Characterizing the Role of the Lateral Orbitofrontal Cortex in Risky Decision Making and Drug Taking Behavior

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Abstract

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Unnecessary risk-taking is a core characteristic of maladaptive behaviors such as addiction and gambling. Risk is defined as possible exposure to an unfavorable outcome which may include danger, loss, or harm. Engaging in risky behaviors despite the uncertainty of consequences alludes to the involvement of the prefrontal cortex as the region is vital for higher-order thinking and executive functioning. Adaptations or changes within the specific subregion of the PFC, known as the Orbitofrontal Cortex, are of particular interest due to a long history of literature supporting the idea that the region is heavily involved in Cocaine Use Disorder and decision making based upon past outcomes/value/risk. To investigate drug use despite negative consequences, a core trait of addiction, developed a punishment-based drug self-administration paradigm with intermittent access. I found that exciting the lateral OFC(lOFC) leads to animals both taking and seeking cocaine despite punishment. In another experiment, I found that inhibiting the region led to the animals being less likely to press the risky lever in a probability discounting paradigm. Together these two studies suggest the lOFC plays a role in various forms of risk-taking, providing a potential target for treatments.

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Hopefully, this work may eventually prove valuable in helping people that suffer from severe substance abuse disorders, such as my late biological father Steve Brooks.

Abstract

For my Dissertation studies, I chose to focus on the anomaly of risk-taking. Unnecessary risk-taking is a core characteristic of maladaptive behaviors such as addiction and gambling. Risk is defined as possible exposure to an unfavorable outcome which may include danger, loss, or harm. Engaging in risky behaviors despite the uncertainty of consequences alludes to the involvement of the prefrontal cortex as the region is vital for higher-order thinking and executive functioning. Adaptations or changes within the specific subregion of the PFC, known as the Orbitofrontal Cortex, are of particular interest due to a long history of literature supporting the idea that the region is heavily involved in Cocaine Use Disorder and decision making based upon past outcomes/value/risk. To investigate drug use despite negative consequences, a core trait of addiction, developed a punishment-based drug self-administration paradigm with intermittent access. I found that exciting the lateral OFC(lOFC) leads to animals both taking and seeking cocaine despite punishment. In another experiment, I found that inhibiting the region led to the animals being less likely to press the risky lever in a probability discounting paradigm. Together these two studies suggest the lOFC plays a role in various forms of risk-taking, with increased risk-taking correlated with higher regional activity and the opposite effect with less regional activity. To expand upon this, I would excite the lOFC, as well as the mOFC in a follow-up probability discounting experiment. In my punished drug-taking experiment, I would do the same specifically targeting lOFC to amygdala reciprocal connections. From this information, I may be able to propose a possible target for future treatments.

Introduction

Characteristics of Addiction

Substance Use Disorders (SUDs) have taken a hefty toll on society in recent years, leaving many communities to suffer severe consequences. In 2017 there were approximately 19.7 million people (about the population of New York) in the United States living with the condition, with 7.5 million using illicit drugs specifically (NIDA;2017). As a result, 700 billion dollars has been lost in productivity, wages, healthcare, and crime. SUDs have proven particularly difficult to "treat" as roughly 85% of individuals relapse within one year of treatment (Sinha 2011). Some have given arguments that SUDs are like other chronic relapsing medical disorders, such as hypertension, diabetes, asthma etc., in the sense that there are individual differences in responses and limited efficacy of treatment in all these disorders (Mclellan et al; 2000). In addition to these reasons, several emotional factors make SUDs difficult to overcome. Elevated levels of stress and trauma are associated with the onset of drug use, in addition to negative mood and anxiety, drug-related cues, boredom, and lack of positive environmental contingencies associated with reasons for relapse. (McKay et al, 1995; Sinha, 2008). In addition, there is the prominent concept of incentive salience. Incentive salience can be defined as the increased value of a particular reward, and thus incentivization of its pursuit, in the face of other factors or information. Thus, past researchers have theorized its implication in relapse, especially in stress or contexts. A final characteristic that is vital to characterizing addictive behavior, and is the nature of my thesis work, is the continued pursuit of a substance despite the possibility of a negative outcome or consequence (NIDA, 2020). This phenomenon adds another factor to what makes addiction so difficult to treat, in addition to asking the question what neurobiological changes have manifested in the individual leading to this self-harming behavior.

Neurobiology of Addiction

Although several potential emotional and environmental factors may lead to addiction behaviors, I would be remiss if I did not mention physiological contributions, particularly in the brain. Certain regions including, but not limited to, the prefrontal cortex (PFC), amygdala, ventral tegmental area (VTA), nucleus accumbens (NAc), ventral striatum (VS), lateral habenula and hippocampus are involved in addiction. Various neural changes occur in the development of addictive behavioral traits linked to goal-directed behavior, impulsivity, compulsivity, motivation, executive functioning, memory, aversion, decision-making, and risk-taking. (Koob and Volkow, 2016) In this section, I shall discuss various occurrences in the cortico-striatal pathway that contribute to SUDs, before narrowing down to my region of interest for risk-taking.

A key factor to discuss first in the neurobiology of addiction is the change in the modulation of certain neurotransmitters, specifically those associated with reward. (Koob, 1997). Dopamine is of interest particularly. Dopamine increases in the ventral striatum while encoding reward from drug use with possible orbitofrontal cortex involvement. (Volkow, 2007; Mitchell, 2012) Additionally, many studies go into detail about how the balance of D1 and D2 receptors, as well as their affinity, can influence addiction. Although D1 receptors are low affinity the increased binding of dopamine to these receptors is correlated with reward in addiction, rather than the higher affinity D2 receptors, as shown in cocaine self-administration. (Caine et al, 2002; Caine et al, 2007) D2 receptors may limit drug reward (Durieux, 2009) In the prefrontal cortex, binding of the D1 receptor increases intracellular cAMP signaling to process reward, and to a lesser degree D3 but not D2. As mentioned, this idea of reward, mediated by dopamine, feeds heavily into incentive salience. Dopamine contributes to the "wanting" aspect of reward in incentive salience, not the

"liking" or "learning" (Berridge 2007). This means there is not as strong a role for dopamine in the hedonic experiencing aspects of reward or the prediction of future rewards and future rewards and linking associations, compared to craving in incentive salience.

Although dopamine cannot explain all aspects of incentive salience, several other neurotransmitters contribute to drug-taking behavior including glutamate. For example, executive functioning is essential in supporting stimulus–response associations and altering goal-directed behavior. Executive functioning is associated with the prefrontal cortex. (Goldstein & Volkow, 2011; Friedman & Robbins, 2021). The PFC sends glutamatergic projections to multiple regions of the brain to influence behavior. The medial orbitofrontal, prelimbic, and infralimbic cortexes of the PFC send projections to mesocortical dopamine neurons in the VTA, as well as the VS to control the striatal-pallidal-thalamic-cortical system (Chudasama & Robbins, 2006; Geiser & Wise, 2008). Eventually, in drug addiction, the behavior becomes less focused on the reward of the drug itself but rather on surrounding associations. (Vorel et al, 2002) Projections from the prelimbic to the VS are affiliated with drug-induced reinstatement and projections of the NAc are modulated by D1 and D2 receptors in the prefrontal cortex. (McFarland& Kalivas, 2001). Projections to the NAc from the PFC are also linked to cued reinstatement. (Everitt and Wolf 2002). Interestingly, stress-induced reinstatement is affiliated with differing neurotransmitters and circuits. Corticotropin Releasing Factor (CRF) is increased in the extended amygdala and VTA in cocaine withdrawal-induced stress (Shalev et al, 2002). In fact, up until this point, we have focused on one major aspect of addiction, impulsive reward-seeking. The stress-induced brain adaptations are more reminiscent of the second aspect, avoiding aversive experiences. Aversion can be related to an increase in stress and a decrease in reward processing in the brain.

Before we continue it would prove beneficial to clarify the difference between impulsivity and compulsivity. Impulsivity is broadly defined as the tendency toward rapid, poorly considered, and disinhibited decisions and actions, despite negative consequences (Thomsen et al, 2018). Compulsivity is the tendency to engage in repeated behavior, usually to avoid an adverse experience. The transition from impulsivity to compulsion is marked by a change from goal-directed behavior to habitual (Wolf, 2010; Luscher, 2020). Incentive salience is more related to impulsivity. When an individual looks past a reward and chooses to engage in a behavior to stave off aversive stimuli, the behavior is no longer purely goal-oriented. Naturally, the pathways and neural substrates shift during this change as well. When a person stops using a drug, they can go through withdrawal. Withdrawal is associated with negative emotional states such as irritability, malaise, dysphoria, and stress. CRF, norepinephrine, and dynorphin are shown in the amygdala at increased levels during these negative emotional states (Koob et al, 2014). CRF is increased during withdrawal from all major drugs of abuse, mainly in the extended amygdala, including cocaine (Richter & Weiss, 1999). Taking drug to limit the continuation of these adverse effects is a form of negative reinforcement and thus, compulsion. Simply put, during withdrawal there is an overactivation of stress systems (anti-reward) and an under activation of anti-stress systems. (Leventhal et al 2008) Neuropeptide Y, nociception, and endocannabinoids are involved in the anti-stress systems, and are usually reduced in withdrawal states (Koob et al, 2014).

Finally, in chronic users, who have constantly engaged in habit-like drug-taking behavior, we see a decrease in D2 receptors, which are thought to slow addictive drug-taking behaviors. (Volkow et al, 2009). This decrease in dopamine function is associated with reduced regional activity in the orbitofrontal cortex (OFC), anterior cingulate gyrus (ACC), and dorsolateral prefrontal cortex (dlPFC). Furthermore, low levels of D2 receptors are associated with less glucose

metabolism, an indicator of brain activity, in the mOFC, ACC, and dlPFC. (Volkow et al, 1993) although researchers have shown the importance of each of the contributing regions mentioned in this chapter. I believe the prefrontal cortex and its respective subregions are of particular importance, as we continue to see this lessening of D2 receptors in cocaine-addictive individuals with hypoactivity of the OFC (Volkow et al, 2001).

Prefrontal Cortex and Cocaine Addiction

Cocaine is amongst the more common illicit substances abused in the United States. In 2020 about 1.3 million people (0.5% of the US (United States) population) had a cocaine use disorder (CUD) in the past 12 months, with about 20,000 people dying from an overdose involving cocaine (NIDA, 2020). Cocaine has potentially harmful effects including neurotoxicity, headaches, seizures, strokes, and gastrointestinal complications. (Riezzo, 2012). Most resulting deaths are caused by resulting seizures or cardiac arrest from drug use (Goldstein, et al 2008). With so many negative consequences possible there should be a reason individuals would continuously partake in cocaine use despite aversive outcomes.

Cocaine produces its effects by acting on the brain's mesolimbic dopamine system. By inhibiting the reuptake of dopamine by transporters, increased dopamine accumulates at the synapse, leading to increased pleasurable effects. Cocaine also severely alters glutamate levels and transmission (Wolf, 2010). As discussed earlier these two neurotransmitters are heavily involved in establishing cocaine-seeking behavior, especially involving the region of the PFC (Wise, 2004). As shown earlier, the PL, IL, and ACC are integral in cocaine use, however, there is an added prefrontal cortex region that is thought to be involved. As mentioned before, the OFC is altered and shows diminished activity in cocaine-addicted individuals as shown by a lower glucose metabolism during acute intoxication and prolonged withdrawal. (Volkow et al 2011). During early abstinence Volkow also found hypermetabolism signifying craving. Researchers have found decreased grey matter volume in the OFC of individuals under chronic cocaine addiction compared to non-cocaine takers, leading to dysfunction of the region. (Franklin et al, 2002; Ersche, 2011).

Grey matter volume reduction in the OFC has been associated with longer dependence on cocaine and greater compulsivity in drug use in humans. Frontal white matter integrity suffers as well (Volkow, 1988). To further confirm if it is the cocaine inducing the change, researchers performed a study where they found after chronic use of cocaine, dendrites increase in density in the mPFC while the opposite happens in the OFC in animal models. All these morphological changes allude to cocaine-induced neuroadaptations in the OFC may influence behavior. The loss of the ability to use information about consequences or prior experiences to stop continued drug use is a core trait of addiction. This inability to guide behavior based on specific information can be found in other behaviors sensitive to OFC dysfunction, where users showed greater impulsive choice and decision-making (Roesch et al, 2007; Simon et al, 2007).

There are two rival systems thought to be at work in the decision-making process associated with the PFC. (Daw et al, 2011) The model-based system and the model-free system coordinate together to manipulate prediction error signaling mechanisms. While the model-based system focuses on dictating actions based on process information of surrounding environmental cues and outcomes, the model-free system limits actual planning involved rather than choosing to experience consequences and outcomes before adjusting decision making. Once again, we see this symbolism between the ongoing balance of impulsivity and compulsivity in the addictive state. OFC dysfunction is shown to affect these two factors. As a next logical step, one must consider both the actual determined function of the OFC, its respective subregions, and individual cell composition.

Orbitofrontal Cortex Function

The orbitofrontal cortex is a subregion of the prefrontal cortex on the lateral aspect of the brain. The previously discussed works have confirmed the mPFC role in impulsive drug taking (Park et at, 2022), but what about compulsion? It is thought that OFC damage is linked to compulsive drug-seeking and taking in cocaine-using individuals. (Volkow & Fowler, 2000). Dysfunction in the OFC is correlated with other compulsive disorders as well. (Hu et al, 2019). There can be several reasons for this finding spanning from its connectivity, morphology, and intrinsic processing functions.

The OFC is heavily connected with multiple brain regions. The primary areas are with other regions of the prefrontal cortex, medial striatum, mediodorsal thalamus, limbic system, sensory systems, hypothalamus, hippocampus, and periaqueductal gray (Rudebeck & Rich 2018). Naturally, this allows the OFC to combine processed sensory information, information about current bodily states, and high-level emotional and social information to form key associations that may influence behavior. Humans with OFC damage showed a reduction in behavioral and emotional inhibition (Rudebeck & Rich, 2018). This inhibitory nature of the OFC is thought to allow humans to resist impulses to engage in activities that may garner negative consequences. Lesions in other species such as primates also showed a lack of emotional inhibition and processing thus resulting in subjects ignoring threatening stimuli (Agustin-Pavon, 2012). This failure to resist impulses begins to portray humans as disinhibited. Simply put one may view the ofc as a "stop" sign. Continuing this train of thought when a response is registered, some subjects may not make a proper change in behavior. This concept is referred to as "reversing".

Reversing refers to the ability to switch or change behavior after an update in information, especially when referring to the expectation of reward or outcomes. (Schoenbaum et al ,1998) Animals who had the OFC inhibited or lesioned show a change in reversal behavior where they are more likely to stay with a behavior despite the less advantageous outcome. Here we have a point of clarification unique to the OFC's role in compulsion. The prefrontal cortex has been shown in the prior mentioned studies to be heavily involved in goal-directed behavior, however, there is an issue here. (Neubert, 2015) The subject continues along a certain path of action despite the fact they aren't reaching their goal or preferred outcome. They form a habitual behavior more like compulsion rather than impulsivity. This transition is key to addiction and shows a possible role for the OFC as the rest of the mPFC is affiliated with impulsivity rather than compulsion.

Odds estimation is another possible feature of the OFC. A key point of addiction is the continuation of the behavior despite uncertainty. This could be a prediction error, valuation error, or optimization behavior. As far as the OFC is concerned there is evidence of a combination of these explanations in terms of decision making. Value signaling also takes into calculation uncertainty and risk, thus presenting as an economic choice. For example, when choosing against varied percentages of reward and choosing optimally the medial OFC is heavily involved. However, probabilistic/contingent learning has been attributed to region 12, the vmPFC (Rudebeck et al, 2017).

Contingent learning is another process associated with the OFC which is characterized by getting information to make predictions about outcomes that will likely occur because of actions taken (Chudasama and Robbina, 2003; Schoenbaum et al, 2003). This process follows the model-free learning theory, which may be put at risk due to OFC dysfunction (Elorette, 2022). Lesions of the OFC in various species are shown to disrupt stimulus award learning, however, this may be

an oversimplification (Rolls,2000; Rudebeck et al,2008; Walton et al). This outcome may be due to the destruction of the surrounding white matter, mainly into ventrolateral PFC(vlPFC) (Rudebeck et al, 2017). However, with damage to the OFC, there has been shown deficits in both stimulus reward learning and emotional responding in humans (Izquierdo et al, 2005; Rolls et al. 1994). Furthermore, it is thought that sub-lesions, and thus subregions, of the OFC may elicit different effects. (Noonan et al, 2010;2017).

Previously we have discussed the possible ramification of emotion dysregulation that comes with OFC dysfunction. It is known that the amygdala plays a significant role in emotion, which happens to be well connected to the lOFC. Lesioning the connection of the OFC to the amygdala resulted in improved performance and reversing while hindering behavior was associated with lesioning the connection from the amygdala to OFC. Lesions also caused a deficit in probabilistic learning (Groman et al, 2019). This tells us that projections from the amygdala are needed to form contingent learning associations, while projections from the OFC are essential in previously learned value associations, more specifically those standing for specific sensory of a reward that will follow a stimulus (Rudebeck and Murray, 2011; Murray et. al 2015). Stimulus-outcome associations may be influenced by the lOFC, not contingent stimulus reward learning (Fiuzat et al, 2017). The mOFC has more connections to the hippocampus which are essential for reward-guided behavior through a mechanism that allows task states to be integrated on going behavior and guide future learning (Wikenheiser et all, 2017; Wikenheiser and Schoenbaum, 2016).

Throughout the past several paragraphs one may notice a dichotomy between the subregions of the mOFC and the lOFC. There is a heterogeneity of function that may supply more insight into how the OFC works. Many authors have found specific discrepancies among the

regions (Rich & Wallis, 2016). Many have theorized that the lOFC processes current task states and sensory information to guide behavior while the mOFC compiles information for future states and thus future learning (Rudebeck & Rich, 2018; Bardfield and Hart, 2020). Furthermore, it has been shown Walkers' areas 11 and 13 are associated with updating and storing outcome-specific information. In contrast, the mOFC role is harder to isolate when it comes to learning behavior (Elorette et al, 2021). Although the literature has shown how regions of the OFC are heterogynous, what about the neurons that compose them?

Implications of IT and PT Neurons

The cortex expresses many types of neurons including interneurons which modulate the activity of other cells locally, and projection neurons which send signals down or upstream from one region to another. These neurons may differ in inputs, projections, morphology, function, signaling, and modulation. As previously mentioned, projections from the PFC to the striatum, mainly the VTA and NAc, are vital to forming goal-oriented and reward-based behavior as well as learning. These projections are referred to as cortico-striatal projections or pathways (CStr). With that in mind, it is pivotal that we investigate specific neurons that contribute to these complex projections. The two primary projection neuron subtypes are Intratelencephalic (IT) and Pyramidal Tract (PT) neurons. CStr neurons can be named as either IT or PT neurons but not both. (Shepherd 2013). While researchers are aware of the presence of these two subtypes, much work needs to be done to fully understand their respective roles and functions, especially in mental and physical disorders. Although projecting to the striatum, there are many discrepancies between the two.

