Feb;25(2):222-9. doi: 10.1177/0269881109348173. Epub 2009 Nov 25. PMID: 19939859.

Hui B, Wang W, Li J. Biphasic modulation of cocaine-induced conditioned place preference through inhibition of histone acetyltransferase and histone deacetylase. Saudi Med J. 2010;31:389–393.

Hunnicutt BJ, Jongbloets BC, Birdsong WT, Gertz KJ, Zhong H, Mao T, A comprehensive excitatory input map of the striatum reveals novel functional organization, ELife. 5 (2016) e19103. https://doi.org/10.7554/eLife.19103.

Jarrard LE. On the use of ibotenic acid to lesion selectively different components of the hippocampal formation. J Neurosci Methods. 1989 Sep;29(3):251-9. doi: 10.1016/0165-0270(89)90149-0. PMID: 2477650.

Joutsa J, Voon V, Johansson J, Niemelä S, Bergman J, Kaasinen V. Dopaminergic function and intertemporal choice. Transl Psychiatry. 2015 Jan 6;5(1):e491. doi: 10.1038/tp.2014.133. Erratum in: Transl Psychiatry. 2015;5:e520. PMID: 25562841; PMCID: PMC4312827.

Jovicich J, Peters RJ, Koch C, Braun J, Chang L, Ernst T. Brain areas specific for attentional load in a motion-tracking task. J Cogn Neurosci. 2001 Nov 15;13(8):1048-58. doi: 10.1162/089892901753294347. PMID: 11784443.

Kenny PJ. Epigenetics, microRNA, and addiction. Dialogues Clin Neurosci. 2014;16:335–344.

Kerekes N, Sanchéz-Pérez AM, Landry M. Neuroinflammation as a possible link between attention-deficit/hyperactivity disorder (ADHD) and pain. Med Hypotheses. 2021 Dec;157:110717. doi: 10.1016/j.mehy.2021.110717. Epub 2021 Oct 20. PMID: 34717072.

Kerns JG, Cohen JD, MacDonald AW, Cho RY, Stenger VA, Carter CS. Anterior cingulate conflict monitoring and adjustments in control. Science. 2004;303:1023–6.

Khera T, Rangasamy V. Cognition and Pain: A Review. Front Psychol. 2021 May 21;12:673962. doi: 10.3389/fpsyg.2021.673962. PMID: 34093370; PMCID: PMC8175647.

Kim MS, Akhtar MW, Adachi M, Mahgoub M, Bassel-Duby R, Kavalali ET, Olson EN, Monteggia LM. An essential role for histone deacetylase 4 in synaptic plasticity and memory formation. J Neurosci. 2012 Aug 8;32(32):10879-86. doi: 10.1523/JNEUROSCI.2089-12.2012. PMID: 22875922; PMCID: PMC3480333.

Kim SY, Giovanello KS. The effects of attention on age-related relational memory deficits: evidence from a novel attentional manipulation. Psychol Aging. 2011 Sep;26(3):678-88. doi: 10.1037/a0022326. PMID: 21707178; PMCID: PMC3193860.

Kim CK, Adhikari A, Deisseroth K. Integration of optogenetics with complementary methodologies in systems neuroscience. Nat Rev Neurosci. 2017 Mar 17;18(4):222-235. doi: 10.1038/nrn.2017.15. PMID: 28303019; PMCID: PMC5708544.

Konrad K, Neufang S, Hanisch C, Fink GR, Herpertz-Dahlmann B. Dysfunctional attentional networks in children with attention deficit/hyperactivity disorder: evidence from an event-related functional magnetic resonance imaging study. Biol Psychiatry. 2006 Apr 1;59(7):643-51. doi: 10.1016/j.biopsych.2005.08.013. Epub 2005 Sep 28. PMID: 16197925.

Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology. 2010;35:217–38.

Krokhine SN, Ewers NP, Mangold KI, Boshra R, Lin CA, Connolly JF. N2b Reflects the Cognitive Changes in Executive Functioning After Concussion: A Scoping Review. Front Hum Neurosci. 2020 Dec 17;14:601370. doi: 10.3389/fnhum.2020.601370. PMID: 33424568; PMCID: PMC7793768.

Laubach M, Caetano MS, Narayanan NS. Mistakes were made: neural mechanisms for the adaptive control of action initiation by the medial prefrontal cortex. J Physiol-Paris. 2015;109:104–17.

Lee JL, Milton AL, Everitt BJ. Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. J Neurosci. 2006 May 31;26(22):5881-7. doi: 10.1523/JNEUROSCI.0323-06.2006. PMID: 16738229; PMCID: PMC6675229.

