The Influence of Environmental Manipulations on Decision-Making and Dopamine Transmission

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Abstract

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The process of educating responses/decisions and controlling our environment can be seen as true liberty. The environment and organisms within it have a bidirectional relationship: the environment affects our internal state which, in turn, affects how an organism navigates through and influences the environment. Decisions require calculation of economic context and utility, and these choices are affected by a myriad of factors. These factors include, but are not limited to, the number of options available, subjective internal states (e.g., stress, fatigue, if the decision has been made before), energy required, magnitude of the outcome (punishment or reward), time to receive outcome, probability of achieving that outcome, and the subjective perspective of the environment in which the decision is made. Although decision-making is complex, the young field of neuroeconomics has sought to reduce these dimensions and empirically test how organisms can assess the utility of decisions. One of the most intensely studied neural mechanisms for reward-guided choice behavior in mammals is the mesolimbocortical dopamine system. Phasic dopamine release in the ventral striatum (nucleus accumbens) from cells projecting from the ventral tegmental area is thought to serve as a learning signal that caches values from a novel rewarding experience and then to any reward-predictive cues for that reward. Disruptions to this monoaminergic system have been applied to theories analyzing the etiology of many psychiatric disorders, such as Major Depressive Disorder

and aberrant habitual behaviors, and the therapeutics designed to manage and ameliorate their associated symptoms. Stress and its biological correlates, via the hypothalamus-pituitary-adrenal axis, have been heavily intertwined with our understanding of these diseases. Consequently, I first aim to understand the interaction of intra-accumbal dopamine or corticotrophin-releasing factor antagonism, chronic stress induction, and economic decision-making. I report that corticotrophin-releasing factor and dopamine act in parallel to affect a concurrent choice, operant conditioning paradigm and that stress augmented the effects of the dopamine (DA) and corticotropin-releasing factor (CRF) antagonists on behaviors that were changed by the drugs. Secondly, I aim to understand how habitual or flexible decision-making in the context of manipulating the effort required to obtain a reward and the reward magnitude influences phasic dopamine release in the nucleus accumbens. Overtraining led to inflexible behavior, a flexible training paradigm reliably led to flexible behavior, and phasic dopamine release reflected reward magnitude, regardless of the animals' choice behavior. Providing more evidence to address these two questions advances our comprehension about the how environmental stressors and the adaptability of economic decisions influences dopamine transmission within the ventral striatum.

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INTRODUCTION

THE NEURAL MECHANISMS OF REWARD AND REINFORCEMENT

Dopamine, Reward, and Reinforcement

Dopamine (DA) serves as one of the most well-studied neural substrates for reward and reinforcement studies in mammals, but this evolutionarily preserved ligand, its related precursors (L-DOPA), and enzymatically produced products (norepinephrine and epinephrine) are found not only in mammals and vertebrates, but also in invertebrates, such as earthworms, clams, and other mollusks (Cotrell, 1967), and have even been identified as pesticides in plants, like algae, legumes, tress, and fruits (Longo et al., 1974; Ingle, 2003; Kulma & Szopa, 2007). Within mammalian brains, limbic and cortical brain regions that receive projections from the ventral tegmental area (VTA) and the substantia nigra (SN), possess receptors for this monoamine. The ubiquitous nature of DA has led to intensive investigations revealing that it interacts in complex ways with other hedonic molecules like endocannabinoids and endogenous opioids to increase circulation of itself through mechanisms that are still unclear but provide targets for further investigation (Wenzel & Cheer, 2018; Peters et al., 2020).

When I took my first neuroscience course during my undergraduate training, I was fascinated by the preceding studies looking into the mechanisms of dopamine on behavior. In addition to reward and reinforcement, it had even applied to complex theories of behavior like consciousness where dopamine-deficient mice became hypoactive and hypophagic (Zhou & Palmiter, 1995; Palmiter & Koch, 2011). The motor effect of dopamine-deficiency could be partially restored by administration of caffeine, an adenosine receptor blocker (Kim & Palmiter,

2003). Caffeine has also been shown to reinvigorate dopamine-deficient mice to consume sucrose on one side of a T-maze task, but not to the point of exercising on a running wheel on the other side of the T-maze (Lopez-Cruz et al., 2018). Interestingly, dopamine played a role in facilitating the learning of conditioned fear responses since DA receptor 1 (D1R) knockout mice showed enhanced fear generalization, evidenced by increased startle responses to novel footshock environments, but also decreased freezing to an extinguished shock-associated cue that should have elicited a startle response (Abraham et al., 2016). This effect of fear generalization could be partially ameliorated through injection of a D1R/D5R agonist (Abraham et al., 2016), and this evidence of dopamine providing a learning signal was later supported via the ability of artificial enhancement of phasic dopamine activity following an induced fear generalization to reverse the effect of fear generalization (Jo et al., 2018).

Through electrophysiological recording of midbrain neurons during behaviors like Pavlovian, operant, and classical conditioning, Phillips and Olds (1969) learned that midbrain neurons fired for food- or water-paired cues and reward retrieval itself, especially when the animal was hungry or thirsty. Consequently, Norman White (1989) was not the first to define the words, ‘reward’ and ‘reinforcement,’ but he gave a more formal definition of these terms as they are related to behavioral neuroscience. He proposes that a reward is any stimuli that elicits an approach response in an organism, and he relates this idea to the ventral striatum, which includes the nucleus accumbens (NAc). Reinforcement would be defined as an increase in stimulus-response associations, and this word would be closely related to the dorsolateral striatum (DLS) and its associated limbic and cortical regions. I learned about the mesolimbocortical dopamine circuitry and its importance in serving as a learning signal in very dynamic ways throughout the brain. Dopaminergic neurons in the primate midbrain, specifically

the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) also conveyed cue-evoked changes in firing (Schultz et al., 1992). In that same year, evidence was revealed that rats would press levers to self-stimulate these neurons and that self-stimulation could be reduced by injection of dopamine antagonists (Wise et al., 1992). Later, it was found that dopamine release in the midbrain also serves as a mechanism to predict rewards, their discrete cues, the absence of the reward, reward magnitude, and the time it takes to receive the reward (Schultz, 1997; Schultz, 2016). These results were also similarly replicated in non-human primates where midbrain DA neuron firing would reflect a better-than-expected signal, called a reward prediction error (RPE), for a juice reward-predictive cue as well as could code for reward magnitude (Tobler et al., 2005; Bayer et al., 2005). In rodents, it was found that the magnitude of burst activity of either DA or GABA neurons in the SN are bidirectionally modulated by the motivational state of the animal (Rossi et al., 2013). It is worth noting that the SN also conveys movement or action initiation patterns and dynamics, such as velocity and movement vector information, when recording during both reward-seeking and harm-reduction (Barter et al., 2015).

In addition to testing the role of midbrain dopamine neurons, the phasic dopamine release that happens downstream in the basal ganglia and the associated behaviors that follow have been a source of many extensive reviews (Yin & Knowlton, 2006; Grillner & Robertson, 2016; Schultz, 2016; Riva et al., 2018). The main region of study for reward-guided behaviors has been the striatum which can be divided into two parts: the dorsal striatum (caudate and putamen) and the ventral striatum (NAc). These regions contain axon terminals from the VTA and SN and receive the tonic and phasic release of DA. This mesolimbic DA system plays a predominant role in prediction error coding since phasic DA release in the NAc occurs with reward predictive cues

in mammals and is sufficient to guide behavior for food and drug rewards (Pagnoni et al., 2002; Phillips et al., 2003). With the advent of optogenetic technology, Tsai and colleagues (2009) showed that induction of phasic dopamine release in the NAc could condition mice for an associated chamber in a conditioned place preference (CPP) paradigm. To support the causal link for dopamine and prediction errors in a more complex behavior than CPP, it was also shown that optogenetically inducing a phasic-like DA burst could serve to augment cue-elicited responses during a conditioned blocking procedure (Steinberg et al., 2013). Phasic activation of VTA DA neurons could also potentiate reward seeking in an operant task and reinstate food-seeking in a reversal learning task, a behavior that is not seen in the absence of food rewards or food-predictive cues (Adamantis et al., 2011). Additionally, dopamine-deficient mice that either had a D1R knockout or phasic-burst activity reduction via knockdown of the NR1 subunit of the NMDA glutamate receptor (Zweifel et al., 2009) also could not learn about event-predictive cues, giving evidence that phasic dopamine release and D1R function are necessary for this learning signal (Wall et al., 2011). The nucleus accumbens continues to be a hub for dopamine and reward related research (Salamone et al., 2003). Papageorgiou and colleagues (2016) also reports the effects of satiation for either food or liquid reinforcer on phasic DA release in the NAc. Dopamine phasic activity was higher for a liquid reward if the animal had been sated on food and vice versa, showing that DA activity was not generalized to reward-cue presentation or retrieval, but that DA continues to be learning signal (Papageorgiou et al., 2016). Others have also supported the idea of subjective state-dependent (sated vs. hungry) phasic DA release, exposing DA as somewhat of a survival-based learning signal (Aitken et al., 2016). The idea of dopamine encoding a reward and cue associated timing was further verified with a study that showed that even when given an abundant amount of trial and cue associations, monkey

midbrain DA neurons would fire to the cues, irrespective of cue identity (Ravel & Richmond, 2006). Unlike the previous studies which attribute dopamine to a general learning signal, some investigators claim dopamine to be a salience signal. This observed stimulus-reward generalization, especially in a habitual or goal-tracking manner, influences whether phasic DA is released. Dopamine release accurately reflected habitual behavioral patterns where an animal continuously presses a lever for food after a conditioned stimulus without parallel exploration of where the food is delivered, or sign-tracking behavior: a behavior that is considered inflexible and resistant to the extinguishment of discrete cues (Fitzpatrick et al., 2019). Additionally, sign-tracking animals that received injections of a non-selective DA antagonist, flupenthixol reduced their conditioned responses, an effect not seen in their goal-tracking counterparts (Flagel et al., 2011; Saunders et al., 2012). It is worth noting that dopamine release is dependent on experience, so that after an extended amount of time experiencing the same cue-reward for either a cue for food or a drug can also reduce the amplitude of phasic DA release (Willuhn et al., 2012; Clark et al., 2013). Providing a novel cue-reward contingency during reversal learning can once again show DA transients, like the initial period of cue-reward association (Radke et al., 2019).

This time dependent change in dopamine dynamics was also seen in the dorsal striatum, with the medial part of the dorsal (DMS) striatum being activated during acquisition of a behavior and lateral part (DLS) facilitating the continuation, or habitual part, of a behavior (Sommer et al., 2014). The nigrostriatal (SN → DLS/DMS) pathway also does not seem to affect the salience of conditioned stimuli in Pavlovian paradigms like the mesoaccumbal (VTA → NAc) pathway, since microinjections of flupenthixol into the NAc could reduce sign-tracking behaviors (Fraser et al., 2017). The role of the DMS and DLS for goal-directed and habitual behavior, respectively, was revealed by lesioning either area and observing behavioral flexibility

during instrumental conditioning in rats by Henry Yin and colleagues (2004, 2005, 2006). Human data from fMRI recordings supports these findings from rodents, showing that there are rodent studies are likely to inform us about human physiology (Balleine & O'Doherty, 2010). Dopamine receptor antagonism in the dorsal striatum could also decrease responding during operant responding for food (Beninger et al., 1993), and specifically dorsal striatum activity occurs most during the actions leading up to food retrieval (London et al., 2018). There is even report of distinct populations of phasically active dorsal striatal neurons coding for separate representations of actions, the value of the action to choose and the value of receiving the chosen reward (Lau & Glimcher, 2008).

The role of the basal ganglia in habit formation is well known and is of much interest since it is assumed that most of our daily rituals/routine are mostly comprised of automatic behaviors (assuming one does not live a very dynamic lifestyle). Automatic behaviors are habitual behaviors that are repetitive and initially start as goal-directed but then become habitual (e.g., like learning to fully chew food or locking the door; Graybiel, 2008; Smith & Graybiel, 2016; Robbins & Costa, 2017). Habits are valuable for their ability to help the body and brain to conserve energy. This sparked the question of how the brain encodes habitual behavior and how do habits develop from the repetition of goal-directed actions. In my experience, habits are harder to break than they are to build like trying not to get off at the same exit for work or my partner's home on the highway when I am going somewhere completely new. In my scenario, I used my habitual or model-free decision system, instead of the goal-directed or model-based reinforcement learning which both rely on DA transmission (Dezfouli & Belleine, 2013; Sharpe et al., 2017).