IT neurons have sparse apical tufts, minimal h-currents, and regular adapting firing while projecting bilaterally to the striatum and contralateral cortex. In contrast, PT neurons have thick

apical tufts, prominent h currents, non-adapting burst firing, and project to the ipsilateral striatum and subcortical structures (Miller et al, 2008). Both are present at L5 of the cortex with neurons in sublayer 2 projecting to IT and neurons in sublayer 3 projecting to PT (Anderson et al, 2010). IT and PT neurons collect information from separate upstream cortical networks to relay to separate downstream striatal regions. This alludes to evidence suggesting IT and PT neurons have distinct roles in planning and movement control as a result (Reiner, 2010). The two subtypes may work in tandem through certain interactions through their connectivity.

Recurrent connectivity proposes cortical circuits amplify, integrate, distribute, and store information amongst subsets of neurons. (Douglas et al 1995) So, depending on the behavior, system involved, brain area, and species, different neurons may be active. Furthermore, Cotricostriatal neurons are known to have hierarchal connections, meaning that certain neurons are influenced by upstream connections. Some studies have found in layer 5, there is evidence of unidirectional IT to PT connectivity (Kiratani 2012). PT neurons receive excitatory signaling from IT neurons but can modulate activity by sending feedback inhibition to IT, using recurrent connectivity. It should also be known that IT is affiliated with excitatory signaling while PT is affiliated with inhibitory signaling. Within the striatum, there is a direct and an indirect pathway, populated by D1 and D2 receptors, respectively. There is some evidence that IT neurons selectively project to the direct pathway while PT neurons selectively project to the indirect pathway (Dembrow et al, 2014). Some have also provided evidence that both the direct and indirect pathway respond IT/PT activation (kress et al, 2013)to Due to these reasons, researchers have theorized IT neurons are used in movement planning, gathering information about body position and the environment, as well as reward-prediction-related information via dopaminergic inputs. The OFC's connections to the DMS (which is populated by IT) are critical for goal-directed behavior.

Lesioning the connection or inhibiting it via DREADDS promoted habitual behavior (Gremel et al, 2013; Wess et al, 2013). On that tangent PT neurons are more concerned with motor control, more specifically slowing down or inhibiting movement in terms of coordination.

Certain disease states are related to the potential IT/PT imbalance. Certain neuropsychiatric disorders are associated with one of the subsets of neurons over the other. For instance, due to their connections, each has a separate set of disease associations. IT only populates the cortex and dorsomedial striatum while the PT neurons connect to these regions and the mid-brain and spinal cord. IT neurons are associated with Schizophrenia, OCD, ASD, and MDD, while PT has been shown in ALS, Parkinson's, and Hereditary Spastic Paraplegia. (Shepherd 2013). These neurons may be implicated in risk-taking and aversion like many CStr projections.

Risk Taking

A common trait of SUDs is an increased likelihood of partaking in unnecessary risk. These acts put one at risk for losing shelter, finances, relationships, and health. Risk-taking is more prone for adolescents. Many attributes this to the delay in the growth of the prefrontal cortex, mainly the lack of myelination and grey matter thinning (Bava et al 2010; Giedd 2015). As a reminder, the PFC is implemented in impulse control by assessing risk and consequences. (Uhl et al, 2019) Dysfunction in this region leads to behavior such as risky sex, gambling, and lastly illicit substance use. Acknowledging this, risk-taking is an unavoidable part of life as we must engage in tasks daily where we are uncertain of all possible outcomes. For example, driving, forming relationships with other humans, etc have potential benefits as well as risks that humans must navigate and ultimately make a decision on.

When it comes to risk-based decision making, more medical aspects of the PFC are involved. Inactivation of the prelimbic mPFC caused an increased likelihood of risky decision-making (Holstein & Floresco, 2019). The prelimbic cortex plays a pivotal role in risk discounting and integrating information about changing reward probabilities. Inactivating the OFC just increased latency while decision making but didn't change choice (St. Onge & Floresco, 2009). However, with the mOFC role in probability and the lOFC role in modulating current state behavior could it be possible for the OFC to have a role in risky decision-making as well? More specifically can we attribute why individuals may take risks with their well-being to obtain drugs. This sets the stage for why we chose to investigate the role of the lOFC in the risky drug-taking behavior of cocaine and risky decision-making using probability discounting as a model.

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Chapter 1

DREADD activation of the lateral orbitofrontal cortex increases cocaine-taking and cocaine-seeking in male and female rats during intermittent access self-administration under risky conditions.

Abstract

Addiction involves the constant pursuit of a substance despite negative consequences. Although the orbitofrontal cortex (OFC) is known to regulate risk-taking more generally and can be critical to the development of addiction, its role in regulating drug use under risk-taking conditions is unknown. To address this, we examined drug-taking and drug-seeking in male and female rats under conditions where cocaine infusions were paired with foot shock punishment 50% of the time and combined this paradigm with cFos immunohistochemistry. We found that rats that showed higher levels of drug-taking and drug-seeking before punishment showed decreased responding during self-administration sessions under risky conditions and lower levels of c-Fos expression in the lateral but not medial OFC. Despite these initial differences, however, all rats showed decreased responding with additional foot shock punishment sessions. We then used chemogenetic viral approaches to examine how altering the activity of the lateral OFC affects drug-taking and drug-seeking during punished drug use. Although there was no effect of Gi/o DREADD-mediated inhibition of the lateral OFC on these behaviors, Gq DREADD-mediated activation increased drug-taking and drug-seeking when drug use was associated with foot shock 50% of the time. Interestingly, this manipulation did not affect non-risky self-administration behavior. These results suggest that the involvement of lateral OFC in cocaine use is context-sensitive and influences decision-making based on negative outcomes.

Introduction

Drug addiction, which can be characterized in part by compulsive drug-seeking and use of a substance despite negative consequences, exerts tremendous costs to both individuals and our society, and these costs are increasing. For example, cocaine use is associated with morbidity and elevated rates of healthcare utilization (Butler et al., 2017), and cocaine-related deaths have doubled between 2011 and 2016 (Kampman, 2019). Furthermore, repeated cocaine use has several long-term, negative health consequences, including hypertension, myocardial infarctions, inflammation, and neurotoxicity (Bachi et al., 2017). Taking drugs despite potential negative outcomes is a defining feature of addiction. Little is known regarding the neural substrates that mediate this phenomenon. Thus, identifying the neural correlates of risky drug-taking behavior will allow us to better understand resilience and susceptibility to addiction, as well as supply potential targets for treatment.

The orbitofrontal cortex (OFC) has been identified as an area fundamental for reward-seeking and critical in addiction development (Guillem and Ahmed, 2018). However, its precise role in regulating addiction-related behaviors is difficult to pinpoint because the role of the OFC can differ depending upon behavior, timing, and context (Orsini and Setlow, 2017). For example, increased OFC activity is associated with acute cocaine use, while decreased OFC grey matter has been linked to chronic cocaine use (Fuchs et al., 2004). In model-based learning paradigms, which normally test goal-directed behavior, OFC lesions disrupt the learning/integrating of the latest information (Mar et al., 2011). The failure to process new information due to OFC dysfunction could explain why cocaine use in the face of negative consequences continues. Indeed, addiction is seen as a compulsive disease, and OFC imbalances/dysregulation have been found to influence

compulsive behaviors, especially in punishment-resistant individuals (Hu et al., 2019). Adding to the complexity, it has been found that the medial and lateral subregions of the OFC can differentially regulate behavior (Rich and Wallis, 2014). For example, the medial OFC is more intensely activated in humans during positive reinforcement whereas the lateral OFC activation has been associated with outcomes that require a behavior change, such as punishment. (Rich and Wallis, 2014)

The main goal of our study was to characterize the role of the lateral OFC in drug use under risk-taking (i.e., variable foot shock punishment) conditions. To carry this out, we expressed Gq- or Gi/o-DREADDs in the lateral OFC to examine how transiently activity or inhibiting lateral OFC activity, respectively, alters cocaine use during periods of drug availability and cocaine-seeking during periods of drug-unavailability when cocaine use is paired with foot shock 50% of the time. In addition, we examined how foot shock punishment changes drug-taking and -seeking over time and as a function of sex.

Methods

Subjects

Outbred Sprague-Dawley rats (n=120; Envigo) weighing 250-274g upon arrival were pair housed in a temperature and humidity-controlled vivarium, on a 12 h light/day cycle. Rats were given 72 hours (about 3 days) to adjust to the environment before any experimental manipulations. Behavioral experiments took place during the light cycle, and all procedures were in accordance with the National Institutes of Health (NIH) Guidelines and approved by the Seattle Children's Research Institute's Institutional Animal Care and Use Committee.

Drugs

Cocaine HCl was obtained from the Drug Supply Program of the National Institute on Drug Abuse (NIDA) and dissolved in sterile saline (0.9% NaCl) to a stock concentration of 15 mg/ml. Syringes were prepared for each subject based upon respective body weight, for administration of 0.4 mg/kg of cocaine per infusion. Clozapine-N-oxide (CNO) was obtained through the Rapid Access to Investigate Drug Program of the National Institute of Neurological Disorders and Stroke (NINDS) of the NIH and was dissolved in dimethyl sulfoxide (DMSO; Sigma Aldrich) in a hot water bath, then further diluted in sterile water to a final concentration of 6% DMSO. CNO was prepared fresh daily at a concentration of 5 mg/ml and administered intraperitoneally (IP) at a dose of 1 ml/kg. Vehicle (VEH) injections consisted of DMSO dissolved in sterile water to a final concentration of 6% DMSO and were administered ip at a volume of 1 ml/kg.

Viral Vectors

Adeno-associated viruses coding for constitutively expressed Gi-coupled (pAAV-hSyn-hM4D(Gi)-mCherry) and Gq-coupled (pAAV-hSyn-hM3D(Gq)-mCherry) DREADDs were obtained from Addgene (viral prep 50475-AAV8 and 50474-AAV8 respectively) and had titers of $\geq 7\times10^{12}$ GC/ml and 2.7^13 GC/ml.

Surgical Procedures

Rats were anesthetized with isoflurane (1–5% inhaled; Patterson Veterinary) and received meloxicam (1 mg/ml, 1 ml/kg subcutaneously; Patterson Veterinary) for analgesia. Rats were monitored for at least 3 days following each surgical procedure.

Stereotaxic surgeries.

Surgeries were performed using standard procedures (Kerstetter et al., 2016). Viral vectors (hsyn-hM4Di or hsyn-hM3Dq) were delivered bilaterally via 33-gauge needles and infused at a volume of 500 nl at a rate of 100 nl/min. Coordinates for the lateral orbitofrontal cortex (lOFC) (in mm, relative to bregma): A/P +3.7, M/L ±2.5, D/V – 5.4.

Catheter Surgeries

Jugular catheterization surgeries were performed as previously described (Kerstetter et al., 2016). Catheters were flushed daily with gentamicin (10mg/ml) and heparin (3 U/ml) in sterile saline (0.2 ml/day, iv) to maintain patency. Catheter patency was checked before the first day of the intermittent access (IntA) stage, Punishment Baseline stage, and Punishment Treatment stage with brevital sodium (10mg/ml in sterile saline, 0.05–0.2 ml/ infusion, iv; methohexital sodium, Patterson Veterinary); rats that became ataxic within 5 s of infusion were considered to have maintained patency. Rats that lost patency or whose catheter was damaged were excluded from the experiments.

Self-administration Training

After three days of recovery from the surgical procedures, animals began self-administration training. Animals were placed in operant boxes on a FR1 paradigm with the active lever present and self-administered cocaine for a maximum of 10 lever presses/infusions. After 5 days of training and at least three successful 10-infusion sessions in a row, animals moved to the next phase of the self-administration experiments.

Intermittent Access Self-Administration Procedure

Rats were trained to self-administer cocaine under an intermittent access (IntA) schedule that produces spikes in brain drug concentration and can rapidly produce addiction-like behavior (Kawa et al., 2016; Garcia et al., 2020; Zimmer et al., 2012). Each SA session (2.5 h/session, 9 sessions) consisted of 5 repeating drug-available (5 min) and drug unavailable (25 min) blocks; the length of each drug-unavailable block ensured that brain concentrations for cocaine were on the descending limb of the pharmacokinetic curves at the beginning of each drug-available block (Garcia et al., 2020). Daily IntA SA sessions were signaled by turning off house lights and extension of two levers into the chamber: lever presses on the active lever resulted in drug delivery (FR1, 0.4 mg/kg/infusion over 2.8 s) and the illumination of the cue light (3 s), while lever presses on the inactive lever had no programmed consequences. At the end of each drug available block, the house lights re-illuminated to signal the beginning of the drug unavailable block and levers remained extended to monitor drug-seeking during signaled-unavailability. During these periods lever presses resulted in neither drug delivery nor cue presentation. Rats that did not average twice as many active lever presses compared to inactive lever presses during this phase were excluded from all analyses (n=2).

Following IntA self-administration, rats moved to the next phase of the paradigm where they received a mild foot shock punishment (0.25 mA) during 50% of their cocaine infusions. During this phase, lever presses during drug unavailable sessions did not result in shocks or drug infusions. Rats underwent a total of 8 punishment sessions, the initial 4 sessions were used to establish a baseline of responding and the additional 4 sessions were used to assess the effects of transient alteration of neuronal activity, as rats received treatments of CNO or vehicle during these sessions.

Classification of Risk-taking Behavior

The average number of active lever presses during the drug-available period of the last four sessions of the IntA phase were compared against the average number of drug-available period active lever presses during the first four sessions of footshock punishment (i.e., punishment baseline (PB)). Individual rats were designated as punishment sensitive (PS) if they decreased the number of active lever presses during the PB compared to the IntA baseline or as punishment resistant (PR) if they increased the number of active lever presses during the PB compared to the IntA baseline.

Immunohistochemistry of CFos and DREADD Expression.

Animals were first perfused with 4 % paraformaladehyde solution. Brains were extracted and stored in paraformaldehyde for 24 hours. Brains were sliced via vibratome at 40um thickness. To visualize c-Fos-positive cells, floating sections were washed (PBS; 3 × 10 min), blocked (0.25% Triton-X, 5% normal goat serum, PBS; 120 min), and incubated with a primary antibody (1:800 rabbit anti-c-Fos, 0.25% Triton-X, 2.5% normal goat serum, PBS; 24 h; Cell Signaling #2250S, RRID: AB_2247211). Sections were then washed (PBS; 3 × 10 min) and incubated with a secondary antibody conjugated to AF-568 (1:500 goat-anti-rabbit, 0.25% Triton-X, 2.5% normal goat serum, PBS; 120 min; Life Technologies #A32733, RRID: AB_2633282). Finally, sections were washed (PBS; 3 × 10 min), mounted on slides, and coverslipped with Vectashield mounting medium with DAPI (Vector Labs). For cells infected with DREADDs, confirmation was determined by a primary antibody against mcherry (1:400 rabbit anti-mcherry) that was paired with a secondary antibody (1:500 goat anti-rabbit 568).

Statistical analysis

Behavioral data were collected using automated procedures (Med Associates) and analyzed using GraphPad Prism (V9.4). Two-way repeated measures (RM) ANOVAs were used to analyze group differences (males versus females, punishment-sensitive (PS) versus punishment-resistant (PR), vehicle versus CNO) in active or inactive lever presses.

Results

Footshock-induced alterations in drug-taking and drug-seeking were similar in males and females. After an initial 5-day training period, male and female rats underwent 9 sessions of IntA self-administration. Both sexes acquired cocaine self-administration and learned to discriminate between the active and inactive levers during drug available (DA) (Two-way RM ANOVA, significant main effect of lever, females: $F(1,34) = 34.50$, $p < .0001$; males: $F(1,30) = 25.03$, $p < .0001$; Figure 1A) and drug unavailable (DU) periods (Two-way RM ANOVA, significant main effect of lever, females: $F(1,34) = 10.23$, $p = .003$; males: $F(1,30) = 8.24$, $p = .007$; Figure 1F). However, there were no sex differences during self-administration in active lever presses during DA (Two-way RM ANOVA, no significant main effect of sex, $F(1,32) = 0.92$, $p = .34$; Figure 1A) or DU periods (Two-way RM ANOVA, no significant main effect of sex, $F(1,32) = 0.59$, $p = .45$; Figure 1F), nor in total cocaine intake (unpaired t-test, $t(32) = 1.11$, $p = .28$, Figure 1B) or total active presses during DU periods (unpaired t-test, $t(32) = .77$, $p = .45$, Figure 1G). Rats then underwent a punishment task to determine how delivery of footshock during some of the cocaine infusions (50% of the time, randomly distributed through the session) altered drug-taking during DA periods and drug-seeking during DU periods. Two-way RM ANOVAs revealed a significant main effect of phase during DA periods ($F(1,32) = 9.72$, $p = .004$) and DU periods ($F(1,32) = 11.00$, $p = .002$), with females but not males showing a decrease in active lever presses during DA periods (females: $p = .02$, males: $p = .22$; Figure 1C) and an increase in active lever presses during DU periods (females $p = .03$, males: $p = .08$, Figure 1H) during the punishment test compared to the self-administration sessions preceding punishment (i.e., baseline). However, there were no

differences in the percent change in active lever presses from baseline between females and males during DA (unpaired t-test, $t(32) = .21$, $p = .84$, Figure 1D) or DU periods (unpaired t-test, $t(32) = .97$, $p = .34$, Figure 1I). Footshock punishment also had no effect on inactive lever presses in males or females during DA (no significant sex x phase interaction: $F(1,32) = 0.28$, $p = .60$, Figure 1E) or DU periods (no significant sex x phase interaction: $F(1,32) = .60$, $p = .44$, Figure 1J). Given the similarities in responses across sexes, male and female data were combined for all subsequent analyses.

Punishment-resistant rats show lower levels of drug-taking and drug-seeking prior to punishment but higher levels of drug-taking and drug-seeking during punishment compared to punishment-sensitive rats.