Leiker AM, Miller M, Brewer L, Nelson M, Siow M, Lohse K. The Relationship Between Engagement and Neurophysiological Measures of Attention in Motion-Controlled Video Games: A Randomized Controlled Trial. JMIR Serious Games. 2016 Apr 21;4(1):e4. doi: 10.2196/games.5460. PMID: 27103052; PMCID: PMC4858597.

Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD. Regulation of histone acetylation during memory formation in the hippocampus. *J Biol Chem.* 2004;279:40545–40559.

Li X, Rubio FJ, Zeric T, Bossert JM, Cates H, Kennedy P, Liu QR, Cimbro R, Nestler EJ, Hope BT, Shaham Y (2015) Incubation of methamphetamine craving is associated with selective increases in expression of BDNF and TrkB, glutamate receptors, and epigenetic enzymes in cue-activated Fos-expressing dorsal striatal neurons. Journal of Neuroscience 35, 8232-8244. PMID: 26019338

Li X, Carreria MB, Witonsky KF, Zeric T, Lofaro OM, Bossert JM, Zhang J, Surjono F, Richie CT, Harvey BK, Son H, Cowan CW, Nestler EJ, Shaham Y (2018) Role of dorsal striatal histone deacetylase 5 in incubation of methamphetamine craving. Biological Psychiatry 84: 213-222. PMID:29397902

Li X, Davis IR, Lofaro OM, Zhang J, Cimbro R, Rubio FJ. Distinct gene alterations between Fos-expressing striatal and thalamic neurons after withdrawal from methamphetamine self-administration. Brain Behav. 2019 Sep;9(9):e01378. doi: 10.1002/brb3.1378. Epub 2019 Jul 31. PMID: 31364821; PMCID: PMC6749486.

Liao DL, Huang CY, Hu S, Fang SC, Wu CS, Chen WT, Lee TS, Chen PC, Li CS. Cognitive control in opioid dependence and methadone maintenance treatment. PLoS One. 2014 Apr 11;9(4):e94589. doi: 10.1371/journal.pone.0094589. PMID: 24727743; PMCID: PMC3984179.

Lier EJ, van Rijn CM, de Vries M, van Goor H, Oosterman JM. The interaction between pain and cognition: on the roles of task complexity and pain intensity. Scand J Pain. 2021 Nov 3;22(2):385-395. doi: 10.1515/sjpain-2021-0119. PMID: 34727588.

Lindsay GW. Attention in Psychology, Neuroscience, and Machine Learning. Front Comput Neurosci. 2020 Apr 16;14:29. doi: 10.3389/fncom.2020.00029. Erratum in: Front Comput Neurosci. 2021 May 26;15:698574. PMID: 32372937; PMCID: PMC7177153.

Lipton DM, Gonzales BJ, Citri A. Dorsal Striatal Circuits for Habits, Compulsions and Addictions. Front Syst Neurosci. 2019 Jul 18;13:28. doi: 10.3389/fnsys.2019.00028. PMID: 31379523; PMCID: PMC6657020.

Mahn, M., Gibor, L., Patil, P. *et al*. High-efficiency optogenetic silencing with soma-targeted anion-conducting channelrhodopsins. *Nat Commun* **9**, 4125 (2018). https://doi.org/10.1038/s41467-018-06511-8

Mailly P, Aliane V, Groenewegen HJ, Haber SN, Deniau J-M. The rat prefrontostriatal system analyzed in 3D: evidence for multiple interacting functional units. J Neurosci. 2013;33:5718–27.

Malvaez M, Greenfield VY, Matheos DP, Angelillis NA, Murphy MD, Kennedy PJ, Wood MA, Wassum KM. Habits Are Negatively Regulated by Histone Deacetylase 3 in the Dorsal Striatum. Biol Psychiatry. 2018 Sep 1;84(5):383-392. doi: 10.1016/j.biopsych.2018.01.025. Epub 2018 Feb 8. PMID: 29571524; PMCID: PMC6082729.

Marchant NJ, Kaganovsky K, Shaham Y, Bossert JM. Role of corticostriatal circuits in context-induced reinstatement of drug seeking. Brain Res. 2015 Dec 2;1628(Pt A):219-32. doi: 10.1016/j.brainres.2014.09.004. Epub 2014 Sep 6. PMID: 25199590; PMCID: PMC4362860.

Marcott PF, Gong S, Donthamsetti P, Grinnell SG, Nelson MN, Newman AH, Birnbaumer L, Martemyanov KA, Javitch JA, Ford CP. Regional Heterogeneity of D2-Receptor Signaling in the Dorsal Striatum and Nucleus Accumbens. Neuron. 2018 May 2;98(3):575-587.e4. doi: 10.1016/j.neuron.2018.03.038. Epub 2018 Apr 12. PMID: 29656874; PMCID: PMC6048973.