Given the evidence, it seems that dopamine dynamics change with experience and the viewed patterns of activity of dopamine-associated structure depend on the region that is being studied. This leads to controversial ideas about the patterning and plasticity of reward-related circuitry in the basal ganglia, but there is evidence for what are called corticostriatal feedback 'loops' where a novel experience is coded in a rostro-medial fashion and as the experience becomes learned, the caudo-lateral region is employed (Yin & Knowlton, 2006; Yin et al., 2008; Peak et al., 2019). The story gets even more complex when considering the distribution of D1R and D2R containing neurons in the dorsal striatum, called medium spiny neurons (MSNs), and these neurons supposedly follow two orthogonal pathways: the direct (D1R; striatonigral; 'go') and the indirect (D2R; striatopallidal; 'no go') pathways (Calipari et al., 2016; Kupchik et al., 2015; Vicente et al., 2016). As addressed in a couple reviews in the topic, the canonical pathways proposed are not that simple (Yager et al., 2015; Soares-Cunha et al., 2016; Cui et al., 2013). This could be reconciled through more extensive studies using more precise genetic manipulations and standardized behavioral tests, assuming we can do so. Nonetheless, it depends on where one is looking, and how one manipulates these brain regions of interest, especially considering the overlap in projection sites and genetic markers for sub-populations and evidence for D1R/D2R heteromers (Perreault et al., 2011).

The importance of understanding the role of the cortical areas in relation to mesoaccumbal and nigrostriatal circuitry, even in the rat brain, has been shown to provide useful evidence that the cortex also conveys learning of reward prediction. Whereas dopamine neurons in the midbrain have been shown to facilitate learning via reward size coding or event prediction error (Bayer & Glimcher 2005), inhibiting neurons in the infralimbic cortex can disrupt the formation of habits (Smith & Graybiel, 2012). Moreover, areas like the medial prefrontal cortex

have been shown to code reward expected value (Lak et al., 2020). Expected reward value could be related to why an animal would expend valuable energy in procuring a reward, especially if the effort/energy to obtain that reward is remarkably high. Consequently, related cortical structures, such as the orbitofrontal cortex (OFC), specifically the medial OFC, have been under investigation. It was demonstrated that D1R blockade in the OFC in rats could reduce the ability of rats to expend energy in and effortful task, the progressive ratio task (Munster et al., 2020). Evidence from lesioning the anterior cingulate cortex is also shown to mediate goal-directed/model-based and effortful reward-seeking behaviors (Walton et al., 2009; Akam et al., 2021). The interaction between corticostriatal synapses, their influences on striatal DA transmission and other relevant molecular loci of function has been reviewed in Bamford et al., 2018. Habits seem to rely on putamen-thalamic-motor regions, whereas a top-down control from the PFC or OFC to the caudate or processing in the premotor cortex seem to facilitate goal-directed actions (Gremel & Costa, 2013[a, b;] Ceceli & Tricomi, 2018).

Our lab has also advanced the technological ability from recording field potentials and the cells themselves to using chronic, carbon-fiber microelectrodes and chemometrics in a process called fast-scan cyclic voltammetry (FSCV) to record phasic DA release in the NAc and DLS on a momentary timescale (Clark et al., 2010; Willuhn et al., 2012; Arnold et al., 2015; Rodeberg et al., 2017). We have employed this technique to advance the understanding of the function of DA at a sub-second timescale in different behaviors, including ‘incentive learning’ in a Pavlovian task, deciphering utility (cost/benefit), and its role in promoting drug-seeking behavior (Flagel et al., 2011; Gan et al., 2010; Phillips et al., 2007; Willuhn et al., 2012). Others have also been able to utilize this technique in primates (Schwerdt et al., 2017) as well as in select human studies, also examining prediction error responses (Kishida et al., 2016).

Monoaminergic Dysfunction

My first exposure to how dopamine dysfunction related to body health was reading about reductions in total brain dopamine receptor 2 (D2R) seen in obese individuals via positron emission topography (PET) studies, where body mass index (BMI) negatively correlates with D2 dopamine receptor (D2R; Wang et al., 2001). The authors found that obese subjects had lower D2R binding availability in the striatum, but this could be due to a higher extracellular dopamine concentration in that region so [11C] raclopride could not mark it. This finding supports the theory that dopamine dynamics in the ventral striatum in compulsive overeating has physiological similarities to that seen in drug addiction (lower D2 receptor availability as well; Volkow et al., 1993, 2009).

Dopamine dysfunction in the basal ganglia can also play a role in diverse symptoms associated with mental and neurodegenerative disorders, especially in people with depression or negative symptoms of schizophrenia. The first person to formally coin the term anhedonia was John Haslam in 1809, who was studying patients with schizophrenia stating that the patients were "indifferent to those objects and pursuits which formerly proved sources of delight and instruction" (Haslam, 1809). The Diagnostic and Statistical Manual of Mental Disorders describes the symptoms of this disorder as "Markedly diminished interest or pleasure at all…nearly every day" and "diminished ability to think or concentrate, or indecisiveness, nearly every day" (American Psychiatric Association, 2013). This could be described as anhedonia and

lack of motivation to obtain a reward which closely relates to the idea of learned helplessness proposed by Martin Seligman in 1972. Perturbations in the monoaminergic system contribute to several psychiatric disorders, namely Major Depressive Disorder, and these effects are not solely dependent on changes in just dopamine (Charney, 1988; Salamone et al., 1997; Nestler & Carlezon, 2006). The dopamine-rich nucleus accumbens (NAc) is thought to serve as an integrator of stress and reward-responses between limbic, motor, and cognitive brain regions, leading to affective action selection (Sporn & Charney, 2002; Nestler et al., 2002; Nestler & Carlezon, 2006).

The importance of understanding the neurobiological influences of the monoaminergic system in depression is emphasized by the lifetime prevalence of mood disorder for the population of 20.8%, with women being most affected by this disease 70% more often than men (Kessler, 2003 & 2005). These numbers show the importance of studying both sexes and point to differences in hormonal profile throughout their lifetimes, especially under stressful conditions. Patients with Major Depressive Disorder (MDD) also have a reduced neural response in the NAc and the caudate compared to their healthy counterparts, and MDD subjective severity was associated with a lower caudate volume (Pizzagalli et al., 2009). The concept of anhedonia is often misconstrued as a general lack of reward but even when testing patients, it is not that these patients do not like what they previously enjoyed, but some investigators state that anhedonia is thought to reflect a reduced motivation to work for rewards, which is not the absence of reward *per se* (comparative to making effortful decisions in a state of malaise or illness; Treadway et al., 2012; Yang et al., 2014). Patients with MDD also have a reduction in performance in a visual probabilistic reward task in which patients received monetary rewards for correct response (Elske et al., 2013), and during a similar reward task, if the patients received a D2/3R antagonist,

investigators could rescue corticostriatal connectivity seen during fMRI (Admon et al., 2018). The challenge for studying depression is the lack of being able to adequately measure depression in preclinical studies in animals, since the severity of MDD is mostly made through qualitative surveys given to human patients. However, there are ways to produce depressive-like behaviors in animals that would show a form of learned helplessness, which usually requires chronically stressing the animal and then testing the animal in a task that requires an affective component and seeing the effects of antidepressants on these behaviors (Czeh et al., 2016; Slattery & Cryan; 2017). The problem with these approaches is that animals cannot tell us how they feel, animals may not be depressed in the same way humans are, and there is a huge social component of depression that we cannot accurately model in animals. For example, although a very clever approach to modeling depressive-like behaviors Tye and colleagues (2013) optogenetically induced VTA → NAc neuron firing which led to increased escape behaviors in the forced swim task and increased sucrose preference. In contrast, another study also activated VTA → NAc DA neurons during a social-defeat task that increased susceptibility to defeat but found opposite results during the sucrose preference task (Chaudhury et al., 2013). To be able to accurately study depressive phenotypes in animals, we need better standardization of methodology, but that will be a difficult task since individual labs want to provide their own way of doing things to seem more novel and outstanding when the time comes to publish and obtain funding (which is not just a problem with preclinical depression studies). To make a more concrete role of dopamine and monoaminergic function, we must also understand its relation to every other molecule of interest in the brain (including targets that have not yet been identified).

THE UTILITY OF STUDYING NEUROECONOMICS

Why Neuroeconomics?

Neuroeconomics is the joint effort of neuroscientists and economists to understand what happens in the brain when we or any other organism makes economic decisions. Admittedly, when I first became interested in neuroscience, dopamine, and reward-related behaviors, I was not interested in the neural mechanisms of decision-making, specifically. I was interested in the effect of psychoactive drugs and other pharmacological agents on behavior, namely in substance use disorders. As my educational career progressed and I learned more about addiction, and I wanted to get to the root of the issue: why did people suffering from addiction make the decisions to engage in those drugs in the first place? I always viewed ads on television for people to 'just say no' to illicit substances (not including alcohol, since that somehow is 'better' than other drugs). Now I understand that just saying no is not as easy as anti-drug commercials make it seem. There are many environmental and affective reasons for people to seek and consume drugs and I found that decision-making was intricately woven throughout addictive behavior and other psychiatric illnesses (Redish, 2004). Thus, I became curious about the neural processes that guide decisions, which is now known as neuroeconomics.

Neuroeconomics is a relatively young field that combines behavioral economic theory and neuroscience, and this is largely due to the advancement of technologies that can monitor awake, behaving animals during value-based decision-making (Platt & Glimcher, 1999). I previously give evidence of how reward and neural correlates of value can affect conditioning behaviors, but economic theory aims to understand seemingly complex behaviors which can

provide more dimensions of information. Economic theories of utility, or cost/benefit calculations in the environment, were first introduced in the early 1900s. The relationship between objective value and the concept of utility is defined as nonlinear: as value increases the subjective utility of the commodity that is valued decreases (von Neumann & Morgenstern, 1944; Kahneman & Tversky, 1979, 1981). For example, unless you have a large amount of money, your conception of buying designer fabrics or expensive automobiles becomes less attractive. Although, this tenet of economics applies to every cost/benefit analysis, such as whether to make a meal in the microwave even though it will not taste as good as if making it in the oven or on the stove. The cost is time, but the benefit is flavor. One popular example would be in delayed discounting tasks, where the subject must choose between an immediate reward or wait an extended period for a larger reward, which most of the time offers real incentives such as food in animals (Mitchell, 2014) or money (Johnson & Bickel, 2002). In human research one can offer different magnitudes of hypothetical monetary rewards that can gauge subjective valuation and state (Johnson & Bickel, 2002). Now that we can record the activity of neurons in the brain, we can look at how the brain encodes the dimensions of decision processing that act in concert with each other and manipulate these dimensions empirically with clever experimental manipulations (e.g., changing task structure or the rewards themselves) to influence subjective valuation and perception (Sugrue et al., 2005). These dimensions include the internal state of the subject, the external state (environment), the courses of action available and the associated utility of each action, and the learning that occurs following the choice (Rangel et al., 2008).

The field is only limited in understanding human decision-making behaviors because only neural *correlates* can be inferred. Human studies do come with a caveat other than not being able to use invasive procedures, and that is the fact that human decision-making is fickle

and variable due to complex internal states such as personality types and metabolic state (Fujiwara et al., 2008; Symmonds et al., 2010). Nonetheless, investigators can use more complex decision-making tasks, like trust games, than in animals to observe neural and behavioral patterns in healthy individuals and compare them to individuals whose behavior deviates from 'normal' (Kishida & Montague, 2013). For the example of delayed discounting, also called temporal discounting, there is evidence that two neural valuation systems, the limbic and dopaminergic circuitry, or fronto-parietal areas, play a role in either the immediate rewards or the delayed rewards, respectively (McClure et al., 2004[a]). This evidence is also irrespective of the amount of time the of hypothetical delay (McClure et al., 2007). Moreover, other investigators found similar patterns of activation in that the NAc, mPFC, and the posterior cingulate cortex (PCC) activity is positively correlated with reward magnitude, but dorsolateral PFC (dlPFC) and the posterior parietal cortex (PPC) activity are negatively correlated with the actual delay of the reward. These findings show neural correlates of impulsivity and subjective value and may suggest therapeutic targets for impulsive individuals with gambling disorders or attentional deficit disorders (Kable & Glimcher, 2007; Ballard & Knutson, 2009). Competing systems are also commonly observed in tasks that have a risk or probabilistic component, where striatal activation is usually associated with the magnitude or expected value of one option and frontal cortices (prefrontal cortex (PFC) or orbitofrontal cortex (OFC)) being associated with probability (Tobler et al., 2007; Knutson et al., 2005, 2008). The medial PFC (mPFC) has been shown to have a negative correlation with paying unreasonable prices for a reward (Knutson et al., 2007), which gives evidence to the neural representation of costs.