We observed considerable variability within both sexes in the effects of footshock punishment on drug-taking. Therefore, we classified rats as punishment-sensitive (PS) if they decreased active lever pressing during DA punishment sessions compared to the baseline (12 of 16 (75%) males and 10 of 18 (56%) females) or punishment-resistant (PR) if they increased responding during the DA punishment sessions (4 of 16 (25%) males and 8 of 18 (44%) females) (Figure 2A). During self-administration, the PS group showed an increase in active lever presses during DA (Two-way RM ANOVA, main effect of phenotype, $F(1,32) = 4.68$, $p = .04$; Figure 2B) and DU periods (Two-way RM ANOVA, main effect of phenotype, $F(1,32) = 4.30$, $p = .05$; Figure 2G), as well as in total cocaine intake (unpaired t-test, $t(32) = 2.57$, $p = .02$, Figure 2C) and total active lever presses during DU periods (unpaired t-test, $t(32) = 2.07$, $p = .05$, Figure 2H) compared to the PR group. As expected, since it was the measure used for classification, the PS group showed a decrease ($p < .0001$) and the PR group showed an increase ($p = .02$) in DA period active lever presses during the punishment test compared to baseline (Two-way RM ANOVA, significant phenotype x phase

interaction, $F(1,32) = 49.95$, $p < .0001$; Figure 2D), and the percent change in active level presses from baseline was much greater in the PR group compared to the PS group (unpaired t-test, $t(32) = 6.14$, $p < .0001$, Figure 2E). Footshock punishment during drug-taking increased active lever presses during DU periods in the PR ($p = .0007$) but not PS ($p = .26$) group (Two-Way RM ANOVA, significant phenotype x phase interaction: $F(1,32) = 5.28$, $p = .03$, Figure 2I) and the percent change in active level presses from baseline was much greater in the PR group compared to the PS group (unpaired t-test, $t(32) = 2.10$, $p = .04$, Figure 2J). However, there were no changes in inactive lever presses in the PS or PR groups during DA (no significant phenotype x phase interaction: $F(1,31) = .34$, $p = .56$, Figure 2F) or DU periods (no significant phenotype x phase interaction: $F(1,31) = .04$, $p = .84$, Figure 2K), suggesting that the effects of punishment were specific to drug-taking and drug-seeking and not generalized motor effects.

Footshock-induced decreases in drug-taking are associated with decreased Fos activation in lateral OFC.

To assess the role of subregions of the OFC in footshock punishment-induced alterations in drug-taking and drug-seeking, rats were sacrificed within 30 min after the fourth risk-taking session where 50% of lever presses resulted in both a cocaine infusion and a footshock, and brains were processed for Fos immunohistochemistry. A two-way RM ANOVA revealed a significant main effect of group ($F(2,9) = 6.33$, $p = .02$; Figure 3) and a significant main effect of OFC region ($F(1,9) = 19.95$, $p = .002$; Figure 3), due to a decrease in Fos expression in the lateral but not medial OFC of rats susceptible to footshock punishment compared to non-shocked controls (lateral OFC: $p = .01$, medial OFC: $p = .38$, Figure 1H). Based on these results, subsequent DREADD manipulations were targeted to the lateral OFC.

Increasing lateral OFC activity during punishment increases drug taking and drug-seeking.

To test how the lateral OFC regulates drug-taking and drug-seeking during footshock punishment, we expressed Gq- or Gi/o DREADDs in lateral OFC neurons (Figure 4). For these experiments, groups underwent a total of 8 punishment sessions (4 sessions to establish a baseline + 4 additional sessions where rats received vehicle or CNO). Analysis of the vehicle-treated PS and PR groups revealed that following additional punishment sessions, there were no longer group differences in active lever presses during DA (no significant phenotype x phase interaction: $F(1,10) = 3.38$, $p = .10$, Supplemental Figure 1A) or DU periods (no significant phenotype x phase interaction: $F(1,10) = .01$, $p = .93$, Supplemental Figure 1B) due to decreases in responding during the additional punishment tests. As a result, we combined the PS and PR groups for the DREADD experiment analyses.

Chemogenetic activation of the lateral OFC following CNO treatment in rats expressing hM3Dq during footshock punishment increased active lever presses during DA (Two-Way RM ANOVA, significant treatment x phase interaction: $F (1,20) = 4.86$, $p = .04$, Figure 5A) and DU periods (Two-Way RM ANOVA, significant treatment x phase interaction: $F (1,20) = 4.67$, $p = .04$, Figure 5D). In addition, the percent change in active level presses from baseline was much greater in the CNO group compared to the VEH group during both DA (unpaired t-test, $t (19) = 3.23$, $p = .004$, Figure 5B) and DU (unpaired t-test, $t (19) = 3.79$, $p = .001$, Figure 5E) periods. These effects are unlikely due to nonspecific effects of CNO on motor behavior as two-way RM ANOVAs comparing active and inactive lever responses during treatment revealed a significant treatment x lever interaction during DA periods ($F(1,20) = 4.39$, $p = .05$, Figure 5A,C) and a significant main effect of lever during DU periods ($F(1,20) = 2.34$, $p = .01$, Figure 5D,F), but no changes in inactive lever presses ($p = 1.0$).

Decreasing lateral OFC activity during punishment does not change drug-taking or drug-seeking.

We next used Gi/o-DREADDs to examine whether decreasing lateral OFC activity is also sufficient to alter drug-taking and drug-seeking during foot shock punishment. In contrast to the effects with Gq-DREADDs, chemogenetic inhibition of the lateral OFC with hM4Di during footshock punishment had no effect on active lever presses during DA (Two-Way RM ANOVA, no significant treatment x phase interaction: $F(1,8) = 1.07$, $p = .33$, Figure 6A) or DU (Two-Way RM ANOVA, no significant treatment x phase interaction: $F(1,8) = .84$, $p = .39$, Figure 6D) periods. In addition, the percent change in active lever presses from baseline did not differ across treatment groups during both DA (unpaired t-test, $t(8) = 1.0$, $p = .35$, Figure 6B) and DU (unpaired t-test, $t(8) = .449$, $p = .65$, Figure 6E) periods.

Activation of the lateral OFC during IntA self-administration does not alter drug-taking or drug-seeking.

Finally, we examined whether increasing activity of the lateral OFC in the absence of punishment during IntA self-administration was able to enhance drug-taking and drug-seeking, like what was observed during footshock punishment. Chemogenetic activation of the lateral OFC with hM3Dq during cocaine self-administration had no effect on active lever presses during DA (Two-Way RM ANOVA, no significant treatment x phase interaction: $F(1,19) = .17$, $p = .68$, Figure 7A) or DU (Two-Way RM ANOVA, no significant treatment x phase interaction: $F(1,19) = .03$, $p = .87$, Figure 7D) periods. In addition, there were no differences in the percent change in active lever presses from baseline between the CNO and VEH groups during DA (unpaired t-test, $t(19) = .29$, $p = .78$, Figure 7B) or DU (unpaired t-test, $t(19) = .20$, $p = .84$, Figure 7E) periods.

Discussion

In this set of experiments, we first assessed risk-taking behavior in rats that had undergone cocaine self-administration by examining drug-taking and drug-seeking under conditions when cocaine infusions were paired with foot shock 50% of the time. Overall, we found similar patterns of drug-taking and drug-seeking in female and male rats during cocaine self-administration as well as during the punishment sessions. However, there was a greater proportion of female to male rats (44% versus 25%) that were designated as punishment-resistant (i.e., increased active lever pressing during DA punishment sessions) and a greater proportion of male to female rats (75% versus 56%) that were designated as punishment-sensitive (i.e., decreased active lever pressing during DA punishment sessions). Our results are consistent with previous work that found no sex differences in the acquisition of cocaine self-administration but higher levels of addiction-like behaviors following self-administration including enhanced motivation for cocaine, increased escalation of drug intake and greater psychomotor sensitization (Algallal et al., 2020; Carr et al., 2020; Kawa and Robinson, 2019). However, it should be noted that other studies have found sex differences during the acquisition of cocaine self-administration, likely due to differences in methodological details (Anker and Carroll, 2011; Becker and Hu, 2008; Fattore et al., 2008; Lynch and Carroll, 1999). In terms of general risk taking or punishment it has been found that females rats are more sensitive to punishment avoidance especially when there is a probabilistic chance of punishment rather than guaranteed (Chowdhury et al., 2019; Orisini and Setlow, 2017; Grissom et al., 2018).

Interestingly, we found that punishment-resistant rats showed lower levels of cocaine intake as well as cocaine-seeking during periods of drug-unavailability during the initial drug self-

administration phase compared to punishment-sensitive rats. These results suggest that the development of addiction-like behaviors, including resistance to punished drug use, are not simply a result of the total amount of cocaine intake. Notably, studies examining the effects of pattern of cocaine self-administration have also found dissociations between total cocaine intake and addiction-like patterns of drug use, including drug-use despite footshock punishment (Garcia et al., 2020; Kawa et al., 2016; Yager et al., 2019; Zimmer et al., 2012). Nonetheless, when we extended punishment sessions to eight as part of the experimental design for our DREADD studies, we observed that all the rats became punishment sensitive with additional sessions. This occurrence is consistent with a recent study where pairing cocaine infusions with a range of footshock intensities resulted in a decrease in lever responding for cocaine in subsequent sessions, even at footshock intensities that were initially ineffective at reducing rates of responding (Durand et al., 2022).

Next, we sought to determine the role of the OFC in regulating cocaine-taking and -seeking during risky (i.e., footshock-punished) cocaine use. We focused on the lateral region of the OFC because we found that punishment-sensitive rats showed decreased c-Fos expression in this region, but not in the medial OFC, following cocaine infusions that were paired with footshock compared to control rats that did not receive footshock. This finding is in-line with previous work that showed that although the medial OFC regulates cocaine-seeking and intake (Kantak et al., 2013), its role is more associated with reinforcement and motivation rather than punishment sensitivity. (O'Doherty et al., 2001). Past literature supports our findings of cFos activity with punishment resistance (Pascoli et al., 2015).

Using a targeted DREADD approach to increase activity of the lateral OFC transiently during cocaine self-administration paired with footshock punishment, we found that this

manipulation led to notable change in risk-taking behavior. Specifically, lateral OFC activation increased drug-taking during drug-available periods and drug-seeking during drug-unavailable periods despite punishment (i.e., animals were less punishment-sensitive). In addition, there were no effects of this manipulation on inactive lever pressing, suggesting that the observed changes in behavior were not a result of general effects on motor activity. Together, these results suggest that lateral OFC activity is associated with the likelihood of pursuing a substance despite negative consequences and increasing activity in this region lowers sensitivity to punishment. This is consistent with the idea that the lateral OFC is highly involved in assessing the value of reward versus potential punishment as well as processing past outcomes, especially those that may elicit an emotional response from aversive experiences through its connection with the basolateral amygdala (Ishikawa et al., 2019; Jean-Richard-Dit-Bressel and McNally, 2016; Shiba et al., 2016). In addition, our findings are in line with prior studies that found that reducing activity in the lateral OFC enhances sensitivity to punishment (Turner et al., 2021).

In contrast to the effects with DREADD activation, we found no notable change in drug-taking or drug-seeking during punished cocaine self-administration sessions following transient inhibition of the lateral OFC via hM4Di DREADDs. Although it is possible that these results are due to a floor-effect, they are not necessarily unexpected as a recent study also found that inactivation of the lateral OFC did not change sensitivity to punishment (Verharen, 2019). In addition, earlier work has found that pharmacological inactivation or lesions of the lateral OFC can have mixed results, with different studies reporting increases, decreases, or no effect on punishment sensitivity (Turner et al., 2021).

Notably, we found no significant effect of lateral OFC activation on drug-taking or drug-seeking behavior during non-punished cocaine sessions. Although it has been shown that lesioning the

OFC results in rats that press for lower doses of cocaine and acquire drug-taking behavior sooner (Grakalic et al., 2010), it is possible that these results are reflective of effects specific to the medial OFC, as inactivation of the medial OFC also alters cocaine intake and cocaine-seeking (Kantak et al., 2013). Taken together, these results suggest that engagement of the lateral OFC may not be occurring during cocaine self-administration when an aversive outcome is not occurring (i.e., when behavior actions are not associated with a risky outcome). Risk taking can be nuanced as there are various forms and interpretations. In this instance we must consider the high chance of punishment, an aversive event. Considering that the lOFC specifically is thought to be heavily involved in punishment I believe that is that risk-taking behavior being studied here rather than probability (Rich & Wallis, 2016). Probabilistic behavior, and thus risky behaviors involving odds, is thought to favor activity within the mOFC, rather than the lOFC subregion that we studied (Rudebeck & Rich, 2018; Bardfield and Hart, 2020).

In conclusion, our experiments support the idea that the lateral OFC regulates the likelihood of engaging in risk-taking behavior. Specifically, activation of the lateral OFC can result in the discounting of negative outcomes, thus increasing behaviors despite their occurrence under risky conditions. In the context of drug use and addiction, this work suggests that cocaine use in susceptible individuals could lead to dysregulation of the OFC such that the negative outcomes associated with drug use are discounted. Thus, continued drug use would be perpetuated, despite any adverse consequences that occur because of this drug use.

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Figure Legends

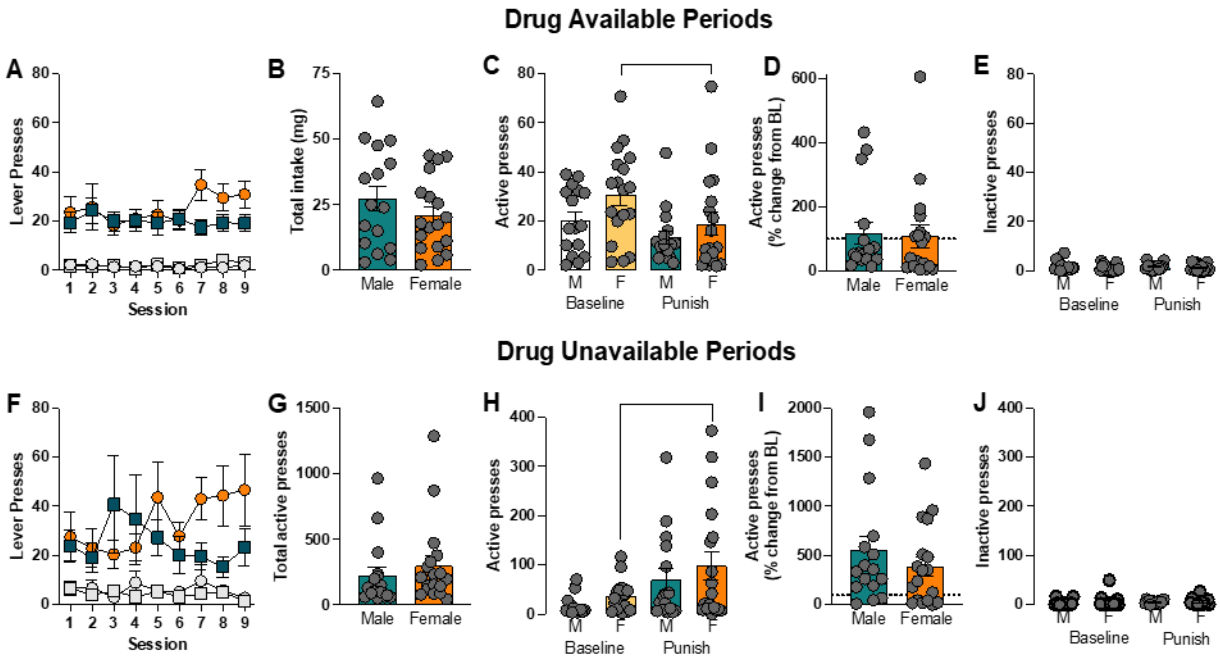


Figure 1. Cocaine self-administration under baseline and risky conditions is similar across sexes. (A-B, F-G) Male and female rats had similar levels of IntA cocaine self-administration, with both groups showing increased active lever presses (females: orange circles, males: blue squares) compared to inactive lever presses (females: gray circles, males: gray squares) during drug available (A) and drug unavailable (F) periods. Male and female rats also had similar amounts of total cocaine intake (B) and total number of presses on the active lever during drug unavailable periods (G). (C-D, H-I) Female (F) but not male (M) rats showed significant decreases in active lever presses during drug available periods (C) and significant increases in active lever presses during drug unavailable periods (H) when comparing footshock punishment sessions with baseline responding. $P < 0.05$. However, male and female rats had similar percent changes in active lever

presses between punishment and baseline sessions during drug available (D) and drug unavailable (I) periods. (E, J) Female and male rats had similar levels of baseline and punishment session inactive lever presses during drug available (E) and drug unavailable (J) periods. N=16-18/group.

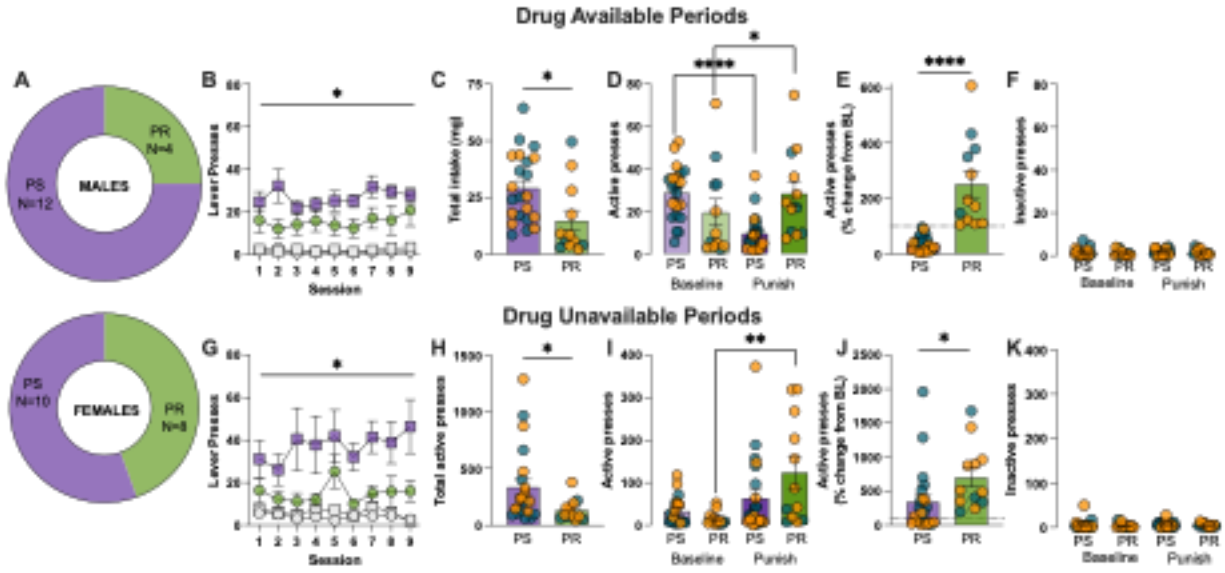


Figure 2. Punishment-resistant rats have lower levels of cocaine-taking and cocaine-seeking during IntA self-administration (A) There were a higher proportion of females in the punishment-resistant (PR) group (green, 8 females, 4 males) and a higher proportion of males in the punishment-sensitive (PS) group (purple, 10 females, 12 males). (B-C, G-H) PS rats (squares) had significant increases in active lever presses during drug available (B) and drug unavailable (G) periods compared to PR rats (circles). There were no group differences in inactive lever presses. PS rats also had significant increases in total cocaine intake (C) and total number of presses on the active lever during drug unavailable periods (H) compared to PR rats. $P < 0.05$. (D-E, I-J) PS showed significant decreases ($P < 0.0001$) and PR rats showed significant increases ($P < 0.01$) in active lever presses during drug available periods (D) and PR rats showed significant increases ($P < 0.05$) in active lever presses drug unavailable periods (I) when comparing footshock punishment sessions with baseline responding. PR rats also had significant increases in the percent change in active lever presses between punishment and baseline sessions during drug available (E, $P < 0.0001$) and drug unavailable (I, $P < 0.05$) periods. (F, K) PS and PR rats had similar levels of

baseline and punishment session inactive lever presses during drug available (F) and drug unavailable (K) periods. N=12-22/group.