Márquez Loza AM, Holroyd KB, Johnson SA, Pilgrim DM, Amato AA. Guillain-Barré Syndrome in the Placebo and Active Arms of a COVID-19 Vaccine Clinical Trial: Temporal Associations Do Not Imply Causality. Neurology. 2021 May 31;96(22):1052-1054. doi: 10.1212/WNL.0000000000011881. PMID: 33824169.

Martínez-Torres NI, Vázquez-Hernández N, Martín-Amaya-Barajas FL, Flores-Soto M, González-Burgos I. Ibotenic acid induced lesions impair the modulation of dendritic spine plasticity in the prefrontal cortex and amygdala, a phenomenon that underlies working memory and social behavior. Eur J Pharmacol. 2021 Apr 5;896:173883. doi: 10.1016/j.ejphar.2021.173883. Epub 2021 Jan 26. PMID: 33513334.

Maurice N, Deniau JM, Glowinski J, Thierry AM. Relationships between the prefrontal cortex and the basal ganglia in the rat: physiology of the corticosubthalamic circuits. J Neurosci. 1998;18:9539–46.

McQuown SC, Wood MA. HDAC3 and the molecular brake pad hypothesis. Neurobiol Learn Mem. 2011 Jul;96(1):27-34. doi: 10.1016/j.nlm.2011.04.005. Epub 2011 Apr 16. PMID: 21521655; PMCID: PMC3111848.

McQuown SC, Barrett RM, Matheos DP, Post RJ, Rogge GA, Alenghat T, et al. HDAC3 is a critical negative regulator of long-term memory formation. *J Neurosci.* 2011;31:764–774.

Minzenberg MJ. Pharmacotherapy for attention-deficit/hyperactivity disorder: from cells to circuits. Neurotherapeutics. 2012 Jul;9(3):610-21. doi: 10.1007/s13311-012-0128-7. PMID: 22718077; PMCID: PMC3441935.

Mitchell MR, Weiss VG, Ouimet DJ, Fuchs RA, Morgan D, Setlow B. Intake-dependent effects of cocaine self-administration on impulsive choice in a delay discounting task. Behav Neurosci. 2014 Aug;128(4):419-29. doi: 10.1037/a0036742. Epub 2014 May 19. PMID: 24841739; PMCID: PMC4107092.

Moon HC, Heo WI, Kim YJ, Lee D, Won SY, Kim HR, Ha SM, Lee YJ, Park YS. Optical inactivation of the anterior cingulate cortex modulate descending pain pathway in a rat model of trigeminal neuropathic pain created via chronic constriction injury of the infraorbital nerve. J Pain Res. 2017 Oct 3;10:2355-2364. doi: 10.2147/JPR.S138626. PMID: 29042811; PMCID: PMC5633286.

Moon HC, Park YS. Optogenetic Stimulation of the Anterior Cingulate Cortex Modulates the Pain Processing in Neuropathic Pain: A Review. J Mol Neurosci. 2022 Jan;72(1):1-8. doi: 10.1007/s12031-021-01898-4. Epub 2021 Sep 10. PMID: 34505976.

Morris RG, Schenk F, Tweedie F, Jarrard LE. Ibotenate Lesions of Hippocampus and/or Subiculum: Dissociating Components of Allocentric Spatial Learning. Eur J Neurosci. 1990;2(12):1016-1028. doi: 10.1111/j.1460-9568.1990.tb00014.x. PMID: 12106063.

Morris MJ, Karra AS, Monteggia LM. Histone deacetylases govern cellular mechanisms underlying behavioral and synaptic plasticity in the developing and adult brain. Behav Pharmacol. 2010 Sep;21(5-6):409-19. doi: 10.1097/FBP.0b013e32833c20c0. PMID: 20555253; PMCID: PMC2923662.

Nahar L, Delacroix BM, Nam HW. The Role of Parvalbumin Interneurons in Neurotransmitter Balance and Neurological Disease. Front Psychiatry. 2021 Jun 18;12:679960. doi: 10.3389/fpsyt.2021.679960. PMID: 34220586; PMCID: PMC8249927.

Narayanan NS, Cavanagh JF, Frank MJ, Laubach M. Common medial frontal mechanisms of adaptive control in humans and rodents. Nat Neurosci. 2013;16:1888.

Nestler EJ. Epigenetic mechanisms of drug addiction. Neuropharmacology. 2014 Jan;76 Pt B(0 0):259-68. doi: 10.1016/j.neuropharm.2013.04.004. Epub 2013 Apr 30. PMID: 23643695; PMCID: PMC3766384.

Newman LA, McGaughy J. Attentional effects of lesions to the anterior cingulate cortex: how prior reinforcement influences distractibility. Behav Neurosci. 2011 Jun;125(3):360-71. doi: 10.1037/a0023250. PMID: 21480690; PMCID: PMC3109123.