In monkeys and rodents, one can directly record neurons in awake, behaving animals, and similar temporal patterns of cellular activity of both striatal and cortical regions are observed,

although the task structures are inherently different in both rodent and primate models (Lee et al., 2015; Kuwabara et al., 2019). Both rodent and primate animal models of decision making often investigate either the striatum or the OFC since similar phenotypes are changed when manipulating either brain structure. In rodents it is shown that model-based decisions are still found in the NAc and OFC, unlike model-free associations, but it depends on how complex the task design is because some task designs guide rodents to one behavioral mechanism or the other (Groman et al., 2018). One can even create scenarios of regret in rodents, where occasionally choosing a low-cost, low-reward choice and skipping a high-cost, high-reward can make animals choose the high-cost, high-reward option on the next trial to reap the benefits of their decision instead of sticking with a low-cost option (Laurent & Balleine, 2015; Steiner & Redish, 2014). These choices and regret are also reflected in the NAc and OFC, where cells are activated during the moment of regret (missing the high-reward choice; Steiner & Redish, 2014). Inhibition of the OFC disrupts learning via alteration of experience-dependent updating (Baltz et al., 2018). However, one study found that inactivation of OFC neurons in rats did not change representation of economic choice in a task with 2 different options but 5 different magnitudes but still impacted effort related decision-making (Gardner et al., 2018). This contrasted with results in monkeys performing on a similar task (Padoa-Schioppa & Assad, 2006). Monkeys, like humans and rodents (Mai et al., 2012), also make state-dependent choices (Yamada et al., 2013) but can develop similar risk-averse patterns of behavior. Additionally, activation or inhibition of OFC neurons reflects good-based (transitive value computed at the time of choice) economic value across multiple studies (Yamada et al., 2018; Cai et al., 2019).

Dopamine and Decision-Making

As mentioned before, DA and DA-rich brains areas influence utility-based decisions, either as a learning signal through phasic burst activity during reward prediction errors (RPEs) (Diederen et al., 2017) or through tonic levels of dopamine which can be manipulated in humans via ingestion of pro-dopaminergic drugs (Westbrook et al., 2020). Kahneman and Tversky (1979, 1981) introduced the use of utility curves where one can use value/benefit and effort/cost to create utility curves for animal behavior that are causally related to the role of dopamine to modulate the energy required to obtain a reward (Phillips et al., 2007). Mesoaccumbal DA reflects reward magnitude, with greater phasic bursts following rewards of larger magnitudes and their associated discrete cues, leading to the idea that dopamine serves as a stored, or cached-value, signal (Gan et al., 2010). What is unclear though is how phasic dopamine release is associated with effort. Wanat and colleagues (2010) showed that either in a fixed ratio or progressive ratio (escalating costs over each trial), DA release in the NAc, measured with FSCV, positively correlates with reward delivery, but not cue presentation, following a larger effort expenditure. In another study, when experimenters altered the "cost" of sucrose reinforcers (number of lever presses for each mg of sucrose to linearly fix price to reward), they found that DA release was negatively correlated with the amount of lever presses the animal had to make (Schelp et al., 2017). This is supported by another rodent study that manipulated either delay or effort to obtain reward (Day et al., 2010). In monkey studies, effort and expected reward value in relation to the effort required to obtain the reward show similar patterns of DA release to the rodent studies above, but also give evidence of norepinergic coding of single-dimensions, scaling effort and reward options (Pasquereau & Turner, 2013; Varazzani et al., 2015). What is unclear

though is when two concurrent choices are offered in a mixed-contingency effort and reward task structure, how does the cached-value DA signal lead to action selection? There is one study, to my knowledge, that does include manipulating multiple dimensions of value-based decision making. Lak and colleagues (2014) altered subjective value in head-fixed mice to use a steering wheel to choose a left or right option when the stimulus (a grating pattern on a screen) is presented. Stimuli for either side represented rewards (water drops) of differing magnitudes led the mouse to move the steering wheel to the center to receive reward, and to manipulate cognitive effort, investigators change the contrast of the stimuli to the screen. They found that DA accurately reflected animals' subjective value and action selection. A more compelling study shows that in a mixed-contingency task that DA cached value does not reflect an animal's choice behavior. It is shown that an animal that prefers a low-reward, low-cost option, still has a greater phasic DA release to a high-reward, high-cost option (Hollon et al., 2014). Because the animals' contingencies change from a right or left lever every session, the next step will be to test if this remains to be true even when an animal is overtrained (non-changing contingencies) or forced to switch every test day on choosing low vs. high effort contingencies or low vs. high reward options. I will provide evidence for this in Chapter 3.

STRESS, ITS BIOLOGICAL CORRELATES, AND THEIR INFLUENCES ON REWARD-RELATED CIRCUITRY AND BEHAVIOR

Biological Mechanisms of Stress

Claude Bernard (1865) was one of the first scientists to provide a formal definition of stress response within an organism, specifically humans. He defined the concept of the 'internal milieu', stating that the goal of physiological systems is to buffer the internal environment from external, environmental perturbations. Walter Cannon (1952) later emphasized the ideas of homeostasis, the stability of the internal milieu, and the flight or fight response, the behavioral correlates of stress seen in both prey and predator species (even though either type of animal responded similarly to different stressors). Stress is a disruption of homeostasis within an organism and can elicit a response to alleviate that stress which follows the theory and considerations proposed by Hans Selye (1952, 1961, 1975). The hypothalamic-pituitary-adrenal (HPA) axis is a major biological mechanism that regulates sympathetic and parasympathetic responses to stressors. The HPA axis secretes glucocorticoids (GC) into the bloodstream, which act on central and peripheral target tissues during heightened stress and provide a negative feedback system to decrease glucocorticoid secretion. Corticotropin-releasing factor (CRF), the molecule released in the paraventricular nucleus of the hypothalamus, is released in a general fashion when an organism experiences any invigorating stimuli, regardless of the affective value (Johnson et al., 1992; Merali et al., 2004; Wang et al., 2004). It is also shown to encode both positive and negative valence signals since CRF manipulation in the paraventricular nucleus of

the hypothalamus (PVN) could induce a conditioned place preference or aversion (CPP or CPA) and show phasic burst-like activity to both aversive and appetitive stimuli (Kim et al., 2019).

CRF was first characterized as a 41-amino acid polypeptide by Spiess and colleagues (1981) and has three distinct receptors distributed all over the body, CRFR1, CRFR2, and CRF binding protein (Bale & Vale, 2004; Behan et al., 1996). Following this characterization, investigators found that CRF and its receptors (CRFR1, CRFR2, CRF-BP) were not solely located in the hypothalamus but distributed all over the brain with dense receptor mRNA localization in major DA-rich brain regions (VTA/SN; Van Pett et al., 2000; Henckens et al., 2016). CRF binding studies showed that the protein bound in similar, though not the same, brain regions, alluding to the trafficking of receptors to dendritic spines (Tan et al., 2017). This evidence pointed to stress and CRF's ability to alter reward-related behaviors (e.g., decision-making and reward sensitivity), especially in a sex-specific manner in humans with Major Depressive Disorder (Starke, 2012; Vrieze et al., 2015). Assuming acute and chronic stress are subjective to the organism studied, some studies purport the heightened susceptibility of females to have stress-induced alterations in CRF receptor interactions compared to their male counterparts (Bangasser et al., 2012; Valentino et al., 2013).

Stress Influences Dopamine Dynamics and Reward-related Behaviors

The induction of chronic stress and trauma is thought to expedite the onset of Major Depressive Disorder (MDD) where a small motivational stressor, such as making a new friend or deciding between different brands of food, becomes a large obstacle for optimal performance (Beck, 2008), giving evidence to the effort component of anhedonia (Treadway et al., 2012).

Lenow and colleagues (2017) showed that both acute stress and chronic stress, experienced via a cold-water hand submersion and questionnaire, respectively, resulted in overexploitation in a human, virtual foraging task. Similar effects on motivation and decision-making accuracy were seen in studies that analyzed depressive human and rat behavior that was rescued by κ-opioid receptor antagonist, concurrently (Beard et al., 2015; Van'T Veer et al., 2012). The same idea follows for rodent models of stress (e.g., pharmacological stressors and physically induced stress) with females having estrous-dependent alterations in behavioral responsivity and cognitive performance to acute and chronic stress (Wood & Shors, 1998; Shansky et al., 2003; ter Horst et al., 2013). For example, endogenous estrogen in females and exogenous estradiol administration to males rescued behavioral and glutamatergic deficits in the prefrontal cortex pyramidal neurons. These deficits could be reversed by aromatase (the enzyme that produces estrogen) and aromatase inhibition in females and males, respectively (Wei et al., 2014; Yuen et al., 2016). One caveat is that females will be food deprived which could affect their estrous cycle, an effect seen in rats (Tropp et al., 2001).

In relation to DA-rich brain regions and reward behaviors, it is found that intra-accumbal injections of CRF can augment motivation for both sucrose-seeking and partner preference (Peciña et al., 2006; Lim et al., 2007). DA is known to facilitate these behaviors (Aragona et al., 2006; Lex & Hauber, 2008). Additionally, work in our lab has shown that CRF administration potentiates DA in the NAc, and this potentiation is abolished by a 2-day, repeated forced swim stress in both male and female mice (Lemos et al., 2012; Steger et al., 2020). Normally, CRF administration in the NAc augments reward sensitivity via an increase in novel object recognition and CRF-paired place preference but following FSS mice switch their behavior from an appetitive state to an aversive state (Lemos et al., 2012). The paradigm of repeated- forced

swim stress used has great predictive validity, since pharmacological agents like antidepressants can increase immobility times (Porsolt, 1978; Petit-Demouliere, 2005; Cryan et al., 2005), but the Porsolt rFSS lacks construct and face validity, since it does not directly compare to human cause of stress and depression. Some state that the immobility seen in rFSS, does not reflect depressive state, but rather learned behavior (Molendijk & de Kloet, 2019). This idea of immobility as a learned behavior, but also a depressive one, has some overlap in the fact that tonic immobility is seen in animals under a great amount of survival stress. For example, animals that play dead, animals that have been caught within the jaws of their predators, and even animals being handled in laboratory environments (holding a mouse still for an injection). This tonic immobility response is regulated in part by CRF release in both the central amygdala (CeA) and the basolateral amygdala (BLA) and can be reversed via injection of the non-selective antagonist, α-helical CRF (Donatti et al., 2011). Interestingly, in one study, microinjection of CRF into the NAc increases swim immobility, decreases sucrose preference, and induces anxiety-like behavior (Chen et al., 2012). To make the effects of the Porsolt test even more complicated, it is shown that when animals are binarily categorized as active (less immobile) or passive (more immobile), passive animals emit more appetitive ultrasonic vocalizations to a rat encounter following social isolation and spend more time in an amphetamine-associated context in CPP compared to their active counterparts (Wislowska-Stanek et al., 2019).

The state-dependence of how glucocorticoids influence dopaminergic transmission is evidenced by peripheral effects of corticosterone injections being able to increase dopamine, measured by microdialysis, only in the dark phase of an animals' light cycle, during eating, and high locomotor activity (Piazza et al., 1996). Conversely, another study in our lab showed that CRF administration in the VTA after acute, 1-hr restraint stress had opposite effects via a

decrease in breakpoint in an operant progressive ratio task to obtain food-reward which was reversed by administration of α-helical CRF (Wanat et al., 2013). The effects of CRF on mesoaccumbal DA must be experience dependent since injections of CRF into the VTA lead to an increase in DA neuron firing via CRFR1 binding (Wanat et al., 2008). These results point to the detrimental effects of stress, so I aim to investigate a more complex decision-making task in mice to verify the slice physiology purported by Lemos (2012). Another group found that acute stress (1 hr. restraint stress) decreased the motivation for rats to work harder for a larger reward as well as increased latencies to decide which was unaffected by dopamine antagonist flupenthixol (Shafiei et al., 2012). Following this study, the same group found that an even shorter (20 min. restraint stress) did not affect the choice for a more effortful choice, but increased latency to obtain that reward. Central and intra-VTA CRF administration decreased motivation to obtain reward which was evidenced by increased latency, reduced breakpoint, and reduced rate of lever-pressing (Bryce & Floresco, 2016). It was later shown that intra-accumbal injections of CRF led to a general flattening of the effort-discounting curve, which is contrary to the effects the investigators predicted since D2/3 agonism led rats to be effort-averse. It is still unclear how NAc CRF impacts complex decision-making behaviors (Bryce & Floresco, 2019). All in all, the effects of stress and CRF on DA transmission and DA-dependent behaviors, especially in decision-making, are not clear since there are so variables to account for. I hope that in the future, studies involving stress and CRF can be more standardized to allow for replicability and I feel that we are extremely far from developing and mutual understanding to design therapeutics for stress-induced psychological states and MDD. The silver lining is that there is much more work to be done and questions to ask.