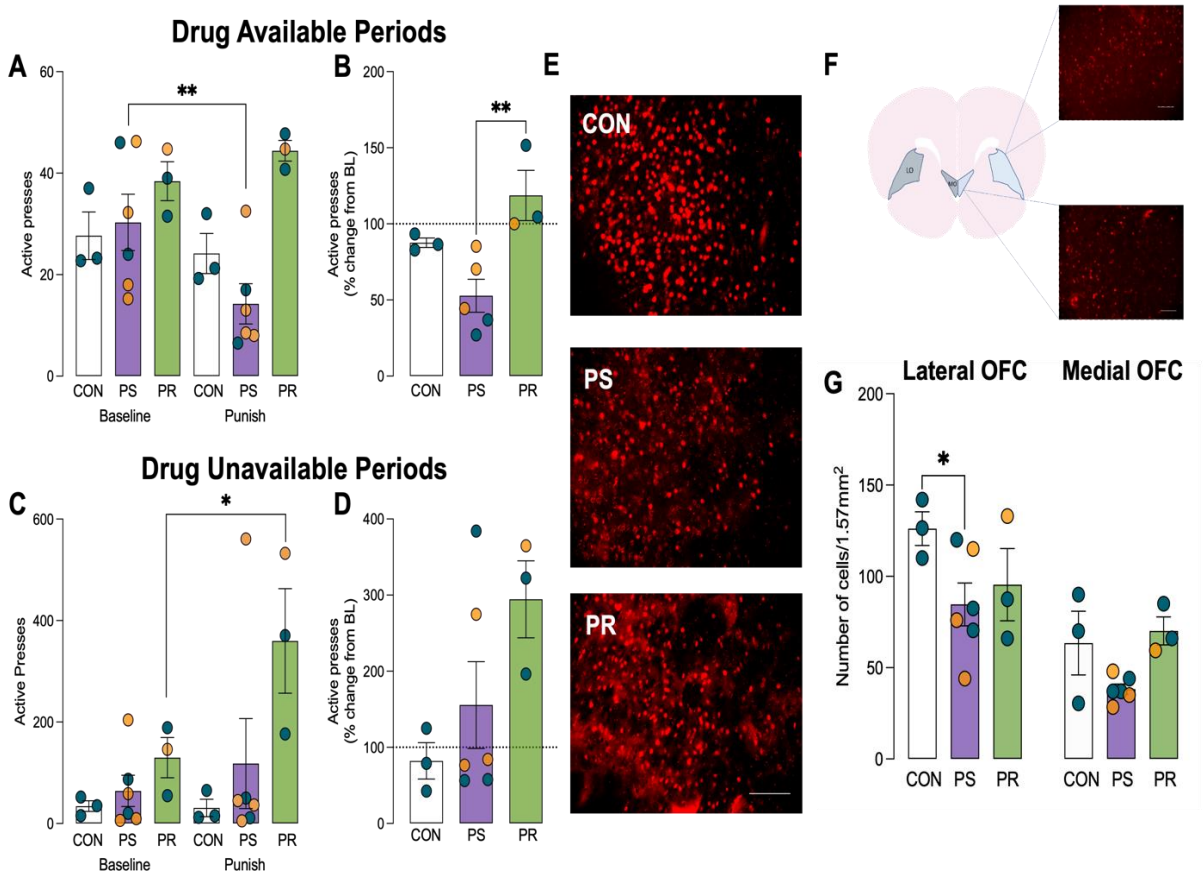


Figure 3. Risky cocaine use decreases c-Fos expression in the lateral but not medial OFC. (A) Representative images of c-Fos expression in control (CON), punishment-sensitive (PS), and punishment-resistant (PR) rats during cocaine self-administration under risky conditions (i.e., when 50% of cocaine infusions were paired with footshocks). (B) PS but not PR rats showed a significant decrease in c-Fos expression in the lateral (left panel, $P < 0.01$) but not medial (right panel, $P > 0.05$) OFC compared to controls. N = 3-6/group, scale bar = 50 □m.

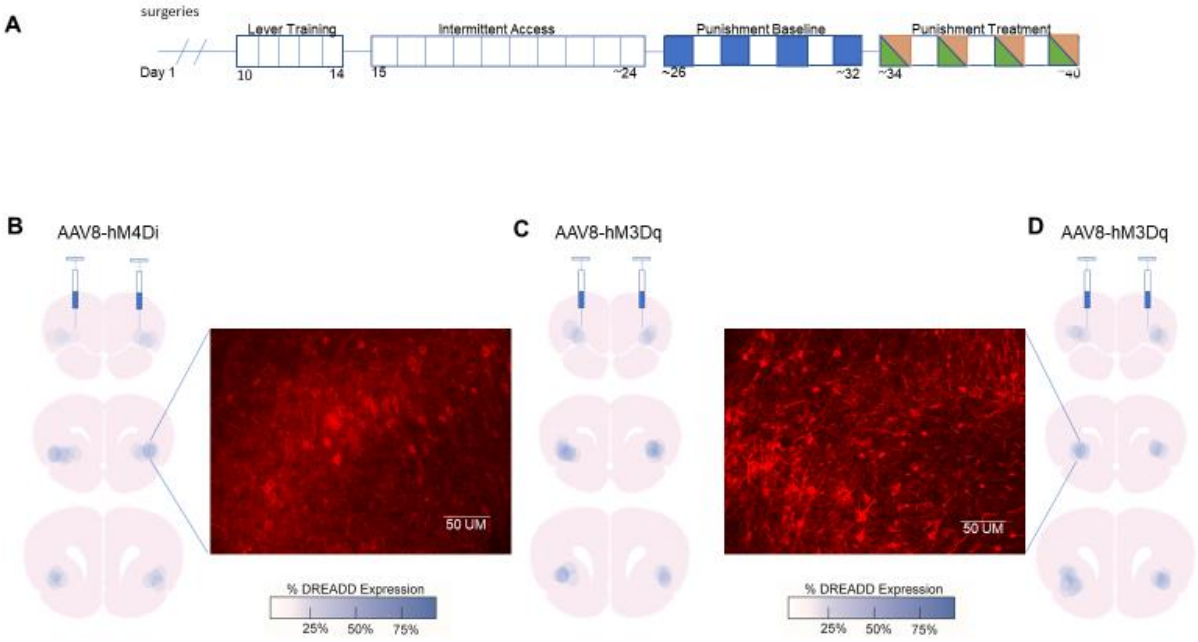


Figure 4. DREADD experimental timeline and viral expression (A) Timeline for chemogenetic modulation of cocaine-seeking and cocaine-taking during punished drug use. (B) Viral strategy, representative histology, and quantification of DREADD expression. AAV8-hM4Di (B) or AAV8-hM3Dq (C,D) was bilaterally infused into the lateral OFC. Scale bar = 50 □m.

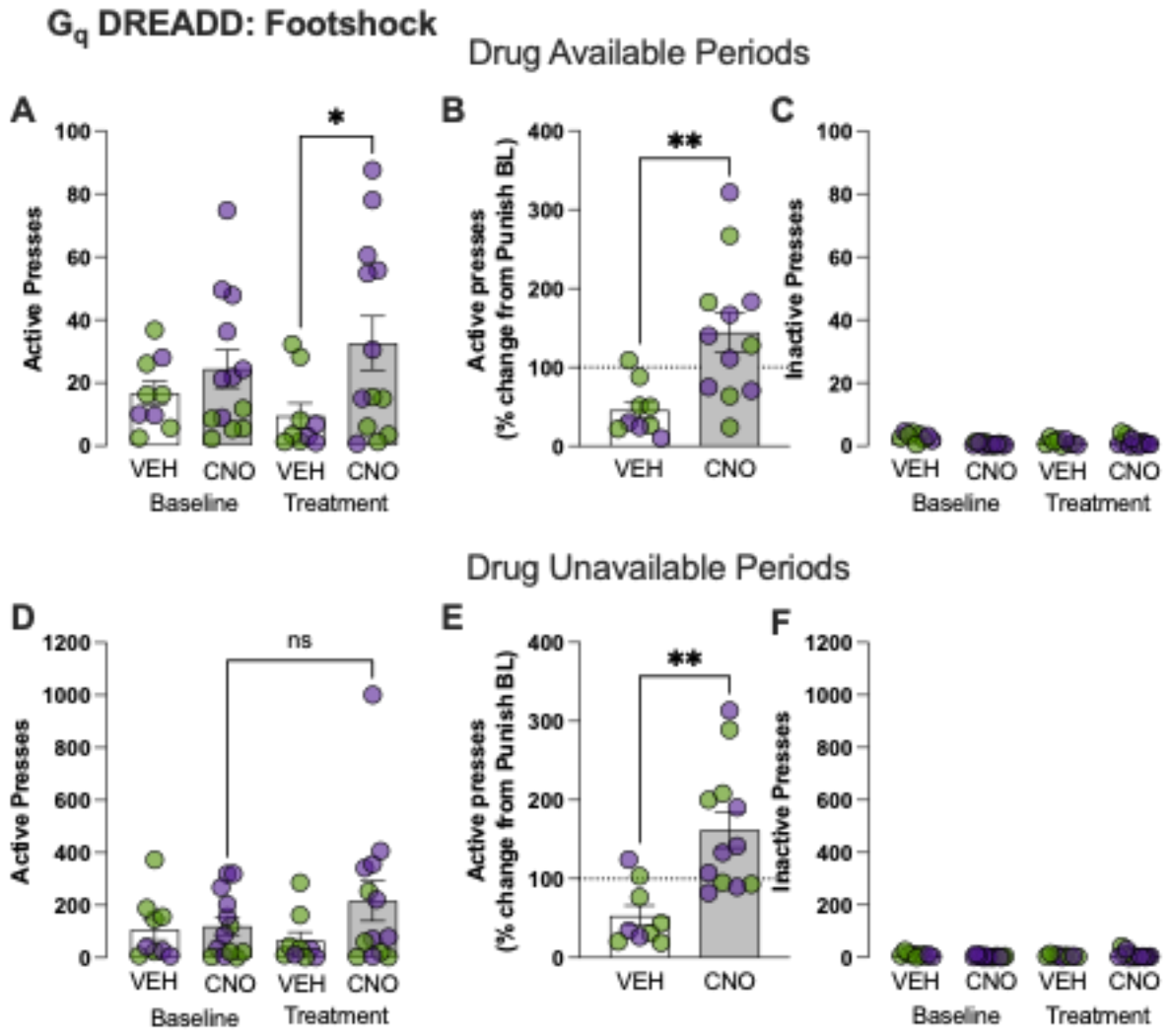


Figure 5. Transient DREADD-mediated activation of the lateral OFC increases punished drug-taking and drug-seeking (A-B, D-E) CNO-treated rats had significant increases in active lever presses during drug-available (A) periods compared to vehicle-treated rats during treatment sessions and significant increases in drug unavailable (D) periods during treatment sessions compared to baseline periods. $P < 0.05$. CNO-treated rats also had significant increases in the percent change in active lever presses between treatment and baseline sessions during drug available (B) and drug unavailable (E) periods. $P < 0.001$. (C, F) Vehicle and CNO-treated rats

had similar levels of baseline and treatment session inactive lever presses during drug available (C) and drug unavailable (F) periods. N=9-13/group.

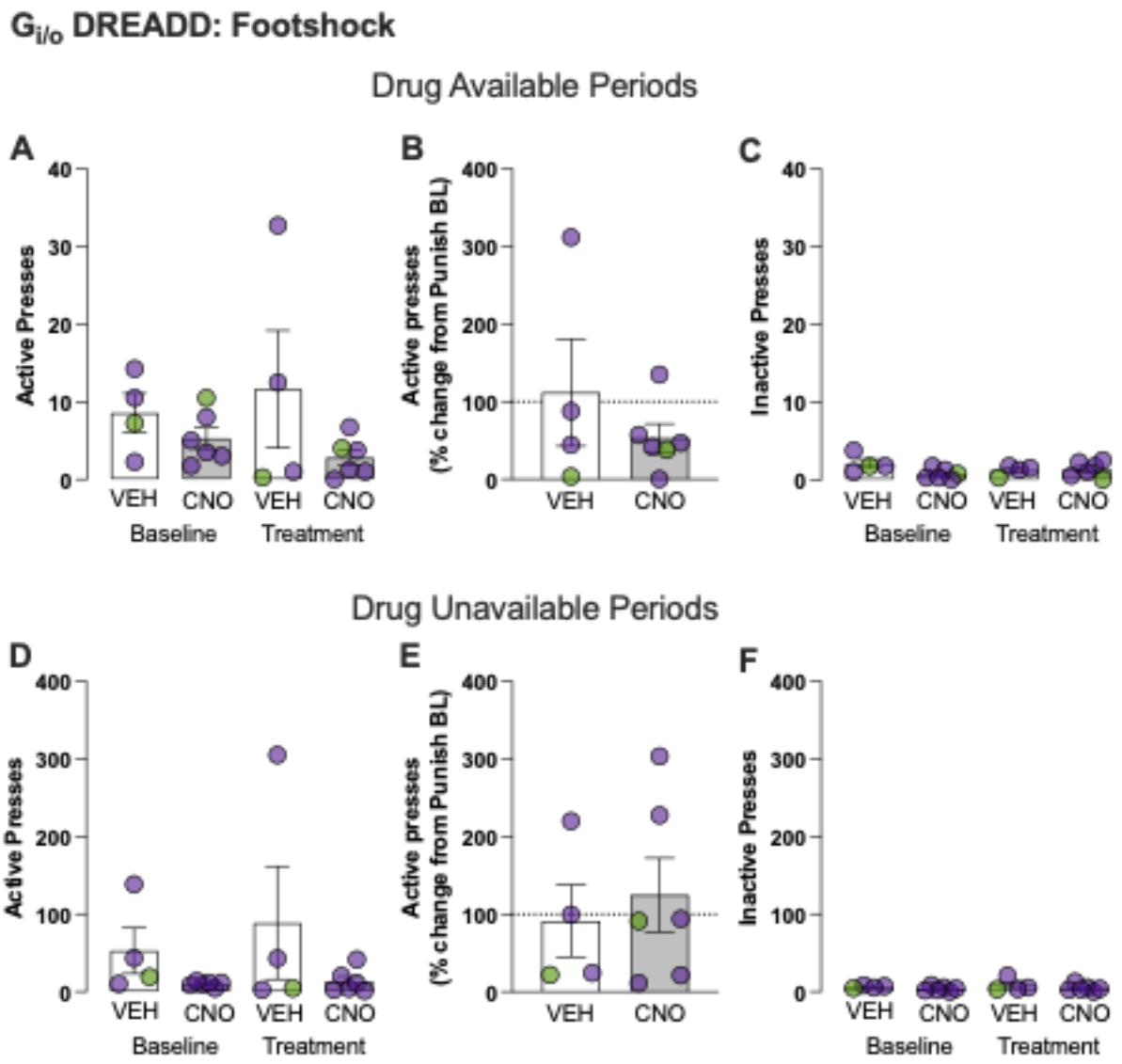


Figure 6. Transient DREADD-mediated inhibition of the lateral OFC had no effect on punished drug-taking and drug-seeking (A-B, D-E) Vehicle and CNO-treated rats had similar levels of active lever presses during drug available (A) and drug unavailable (D) periods during treatment sessions compared to baseline periods. The percent change in active lever presses between treatment and baseline sessions during drug available (B) and drug unavailable (E) periods also did not differ across groups. (C, F) Vehicle and CNO-treated rats had similar levels of baseline

and treatment session inactive lever presses during drug available (C) and drug unavailable (F) periods. N=4-6/group.