Newman LA, Creer DJ, McGaughy JA. Cognitive control and the anterior cingulate cortex: how conflicting stimuli affect attentional control in the rat. J Physiol Paris. 2015 Feb-Jun;109(1-3):95-103. doi: 10.1016/j.jphysparis.2014.06.004. Epub 2014 Jul 19. PMID: 25051488; PMCID: PMC4298471.

Oliveira FT, McDonald JJ, Goodman D. Performance monitoring in the anterior cingulate is not all error related: expectancy deviation and the representation of action-outcome associations. J Cogn Neurosci. 2007 Dec;19(12):1994-2004. doi: 10.1162/jocn.2007.19.12.1994. PMID: 17892382.

Opitz A, Falchier A, Linn GS, Milham MP, Schroeder CE. Limitations of ex vivo measurements for in vivo neuroscience. Proc Natl Acad Sci U S A. 2017 May 16;114(20):5243-5246. doi: 10.1073/pnas.1617024114. Epub 2017 May 1. PMID: 28461475; PMCID: PMC5441777.

Overmeyer S, Bullmore ET, Suckling J, Simmons A, Williams SC, Santosh PJ, Taylor E. Distributed grey and white matter deficits in hyperkinetic disorder: MRI evidence for anatomical abnormality in an attentional network. Psychol Med. 2001 Nov;31(8):1425-35. doi: 10.1017/s0033291701004706. PMID: 11722157.

Pace-Schott EF, Morgan PT, Malison RT, Hart CL, Edgar C, Walker M, Stickgold R. Cocaine users differ from normals on cognitive tasks which show poorer performance during drug abstinence. Am J Drug Alcohol Abuse. 2008;34(1):109-21. doi: 10.1080/00952990701764821. PMID: 18161649.

Pandeya SR, Nagy JA, Riveros D, Semple C, Taylor RS, Sanchez B, Rutkove SB. Relationships between in vivo surface and ex vivo electrical impedance myography measurements in three different neuromuscular disorder mouse models. PLoS One. 2021 Oct 29;16(10):e0259071. doi: 10.1371/journal.pone.0259071. PMID: 34714853; PMCID: PMC8555802.

Paraskevopoulou M, van Rooij D, Schene AH, Batalla A, Chauvin RJ, Buitelaar JK, Schellekens AFA. Effects of family history of substance use disorder on reward processing in adolescents with and without attention-deficit/hyperactivity disorder. Addict Biol. 2022 Mar;27(2):e13137. doi: 10.1111/adb.13137. PMID: 35229951; PMCID: PMC9285350.

Patel SH, Azzam PN. Characterization of N200 and P300: selected studies of the Event-Related Potential. Int J Med Sci. 2005;2(4):147-54. doi: 10.7150/ijms.2.147. Epub 2005 Oct 1. PMID: 16239953; PMCID: PMC1252727.

Paus T. Primate anterior cingulate cortex: where motor control, drive and cognition interface. Nat Rev Neurosci. 2001;2:417–24.

Peak J, Chieng B, Hart G, Balleine BW. Striatal direct and indirect pathway neurons differentially control the encoding and updating of goal-directed learning. Elife. 2020 Nov 20;9:e58544. doi: 10.7554/eLife.58544. PMID: 33215609; PMCID: PMC7707820.

Pearce JM, Hall G. A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli. 1980;87:532–552.

Perry CJ, Zbukvic I, Kim JH, Lawrence AJ. Role of cues and contexts on drug-seeking behaviour. Br J Pharmacol. 2014 Oct;171(20):4636-72. doi: 10.1111/bph.12735. Epub 2014 Jul 2. PMID: 24749941; PMCID: PMC4209936.

Pliszka SR, Glahn DC, Semrud-Clikeman M, Franklin C, Perez R 3rd, Xiong J, Liotti M. Neuroimaging of inhibitory control areas in children with attention deficit hyperactivity disorder who were treatment naive or in long-term treatment. Am J Psychiatry. 2006 Jun;163(6):1052-60. doi: 10.1176/ajp.2006.163.6.1052. PMID: 16741206.

Pribut HJ, Vázquez D, Wei AD, Tennyson SS, Davis IR, Roesch MR, Li X. Overexpressing Histone Deacetylase 5 in Rat Dorsal Striatum Alters Reward-Guided Decision-Making and Associated Neural Encoding. J Neurosci. 2021 Dec 8;41(49):10080-10090. doi: 10.1523/JNEUROSCI.0916-21.2021. Epub 2021 Oct 29. PMID: 34716230; PMCID: PMC8660049.