CHAPTER 2

The Influence of Chronic Stress on Decision-Making: Effects of CRF and DA Antagonism in the Nucleus Accumbens

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ABSTRACT

In the core of the nucleus accumbens, corticotropin-releasing factor (CRF) increases evoked dopamine (DA) release and produces conditioned place preference in stress-naïve animals. However, following two-day, repeated forced swim stress (rFSS), neither of these effects are present, indicating an interaction between CRF and DA. To ascertain the degree to which this mechanism influences integrated, reward-based decision making, we developed a novel operant concurrent-choice task where mice could choose between two liquid receptacles containing a sucrose solution or water delivery. Following initial training, either a CRF or DA antagonist, α-helical CRF (9-41) and flupenthixol, respectively, or vehicle was administered intracranially to the nucleus accumbens core (NAcc). Next, the animals underwent rFSS, were reintroduced to the task, and were retested. Antagonizing CRF and DA should reduce overall response vigor which is represented by reduced preference and activation metrics of our concurrent choice task. Prior to stress, mice exhibited a significant preference for sucrose over

water and made more total nose pokes into the sucrose receptacle than the water receptacle throughout the session. There were no observed sex differences. Stress did not robustly affect preference metrics but did increase the number of trial omissions compared to their stress-naïve, time-matched counterparts. Interestingly, flupenthixol administration did not affect sucrose choice but increased their nosepoke preference during the inter-trial interval, increased trial omissions, and decreased the total nosepokes during the ITI. Microinjections of α-helical CRF (9-41) did not starkly affect preference metrics compared to vehicle injection, but during the retest period following stress, it differentially affected choice and preference. These data suggest that behavioral vigor during choice selection is dopamine- and stress-dependent, but also has separate components that are sensitive to CRF antagonism. Specifically, effects of either CRF or DA antagonism within the ventral striatum were larger following stress. Understanding the biological interactions between stress, reward responsivity, and decision-making can elucidate therapeutic targets for individuals with aberrant decision-making patterns, especially in a chronically stressed state.

INTRODUCTION

Perturbations in the DA monoaminergic system contribute to several psychiatric disorders, namely MDD. The dopamine-rich nucleus accumbens (NAc) is thought to serve as an integrator of stress and reward-responses between limbic, motor, and cognitive brain regions, leading to affective action selection (Sporn & Charney, 2002; Nestler et al., 2002; Nestler & Carlezon, 2006). The induction of chronic stress and trauma is thought to expedite the onset of Major Depressive Disorder (MDD) where a small motivational stressor, such as making a new

friend or deciding between different brands of food, becomes a large obstacle for optimal performance (Beck, 2008). In humans, it is thought that patients with MDD also have a reduced motivation to work for rewards, which isn't the absence of reward *per se* (Treadway et al., 2012; Yang et al., 2014). For example, Lenow and colleagues (2017) showed that both acute stress and chronic stress experience via a cold-water hand submersion and questionnaire, respectively, resulted in overexploitation in a human, virtual foraging task. Similar effects on motivation and decision-making accuracy were seen in studies that analyzed depressive human and rat behavior that was rescued by κ-opioid receptor antagonist, concurrently (Beard et al., 2015; Van'T Veer et al., 2012). The same idea follows for rodent models of stress (e.g., pharmacological stressors and physically induced stress) with females having estrous-dependent alterations in behavioral responsivity and cognitive performance to acute and chronic stress (Wood & Shors, 1998; Shansky et al., 2003; ter Horst et al., 2013). Additionally, male rodent decision-making behavior has been shown to be disrupted by stress (Shafiei et al., 2012; Wanat et al., 2013). Therefore, the interaction between biological correlates of stress in the hypothalamic-pituitary-adrenal (HPA) axis and mesolimbic dopamine (DA) that facilitates decision-making has been of interest.

Corticotropin-releasing factor (CRF), the molecule released in the paraventricular nucleus of the hypothalamus, is released in a general fashion when an organism experiences any invigorating stimuli, regardless of the affective value (Johnson et al., 1992; Merali et al., 2004; Wang et al., 2004). Interestingly, it was found that CRF and its two receptor subtypes, CRFR1 and CRFR2, were not only found in the anterior pituitary, but were also distributed throughout the brain, including dopamine-rich areas (Van Pett et al., 2000; Henckens et al., 2016). Therefore, there should be an interaction between CRF and dopamine in areas like the ventral tegmental area (VTA) and NAc. Other evidence shows CRF's ability to alter reward-related

behaviors (e.g., decision-making and reward sensitivity), especially in a sex-specific manner in humans with Major Depressive Disorder (Starke & Brand, 2012; Vrieze et al., 2015). Assuming acute and chronic stress are subjective to the organism studied, some studies purport the heightened susceptibility of females to have stress-induced alterations in CRF receptor interactions compared to their male counterparts (Bangasser et al., 2012; Valentino et al., 2013). The importance of understanding the neurobiological influences of the monoaminergic system in depression is further emphasized by the lifetime prevalence of mood disorder for the population of 20.8%, with women being most affected by this disease 70% more than men (Kessler, 2003 & 2005). These numbers show the importance of studying both sexes, and points to differences in hormonal profile throughout their lifetimes, especially under stressful conditions.

In earlier studies, it was found that intra-accumbal injections of CRF can augment motivation for both sucrose-seeking and partner preference (Peciña et al., 2006; Lim et al., 2007). DA is known to facilitate these behaviors (Aragona et al., 2006; Lex & Hauber, 2008). Additionally, work in our lab has shown that CRF administration potentiates DA in the NAc, and this potentiation is abolished by a 2-day, repeated forced swim stress (rFSS). Normally, CRF administration in the NAc augments reward sensitivity via an increase in novel object recognition and CRF-paired place preference, but following rFSS, mice switch their behavior from an appetitive state to an aversive state (Lemos et al., 2012). In other complex decision-making behaviors, CRF and stress increase choice latencies, reduce progressive-ratio breakpoints to choose, and reduce rates of lever pressing (Shafiei et al., 2012; Bryce & Floresco, 2016).

Therefore, using a novel concurrent choice to test affective decision-making in male and female mice, we hypothesized that rFSS should reduce behavioral vigor (e.g., choice preference and behavioral activation). If CRF affects the task by modifying dopamine transmission, then

CRF antagonist effects should be qualitatively like those of dopamine antagonists in our behavior. Surprisingly, we found no effects of stress alone on this task. Also, CRF antagonism, via local microinjection of α-helical $CRF_{(9-41)}$ mainly affected preference metrics of our behavior in a stress-dependent manner since no effects were observed in animals who did not receive stress or received a lower dose. Inhibition of DA receptors, via intra-accumbal injection of fluphenthixol, reduced behavioral activation in our task, as we thought, but stress amplified the effects of antagonism. We believe that CRF is not acting serially, but in parallel, with DA in the NAc core (NAcc) through mechanisms unknown.

METHODOLOGY

Subjects

Eighty-eight male (n=46) and female (n=42) C57/Bl6 mice in separate cohorts were used for all experiments and weighed between 17 and 25 g prior to food restriction and training. All mice were single-housed in a temperature- and humidity-controlled vivarium on a 12L:12D cycle (lights on at 07:00) and food restricted above 85% of free-feeding body weight after at least one week of acclimation and surgery recovery. Animals who did not receive cannulation surgery (n=20) were food restricted one week after acclimation to being house individually. Water was provided ad libitum. Animals were weighed before each training or testing session and provided with standard rodent chow after the session. All experiments were performed in accordance with the University of Washington Institutional Animal Care and Use Committee.

Apparatus

Testing was conducted in Plexiglas operant chambers (24 x 20 x 21 cm; ENV-307W; Med Associates, St. Alban, VT, USA) that were contained in in-house built, sound attenuating cubicle. Each chamber contained two liquid receptacles (ENV-300R1AM) on the right and left side that were equipped with infrared (IR) sensing, head entry detectors (ENV-303HDW) for the animal to nosepoke for either water or a 0.1M sucrose solution located 4cm above the grid floor. The liquid delivery system was controlled by a solenoid opening (100ms) and pressurized air to deliver 5µL of liquid for each reinforced choice.

Either a flashing (1Hz) or a solid, LED stimulus light (ENV-321W) was positioned over each receptacle 8cm above the grid floor. A mounted speaker and audio generator (ENV-223) positioned on the rear wall of the chamber was used to signal the availability of either liquid at either 8 or 15 kHz noise for forced-choice trials. Free-choice trials where both liquids are available were signaled by illumination of both visual cues and a 4kHz noise. Which liquid associated with visual and auditory cues was counterbalanced across animals. These behavioral protocols were controlled by Med-PC software and the data was stored for later analysis at the end of each day. Video was recorded using an IR camera and DVR system (Zosi ZG2111C) and analyzed using Ethovision XT video analysis software (Noldus Information Technology).

Nosepoke Training

Our novel concurrent choice operant task required magazine and fixed-ratio 1 training, side bias assessment, sucrose preference training, and the 8-day task itself. Animals were introduced to sucrose (10 sucrose pellets) in their home cage a day before nosepoke training following at least 5 days of food restriction. Magazine training exposed the animal to the discrete

cues associated with either the left or right liquid receptacle by continuous visual and auditory stimulus presentation with free deliveries of sucrose solution over 90 minutes. The animals also had the option of nosepoking into the receptacle to earn extra liquid following dispense of free reward which turned off the stimulus for one second before starting again. Behavior was further shaped for the first two days by adding half a crushed sucrose pellet into the receptacle. The number of free rewards during magazine training decreased over 4 days from 48 for the first two days to 24 to 12 to increase the number of responses required to earn a maximum number of 48 earned rewards to emulate the 48 trials required to complete during the task. The criterion was to earn at least 40 of the 48 maximum earned rewards to transition into fixed-ratio 1 (FR1) training. The FR1 training consisted of alternating, psuedorandom forced-choice trials which started with discrete cue (visual and auditory) presentation for either the left or right side until the animal successfully nosepoked into the receptacle to retrieve the reward (0.1s, 0.1M sucrose delivery) or the time to initiate ran out. These training days took 7-10 sessions for all animals to complete at least 24 trials with optimal performance of completing 40-48 trials. Over the sessions the time to initiate the reward delivery before going to the next trial went from an infinite time to 40s (∞, 120s, 60s, 40s) and the inter-trial interval (ITI) went from 5±2s to 40±10s (5±2s, 10±5s, 20±5s, 30±10s, 40±10s). Sessions were completed when the animal reached 48 reinforced trials or when the 90 minutes was over. Once animals reached criterion, their side bias was tested by replacing 24 of the forced-choice trials with free-choice trials. Animals were then assigned sucrose solution to either the left or right side for the remainder of the experiment in a counterbalanced fashion. For example, half of the animals that preferred the right side would be assigned sucrose in the right receptacle and the other half assigned water in the right receptacle (the same applies to left-preferring animals).

Concurrent Choice Decision-Making Task

Following magazine and FR1 training on sucrose solution, animals then were introduced to 60-minute sessions where the animal could choose between sucrose and water. The session consisted of 48 trials separated into 6 blocks of 8 trials that began with pseudorandom presentation of four forced-choice trials (visual and 8 or 15 kHz auditory cues for left or right receptacle availability) and 4 free-choice trials (visual cues on both sides and 4kHz noise). Mice had 40s to respond to either side before the cues turned off and a 40±10s ITI commenced. As a group, 5-7 days of session completion was required for animals to reach a 75% preference for the sucrose receptacle in free-choice trials. After reaching criteria, the experiment included four days of the same task, the 2-day repeated forced swim stress (rFSS), and 4 more days of the task before animals were sacrificed. For this within-subjects design mice receiving intra-accumbal microinjections were injected on test days 1 and 3 with no injections on days 2 and 4 to allow the drug to diffuse and/or be metabolized. A day following rFSS, mice received injections on test days 5 and 7, in the same fashion, with no injections on days 6 and 8. Mice in our stress-naïve conditions (n=13) performed the same task, but with no rFSS, to test time-dependent effects of the behavior and/or repeated drug exposure.