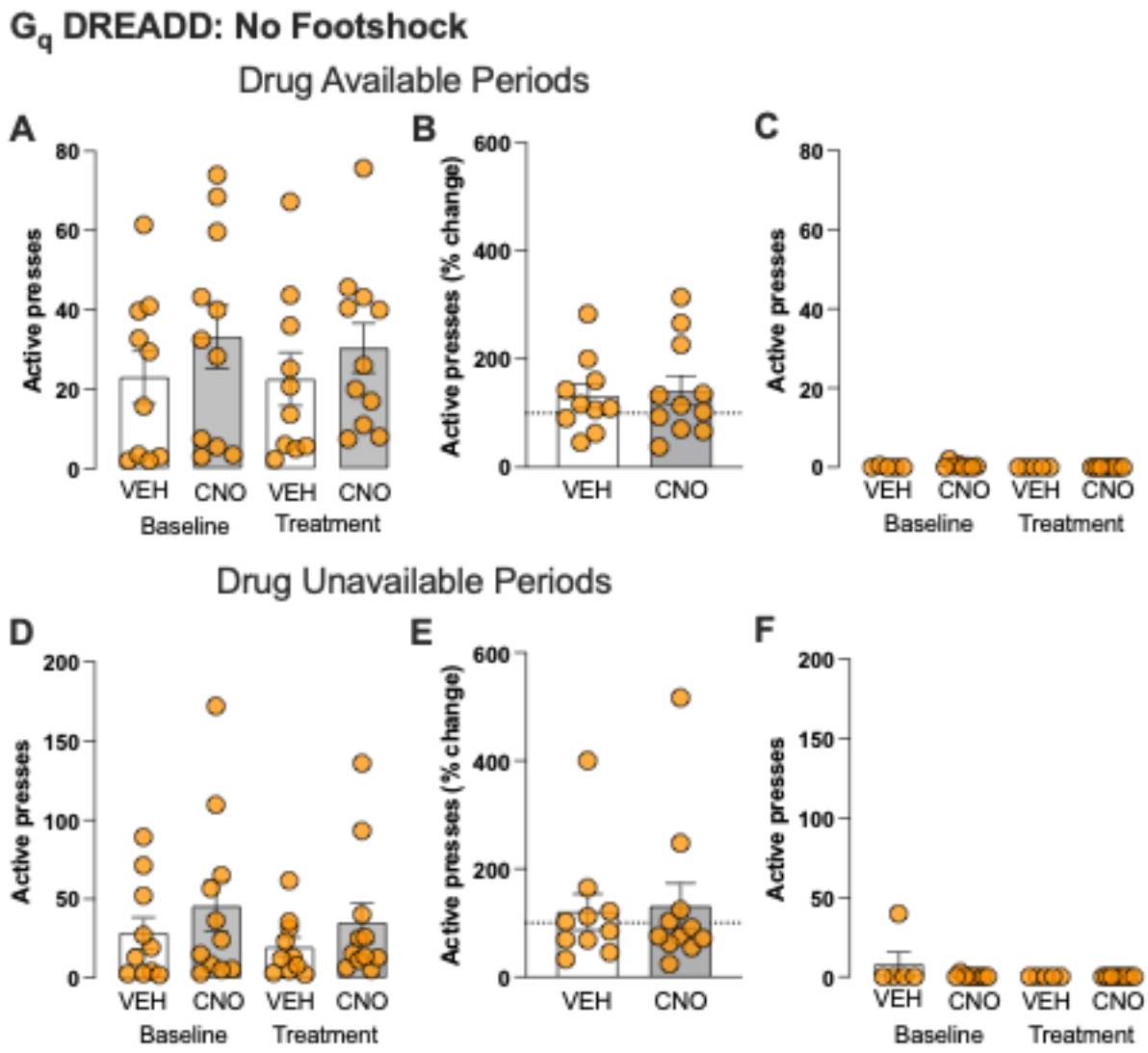
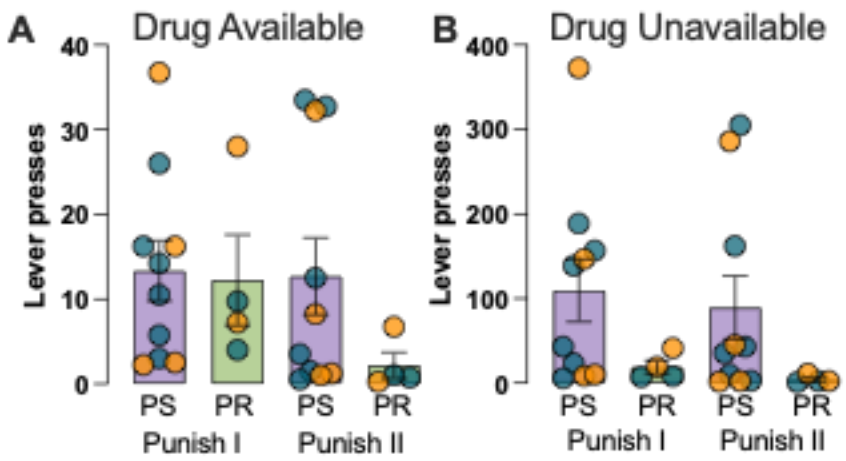


Figure 7. DREADD-mediated activation of the lateral OFC during IntA self-administration has no effect on drug-taking and drug-seeking (A-B, D-E) Vehicle and CNO-treated rats had similar levels of active lever presses during drug available (A) and drug unavailable (D) periods during treatment sessions compared to baseline periods. The percent change in active lever presses between treatment and baseline sessions during drug available (B) and drug unavailable (E) periods also did not differ across groups. (C, F) Vehicle and CNO-treated rats had similar levels

of baseline and treatment session inactive lever presses during drug available (C) and drug unavailable (F) periods. N=10-11/group.



Supplemental Figure 1. Extended IntA self-administration under risky conditions decreases cocaine-taking and cocaine-seeking across groups. (A-D) Following 8 punishment cocaine self-administration sessions, there were no differences in PS and PR rats in active lever presses during drug available (A) and drug unavailable (B) periods. The percent change in active lever presses between the first four and last four punishment sessions during drug available (C) and drug unavailable (D) periods also did not differ across groups. N=4-10/group.

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Chapter 2

Orbitofrontal cortical projection neurons do not affect probabilistic decision making

Abstract

Risk-taking behaviors, which are a hallmark of neuropsychiatric diseases including substance use disorders and ADHD, are regulated by cortical circuits. However, studies examining the contributions of these circuits to decision-making strategies have largely focused on regional activity rather than delineation of cell-type specific contributions. The cortex is primarily formed of two highly heterogenous but physically intermingled populations of glutamatergic projection neurons, intratelencephalic (IT) and pyramidal tract (PT), and the role of these cell types in complex decision making has not been investigated. To begin to address this, we used combinatorial, chemogenetic viral approaches to assess how IT and PT neurons regulate performance during probability discounting and reversal tasks. The ventral and lateral orbitofrontal cortex (vOFC and lOFC) were targeted for neuronal manipulation because Fos activity was elevated in these areas following probabilistic choice. There was no effect of chemogenetic inhibition of IT or PT neurons on probability discounting, though general OFC inhibition reduced overall choice of riskier rewards. Together these results suggest that, while OFC glutamatergic projection neurons are not necessary for regular choice selection in risk-based models other populations of neurons may mediate aspects of probabilistic choice within OFC. This study, therefore, provides further evidence of OFC activity involved in probabilistic decision-making but does not suggest that IT or PT projection neurons are necessary for risk-based valuations in this paradigm.

Introduction

Decision-making strategies, including those involved in risk valuation, are largely mediated by the executive functions of the prefrontal cortex (PFC) (Floresco et. al, 2008; Knutson et al., 2005; Wrase et al., 2007). Alterations in decision-making processes are common among neuropsychiatric disorders, including substance use disorders and ADHD, but how computations of value and risk are normally regulated by the PFC and become dysregulated in these diseases is still not fully understood. Manipulations to several regions of PFC alter decision making in various risk and impulsivity models, including medial PFC (Knutson et al., 2005; Stopper et al., 2014) and orbitofrontal cortex (OFC) (Pais-Vieira et al., 2007; Wrase et al., 2007). However, differences in task structure and regional targeting have produced conflicting results (Mai & Hauber, 2012; Mobini et al., 2002; St. Onge & Floresco, 2010), suggesting that the cortical processes underlying decision-making are complex and not fully understood. Adding to this complexity, the PFC is primarily made up of two distinct classes of glutamatergic projection neurons; intratelencephalic (IT) neurons which project ipsi- and bi-laterally within the cortex and to the striatum, and pyramidal tract (PT) neurons which project ipsilaterally to the striatum and brainstem structures. IT and PT neurons are morphologically distinct, have distinguishable firing patterns, and are differentially affected by various neuromodulators (Dembrow et al., 2010; Shepherd, 2013; Stephens et al., 2014). In addition, IT neurons are present across layers II-VI of the cortex, with layers II and III characterized by cortico-cortical projections and layers V-VI characterized by both cortico-cortical and cortico-striatal projections. PT neurons are restricted to layer VB but are notable for branching axons that innervate multiple targets in the striatum, midbrain, and brainstem (for review, see Shepherd, 2013). To date, the contributions of these different cortical projection populations in decision making processes have not been assessed. Accordingly, this study's purpose was to investigate how these subsets of PFC neurons regulate choice patterns. We first used Fos labeling following probabilistic choice for sucrose rewards to decide regions of interest for subsequent cell-type neuronal manipulation. Then, using an inhibitory chemogenetic approach, the role of IT and PT cells were assessed during probabilistic choice tasks.

Methods

Animals. All experiments were approved by the Seattle Children's Research Institute Institutional Animal Care and Use Committee and were conducted in accordance with National Institutes of Health guidelines.

Male Long Evans rats (N=59, Charles River) weighing 251-275g upon arrival were pair-housed in a temperature- and humidity-controlled vivarium on a 12-hour light/dark cycle. Rats were acclimated for at least three days prior to any experimental manipulations. Rats were food restricted and maintained between 85-90% of their free-feeding bodyweight throughout the study with 20g per cage of chow pellets given daily. Water was provided ad libitum. Drugs Clozapine-N-Oxide (CNO) was obtained from the National Institute of Health through the Rapid Access to Investigate Drug Program and prepared by dissolving in dimethyl sulfoxide (DMSO; Sigma Aldrich, D650) in a hot water bath, then further diluted in sterile water to 6% DMSO. CNO was prepared prior to test sessions at a concentration of 5 mg/ml and administered at 1 mg/kg ip. Vehicle injections were 6% DMSO in sterile water administered at a volume of 1 mL/kg ip.

Surgery. Rats were anesthetized with isoflurane (2-5% inhalation, Patterson Veterinary) and were administered meloxicam (0.2mg/kg sc, Patterson Veterinary) for analgesia prior to stereotaxic surgery. Rats underwent post-procedure monitoring a minimum of three days following surgery.

Viral strategy. Given that IT neurons have both ipsilateral and contralateral projections, to achieve selective DREADD expression in IT neurons, two recombinase strategies were utilized for selective, bilateral targeting of IT neurons. Rats received unilateral infusions of CAV-CRE (from the lab of John Neumaier, University of Washington) and AAVrg-EF1α-FLPO (Addgene #55637) into different hemispheres of the NAc (NAc: A/P: +1.2, M/L: ±2.5, D/V: -7.4 mm relative to bregma; 1000 nl/side, 200 nl/min) along with infusions of the complementary CRE- and Flp- dependent AAV-hM4Di into the contralateral OFC (A/P +3.7, M/L ± 2.5, D/V -5.4 mm relative to bregma; AAV1-DIOFRT- hM4Di-EYFP from the lab of Larry Zweifel, University of Washington, AAV8-DIOhM4Di-mCherry from Addgene #44362; 750 nl/side, 150 nl/min) (Figure 2C). To achieve selective DREADD expression in PT neurons, rats received bilateral infusions of CAV-CRE into the caudal pontine nucleus (PNc: A/P: -9.2, M/L: ±0.2 or 0.5, D/V: -9.4 or -9.8 mm relative to bregma; 750 nl/side, 150 nl/min), along with bilateral infusions of AAV8-DIO- hM4Di-mCherry into the OFC (750 nl/side, 150 nl/min) (Figure 2D). To target DREADD expression non-selectively to all OFC neurons, rats received bilateral infusions of AAV8-hSyn-hM4Di-mCherry into OFC

(Addgene #50475; 750 nl/side, 150 nl, min) (Figure 2B). For targeting DREADD expression to both IT and PT CAV CRE for IT was injected in contralteral and PT, then AAVrgpgk-CRE for PT neurons, the same regions were targeted as described for the selective expression, with the exception that FLPO was unilaterally infused into one hemisphere of the PNc ipsilateral to the Flp-dependent DREADD, while CAV-CRE was unilaterally infused into the other hemisphere of the PNc ipsilateral to the corresponding Cre-dependent DREADD (Figure 2C).

Behavior Apparatus. Behavioral testing occurred in standard operant self-administration chambers equipped with retractable levers, stimulus lights, a house light, a feeder, and a metal grid floor (Med Associates ENV-007CT). The front wall housed two white stimulus lights, one located above each lever, with an additional green stimulus light above the white stimulus light on one side. The back wall contained a white house light. Lever Press training A representative timeline of all behavioral testing is outlined in Figure 2A. Rats were trained under a fixed-ratio 1 schedule to a criterion of 100 pellets obtained in a single, 30-minute session on one lever before undergoing training on the opposite lever (~3 days to train both levers), as in previous studies (Cardinal & Howes, 2005; St. Onge & Floresco, 2009; Stopper et al., 2014).

Probability training. Sessions began with levers retracted and operant chambers in darkness. The food receptacle and house lights were presented at the beginning of each trial (every 40s) to signal a response (nose poke) to occur within 10s. If no respond was recorded within this timeframe, the lever was retracted and the trial was counted as an omission. If a response was made, a single pellet was delivered with 50% probability and continuous illumination of the food receptacle light and house light (3s) up to a 10s collection time limit. In every trial, the left or right lever was presented once in a randomized fashion. Training occurred over a five to six-day period to a criterion of ten omissions or fewer in a session (Cardinal & Howes, 2005; St. Onge & Floresco, 2009).

Probability discounting. Rats were run five to seven days a week. Sessions consisted of 96 trials, separated into four blocks of 26 trials (~70-minute session time) and began in darkness with both levers retracted.

Trials began every 40 s with the house light and food receptacle light illuminated. To initiate forced or choice trials, rats nose-poked into the illuminated food receptacle. If a rat successfully initiated the trial, either one lever (forced choice) or both levers (choice trials) were extended. Pressing a lever resulted in cue light presentation (3 s illumination) and food pellets dispensed into the food. If a press resulted in no reward administration, a green cue light above the white cue light associated with the risky lever was illuminated to indicate reward omission. If a rat did not nosepoke into the food receptacle to initiate the trial within 10 s, or if a rat did not press a lever within 10 s, the trial was scored as an omission. The risky lever was associated with descending probability of administration of four sucrose pellets across the four trial blocks: 100%, 50%, 25%, and 12.5% probability of reward delivery, whereas the safe lever always resulted in administration of one food pellet (St. Onge & Floresco, 2009). After a minimum of 25 sessions on the full discounting task, training continued until stable performance was achieved, as determined statistically with no interaction between session and performance across three sessions. Following training, CNO (5 mg/kg in 6% DMSO in sterile water) or vehicle (6% DMSO) was administered 30 minutes prior to testing in a counterbalanced fashion, followed by two days of reestablishing baseline responding in between each test session. The discounting rate per block for the risky reward was calculated using the following equation, where px represents the percent of risky choices at a given probability x during choice trials: $Discounting\ (rate\ per\ block) = |(p100 + p50)/2 - p12.5|\ 3$ The percentage of optimal choices was obtained by taking the average percent choices for the risky reward at p100 and p50 and the safe choice at p12.5. The 25% probability block was not included, as both options were equally valued (adapted from Verharen et al., 2018). To examine neuronal activity during probabilistic choice, rats (N=20) were trained as described above. On the final behavioral session, the discounting session was altered to maintain a constant probability for the larger reward (100%, 50%, 25%, and 12.5%) across all four blocks. Rats were perfused 75 minutes after the start of the discounting session to examine cFos activity. Reversal task Following discounting tests, rats underwent a within-session reversal task (adapted from Verharen et al., 2018) consisting of 150 total trials of FR1 pressing, where an active and inactive lever were extended. Initial

active levers were chosen based on the "safe" lever association from the probability discounting task. Selection of the active lever resulted in delivery of one sucrose pellet, illumination of the white cue light above the active lever for 3s, and retraction of both levers. Selection of the other, inactive lever resulted in illumination of the green cue light for 3s and an additional 8s time-out period whereby the house light was turned off and the levers were retracted. A new trial began 8s after the last response, signaled by illumination of the house light. After five consecutive responses on the active lever, the "active" lever was switched to the previously inactive one, prompting a shift in behavioral strategies to obtain sucrose pellet rewards. A minimum of six sessions was given on this task to assure stable performance before undergoing test sessions. During testing, CNO or vehicle was administered 30 minutes prior to testing in a counterbalanced fashion, followed by one to two days of re-baselining in between each test session. Immunohistochemistry Following completion of behavioral paradigms, rats were anesthetized with Beuthanasia-D and transcardially perfused with 1x phosphate-buffered saline (PBS; pH = 7.4 on ice), followed by 4% paraformaldehyde (PFA) in PBS. Brains were extracted and fixed overnight in PFA and subsequently stored in 30% sucrose (in PBS) prior to being coronally sectioned on a vibrating microtome (40 µm thickness). To confirm DREADD expression, tissue sections were washed in 1x PBS (3 x 10 minutes) prior to incubating in blocking buffer consisting of normal goat serum (5% NGS), Triton-X (0.25%), and PBS (120 minutes). Sections were then transferred from blocking solution to primary antibody solution (2.5% NGS, 0.25% Triton-X, 1:1000 rabbit polyclonal anti-GFP, ThermoFisher # A-11122, 1:400 mouse monoclonal anti-mCherry, Clontech # 632543, or 1:800 rabbit polyclonal anti-cFos MilliporeSigma # ABE457, 24 hours), before being washed in PBS (4 x 10 minutes) and subsequently incubated in secondary antibody solution (2.5% NGS, 0.25% Triton-X, 1:500 Alexa Fluor 488 goat anti-rabbit ThermoFisher# A-11034, 1:400 Alexa Fluor 568 goat anti-mouse ThermoFisher# A-11004, 120 minutes). Finally, sections were washed in PBS (3x10 minutes), mounted on slides, and coverslipped with Vectashield mounting medium with DAPI (Vector Laboratories H-1500). Slides were imaged with a Zeiss LSM 710 confocal microscope. For cFos imaging, N=3 images were collected from the following cortical regions, with at least one image

from each hemisphere obtained: anterior cingulate (ACC), infralimbic (IL), prelimbic (PL), medial OFC (mOFC), ventral OFC (vOFC), and lateral OFC (lOFC). Image collection of z stacks was set to 20x magnification for 9µm range and cFos counts were obtained using Image J (NIH) analyze particles (thresholded to 2% signal and using parameters for sphericity=0.15-1 and size=250-1500 pixels2) and Adobe Photoshop software.

Experimental design and statistical analysis. Data was processed using customized python scripts (available upon request) and analyzed using GraphPad Prism 8.4.3. cFos counts were averaged (N=3 images for each region for each subject) and analyzed using two-way ANOVA of cortical region by probability of the risky reward. Pearsons correlations were analyzed at each reward probability for associations between average cFos counts and the percentage of choice selections for the larger reward in each region. For probability discounting experiments, the primary dependent variable was choice on the risky lever on free choice trials (number of responses on risky lever/total number of choice trials in a block). For reversal experiments, the primary dependent variable was the number of completed reversals within a session, with additional variables examined including the number of trials required to reverse the rewarded lever, and preservative responding (defined as the average number of incorrect choices made after a reversal occurs before selecting the correct lever in a session). Choice across probabilities was analyzed using two-way repeated measures (RM) ANOVA with Geisser-Greenhouse's correction for potential differences in variance. Discounting rate, optimal choice, number of reversals, preservative responding, and trials to first reversal were calculated as previously described (Verharen et al., 2018) and analyzed using a two-tailed paired t-test comparing performance between control and test treatment sessions. Choice patterns (based on if the selection resulted in a reward (win) or no reward (loss), and if this were followed by selection of the same lever (stay) or switching to the other lever (shift) during discounting) were analyzed with a three-way ANOVA with Geisser-Greenhouse's correction. Trials to reversal based on outcome at the start of a reversal block were analyzed with a restricted maximum likelihood (REML) mixed-effect analysis. All post hoc comparisons were Bonferroni corrected for multiple comparisons.