Ravi B, Kannan M. Epigenetics in the nervous system: An overview of its essential role. Indian J Hum Genet. 2013 Oct;19(4):384-91. doi: 10.4103/0971-6866.124357. PMID: 24497700; PMCID: PMC3897130.

Rein B, Conrow-Graham M, Frazier A, Cao Q, Yan Z. Inhibition of histone deacetylase 5 ameliorates abnormalities in 16p11.2 duplication mouse model. Neuropharmacology. 2022 Feb 15;204:108893. doi: 10.1016/j.neuropharm.2021.108893. Epub 2021 Nov 22. PMID: 34822816.

Renthal W, Maze I, Krishnan V, Covington HE 3rd, Xiao G, Kumar A, Russo SJ, Graham A, Tsankova N, Kippin TE, Kerstetter KA, Neve RL, Haggarty SJ, McKinsey TA, Bassel-Duby R, Olson EN, Nestler EJ. Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. Neuron. 2007 Nov 8;56(3):517-29. doi: 10.1016/j.neuron.2007.09.032. PMID: 17988634.

Renthal W, Nestler EJ. Histone acetylation in drug addiction. Semin Cell Dev Biol. 2009;20(4):387-394. doi:10.1016/j.semcdb.2009.01.005

Reolon GK, Maurmann N, Werenicz A, Garcia VA, Schröder N, Wood MA, Roesler R. Posttraining systemic administration of the histone deacetylase inhibitor sodium butyrate ameliorates aging-related memory decline in rats. Behav Brain Res. 2011 Aug 1;221(1):329-32. doi: 10.1016/j.bbr.2011.03.033. Epub 2011 Mar 21. PMID: 21421011; PMCID: PMC3093300.

Rescorla RA, Wagner AR. A theory of pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In AH Black, & WF Prokasy, editors. Classical Conditioning II: Current Research and Theory. New York: Appleton- Century-Crofts; 1973. 64–99.

Robbins TW. The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology (Berl). 2002 Oct;163(3-4):362-80. doi: 10.1007/s00213-002-1154-7. Epub 2002 Aug 9. PMID: 12373437.

Rogge GA, Wood MA. The role of histone acetylation in cocaine-induced neural plasticity and behavior. Neuropsychopharmacology. 2013 Jan;38(1):94-110. doi: 10.1038/npp.2012.154. Epub 2012 Aug 22. PMID: 22910457; PMCID: PMC3521972.

Roesch MR, Calu DJ, Schoenbaum G. Dopamine neurons encode the better option in rats deciding between differently delayed or sized rewards. Nat Neurosci. 2007 Dec;10(12):1615-24. doi: 10.1038/nn2013. Epub 2007 Nov 18. PMID: 18026098; PMCID: PMC2562672.

Roesch MR, Takahashi Y, Gugsa N, Bissonette GB, Schoenbaum G. Previous cocaine exposure makes rats hypersensitive to both delay and reward magnitude. J Neurosci. 2007 Jan 3;27(1):245-50. doi: 10.1523/JNEUROSCI.4080-06.2007. PMID: 17202492; PMCID: PMC2249703.

Roesch MR, Bryden DW. Impact of size and delay on neural activity in the rat limbic corticostriatal system. Front Neurosci. 2011 Dec 7;5:130. doi: 10.3389/fnins.2011.00130. PMID: 22363252; PMCID: PMC3277262.

Roesch MR, Esber GR, Li J, Daw ND, Schoenbaum G. Surprise! Neural correlates of Pearce-Hall and Rescorla-Wagner coexist within the brain. Eur J Neurosci. 2012 Apr;35(7):1190-200. doi: 10.1111/j.1460-9568.2011.07986.x. PMID: 22487047; PMCID: PMC3325511.

Roesch MR, Esber GR, Bryden DW, Cerri DH, Haney ZR, Schoenbaum G. Normal aging alters learning and attention-related teaching signals in basolateral amygdala. J Neurosci. 2012 Sep 19;32(38):13137-44. doi: 10.1523/JNEUROSCI.2393-12.2012. PMID: 22993430; PMCID: PMC3461330

Rossmann C, Haemmerich D. Review of temperature dependence of thermal properties, dielectric properties, and perfusion of biological tissues at hyperthermic and ablation temperatures. Crit Rev Biomed Eng. 2014;42(6):467-92. doi: 10.1615/critrevbiomedeng.2015012486. PMID: 25955712; PMCID: PMC4859435.

Rubio FJ, Li X, Liu QR, Cimbro R, Hope BT. Fluorescence activated cell sorting (FACS) and gene expression analysis of fos- expressing neurons from fresh and frozen rat brain tissue. J. Vis. Exp. 2016;114:54358

Rudebeck PH, Murray EA. The orbitofrontal oracle: cortical mechanisms for the prediction and evaluation of specific behavioral outcomes. Neuron. 2014 Dec 17;84(6):1143-56. doi: 10.1016/j.neuron.2014.10.049. PMID: 25521376; PMCID: PMC4271193.