Repeated Forced Swim Stress (rFSS)

To induce chronic stress, we exposed mice to a 2-day, repeated forced swim stress. This was a modified Porsolt forced-swim paradigm described in by McLaughlin and colleagues (2003; Porsolt et al., 1977). Mice swam in 30ºC water for 15 minutes on the first day, but the second day included 4, 6-minute swims to increase immobility times for every swim. There were

no opportunities for the animals to escape by making sure the water was 10 cm from the rim of the bucket and high enough for the tails to never reach the bottom of the 5L bucket. During the stress, we measured immobility times for all animals to observe if there were any effects of sex, cannulation surgery, or drug pre-exposure. All animals were sufficiently dry before returning to their home cages.

Cannulation Surgery

Mice were fully anaesthetized with isoflurane gas in an induction chamber with 5% isoflurane for 2 minutes before their shaving and injection with analgesic (Meloxicam, 5mg/kg s.c. in 1mL of saline). Weights were taken before anesthesia induction. Animals were then secured in a stereotaxic frame with ear and bite bars to maintain a stable and flat skull surface. For the duration of the aseptic surgery animals were kept on a 1.5-2% isoflurane in oxygen gas for a reliable surgical plane. Bilateral cannula was then implanted to 1mm above the nucleus accumbens core (NAcc; coordinates, anteroposterior (AP) +1.2mm from bregma, mediolateral (ML) +1mm from bregma, and dorsoventral (DV) -3.5mm below the skull). Double-guide cannulas (26 gauge, 3.5mm from the pedestal, 2mm separation; Plastics One) were anchored using a skull screw and dental acrylic cement. Dummy internal cannulas were kept inside the cannula until injection using a 33-gauge internal cannula (Plastics One). For post-operative care, analgesic (Meloxicam, 5mg/kg in dissolved in 1mL of saline) was administered 24 hours after the surgery and weights were taken daily.

Drug and microinjection protocol

Before injection day 1 of our experimental design, animals were habituated to handling and the microinfusion procedure via insertion of internal cannula and allowing the animal to roam in a separate, clean Plexiglas homecage following their sucrose preference training session without injecting any liquid to emulate the time (~5 min.) and handling it requires for drug administration. In this within-subjects design, animals were injected with drug or vehicle on test day 1, 3, 5, and 7, in a fashion that they would receive two injections before the rFSS (2 days break for stress naïve animals) and two injections following stress-induction. For example, if an animal was injected with drug on day 1, they would be injected with vehicle on day 3. After the 2-day rFSS, they would be injected with drug on day 5 and vehicle on day 7. The other half of the animals received vehicle on day 1 and 5 and injected with drug on days 3 and 7. As a control, animals who did not receive surgery were habituated to handling.

We used a non-selective CRF antagonist, α-helical CRF $_{(9\text{-}41)}$ (500ng/200nL; Tocris Bioscience; n=17) or its vehicle (0.01% acetic acid in lactated ringer's solution; n=17), to test its effects on decision-making. We chose the 500ng/200nL since our lab has shown that they can reduce stress-induced changes in novel object exploration (in stress-naïve mice) and progressive ratio breakpoints (Lemos et al., 2012; Wanat et al., 2013). Regarding DA antagonism, we locally infused flupenthixol (20µg/0.5µL; n=19), to see what components of our behavior would be influenced compared to its vehicle (physiological saline; n=19). This dose was chosen since it was shown to decrease sign-tracking behaviors, reduce lever pressing probability, and increase lever-pressing latency in rats (Fraser & Janak, 2017; Saunders & Robinson, 2012). It has also been shown to decrease rates of food-operant responding when injected into the NAc (Beninger & Ranaldi, 1993). This dose was also the only effective dose used in a disconnection experiment

to influence ethanol CPP, although it was unilaterally injected into the amygdala (Gremel & Cunningham, 2010). All solutions were microinjected at a rate of 125nL $min^{-1,}$ and the internal cannula was left in for an additional minute to diffuse into the tissue. Mice were then left in their homecages for 15 min prior to their session.

Histology

Mice were deeply anesthetized with a ketamine/xylazine cocktail (ketamine 75.8mg/mL, xylazine 4.8mg/mL) at a volume of 0.1mL/20g for a total of 7.58mg ketamine before intracardial perfusion. We microinfused Chicago SkyBlue to verify injection sites before brain removal, fixation in 4% paraformaldehyde, and preservation in 30% sucrose solution in phosphate-buffered saline. Brains were sectioned at 40µm, mounted on slides. Animals without correct placement or had surgical complications were not included in histology or data analysis (n=15). Figure (see in Supplemental Fig. 1) represents the ventral portion of the injection sites in the NAc. All cannula traces and dye stains were demarcated in a blind fashion.

Data analysis

To verify that the animals were following the cues in a manner that reflected more than just exploratory behavior and baseline responding, we compared the average free- and forced-choice latencies to %50 of the ITI response interval. To calculate the response interval, we multiplied 48 trials by the 40s average ITI then divided that number (1920 s) by the total amount of nosepokes an animal made during the session to get the average interval of nosepokes made during the ITI (NPI). A two-way ANOVA was used comparing the session day and the time calculated from each metric.

To assess performance during testing, we measured reinforced choice (percentage of completed free-choice trials where mice selected sucrose), omissions (number of trials where mice did not respond during cue presentation), baseline responding (number of responses during intertrial intervals), and baseline preference (percentage of total responses during intertrial intervals that were in the sucrose receptacle). These metrics were grouped into preference (reinforced choice and baseline preference) and engagement (omissions and baseline responding). Analyses of response latencies did not prove to be especially informative but are included in the supplementary figures for transparency. We also measured and analyzed immobility times during the rFSS across cohorts (see in Supplemental Fig. 6). Sex-differences between male and female mice were tested for the aforementioned dependent variables.

The concurrent-choice data was analyzed using two-way, repeated measures ANOVA, with drug treatment and stress-state (pre-rFSS and post-rFSS) as two within-subjects factors. For, animals without surgery, factors were stress treatment and stress-state in individual days (i.e., 1-4 vs 5-8). To compare sex-differences within the task three-way, repeated measures ANOVA was used with sex as the third factor, but a final comparison of males and females in a stress-naïve condition were compared using an unpaired t-test. Mixed-effects analysis was performed where there were missing data points (e.g., if an animal did not choose the water option for the whole session). Multiple comparisons using Sidak's post hoc test were used when applicable. For analysis of immobility, two-way, repeated measures ANOVA was used, with sex and rFSS block as factors or surgical condition and block as factors. All statistical analysis was carried out using Prism 9 (GraphPad).

RESULTS

During training, mice acquired a preference for sucrose, as ascertained from free-choice trials (Fig 2a). They also acquired a preference for the sucrose-associated nose-poke port when making exploratory (non-reinforced) nose pokes during the intertrial interval (Fig 2a). The rate of nose-poke responses during the intertrial interval between the 48 trials during the training phase was once every 25.57 +/- 9.66 s. If responding during sucrose or water cue presentation (i.e., during a forced- or free-choice trial) was simply due to baseline responding, then we would anticipate the average latency to respond following cue presentation be half of this time. However, this latency was significantly shorter (Fig 2b-d) for free-choice responses ($F_{(1.38)} = 42.39$, $p < 0.0001$) with a significant interaction ($F_{(4,152)} = 3.803$, $p = 0.0056$) indicating that mice were engaging to the cue presentation. The same was true for force-choice trials: latencies were significantly shorter than half of the average time between responses during the ITI for their respective receptacle (sucrose: $F_{(1,38)} = 45.79$, $p < 0.0001$; interaction: $F_{(4,152)} = 2.66$, $p = 0.034$; water: $F_{(1,38)} = 25.70$, $p < 0.0001$; interaction: $F_{(4,152)} = 3.59$, $p = 0.0081$).

Sex differences were not observed when assessing behavior with the primary performance metrics (reinforced choice, $p = 0.8825$; baseline preference, $p = 0.9674$; omissions, $p = 0.6445$; baseline responding, $p = 0.7173$; Fig 2e-h). In fact, the only significant sex difference observed in task performance was that males had longer free-choice latencies than females for both sucrose ($p < 0.05$) and water ($p < 0.01$), but not forced-choice trial latencies (see Supplemental Fig. 2).

We next tested whether performance was sensitive to stress exposure. Following one testing period (Week 1) animals underwent repeated forced-swim stress and were retested during

a second period (Week 2). Stress-naïve control animals were tested during weeks 1 and 2 but were not exposed to swim stress in the interim. Reinforced choice, baseline preference and baseline responding were not significantly different between weeks 1 and 2 for either control or stress groups ($p > 0.05$; Fig. 3a, b, d). There was a significant main effect of time for omissions ($F_{(1,18)} = 13.92$, $p = 0.0015$) and this metric was significantly increased between weeks 1 and 2 for the stress group ($p = 0.0133$; Fig. 3b). However, there was no stress-by-time interaction ($F_{(1,18)} = 0.3621$, $p = 0.5549$), suggesting that this result was not an effect of stress but driven by a general increase in omissions over time. Likewise, there were no detectable differences between the control or stressed groups for either free- and forced-choice latencies (see Supplemental Fig.3). Furthermore, there were no significant effects ($p > 0.05$) of stress across sexes as indicated by the lack of significant interactions pertaining to sex (sex x stress, sex x time, sex x stress x time) when analyzed by three-way ANOVA with sex, time, and stress as factors (data not shown).

While there were no apparent effects of stress on the task performance, we do know that this same stressor exposure robustly changes the ability for CRF to increase dopamine (Lemos et al, 2012; Lemos et al 2019; Steger et al, 2020). Therefore, we tested the roles of dopamine and CRF on the decision-making task before and after stress.

Administration of the dopamine-receptor antagonist, flupenthixol (20 µg in 500 nl), into the NAc had modest effects on preference. It increased baseline preference compared to vehicle administration ($F_{(1,36)} = 5.544$, $p = 0.0241$; Fig. 4b), but did not significantly change reinforced choice ($p > 0.05$). However, this treatment robustly affected engagement metrics. Flupenthixol increased the number of omissions with a main effect of drug ($F_{(1,36)} = 34.39$, $p < 0.0001$) and an interaction between drug and time ($F_{(1,35)} = 8.031$, $p = 0.0076$). The effect of DA antagonism on

omissions appeared more robust after stress ($p < 0.001$) compared to pre-stress conditions ($p = 0.0059$; Fig. 4c). Baseline responding was decreased by flupenthixol with a main effect of drug ($F_{(1,36)} = 7.172$, $p = 0.0111$) and the effect was stronger following stress ($p = 0.0031$; Fig. 4d). Similarly, flupenthixol significantly increased free-choice latencies of both sucrose ($F_{(1,36)} = 8,629$, $p = 0.0057$) and water ($F_{(1,31)} = 9.469$, $p = 0.0043$) choices, with a stronger effect following stress for both ($p < 0.01$). Forced-choice trials were affected by drug treatment for sucrose, but not water, trials ($F_{(1,36)} = 7.946$, $p = 0.0078$) an effect driven by stress ($p < 0.05$; see in Supplemental Fig. 4).

Once again, these effects were not sexually dimorphic as indicated by the lack of interaction ($p > 0.05$) pertaining to sex (sex x drug, sex x time, sex x drug x time) when analyzed by three-way ANOVA with sex, time, and drug as factors (data not shown).

In contrast to the effects of flupenthixol, administration of the CRF antagonist, α-helical CRF (500 ng in 200 nl), affected preference rather than engagement. There was a significant interaction between stress and drug for either reinforced choice ($F_{(1,32)} = 4.447$, $p = 0.0429$) or baseline preference ($F_{(1,32)} = 6.116$, $p = 0.0189$; Fig. 5a, b). Interestingly, there was a main effect of time (before and after stress; $F_{(1,32)} = 15.17$, $p < 0.001$) where α-helical CRF administration following stress elicited a reduced preference compared to microinjection during the stress naïve state ($p < 0.001$). However, neither of the engagement metrics were significantly affected by CRF antagonism (Fig. 5c, d).

When analyzing additional performance metrics, only the water free-choice latency had an interaction between drug and stress ($F_{(1,24)} = 7.236$, $p = 0.0128$) where drug administration after stress led to a higher latency compared to before-stress conditions ($p = 0.0138$). None of the other latency types had an effect from either stress or drug (see in Supplemental Fig. 5).