Results

To determine the cortical areas activated during probabilistic decision making, rats were trained on the probability discounting task and split into one of four groups: A group receiving the larger reward on 100% of trials, 50% of trials, 25% of trials, or 12.5% of trials (N=5 for each group). cFos was used as a correlate for neuronal activation during the task (Dragunow & Robertson, 1987), and was quantified in seven potential regions of interest known to be involved in affective and motivated behaviors: ACC, IL, PL, mOFC, vOFC, lOFC, and insula. cFos expression was highest in vOFC and lOFC across all probabilities (Figure 1A, Two-way ANOVA main effect of region: $F(6, 112) = 19.08$; $pp<0.0001$), and significantly higher in post hoc comparisons to all regions but the ACC (Table 1). However, activity did not differ based on the probability of the larger reward, but was higher at the 50% reward probability, suggesting overall activity was higher when it was advantageous to choose the larger, riskier option (Figure 1A, Two-way ANOVA main effect of probability: $F(3, 112) = 5.739$, $p=0.0011$), but no interaction between region and probability was found (Figure 1A, Two-way ANOVA probability x region: $F(6, 112) = 19.08$, $p=0.2167$). To determine if activity was related to preference for the "riskier" option, regional cFos at each probability was compared to the frequency of choosing the larger reward when given a choice between the risky and safe options (Figure 1 I-O). Interestingly, IL activity at 50% probability revealed significant associations between risk preference and cFos activity, but no other significant associations were found. Together, these results suggest that, while not exhibiting changes in activity based on probability, ventral and lateral OFC are engaged during decision making with probabilistic options. Based on these results, we targeted subsequent manipulations to these cortical areas. Rats underwent stereotaxic surgery to target DREADD expression to either ventral and lateral OFC (referred to generally as OFC), which exhibit overlapping connectivity (Hoover & Vertes, 2011), or selectively to IT, PT or both neuronal populations in OFC (Dual IT and PT; Figure 2C-E). Once rats reached stable performance on the full probability-discounting task (minimum of 25 sessions with no session effect for final three sessions; Figure 2A) they were administered

a pretreatment of 5 mg/kg CNO or vehicle 30 minutes prior to the start of the probability-discounting task, in a counterbalanced fashion.

All groups exhibited sensitivity to the change in probability of the larger reward across a session (Figure 3A-D: Repeated Measures (RM) two-way ANOVA, Main effect of probability of risky choice: Nonspecific: $F(1.960, 9.799) = 14.35$, pp=0.7343). Additionally, none of the manipulations increased omissions during the task (Extended Figure 3-1A-D, Two-tailed paired t-test: Nonspecific: t=0.2745, df=5, p=0.7946; Dual IT and PT: t=0.6195, df=6, p=0.5583; IT: t=0.000, df=10, p=0.9999; PT: t=0.9856, df=12, p=0.3438). While inactivation of OFC neurons altered discounting of the risky option (Figure 3A, Nonspecific, RM two-way ANOVA, Main effect of treatment: $F(1, 5) = 13.89$, p=0.0136), inhibiting either IT and/or PT neuronal subtypes did not alter probabilistic discounting (Figure 3B-D: RM two-way ANOVA, Main effect of Treatment: Dual IT and PT: $F(1.000, 6.000) = 0.07080$, p=0.7991; IT: $F(1, 10) = 2.366$, p=0.1551; PT: $F(1, 12) = 0.1522$, p=0.7032), nor were there effects specific to the probability of obtaining the larger reward for any OFC manipulations (Figure 3A-C, 3E: RM two-way ANOVA, Interaction of Probability x Treatment: Nonspecific: $F(2.383, 11.92) = 0.4954$, p=0.6525; Dual IT and PT: $F(1.672, 10.03) = 1.049$, p=0.3726; IT: $F(2.080, 20.80) = 1.027$, p=0.3782.; PT: $F(1.983, 23.79) = 0.1193$, p=0.8865.). This result is consistent with the absence of effects found for discounting rate of the risky option (Figures 3E-H, Two-tailed paired t-test, Nonspecific: t=0.1085, df=5, p=0.9179; Dual IT and PT: t=0.6378, df=6, p=0.5472; IT: t=1.381, df=10, p=0.1975; PT: t=0.3954, df=12, p=0.6995) and optimal choice (Figures 3I-L, Two-tailed paired t-test: Nonspecific: t=0.4443, df=5, p=0.6754; Dual IT and PT: t=0.4201, df=6, p=0.6891; IT: t=0.05814, df=10, p=0.9548; PT: t=0.3475, df=12, p=0.7343). Additionally, none of the manipulations increased omissions during the task (Extended Figure 3-1A-D, Two-tailed paired t-test: Nonspecific: t=0.2745, df=5, p=0.7946; Dual IT and PT: t=0.6195, df=6, p=0.5583; IT: t=0.000, df=10, p=0.9999; PT: t=0.9856, df=12, p=0.3438

Choice patterns, however, appear to involve OFC activity, as general inhibition of OFC alters choice depending on prior outcomes (Figure 3M, Three-way ANOVA, Nonspecific: Treatment x Outcome:

F(1.000, 5.000) = 7.619, p=0.0398) and trends towards alterations in subsequent choices to either stay on the previously selected lever or shift to the alternative option (Figure 3M, Threeway ANOVA, Nonspecific: Treatment x Choice: F(1.000, 5.000) = 5.183, p=0.0718). Chemogenetic inhibition of both IT and PT OFC neurons trends towards changes to choice patterns based on outcome and an interaction between outcome and subsequent choice (Figure 3N, Three-way ANOVA, Dual IT and PT: Treatment x Outcome: F(1.000, 6.000) = 4.158, p=0.0876; Treatment x Outcome x Choice: F(0.5954, 3.572) = 6.337, p=0.0759). However, neither subtype is predominantly implicated as manipulations specific to each population had no effect (Figures 3O-P: IT: Treatment x Outcome: F(1.000, 10.00) = 0.2880, p=0.6032; Treatment x Choice: F(1.000, 10.00) = 0.04736, p=0.8321.;Treatment x Outcome x Choice: F(0.5047, 5.047) = 0.6497, p=0.3498; PT: Treatment x Outcome: F(1.000, 12.00) = 0.9953, p=0.3381.; Treatment x Choice: F 1.000, 12.00) = 0.09529, p=0.7629.; Treatment x Outcome x Choice: F(0.4839, 5.807) = 0.004099, p=0.800). Taken together, these data suggest that although OFC activity is involved in probabilistic decision making, activity of each individual glutamatergic subtype is not necessary. Nonetheless, the combination of connectivity between both subtypes to other neuronal subtypes within OFC and/or to other projection regions may impact outcome-dependent choice

Discussion

The PFC is heavily involved in value-based decision making and behavioral flexibility (Li et. al, 2016; McAlonan & Brown, 2003; McDannald et al., 2014; Padoa-Schioppa & Assad, 2006). For example,

recordings of OFC neurons during operant tasks have found that these neurons exhibit changes in background firing reflective of outcome-based modifications to signal firing (Kravitz & Peoples; 2008). These changes are particularly evident with established action-outcome contingencies (Shoenbaum et al., 1999), and are also seen in tasks involving risk-based subjective value (Jo & Jung, 2016). Nonetheless, the precise role of cortical subregions remains to be fully characterized, and the contribution of different cortical projection populations has not been investigated. Here, we utilized an inhibitory chemogenetic viral approach previously shown in our lab to reduce cortical neuronal activity (Kerstetter, et al., 2016) to determine the role of the two major subtypes of glutamatergic projection neurons (IT and PT) in probabilistic valuation of a food reward (St. Onge & Floresco, 2009), as well as in response adaptability in a within-session reversal task (Verharen et al., 2018). We utilized fos activity following exposure to different probabilities of food reward to determine targeting regions. Though activity could be impacted by reward prediction, changes in food consumption, or other factors, we focused our neuronal manipulations on the vOFC and lOFC, because these regions were most active across all probabilities for the larger reward and did not depend on changes in probability, reducing the likelihood of these caveats posing significant confounds to this finding (Figure 1). Overall, we found that general inhibition of OFC impacted probabilistic risk discounting, but that IT and PT inhibition did not affect discounting and optimal response selection. Prior studies using pharmacological or lesion approaches to examine the role of the OFC in riskbased decision making have yielded mixed results, with reports of increases, decreases and no changes in task performance (Mobini et al., 2002; Orsini et. al, 2015; Pais-Vieira et. al, 2007; Stopper et. al, 2014; St. Onge & Floresco, 2010). Consistent with some of this work, we found that transient inhibition of OFC reduced risk preference across reward probabilities (Figure 3A) with OFC inhibition altering subsequent choice (Figure 3M). Importantly, the alteration to choice preference that we observed is not indicative of attentional deficits reducing task engagement, as no differences were seen in omissions or response latencies when administering CNO compared to control treatments in any of the manipulations (Extended Figure 3-1). However, these results are contrary to previous work using the same discounting paradigm (St.

Onge & Floresco, 2010). The differences in these results could be based on the type of inactivation strategy, as the previous study used GABA agonists to decrease OFC activity whereas we utilized activation of a Gi-coupled pathway, as well as differences in cues relating to reward presentation and omission. Nonetheless, these results suggest that the role of the OFC in decision making may involve interactions with inhibitory microcircuitry as well as additional projection-types. Surprisingly, neither IT or PT neuronal activity seems to be necessary for the regulation of these alterations to probabilistic valuation, suggesting that other neuronal subtypes within the OFC are regulating outcome valuation and subsequent choice. However, the trend effects observed on subsequent choice following inactivation indicates that both IT and PT neurons may have some role in this processing. It is also possible that the cell-type specific targeting strategies that we used preserved some cortico-cortical or cortico-subcortical connections. For example, the value information that is encoded by OFC neurons is subsequently sent to anterior cingulate cortex, a region that interfaces with sensory and limbic processing centers and is found to exhibit both value and action-based representations (Rolls, 2019). Investigating this cortico-cortical connection, particularly with respect to IT neurons, may provide greater clarity into their role in choice evaluation and selection. Additionally, as mOFC manipulations have been shown to increase risky preference in the task that we used (Stopper et al., 2014), examining IT and PT neurons within mOFC may reveal further clarify the roles of these two neuronal populations in probabilistic discounting and risk-based decision making. How the OFC regulates decision making and choice processes varies across tasks and task structure. For example, the OFC has a differential role (i.e. increases or decreases to risk sensitivity) in regulating risk assessment under ambiguous conditions versus established probabilities (Floresco et al., 2008), or types of reward associations (i.e. instrumental vs. cuebased pairings). In addition, recent work recording OFC activity during choice tasks suggests that lOFC is critical for initial learning of economic choice and updating subjective preference but is not required when these values are already established (Gardner et al., 2017, 2019, 2020). Although we did not observe a role for IT and PT neurons in probability-discounting, the task that we used requires extensive training to establish the probabilities presented in each block (St. Onge & Floresco, 2009; Stopper et al, 2014). Thus,

it is possible that IT and PT neurons are important in regulating choice during the initial acquisition of discounting baselines, rather than once these risk-based decision-making processes have been established.

In conclusion, our findings build on previous research establishing a significant role of OFC activity in complex decision making. In addition, this work is the first to assess the role of specific populations of glutamatergic projection neurons (IT and PT) in spatial-discrimination risk-based decision making and behavioral flexibility. Given the complexity of cortico-cortico and corticostriatal networks, it will be critical to continue to tease apart how subsets of cortical and striatal neurons interface with one another to regulate decision-making processes, and how these different networks become altered in neuropsychiatric disorders.

Figures:

Figure 1: Ventral and lateral OFC fos activity is higher across probability discounting. Bonferroni post-hoc corrections for main effect of regional fos. AI – Anterior Insular Cortex, ACC – Anterior Cingulate Cortex, IL – Infralimbic Cortex, lOFC – lateral Orbitofrontal Cortex, mOFC – medial Orbitofrontal Cortex, vOFC – ventral Orbitofrontal Cortex, PL – Prelimbic Cortex. Data are presented as Mean ± Standard Error of the mean (SEM). *p< 0.05; Data is presented as mean ± SEM (A-D, M-P) or mean with individual datapoints before-after treatments (E-L). Nonspecific, N=6; Dual IT/PT, N=7; IT, N=11; PT, N=13. Figure 4: OFC IT neuronal inhibition increases adaptive responding in a within-session reversal task. Nonspecific OFC inhibition (A), Dual IT/PT inhibition (B), and PT inhibition (D) did not significantly change the number of reversals occurring within a session whereas IT inhibition (C) increased total number of reversals (2-sided paired t-test). When examining the number of responses on the previously rewarded lever prior to switching to the new active lever (a measure of preservative responding), none of the manipulations (E-H) altered the average number of preservative responses, though there is a trend towards significantly reduced preservative responding with IT inhibition (G). The number of trials required to initiate the first reversal was not significantly altered with any of the neuronal manipulations (I-L) although it trended towards significance with nonselective OFC inhibition (I). Following the first reversal, the subsequent trials to reversal to reach criterion for subsequent reversals were not altered with nonselective (M), Dual IT/PT (N), IT (O), or PT inhibition (P). Data is presented as mean with individual datapoints before-after treatments (A-L). mean ± SEM (M-P). * - p < 0.05; Nonspecific, N=7; Dual IT/PT, N=8; IT, DREADD N=11; PT, DREADD N=13

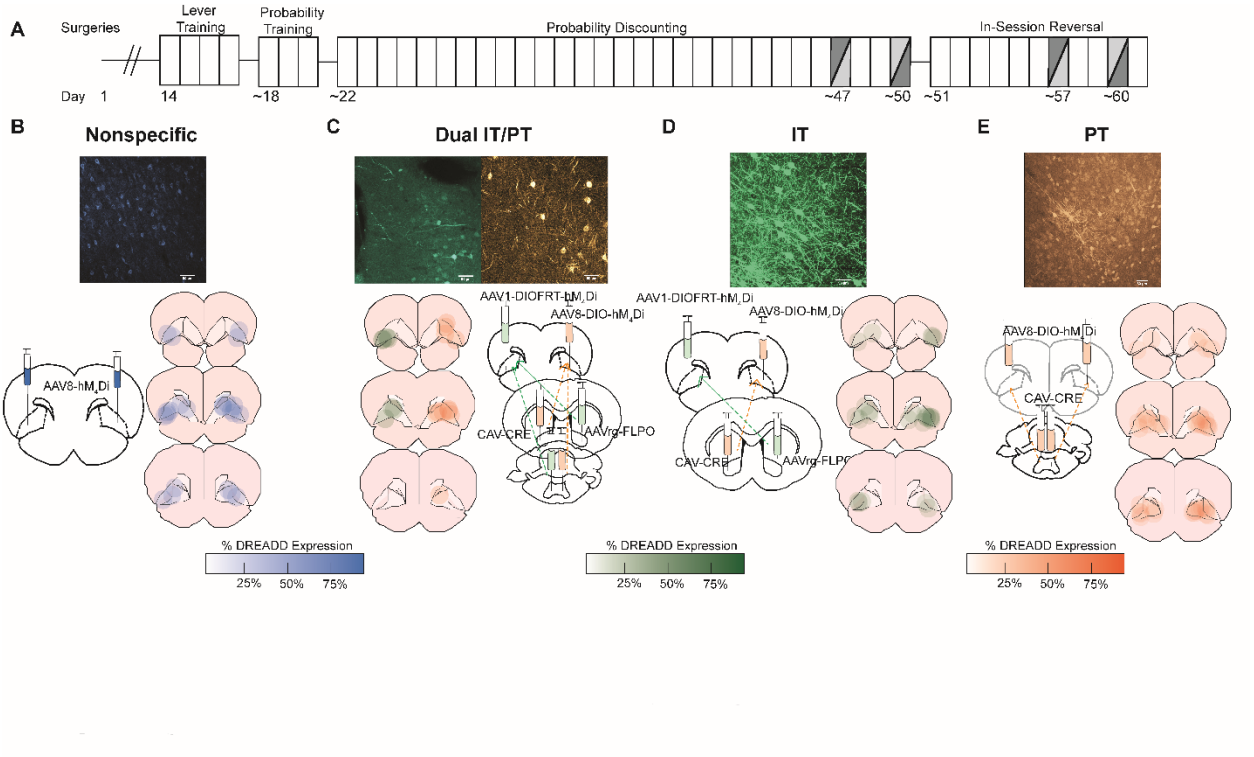


Figure 2: Viral strategy for chemogenetic inhibition of OFC cortical neurons. A. Experiment timeline with test days (CNO/VEH) highlighted in gray. B. To target all OFC neurons (Nonspecific), a constituitive DREADD (AAV8-hSyn-Hm4Di) was injected bilaterally into ventral and lateral OFC. C. To target both IT and PT OFC neurons (Dual IT/PT), a retrograde FLP (AAVrg-EF1a-FLP) was injected into the left hemisphere pontine nucleus and the right hemisphere nucleus accumbens (NAc), while CAV2-CRE was injected into the right hemisphere pontine nucleus and left hemisphere NAc. Inhibitory DREADDS dependent on FLP (Left hemisphere, AAV1-DIOFRTHm4Di-EYFP) or CRE (Right Hemisphere, AAV8-hSyn-DIO-Hm4Di-mCherry) were injected into the OFC. D. To selectively target IT OFC neurons, CAV2-CRE and a retrograde FLP were unilaterally injected into opposite hemispheres of the NAc whereas the corresponding CRE-or FLP-dependent DREADDs were injected unilaterally into the contralateral OFC. E. To selectively target PT neurons, CAV2-CRE was bilaterally injected into the pontine nucleus, while a CREdependent DREADD was injected bilaterally into the OFC. Representative maps of viral spread (% by number of subjects) are included for each targeting strategy. Scale bars= 50 µM

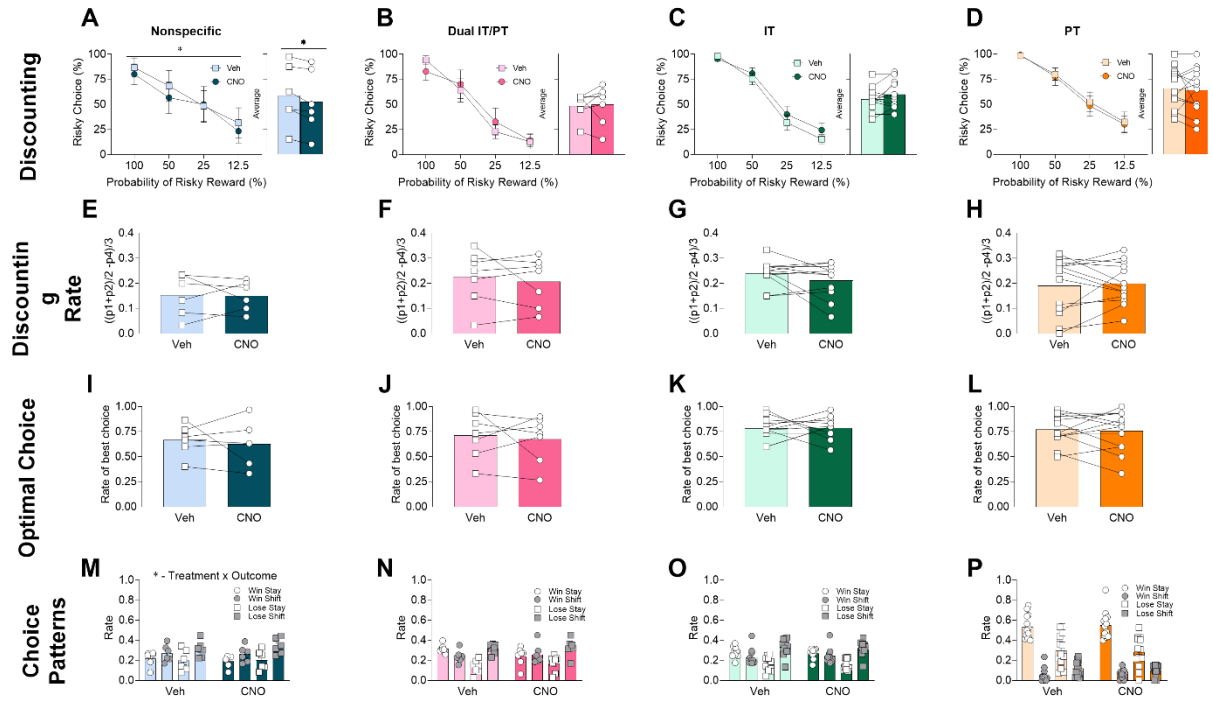


Figure 3: Nonspecific chemogenetic inhibition of OFC alters risk preference. Discounting curves for percent choice of the risky reward option across blocks in a probability discounting task corresponding to 100%, 50%, 25%, and 12.5% probability of receiving the larger "risky" reward. General OFC inhibition (A, blue) reduced overall choice for the risky reward (two-way ANOVA Treatment x Block main effect of Treatment) while Dual IT/PT inhibition (B, pink), IT inhibition (C, green), and PT inhibition (D, orange) did not significantly alter the discounting curve. Neither the discounting rate (E-H, rate per block of risky choice) nor the percentage of optimal choices selected during discounting (I-L) were significantly altered with any of the chemogenetic manipulations. When examining patterns of choice based on the outcome (win or lose) and subsequent pattern (stay on option or shift to alternative), nonspecific OFC inhibition (M) alters choice based on outcome (three-way ANOVA Treatment x Outcome), while Dual IT/PT inhibition (N) alters rates based on both Outcome and whether a choice was on the same lever or shifted to the alternative option from the previous action (three-way ANOVA Treatment x Outcome x Choice). However, neither selective inhibition of IT (O) nor PT (P) neurons altered choice patterns during probabilistic

decision-making. * - $p < 0.05$; Data is presented as mean ± SEM (A-D, M-P) or mean with individual datapoints before-after treatments (E-L). Nonspecific, N=6; Dual IT/PT, N=7; IT, N=11; PT, N=13.