Saddoris MP, Wang X, Sugam JA, Carelli RM. Cocaine Self-Administration Experience Induces Pathological Phasic Accumbens Dopamine Signals and Abnormal Incentive Behaviors in Drug-

Abstinent Rats. J Neurosci. 2016 Jan 6;36(1):235-50. doi: 10.1523/JNEUROSCI.3468-15.2016. PMID: 26740664; PMCID: PMC4701963.

Sallet J, Quilodran R, Rothé M, Vezoli J, Joseph JP, Procyk E. Expectations, gains, and losses in the anterior cingulate cortex. Cogn Affect Behav Neurosci. 2007 Dec;7(4):327-36. doi: 10.3758/cabn.7.4.327. PMID: 18189006; PMCID: PMC2271114.

Schneider KN, Sciarillo XA, Nudelman JL, Cheer JF, Roesch MR. Anterior Cingulate Cortex Signals Attention in a Social Paradigm that Manipulates Reward and Shock. Curr Biol. 2020 Oct 5;30(19):3724-3735.e2. doi: 10.1016/j.cub.2020.07.039. Epub 2020 Aug 6. PMID: 32763169; PMCID: PMC7541607.

Schweimer J, Hauber W. Involvement of the rat anterior cingulate cortex in control of instrumental responses guided by reward expectancy. Learn Mem. 2005 May-Jun;12(3):334-42. doi: 10.1101/lm.90605. PMID: 15930509; PMCID: PMC1142463.

Seidman LJ, Valera EM, Makris N. Structural brain imaging of attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005 Jun 1;57(11):1263-72. doi: 10.1016/j.biopsych.2004.11.019. Epub 2005 Jan 22. PMID: 15949998.

Seidman LJ, Valera EM, Makris N, Monuteaux MC, Boriel DL, Kelkar K, Kennedy DN, Caviness VS, Bush G, Aleardi M, Faraone SV, Biederman J. Dorsolateral prefrontal and anterior cingulate cortex volumetric abnormalities in adults with attention-deficit/hyperactivity disorder identified by magnetic resonance imaging. Biol Psychiatry. 2006 Nov 15;60(10):1071-80. doi: 10.1016/j.biopsych.2006.04.031. Epub 2006 Jul 28. PMID: 16876137.

Seto E, Yoshida M. Erasers of histone acetylation: the histone deacetylase enzymes. Cold Spring Harb Perspect Biol. 2014 Apr 1;6(4):a018713. doi: 10.1101/cshperspect.a018713. PMID: 24691964; PMCID: PMC3970420.

Smith ML, Asada N, Malenka RC, Anterior cingulate inputs to nucleus accumbens control the social transfer of pain and analgesia, Science. 371 (2021) 153–159.

Sprenger C, Eippert F, Finsterbusch J, Bingel U, Rose M, Büchel C. Attention modulates spinal cord responses to pain. Curr Biol. 2012 Jun 5;22(11):1019-22. doi: 10.1016/j.cub.2012.04.006. Epub 2012 May 17. PMID: 22608507.

Stalnaker TA, Calhoon GG, Ogawa M, Roesch MR, Schoenbaum G. Reward prediction error signaling in posterior dorsomedial striatum is action specific. J Neurosci. 2012 Jul 25;32(30):10296-305. doi: 10.1523/JNEUROSCI.0832-12.2012. PMID: 22836263; PMCID: PMC3419368.

Stolyarova A, Rakhshan, M, Hart E, O'Dell TJ, Peters MAK, Lau H, et al. Dissociable roles for anterior cingulate cortex and basolateral amygdala in decision confidence and learning under uncertainty. BioRxiv. 2019. https://doi.org/10.1101/655860.

Soltani A, Izquierdo A. Adaptive learning under expected and unexpected certainty. Nat Rev Neurosci. 2019. https://doi.org/10.1038/s41583-019-0180-y.

Stalnaker TA, Calhoon GG, Ogawa M, Roesch MR, Schoenbaum G. Neural correlates of stimulus-response and response-outcome associations in dorsolateral versus dorsomedial striatum. Front Integr Neurosci. 2010 May 19;4:12. doi: 10.3389/fnint.2010.00012. PMID: 20508747; PMCID: PMC2876878.