These effect of α-helical CRF $_{(9-41)}$ did not differ between sexes as indicated by the lack of interaction ($p > 0.05$) pertaining to sex (sex x drug, sex x time, sex x drug x time) when analyzed by three-way ANOVA with sex, time, and drug as factors (data not shown).

DISCUSSION

In our behavioral paradigm, animals must follow discrete cues to choose between either a sucrose or water reward in two separate receptacles in a psuedorandom order of forced- and free-choice trials. CRF antagonism should reduce vigor in a stress-naïve state but have no effect following stress if there is no endogenous CRF potentiating DA in the NAc. Of course, if we assume if our behavior is DA dependent and that CRF is serially affecting DA release, then inhibiting DA receptors in the NAc should reduce sucrose preference and lower motivation, regardless of stress state. Therefore, if CRF affects the task by its actions on dopamine transmission then dopamine and CRF antagonists should have qualitatively similar effects in naïve animals but diverge following stress. Contrary to our working hypothesis, CRF and dopamine appear to act independently in the nucleus accumbens core during the decision-making task, regulating different aspects of the behavior.

CRF antagonism in our behavioral remained largely ineffective in our concurrent-choice task. However, we have previously shown that this same administration (500ng/200nL into NAc of mice) had significant effects on other behaviors (CPP and novel object exploration; Lemos et al., 2012). Subtle effects of α-helical CRF $_{(9-41)}$ were detected by comparing naïve and stressed animals where a significant stress x drug interactions were observed for the preference metrics (reinforced choice and baseline preference) but not the activation metrics (omissions and

baseline responding). Moreover, baseline preference affected by α-helical CRF $_{(9\text{-}41)}$, was reduced following stress compared to CRF antagonism in a stress-naïve state. Although not congruent to the task structure used in our study in mice, it was shown that CRF central injection in rats did not influence reward magnitude discrimination but did increase choice latencies and pre-stress administration of α-helical CRF $_{(9\text{-}41)}$ rescued the effects of stress on effortful responding (Bryce & Floresco, 2016). In contrast to the CRF-related effects on latency metrics, we found no stark influences of CRF antagonism on our latency results. It is unclear what CRF is communicating in the NAc, but other studies show the possibility of CRF encoding a learning signal in both appetitive and aversive conditioning (Kim et al., 2019). A lower dose of 50ng/200nL was used to test the possible dose-dependent effects of α-helical CRF $_{(9\text{-}41)}$ depending on the loci of interest (reviewed in Rivier & Rivier, 2013). It was also of interest to see if a smaller dose of drug would be sufficient to influence behavior in a reduced, but similar fashion as the high dose. There were no effects of this lower dose on behavior, even in a stressed state (data not shown).

Dopamine (DA) serves as a mechanism to predict rewards, their discrete cues, the absence of the reward, reward magnitude, and the time it takes to receive the reward (Schultz, 1997; Schultz, 2016). This mesolimbic DA system also plays a predominant role in expectation of reward since phasic DA release occurs with reward predictive cues in mammals and is sufficient to guide behavior for food and drug rewards (Pagnoni et al., 2002; Phillips et al., 2003; Tsai et al., 2009). Our lab has extensively studied and advanced the understanding of the function of DA in a variety of behaviors, including 'incentive learning' in a Pavlovian task, deciphering utility (cost/benefit) during decision-making, and its role in promoting drug-seeking behavior (Flagel et al., 2011; Gan et al., 2010; Phillips et al., 2007; Willuhn et al., 2014). Interestingly, the locally injected flupenthixol most robustly affected activation metrics,

indicating a qualitative disassociation between the modes/domains of CRF and dopamine actions on task performance. DA antagonism decreased baseline responding and increased the number of omissions during this task, but the animals' baseline preference was increased. We think this may be due to fluphenthixol decreasing exploratory behaviors so that they did not exert effort to respond for water cue-guided responses. Latencies to choose during most types of trials also were increased by flupenthixol and this effect was significant between drug treatment groups following stress. DA antagonism in the NAc has also been shown to impair sign-tracking, but not goal-tracking behaviors during Pavlovian conditioning (Saunders & Robinson, 2012), and was shown to not alter stress-induced reductions in effortful responding but did rescue effects seen in choice latency (Shafiei et al., 2012). Similarly, it was shown that intra-accumbal, but not dorsal striatal, injections of flupenthixol did not alter total responses for food in an operant conditioning task but did initially decrease rates of responding (Beninger et al., 1993). DA receptor 2 antagonism also does not affect food intake or preference in a touch screen procedure task, whereas food restriction did (Yang et al., 2019). This conveyed the idea that DA antagonism reduces general motivation in a way that does not reflect a reduction in reward retrieval essential to hedonic valuation, and further verifies that the drug remained local to the ventral striatum.

In the current work, the net effect of prior stress on the decision-making task was minimal based upon the behavioral metrics we tested. This finding is not unprecedented as Shafiei and colleagues (2012) reported negligible effects of restraint stress on choices between objectively smaller and larger rewarding outcomes in a reward magnitude task, where animals press a lever once for either option (2 or 4 pellets). However, stress increases the latency to choose between the two options (Shafiei er al., 2012), an effect that we did not see in our study for neither forced- nor free- choice trials. Additionally, other work in our lab purported no effects

of rFSS on anxiety- and depression- related behaviors: 3-Chamber social approach, tail suspension test, and elevated plus maze (Steger et al., 2020). Interestingly, we did see stress dependent effects of how either CRF or DA antagonism affected behavior since there were stress and drug interactions for the activation and preferences metrics, but also in DA-dependent effects on latencies. Another limitation to consider is that we tested our animals during the light-phase of their light-dark cycle. It was previously shown that the interaction between glucocorticoid signaling and dopamine was especially relevant during the animals' dark phase (Piazza et al., 1996).

The rFSS (Porsolt et al., 1978; McLaughlin et al., 2003; Bruchas et al., 2007) used in this study was supposed to be a way to induce a depressive-like phenotype that induced a stress that could lead to sex-specific effects. rFSS has been shown to increase cocaine-paired place preference, an increase in cocaine valuation, induction of long-term changes in the dopaminergic phenotype, as well as evidence of a depressive-like state in rodents (Bruchas et al., 2007 & 2009; Groblewski et al., 2015; Kreibich et al., 2009; Wichmann et al., 2017). It was shown that rFSS could lead to long-lasting alterations in how DA and CRF interact within the nucleus accumbens or reward-related area of female mice (Wichmann et al., 2017; Steger et al., 2020). Unlike reports of sex differences in CRF signaling and stress-responsivity (Bangasser et al., 2018; Bangasser & Valentino, 2012, 2013, 2014; Shansky et al., 2004; Yuen et al., 2017; ter Horst et al., 2013), we found no effects of sex and stress on our task, except for in free-choice latencies where stress-naïve males took longer to make choices than their female counterparts (see Supplemental Figure (x)). Even when using a 5-day rFSS stress paradigm, other investigators found no effects on depressive like behaviors in a sucrose preference test, an open field test, and a tail suspension test, but they did find evidence for depressive-like behavior following a social

defeat paradigm (which is unfeasible in female mice; Mul et al., 2016). This points to the possibility of immobility time, or learned helplessness (Seligman, 1972), in the rFSS as more of an adaptive behavior, like tonic immobility (Donatti et al., 2011), instead of a depressed state (Molendijk & de Kloet, 2014). To complicate matters even more, the effects of stress also depend on the strain used (Petit-Demouliere et al., 2005; Mul et al., 2015). The effects of stress also depend on the subjective state of the animal that we cannot account for. How much of the process of receiving surgery, handling, and receiving injections where the investigator completely controls the animal plays a part in stressing the animal. These manipulations could undermine the efficacy of rFSS.

In summary, much is still unknown about the interaction between CRF, DA, and stress. There exists evidence to support the idea of parallel mechanisms of DA and CRF which show that the cholinergic interneurons of the striatum influence depressive behaviors (; Warner-Schmidt et al., 2012; Cheng et al., 2019). These neural substrates have also been shown to mediate plasticity and function of dopaminergic neurons in the NAc (Wang et al., 2006; Collins et al., 2016; Yorgason et al., 2017; Hanada et al., 2018). And particularly, it is shown that cholinergic interneurons possess CRF receptors which, when the neuropeptide binds, can increase firing of these neurons to lead to augmentation of dopamine release (Lemos et al., 2019). This novel target for CRF and DA convey a new target for testing the interaction of the two molecules. And although rFSS may not be the right chronic stressor to induce depressive phenotypes in mice, the role of the striatum and endogenous CRF and DA signaling is still of importance (Beard et al., 2015). Clinical studies showed that depressed individuals have a reduced response of the NAc to rewards, have a more sensitive response to negative stimuli in

the NAc, and have reduced reward learning (Pizzagalli et al., 2009; Admon et al., 2015, 2017; Vrieze et al., 2015).

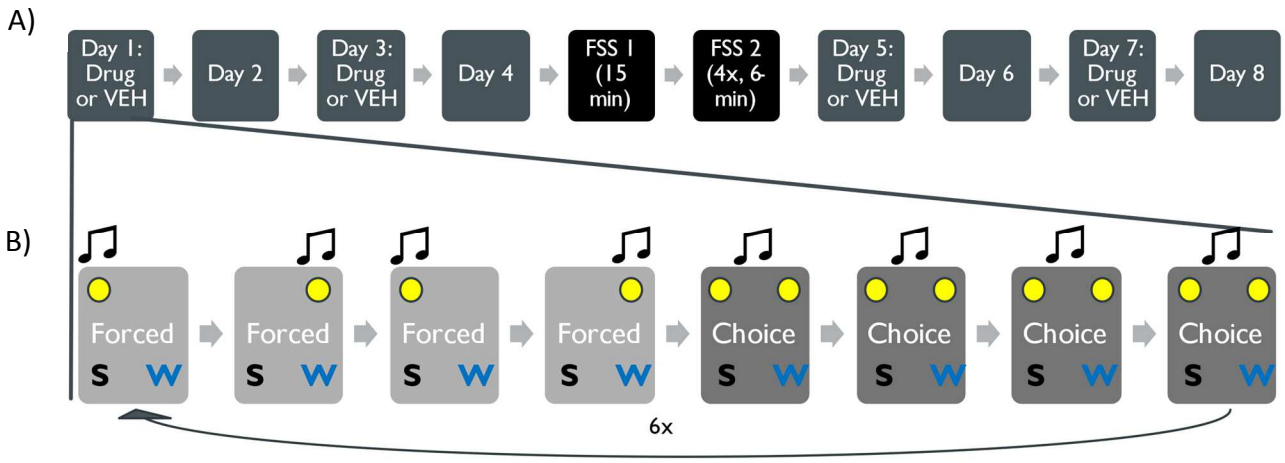


Figure 1. Structure of concurrent-choice operant conditioning task in a within-subjects design. A) Following conditioning, mice underwent 8 days of decision-making task: stress naïve behavior was recorded for 4 days before rFSS (or no stress) and 4 days after the stress. On days 1, 3, 5, and 7, in a counterbalanced fashion, animals designated to receive drug microinjection were injected with drug into the NAc via bilateral cannula implantation 15 minutes before the start of the task. The rFSS was conducted over two days. The first day, animals swam for 15 minutes, and the second day, animals swam 4 times for 6 minutes each time. Animals in a no stress condition could rest for 2 days. **B)** Within a single session, there were 48 trials, half of which were forced choice trials organized in a pseudorandom order. Following 4 trials of forced-choice for either water or sucrose with audio (8kHz or 15kHz) and visual cues (cue light directly above the associated receptacle), 4 free-choice sessions were available (4kHz noise and both cue lights on). This was repeated 6 times throughout the session for a total of 48 trials.