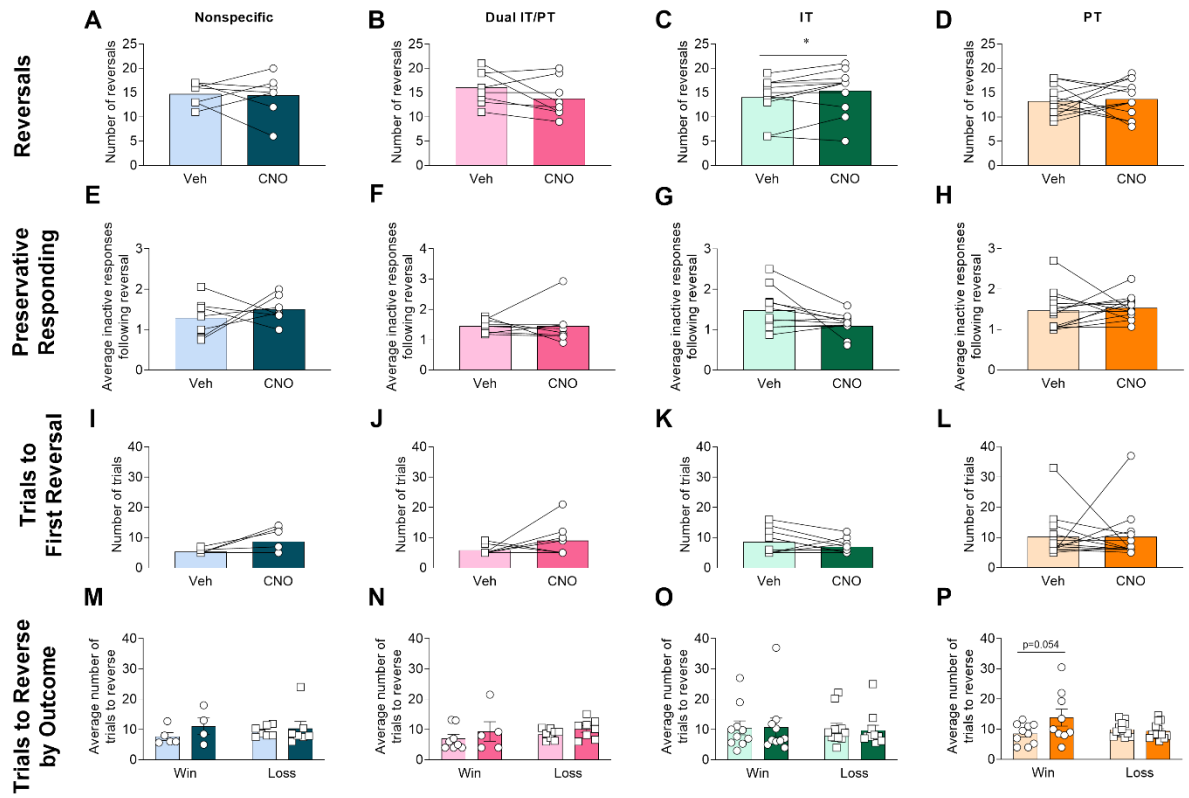
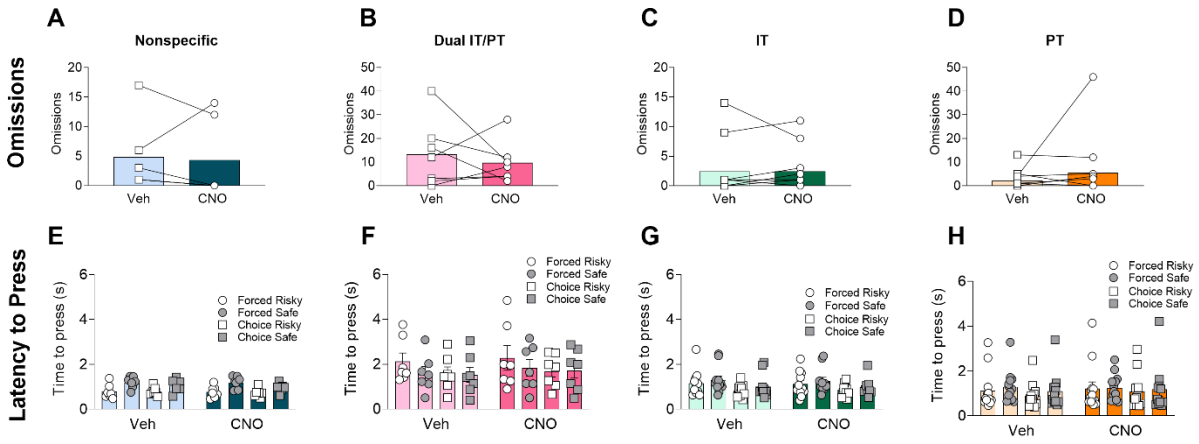
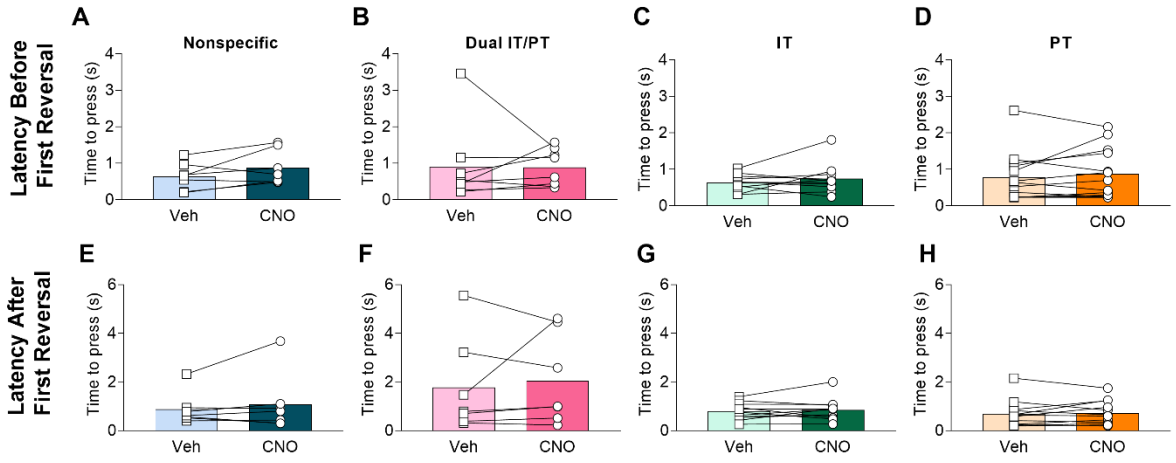


Figure 4: OFC IT neuronal inhibition increases adaptive responding in a within-session reversal task. Nonspecific OFC inhibition (A), Dual IT/PT inhibition (B), and PT inhibition (D) did not significantly change the number of reversals occurring within a session whereas IT inhibition (C) increased total number of reversals (2-sided paired t-test). When examining the number of responses on the previously rewarded lever prior to switching to the new active lever (a measure of preservative responding), none of the manipulations (E-H) altered the average number of preservative responses, though there is a trend towards significantly reduced preservative responding with IT inhibition (G). The number of trials required to initiate the first reversal was not significantly altered with any of the neuronal manipulations (I-L) although it trended towards significance with nonselective OFC inhibition (I). Following the first reversal, the subsequent trials to reversal to reach criterion for subsequent reversals were not altered with nonselective (M), Dual IT/PT (N), IT (O), or PT inhibition (P). Data is presented as mean with individual datapoints

before-after treatments (A-L). mean ± SEM (M-P). * - $p < 0.05$; Nonspecific, N=7; Dual IT/PT, N=8; IT, DREADD N=11; PT, DREADD N=13



Supplemental Figure 3-1. OFC neuronal inhibition has no effect on omissions and responding. Nonspecific (A), Dual IT/PT (B), IT (C), and PT (D) inhibition had no effect on responding during discounting sessions. These manipulations (E-H) also had no effect on the average time to respond during forced trials or on selection of the risky or safe option. Data is presented as mean with individual datapoints before-after treatments (A-D), or mean ± SEM (E-H). Nonspecific, N=6; Dual IT/PT, N=7; IT, N=11; PT, N=13.



Supplemental Figure 4-1. OFC neuronal inhibition has no effect on response times. Nonspecific (A), Dual IT/PT (B), IT (C), and PT (D) inhibition had no effect on average time to respond following extension of levers before completing criterion for the first reversal. These manipulations (E-H) also had no effect on the average time to respond following completion of the first reversal. Data is presented as mean with

individual datapoints before-after treatments. Nonspecific, N=7; Dual IT/PT, N=8; IT, DREADD N=11; PT, DREADD N=13.

Chapter 3

Role of Orbital Frontal Cortex in Risky Decision Making and Drug Taking Behavior

Discussion

As shown, the nature of the OFC in risk taking and decision making is quite nuanced. Prior literature has shown that there are some commonalities as well as differences among the other regions of the PFC. Furthermore, there is the opportunity to further characterize function based on varying subregions and cell populations. Finally, it would be wise to remember that the OFC may influence behavior differently depending on what type of risky behavior is being seen and the resulting context. We shall discuss how the region plays a role in risk designation, sex differences, cocaine self-administration, administration under threat of punishment, and probabilistic decision making based upon neuron subtype.

Brain Region Individual Differences

Starting with individual differences in subjects, more specifically amongst those with cocaine use disorder, there have been several findings alluding to connections between the OFC and the use of the substance. For example, cfos expression has been found in animals after acute intoxication within the region. We tried to see if this activity could be correlated with how likely one would be to pursue cocaine despite negative consequences. We found that there was a significant difference between the phenotypes of Controls, PR (punishment resistant), and PS (punishment sensitive) in cfos count. Furthermore, we saw a discrepancy between the subregions. While the PS rats showed a significantly lower level of cfos expression, this was only seen when compared to the control rats who weren't exposed to foot shock. This finding is promising because overall these rats took substantially less of the drug when exposed to punishment, while the other groups perseverated. We are led to believe higher activation within the region is needed to perseverate.

Risk itself can be a complex topic, as there are various forms not all of which are maladaptive. Researchers have begun to focus on the outcome variance aspect of risk (Mishra, Barclay & Sparks, 2016). With that said "risky" behavior should not be confused with pure uncertainty. Uncertainty involves decision-making with unknown outcomes, while risky decision-making involves a choice where all possible outcomes and probabilities are known (Tversky & Fox, 1995). It should also be noted there are risky behaviors that are viewed in a more positive light (prosocial and non-antisocial) and those in a negative light (antisocial). Positive risk-taking may include thrill-seeking, firefighting, and stock market speculation (Holton, 2004; Mishra, 2014). Negative risk-taking may include crime, pathologic gambling, and violent behavior. For the sake of this study, I am associating drug use with the latter, as in humans it is considered a crime and can be seen as self-isolating behavior.

Our theory behind this is that hypoactivation of the lOFC makes one less likely to engage in risk. Only the lOFC activity would lead to this result as mOFC activity was consistent amongst all three groups. Animals who were more sensitive showed less expression, showing an intrinsic predisposition as these animals were not manipulated chemogenetically. It may be possible that the amygdala, which is connected to the lOFC, may be inhibiting the lOFC and decreasing the drug-taking nature of these rats as they have experienced a negative/aversive outcome. In addition, the lOFC is more associated with referring to past experiences in the current state and thus consequences, so there may be an interaction with reward discounting as the animal is now calculating if the reward value is worth the chance of an aversive event (Wang, Schoenbaum, & Kahnt, T, 2020).

Confounding this proposed theory is the fact we did not see any significant difference between the PS and PR rats. An alternative way of viewing this is acknowledging that while PR

rats decreased responding a small amount, they were more similar to rats that were not punished at all (controls) and continued to not change drug taking between stages when exposed to punishment. The higher expression of cfos shown by these two groups suggests that higher activation is needed to consume excessive amounts of cocaine. However, our later experiment addresses that hyperactivation alone does not ensure higher drug taking or seeking despite earlier literature stating increased activity in the OFC shown with cocaine exposure. We also saw that despite control and PR rats having similar cFos expression, PR rats had a significant increase in seeking during drug-unavailable periods. There exists a possible interaction between exposure to punishment and increased seeking, feasible craving. As mentioned, researchers have found increased glucose metabolism in the OFC during bouts of cocaine craving (Franklin et al, 2002; Ersche, 2011; Volkow et al ,2011). In the twenty-five-minute drug unavailable time points, subjects may increasingly want the drug despite its inaccessibility and thus crave it, leading to more cfos expression.

To be able to confirm this a timed perfusion experiment would need to be done with each of the phenotypes in both the DA and DU period to compare for cfos. Fiber photometry would also supply valuable insight into the timing of the lOFC activity, with the resulting behavior. Expanding upon this idea using Cre-dependent viruses we should be able to isolate pathways involving the lOFC leading to and from the amygdala, seeing if one may be exciting or inhibiting the other.

Designation Differences in Behavior

As we became confident in our categorization based upon punishment sensitivity, we were able to go back and retroactively appoint any subject that went through the baseline intermittent access and punishment stages to the designations of PR and PS, based upon the degree of decreased responding during DA. Some people may theorize the increased drug-seeking and taking behavior of the PR rats is due to an extraneous variable, as it may be a result of PR rats being naturally more likely to lever press regardless of context. However, this was not shown to be the case. Surprisingly, PR rats pressed less than PS rats in the baseline IntA phase (prior to punishment). While after exposure to punishment, the PR rats lever pressed more in DA and DU periods. In summary, the phenotypes switched behavior between stages.

There can be many reasons for the switch in higher lever pressing between the sensitivity groups as the progressed from the unpunished IntA to the punishment phase. but we will try to attribute the discrepancy due to OFC subregion activity. As we know there are certain factors that differentiate the mOFC and lOFC. The hyperactivity of the lOFC may drive the risky decision-making process during that specific current state of the animal based on information gathered while mOFC may be more involved in future planning and learning. So theoretically the mOFC may be more involved in the initial drug-taking stages. Also, the more medial aspects of the PFC. especially the vmPFC, which is more involved in incentive salience and contingent learning. When it comes to pure goal-directed behavior, we theorize the mOFC would be more involved compared to the lOFC. When it comes to processing negative stimuli, consequences, and sensory information experienced during a current state the lOFC is shown to be involved. With that said there may be some mOFC : lOFC imbalance influencing the behavior switch. As lOFC expression seems needed

for persisting through punishment to pursue drug, too much may be detrimental to initial drug taking behavior. PS rats have less of a distinction between the OFC subregions and thus a closer ration of mOFC: lOFC activation. As priorly stated, mOFC activity may be more correlated with learning without experiencing negative outcomes so slightly more expression may be leading PS rats to initially taking more before punishment is introduced. To challenge this Bal has suggested the ventral and lateral OFC, but not medial OFC, are needed for cue-drug association, however this was in reinstatement after abstinence [or extinction?] and not during self-administration (Bal et al, 2019). Once again having a timed perfusion immediately after non-punished self-administration in PS and PR rats would allow us to see if there is an interaction with the mOFC activity, or other medial PFC regions, and high drug taking. A final theory may be that because the PS rats initially took more excessive amounts of cocaine may have changed their OFC region and thus lesser activity in the lOFC. When it came to the punished sessions, due to their now hypoactive OFC they chose not to pursue the drug in the face of the aversive stimuli. Vice versa can be true for the PR who initially took less.

When stratifying for sex I did find there was a difference in unpunished self-administration of male and female rats, however there was a difference in punishment sensitivity, when footshock was introduced and resulting behavior. There was no significance between males and females over the nine-day IntA period. This is surprising, considering many previous reports suggested that females have higher levels of addiction-like behaviors following self-administration, including enhanced motivation for cocaine, increased escalation of drug intake, and greater psychomotor sensitization. (Kawa and Robinson, 2019, Algallal et al., 2020, Carr et al., 2020). Previous reports also found that females show increased sensitivity to punishment and reduce drug intake especially when there is a uncertain likelihood of punished responding (Chowdhury et al ,2019; Orsini & Setlow ,2017;

Grissom & Reyes ,2018). When exposed to punishment only the females showed a change in their behavior. Female rats took less cocaine but sought more in the punishment stage compared to their baseline. Behavior in males saw no such change. As a result, more females were given the PR designation (44%) compared to males (25%). There are several explanations for this finding.