Stawicka ZM, Massoudi R, Oikonomidis L, McIver L, Mulvihill K, Quah SKL, Cockcroft GJ, Clarke HF, Horst NK, Wood CM, Roberts AC. Differential Effects of the Inactivation of Anterior and Posterior Orbitofrontal Cortex on Affective Responses to Proximal and Distal Threat, and Reward Anticipation in the Common Marmoset. Cereb Cortex. 2022 Mar 30;32(7):1319-1336. doi: 10.1093/cercor/bhab240. PMID: 34494095; PMCID: PMC8971078.

Stefanko DP, Barrett RM, Ly AR, Reolon GK, Wood MA. Modulation of long-term memory for object recognition via HDAC inhibition. Proc Natl Acad Sci U S A. 2009 Jun 9;106(23):9447-52. doi: 10.1073/pnas.0903964106. Epub 2009 May 26. PMID: 19470462; PMCID: PMC2695069.

Stray LL, Kristensen Ø, Lomeland M, Skorstad M, Stray T, Tønnessen FE. Motor regulation problems and pain in adults diagnosed with ADHD. Behav Brain Funct. 2013 May 3;9:18. doi: 10.1186/1744-9081-9-18. PMID: 23642255; PMCID: PMC3652792.

Takahashi YK, Stalnaker TA, Marrero-Garcia Y, Rada RM, Schoenbaum G. Expectancy-Related Changes in Dopaminergic Error Signals Are Impaired by Cocaine Self-Administration. Neuron. 2019 Jan 16;101(2):294-306.e3. doi: 10.1016/j.neuron.2018.11.025. PMID: 30653935; PMCID: PMC6338441.

Taniguchi, M., Carreira, M.B., Smith, L.N., Zirlin, B.C., Neve, R.L., and Cowan, C.W. (2012). Histone deacetylase 5 limits cocaine reward through cAMP-induced nuclear import. Neuron 73, 108-120.

Taniguchi M., Carreira M.B., Cooper Y.A., Bobadilla A.C., Heinsbroek J.A., Koike N., Larson E.B., Balmuth E.A., Hughes B.W., Penrod R.D., et al. HDAC5 and Its Target Gene, Npas4, Function in the Nucleus Accumbens to Regulate Cocaine-Conditioned Behaviors. Neuron. 2017;96:130–144.e136. doi: 10.1016/j.neuron.2017.09.015.

Thomas EA. Involvement of HDAC1 and HDAC3 in the Pathology of Polyglutamine Disorders: Therapeutic Implications for Selective HDAC1/HDAC3 Inhibitors. Pharmaceuticals (Basel). 2014 May 26;7(6):634-61. doi: 10.3390/ph7060634. PMID: 24865773; PMCID: PMC4078513.

Thorn CA, Atallah H, Howe M, Graybiel AM. Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. Neuron. 2010 Jun 10;66(5):781-95. doi: 10.1016/j.neuron.2010.04.036. PMID: 20547134; PMCID: PMC3108575.

Umemoto A, Lukie CN, Kerns KA, Müller U, Holroyd CB. Impaired reward processing by anterior cingulate cortex in children with attention deficit hyperactivity disorder. Cogn Affect Behav Neurosci. 2014 Jun;14(2):698-714. doi: 10.3758/s13415-014-0298-3. PMID: 24874420.

van Son D, Wiers RW, Catena A, Perez-Garcia M, Verdejo-García A. White matter disruptions in male cocaine polysubstance users: Associations with severity of drug use and duration of abstinence. Drug Alcohol Depend. 2016 Nov 1;168:247-254. doi: 10.1016/j.drugalcdep.2016.09.023. Epub 2016 Oct 6. PMID: 27736678.

van Veen V, Carter CS. The anterior cingulate as a conflict monitor: fMRI and ERP studies. Physiol Behav. 2002 Dec;77(4-5):477-82. doi: 10.1016/s0031-9384(02)00930-7. PMID: 12526986.

Vázquez D, Schneider KN, Roesch MR. Neural signals implicated in the processing of appetitive and aversive events in social and non-social contexts. Front Syst Neurosci. 2022 Aug 3;16:926388. doi: 10.3389/fnsys.2022.926388. PMID: 35993086; PMCID: PMC9381696.

Vecsey CG, Hawk JD, Lattal KM, Stein JM, Fabian SA, Attner MA, et al. Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. *J Neurosci.* 2007;27:6128–6140.

Villemure C, Bushnell CM. Cognitive modulation of pain: how do attention and emotion influence pain processing? Pain. 2002 Feb;95(3):195-199. doi: 10.1016/S0304-3959(02)00007-6. PMID: 11839418.

Vogt BA. Cingulate impairments in ADHD: Comorbidities, connections, and treatment. Handb Clin Neurol. 2019;166:297-314. doi: 10.1016/B978-0-444-64196-0.00016-9. PMID: 31731917.