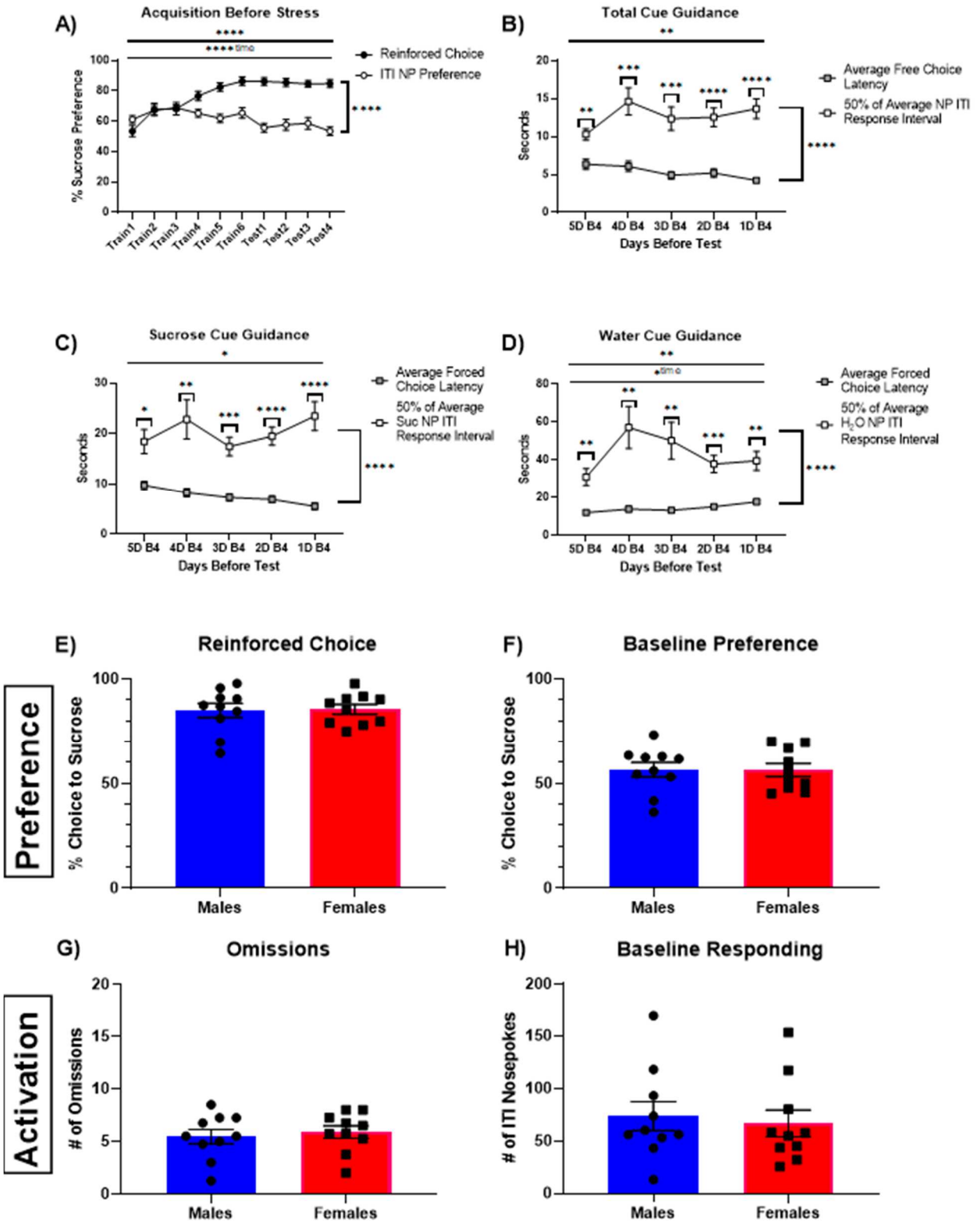


Figure 2. Acquisition behavior, cue-guidance verification, and sex comparisons of behavior in a stress-naïve state. A) Percent sucrose receptacle preference: reinforced choice percentage

vs. baseline preference represented by nosepokes made during the ITI (±SEM) up to the day of stress (or no stress) for animals not receiving drug manipulation (n=20). Criteria for advancement to test days was for each animal to reach >75% preference for sucrose during free-choice trials. **B)** Total cue-guidance verification represented by comparing the average free-choice latency (±SEM) to choose to 50% of the average nosepoke ITI response interval (±SEM) in the days leading up to the first test day. **C)** Cue guidance verification of responses made for the discrete cues associated with sucrose forced choices. **D)** Cue guidance verification of responses made for the discrete cues associated with water forced choices. **E, F, G, H)** Comparison between male (n=10) and female (n=10) mice for reinforced choice (E), baseline preference (F), number of trial omissions (G), and baseline responding (H). Error bars are (±SEM). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

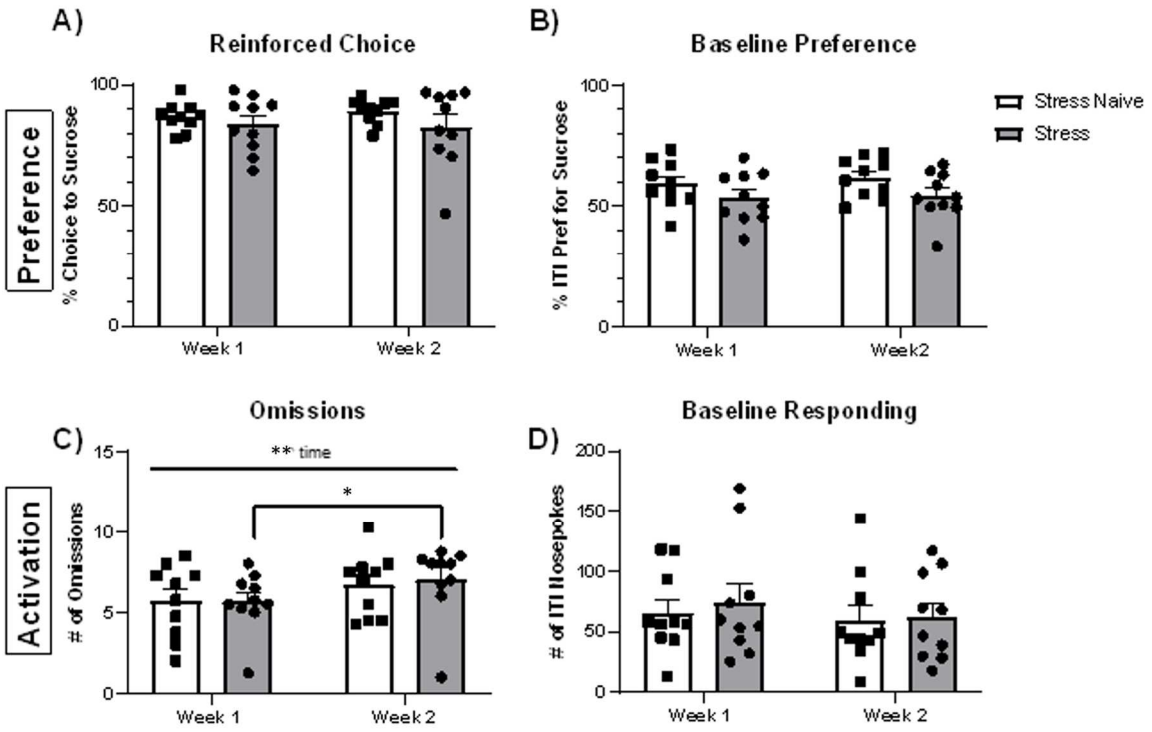


Figure 3. Stress vs stress-naïve behaviors. A) Averaged percentage of reinforced choice for the sucrose liquid receptacle in free-choice trials for animals in sessions before (week 1) and after (week 2) the rFSS (n=10) or 2 rest days (n=10). **B)** Percentage of ITI nosepokes made during the session for the sucrose liquid receptacle compared to water receptacle nosepokes during the ITI. **C)** Total number of omissions made during the sessions. **D)** Total number of ITI nosepokes made at all during the sessions. Error bars are (±SEM). *P<0.05, **P<0.01

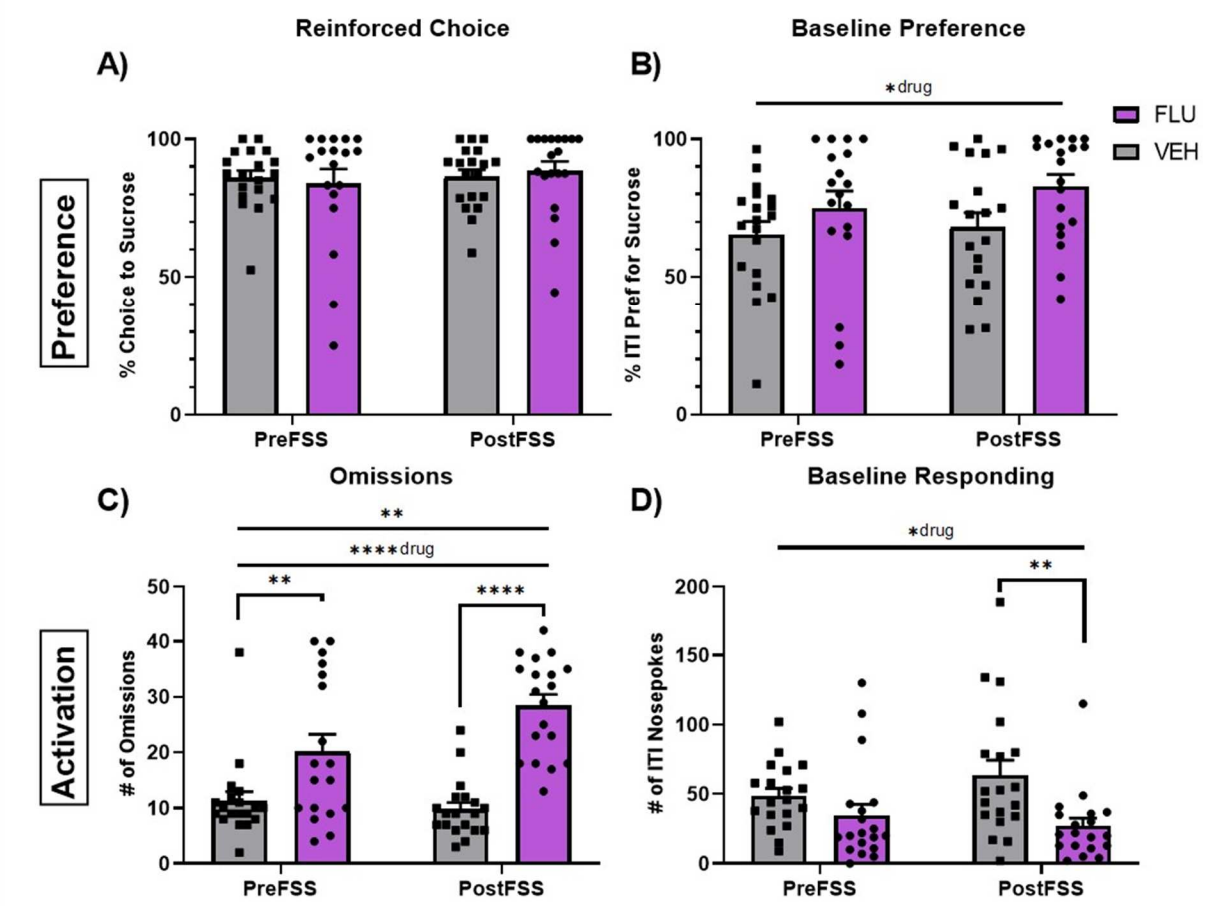


Figure 4. Influence of DA antagonism on decision-making behaviors. A) Averaged percentage of reinforced choice for the sucrose liquid receptacle in free-choice trials in sessions before (PreFSS) and after (PostFSS) the rFSS for animals receiving either flupenthixol (20µg/0.5µL; n=19) or vehicle (physiological saline; n=19). **B)** Percentage of ITI nosepokes made during the session for the sucrose liquid receptacle compared to water receptacle nosepokes during the ITI. **C)** Total number of omissions made during the sessions. **D)** Total number of ITI nosepokes made at all during the sessions. Error bars are (±SEM). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