In human studies it has been shown that women are more susceptible to craving and this likely increases the risk for relapse to cocaine seeking after a period of abstinence from cocaine (Robbins et al, 1999; Kennedy et al, 2012; Fox et al, 2014). Furthermore, women are thought to be more sensitive to the rewarding effects of drugs, in part due to sex hormones directly altering drug reward mechanisms (Evans & Foltin, 2006; Anker & Carroll, 2011). This leads to a possible interpretation that due to physiological differences; the female rats would lever press less than their baseline during DA to avoid punishment but when foot shock is no longer a possibility in DU that would increase pressing due to constant craving. Due to this behavior, we see the sex-based change in risk designation.

Resulting behavior from OFC manipulation

I was able to alter risk taking behavior via the DREADD viral targeting technique. It must be kept in mind that the OFC may have distinct roles or functions depending on context. Risk taking can have many forms, however I have chosen to investigate risky behavior from the negative consequence of drug taking perspective(punishment) and from a probabilistic risk-taking behavior involving reward (probability discounting). I will start by discussing the punishment studies involving the lOFC.

Researchers have shown that deactivating, or lesioning, both the lOFC and mOFC can have varied results on influencing risk sensitivity. Studies have found that manipulation can increase, decrease or not change the sensitivity to punishment. (Turner et al, 2021). Guided by my cFos result, I chose to target the lOFC specifically, because that is the region, I noticed a significant difference in expression. I first chose to inhibit the region using a hM_4Di virus, however I didn't find a significant difference in lever pressing in either DA or DU between vehicle and CNO-treated rats. This is a contradiction to Ishikawa who found that inactivation of the lOFC increased punishment sensitivity and caused animals to respond less (Orsini et al, 2015; Ishikawa et al, 2020). However, that study used baclofen rather than chemogenetic making for a methodical difference. My thought process is that in this experiment decreasing lOFC activity can't drive behavior below a certain threshold, meaning there's no way to eliminate risk taking behavior completely. Of course, there still exists some ambiguity amongst the two regions as the inactivation of the mOFC and lOFC subregion has both led to decreased punished sensitivity and increased compulsive

behavior. (Jean-Richard-dit-Bressel & McNally, 2016; Verharen, 2019). This is surprising considering that the lOFC is to be more associated with punishment (O'Doherty, 2001) while the mOFC is affiliated more with reinforcement (Arnan et al, 2003). With that said the method of punishment differed amongst all these studies. Some were centered around differing predictive cues, time periods, reward, and probabilities. Due to the OFC's other functions in processing aversive stimuli (Morrison & Salzman, 2011), encoding value of reward (Schoenbaum & Roesch 2005), and inhibition of choice (Hodgson, 2001; O'Doherty, 2003), there may be different mechanisms mediating interactions between these proposed functions depending on the type of punishment task.

With this in consideration, I decided to use an excitatory hM_3Dq DREADD instead of another inhibition-style study. Furthermore, I figured it would prove helpful to build upon information and data given from within my own punishment model and context. Imaging showed increased cfos in the PR rats compared to PS rats, so an excitatory DREADD may have been able to mimic this activity and thus the behavior. I found that rats in which I activated the DREADD using CNO, compared to ones who were given the vehicle as a control, showed significantly increased drug taking and drug seeking. I can think of three main theories to explain this. As discussed, acute cocaine intoxication is linked to decreased functioning of the OFC as shown by lower glucose metabolism. However, increased glucose metabolism is shown in craving episodes. Perhaps by increasing lOFC activity we are increasing this craving and drive to obtain cocaine leading to increased lever pressing despite the threat of punishment. This would add greater insight into why drug seeking is exaggerated to such a degree as drug is no longer available, simulating acute withdrawal. Another theory is that activation increases compulsion. As I mentioned before, compulsion is characterized by habitual behavior despite integrated information about experienced

outcomes. This disruption in the model-free system can lead to repetitive action despite consequences or even lack of reward, as shown in DU periods with continued seeking. My final theory is there is an interaction between processing punishment and choosing to obtain the reward. Simply put, as the OFC became more active it is possible it put a higher value on reward than the possible adverse experience of foot shock. This calculation of predicted outcomes led to the impulsive pursuit of cocaine. Also, it must be remembered that the lOFC has connections to the basolateral amygdala, so it may be overriding aversive/emotional feedback from the region.

To better understand which of these ideas may be most correct, I decided to repeat the experiment except without a punishment phase. This time there was no significant difference in the CNO group when compared to the vehicle group. The results in the none punished study combined with the results from the punished study show increasing lOFC activity is punishment specific as no increase in cocaine self-administration was seen when footshock was absent. Both drug taking and seeking stayed rather consistent. I think this strengthens the argument of the lateral's role in processing consequences in the pursuit of a reward. If it was dependent solely on the former two explanations of excessive compulsion and craving, I would expect to see a change at least in DU. Compulsion and craving should still be active contributors as the unavailable phase would assure a no-drug period, where excessive pressing could take place despite no reward, however, this was not the case. Thus, excitation of the lOFC in context sensitive to punishment resistance, not overall drug-taking behavior.

Risk-taking can have many forms. Besides physically aversive stimuli, such as punishment, there is also the possibility of a loss of reward resulting from the variable probability of receiving reward as shown in probability discounting. Reminiscent of gambling, there is a phenomenon where individuals value potentially large rewards at high probabilities. This begs the question that if there

is possible dysfunction in the PFC that would lead to risky behavior? Furthermore, I asked if neuron subpopulations can alter behavior differently depending on which cortico-striatal projections I inhibit. I made two main groups for the study including a nonspecific lOFC group and an IT/PT dual-targeted group. Inhibiting the nonspecific lOFC, led to rats being less likely to seek the risky lever which gives varying probabilities for 3 food pellets compared to the safe lever that only gives 1 food pellet 100 percent of the time. The OFC has a significant role in outcome-directed behavior and adjusting behavior when experiencing outcome-related stimuli. (Schoenbaum & Esber, 2010). So, inhibiting the lOFC may be making the subject more sensitive to missing an expected reward, especially when considering the connection to the BLA, which may be encoding "aversive" outcomes. Also inhibiting the lOFC may allow for a change in behavior after receiving the latest information rather than persisting along a particular behavior course. (Groman et al, 2019). I also theorize that inhibiting the mOFC will have a different result as it is the region more affiliated with probability and reinforcement. The mOFC is more connected with the hippocampus, rather than the BLA, meaning it may refer to information encoded from prior outcomes to create a model capable of predicting future reward. (Wang, Schoenbaum, & Kahnt, 2020).

Dual inhibition of IT and PT neurons simultaneously had a separate set of effects during the trials. There wasn't an observed effect on the probability discount rate nor in subsequent choice operation. With there being an effect from nonspecific lOFC inhibition, I'm led to believe that there may be other, non-glutamergic, neurons that influence behavior. Also, general inhibition alters choice depending on prior outcomes. The vOFC and lOFC showed increased cfos expression compared to the ACC, IL, PL, and mOFC across all probabilities. Observed increased activity at the 50% probability leads to the assumption the lOFC is active when the choice of risky large

award is advantageous. It should be noticed the repeated trend of having higher expression of cfos in the lOFC when risk-taking, or risky decision-making is seen in both the probability discounting and punishment studies. With that said in this study I would like to excite the region, rather than inhibit to see if the subjects would pursue the risky choice more like in the punishment task.

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Conclusion

As I have discussed at length the OFC has many possible functions including, but not limited to, flexibility in behavior, reversal, associative learning, outcome directed behavior, reinforcement, contingent learning, compulsion, sensory information encoding, odds estimation, value representation, risk reward calculations etc. Also, I have shown the ways in which the OFC is both affected by and influences CUD. All these factors may contribute to the phenomenon of individuals choosing to pursue drug despite possible negative consequences. I was able to show that increased lOFC activity is involved in punishment sensitivity, as confirmed by outcomes from similar studies, as Increased cfos expression was associated with subjects more likely to take risks. Following this finding I upregulated the lOFC and found it increased both drug seeking and drug taking despite the uncertainty of a potential foot shock as an outcome for the pursuit of cocaine. However, what makes this finding particularly standout is the fact upregulating the lOFC did not change unpunished cocaine self-administration. While there are many possible explanations, I believe that increasing activity in the respective region allows for the animal to calculate risk versus reward, placing a higher value on the infusions of cocaine compared to a 50% chance of foot shock, thus seeing it as a favorable outcome. This theory supports the idea of the lOFC role in a model-based system. (Clark, Hollin, & Philips, 2012)

There is also a possibility of overlap of the function of the lOFC across other forms of "risk". During my probability discounting study, I inhibited the lOFC and soon found that as a result subjects were less likely to press the risky lever. The risky lever assured a larger reward at varying probabilities. Inhibiting this region also changed choice based on prior outcomes. I would like to believe this alteration in choice shows disruption in compulsion as the subject doesn't

simply stay with the same behavioral choice habitually. In a cfos visualization study, I noticed that during trials of probability, I saw increased activation in the lOFC, especially at the 50% probability. I also saw more cfos activity in rats willing to engage in the punished cocaine self-administration task, which involved a foot shock on 50% of DA active lever presses.

It is my belief that lOFC activity can influence one's likelihood in engaging in risky behaviors due to its designated functions and unique connectivity to other regions within the brain, mainly the BLA. Utilizing DREADDS, increasing lOFC activity seems to make subjects more risk tolerant while inhibiting it seems to make subjects more risk-averse. To get the full characterization of the region, I would plan to repeat the experiments with manipulations in the mOFC, within the IT/PT subpopulation in punishment, and/or specifically target reciprocal connections between the lOFC and the BLA.

ZACKARI D. MURPHY

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EDUCATION

MD.: Doctor of Medicine, Expected in 06/2025
Morehouse School of Medicine - Atlanta, GA

Ph.D.: Doctor of Philosophy, Molecular & Cellular Biology, Expected in 06/2023
University of Washington - Seattle, WA

Bachelor of Science: Psychology, 05/2016
Louisiana State University - Baton Rouge

RESEARCH EXPERIENCE

Undergraduate Research Assistant, 11/2012 to 05/2016
Louisiana State University – Baton Rouge, LA

- Contributed to 750+ hours of research in a biomedical engineering lab under Dr. Daniel Hayes.
- Utilized chemical polymers to form composite material medical scaffolds.
- Initiated scientific tests based upon chemical and physical properties of scaffolding.
- Assisted in writing scientific literature, which resulted in publication in scientific journal.
- Trained in use of Instron, Pico green, Alamar blue, PCR, cell culturing, mass spectrometry, miRNA.

PRESENTATIONS

- Emerging Researchers National Conference (Feb 2013) Presented research on Bone Scaffolds Utilizing Frontal Polymerization. Won first place in Biomedical Engineering
- ABRCMS (Nov 2014) Presented research on Bone Scaffolds Utilizing Frontal Polymerization.
- Presented at URC and LSU Discover day. Research was selected for presentation in front of the LSU Board of Supervisors. Less Than 20 people were chosen for this honor
- Adekunle O, Gaffey J, Gentry J, Gibson D, Hayes D, Mabra D, Murphy Z, Perez HC, Pinzon J, Richardson G, Sonkarley K, Thornton K, Tummings B, Bayer CR, Hernandez N, Jackson M, "Road to Collaborative Intervention: Evaluating the Social and Behavioral Health of Paul L. Dunbar Elementary School Students", poster presentation, Kennedy-Satcher Mental Health Equity Conference, Atlanta, GA, November 28, 2017
- Roth Bayer, C, Gibson, D, Murphy, Z, Thornton, K, "Morehouse School of Medicine & Dunbar Elementary: 5 years of Community Health Partnership", Nation Builders Conference, Atlanta, GA, March 24, 2018
- Adekunle O, Gaffey J, Gentry J, Gibson D, Hayes D, Mabra D, Murphy Z, Perez HC, Pinzon J, Richardson G, Sonkarley K, Thornton K, Tummings B, Bayer CR, Hernandez N, Jackson M, "Is Kindness the Answer?: Implementing a Random Acts of Kindness Campaign to Address Social Emotional Learning Needs", abstract and poster presentation, Dr. Daniel S. Blumenthal Public Health Conference, Atlanta, GA, April 4, 2018
- Adekunle O, Gaffey J, Gentry J, Gibson D, Hayes D, Mabra D, Murphy Z, Perez HC, Pinzon J, Richardson G, Sonkarley K, Thornton K, Tummings B, Bayer CR, Hernandez N, Jackson M, "Is Kindness the Answer?: Implementing a Random Acts of Kindness Campaign to Address Social Emotional Learning Needs", poster presentation, Gloster Society, Atlanta, GA, April 19, 2018

- Murphy Z, Chemogenetic Inhibition of Orbitofrontal Cortex Reveals Distinct Role of Subtypes of Cortical Projection Neurons in Regulating Decision Making in a Probability Discounting Task. Poster Presented at: American College of Neuropsychopharmacology Conference 8 December 2020; virtual
- O'Neal T, Murphy Z, Female Rats Express a More Addictive Phenotype Than Male Rats During Intermittent Access Heroin-Self Administration. Poster Presented at: Winter Conference on Brain Research 27 January 2021; Snowbird, Utah
- Crummy E, Murphy Z, Intratelencephalic and Pyramidal Tract Neurons Differentially Mediate Cocaine Sensitization and Conditioned Taste Aversion. Poster Presented at: Winter Conference on Brain Research 28 January 2021; Snowbird, Utah
- Murphy Z, Chemogenetic Excitation of the Lateral OFC Increases Likelihood of risky Drug Taking under Threat of Punishment. Poster Presented at: Winter Conference on Brain Research 24 January 2023; Snowbird, Utah

PUBLICATIONS

- Totaro NP, Murphy ZD, Burcham AE, King CT, Scherr TF, Bounds CO, Dasa V, Pojman JA, Hayes DJ. 2015. In vitro evaluation of thermal frontally polymerized thiol-ene composites as bone augments. J Biomed Mater Res Part B 2015

FELLOWSHIPS

- R01 Diversity Supplement Fellowship
- ARCS Seattle Fellowship
- TL1 Translational Medicine Fellowship
- June Jackson Christmas Fellowship

PRESTIGIOUS HONORS AND POSITIONS

- Chancellors List
- Dean's List
- Ogden Honors College
- LASTEM Research Scholar
- Ronald E. McNair Scholar
- IMSD Biomedical Research Intern
- Black Caucus Scholar
- Ogden Leadership Scholarship Recipient
- LSU Residential Assistant
- BMLI Kerry Pourciau & Kirk Bennett Scholarship Recipient
- Leadership LSU Cohort 2016
- Alpha Phi Alpha Fraternity Inc.
- Morehouse School of Medicine Merit Scholarship

UNDERGRADUATE EXTRACURRICULAR ACTIVITIES

My OP Program Coordinator, 05/2015 to 05/2016

- Wrote proposal for a mentorship program geared towards adolescent underprivileged minorities.
- Secured funding of $5000 from the Ogden Honors College to support activities and resources, establishing a nonprofit partnership with the Baton Rouge Youth Coalition.
- Created presentations while leading discussions based on topics in cultural awareness, professionalism, and character development.
- Volunteered over 100 hours towards making the program successful.

Minority Science Pre Professional Society President, 05/2015 to 05/2016

- Allocated tasks for Executive board members to assure successful programming.
- Organized events, presentations, and guest speakers for minority students in underrepresented.
- Advised students personally on how to become better applicants for professional school.
- Secured resources for members' individual needs as well as mentoring and networking opportunities.

Baton Rouge General Hospital Volunteer, 05/2015 to 12/2015

- Worked in Outpatient Infusion as a student volunteer worker.
- Assisted nurses with administering aid and refreshments during treatment.
- Communicated with patients to better serve their individual needs and concerns.
- Garnered over 50 hours of direct patient contact.

Black Male Leadership Initiative Success Coach Coordinator, 08/2013 to 05/2016

- Involved in an organization focused on improving the retention of black males in college.
- Participated in various meetings focused on professionalism, diversity, and academic achievement.
- Collaborated and worked alongside peers to improve programming.
- Took active role in aiding minority, underprivileged, and first-generation students in succeeding in college.
- Matched fellows of the organization with local professionals who would serve as mentors.

GRADUATE EXTRACURRICULAR ACTIVITIES

MCB Steering Committee, 12/2020 to Current

- Serve on the steering committee focused on leading the direction of the graduate program.

MSTP Admissions Committee, 11/2020 - Current

- Interview students both individually and in a group setting.
- Responsible for forming questions and dictating the dialogue and trajectory of the interview conversation.
- Meet with the rest of the committee to grade and rank students respectively.

Diversity, Equity, and Inclusion Committee Chair, 01/2020 to Current

- Facilitate meetings between faculty, staff, and students monthly.
- Create initiatives based on community building, engagement, outreach, and logistics.

Student National Medical Association President, 08/2018 to 2019

- Led the organization throughout the year while focusing on mentoring and community outreach.
- Established MAP chapter at Kennesaw State University.
- Visited chapters at Clark Atlanta University, Spelman College, Morehouse College, Georgia Tech, and Georgia State University.
- Collaborated with the school to host the annual First Look Program.
- Participated in the "Why We Cant Wait Breakfast" programs at MSM.
- Established a class shadowing opportunity for visiting undergraduate students.
- Secured mentors for over 60 undergraduates students and matched them with current medical students based on interest.

Psychiatry Interest Group Vice-President, 08/2018 to 08/2019

- Provided input to interested students on how to best secure a career in psychiatry.
- Built andcSecured shadowing for the program.

June Jackson Christmas Fellowship, 07/2018 to 08/2019

- Completed an internship at Columbia University for 6 weeks.
- Rotated through the psychiatry departments of Emergency Psychiatry, Consultation Liason, Inpatient, and Outpatient.
- Participated in New York Mobile Crisis Team and New York HIV Clinic.
- Went on weekly excursions throughout social institutions in NYC.

OSLER Tutor, 07/2017 to 06/2019

- Aided graduate level students in their respective classwork.
- Taught students new effective studying techniques and how to use resources effectively.
- Led sessions based on integrative learning styles.

Morehouse School of Medicine Admissions Committee, 11/2017 to 03/2019

- Gave students tours of the campus while answering their question and assessing their readiness for medical school.
- Reported back to the committee thoughts of the students and ranked them accordingly.

Student National Medical Association Vice President, 10/2017 to 08/2018

- Aided in producing programs for the greater student body.
- Mentored disadvantaged males on education and how to engage in STEM programs.
- Proposed new ideas on how to improve outcomes for minority students applying to medical school.
- Volunteered in the community in order to encourage outreach.

Psychiatry Interest Group Treasurer, 10/2017 to 08/2018