Walton ME, Bannerman DM, Alterescu K, Rushworth MF. Functional specialization within medial frontal cortex of the anterior cingulate for evaluating effort-related decisions. J. Neurosci. 2003;23:6475–6479.

Wang XJ. Decision making in recurrent neuronal circuits. Neuron. 2008 Oct 23;60(2):215-34. doi: 10.1016/j.neuron.2008.09.034. PMID: 18957215; PMCID: PMC2710297.

Weissman DH, Gopalakrishnan A, Hazlett CJ, Woldorff MG. Dorsal anterior cingulate cortex resolves conflict from distracting stimuli by boosting attention toward relevant events. Cereb Cortex. 2004;15:229–37.

Winn P, Tarbuck A, Dunnett SB. Ibotenic acid lesions of the lateral hypothalamus: comparison with the electrolytic lesion syndrome. Neuroscience. 1984 May;12(1):225-40. doi: 10.1016/0306-4522(84)90149-0. PMID: 6462446.

Wu, D., Deng, H., Xiao, X. *et al.* Persistent Neuronal Activity in Anterior Cingulate Cortex Correlates with Sustained Attention in Rats Regardless of Sensory Modality. *Sci Rep* **7**, 43101 (2017). https://doi.org/10.1038/srep43101

Wu H, Miller KJ, Blumenfeld Z, Williams NR, Ravikumar VK, Lee KE, Kakusa B, Sacchet MD, Wintermark M, Christoffel DJ, Rutt BK, Bronte-Stewart H, Knutson B, Malenka RC, Halpern CH. Closing the loop on impulsivity via nucleus accumbens delta-band activity in mice and man. Proc Natl Acad Sci U S A. 2018 Jan 2;115(1):192-197. doi: 10.1073/pnas.1712214114. Epub 2017 Dec 18. PMID: 29255043; PMCID: PMC5776799.

Yang XJ, Grégoire S. Class II histone deacetylases: from sequence to function, regulation, and clinical implication. Mol Cell Biol. 2005 Apr;25(8):2873-84. doi: 10.1128/MCB.25.8.2873-2884.2005. PMID: 15798178; PMCID: PMC1069616.

Yin HH, Ostlund SB, Knowlton BJ, Balleine BW. The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci. 2005 Jul;22(2):513-23. doi: 10.1111/j.1460-9568.2005.04218.x. PMID: 16045504.

Yin HH, Knowlton BJ, Balleine BW. Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. Eur J Neurosci. 2005 Jul;22(2):505-12. doi: 10.1111/j.1460-9568.2005.04219.x. PMID: 16045503.

Yin HH, Mulcare SP, Hilário MR, Clouse E, Holloway T, Davis MI, Hansson AC, Lovinger DM, Costa RM. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. Nat Neurosci. 2009 Mar;12(3):333-41. doi: 10.1038/nn.2261. Epub 2009 Feb 8. PMID: 19198605; PMCID: PMC2774785.

Yu M, Gao X, Niu X, Zhang M, Yang Z, Han S, Cheng J, Zhang Y. Meta-analysis of structural and functional alterations of brain in patients with attention-deficit/hyperactivity disorder. Front Psychiatry. 2023 Jan 6;13:1070142. doi: 10.3389/fpsyt.2022.1070142. PMID: 36683981; PMCID: PMC9853532.

Zhang F, Wang LP, Brauner M, Liewald JF, Kay K, Watzke N, Wood PG, Bamberg E, Nagel G, Gottschalk A, Deisseroth K. Multimodal fast optical interrogation of neural circuitry. Nature. 2007 Apr 5;446(7136):633-9. doi: 10.1038/nature05744. PMID: 17410168.

Zhang C, Yang S, Flossmann T, Gao S, Witte OW, Nagel G, Holthoff K, Kirmse K. Optimized photo-stimulation of halorhodopsin for long-term neuronal inhibition. BMC Biol. 2019 Nov 27;17(1):95. doi: 10.1186/s12915-019-0717-6. PMID: 31775747; PMCID: PMC6882325.

Zhuang X, Huang L, Gu Y, Wang L, Zhang R, Zhang M, Li F, Shi Y, Mo Y, Dai Q, Wei C, Wang J. The anterior cingulate cortex projection to the dorsomedial striatum modulates hyperalgesia in a chronic constriction injury mouse model. Arch Med Sci. 2019 May 15;17(5):1388-1399. doi: 10.5114/aoms.2019.85202. PMID: 34522268; PMCID: PMC8425248.

Zulauf CA, Sprich SE, Safren SA, Wilens TE. The complicated relationship between attention deficit/hyperactivity disorder and substance use disorders. Curr Psychiatry Rep. 2014 Mar;16(3):436. doi: 10.1007/s11920-013-0436-6. PMID: 24526271; PMCID: PMC4414493.