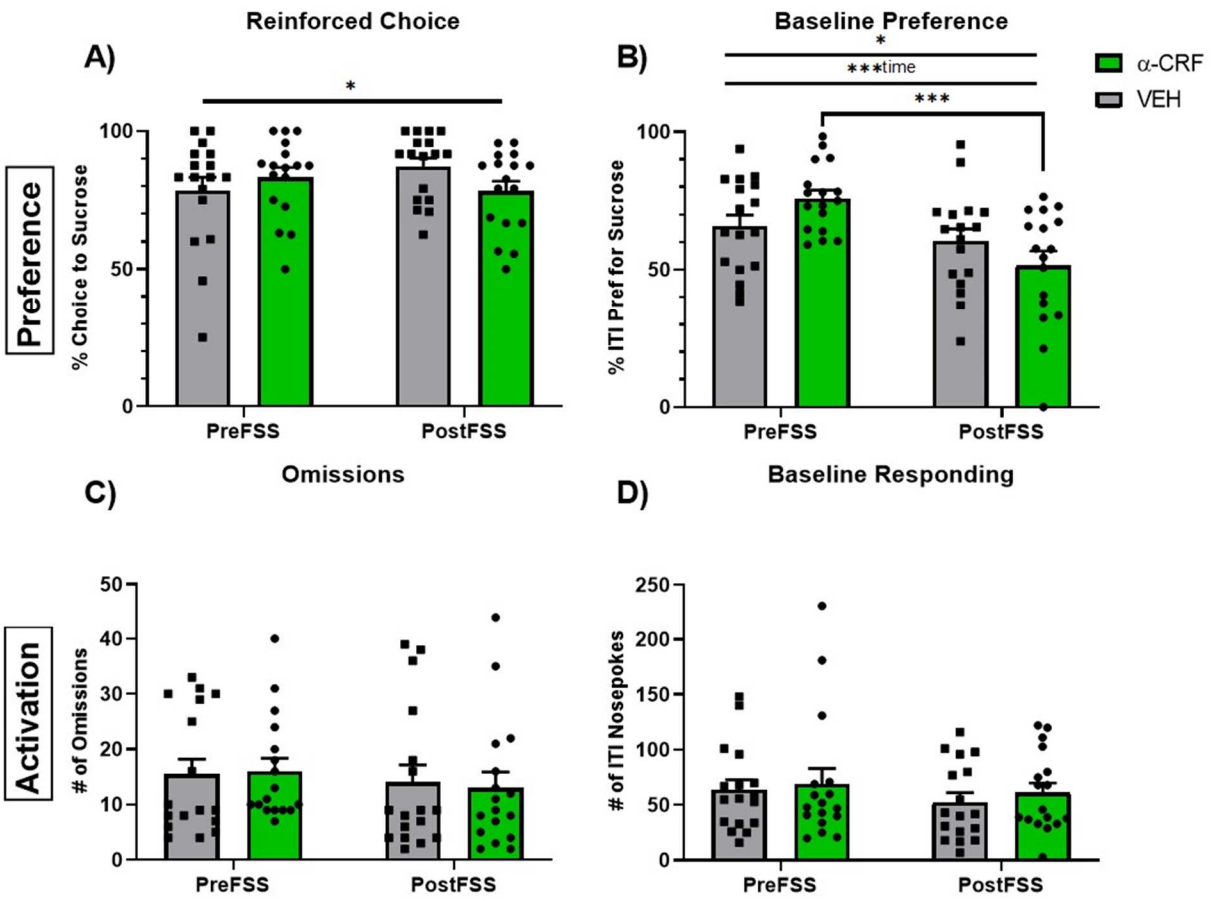
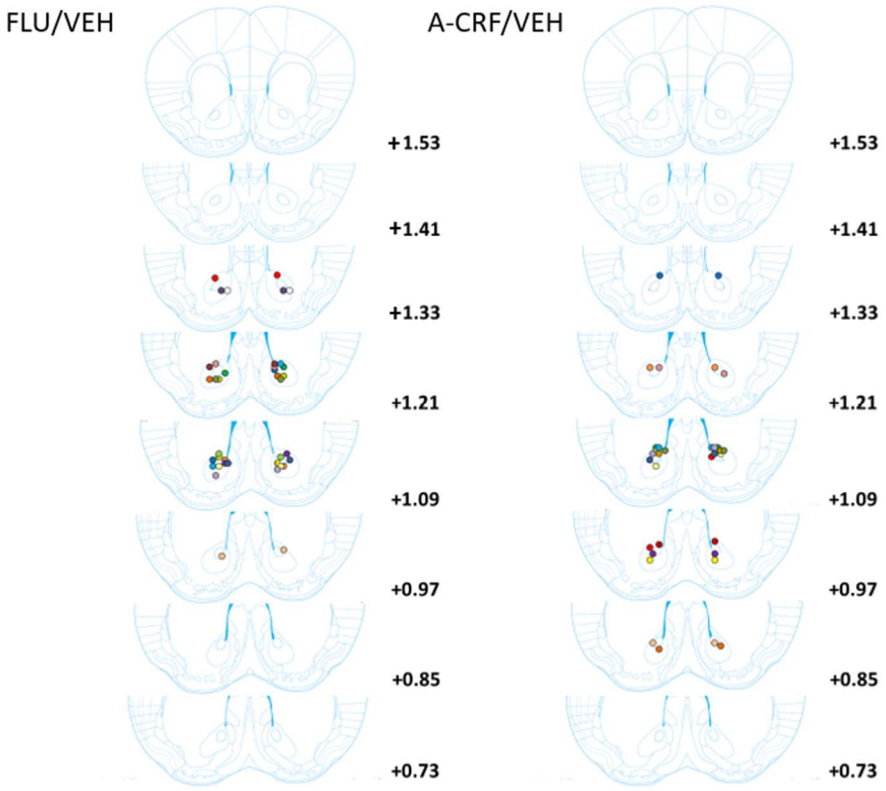
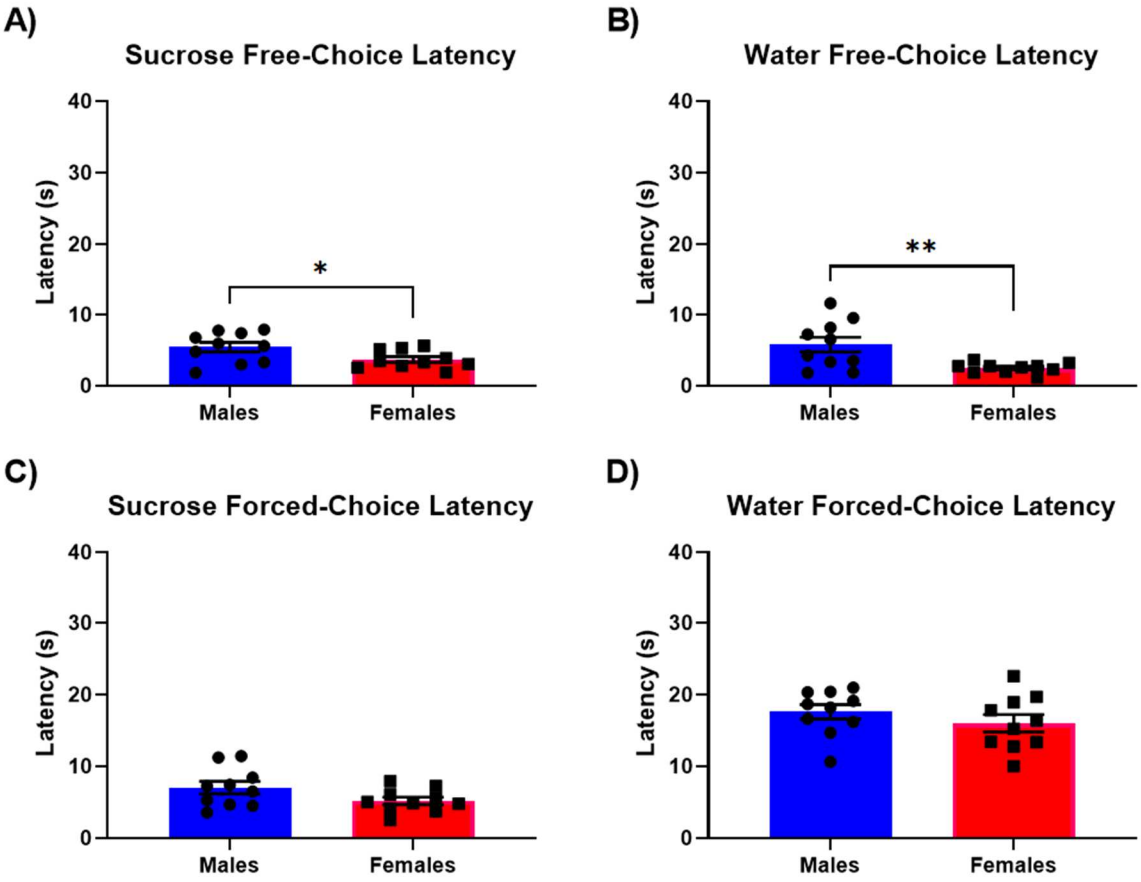


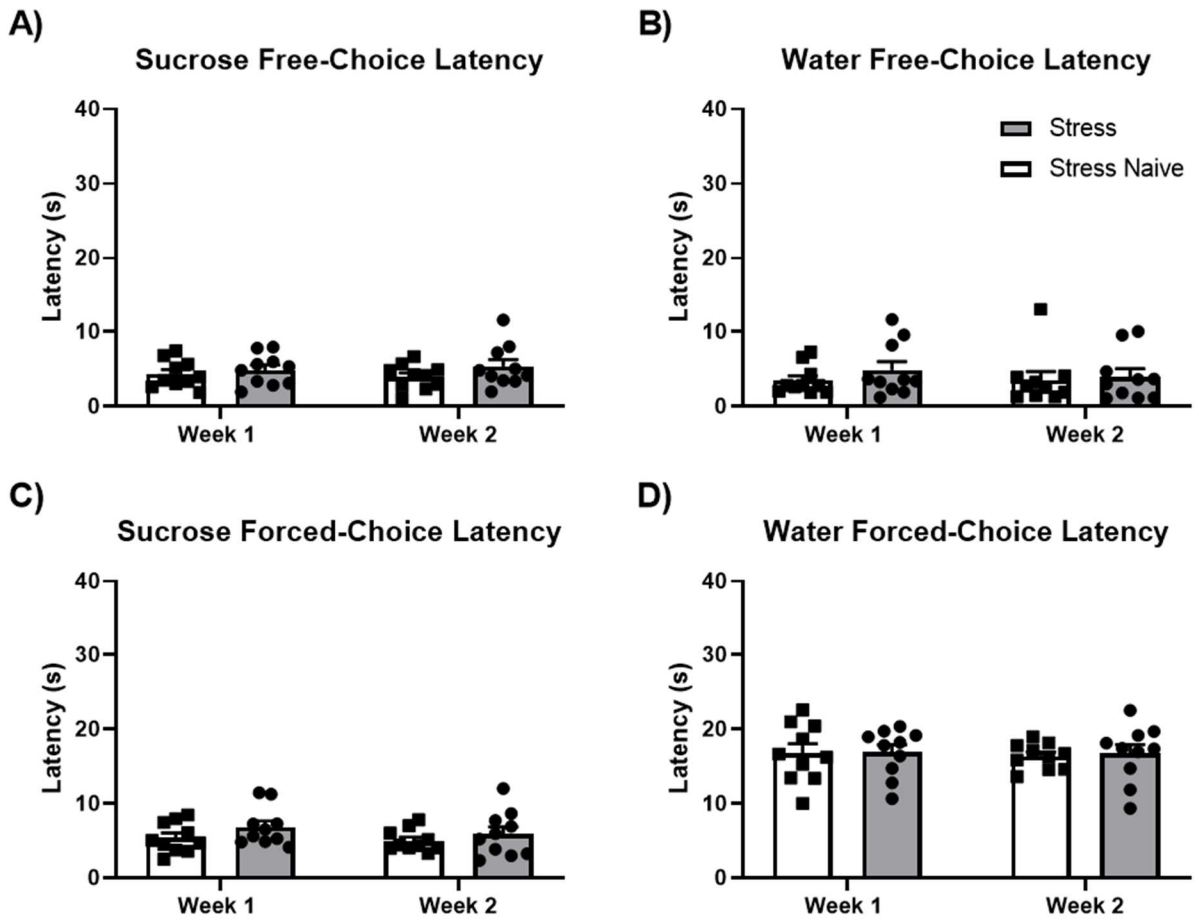
Figure 5. Influence of CRF antagonism on decision-making behaviors. A) Averaged percentage of reinforced choice for the sucrose liquid receptacle in free-choice trials in sessions before (PreFSS) and after (PostFSS) the rFSS for animals receiving either α-helical CRF $_{(9\text{-}41)}$ (500ng/200nL; Tocris Bioscience; n=17) or its vehicle (0.01% acetic acid in lactated ringer's solution; n=17). **B)** Percentage of ITI nosepokes made during the session for the sucrose liquid receptacle compared to water receptacle nosepokes during the ITI. **C)** Total number of omissions made during the sessions. **D)** Total number of ITI nosepokes made at all during the sessions. Error bars are (±SEM). *P<0.05, ***P<0.001



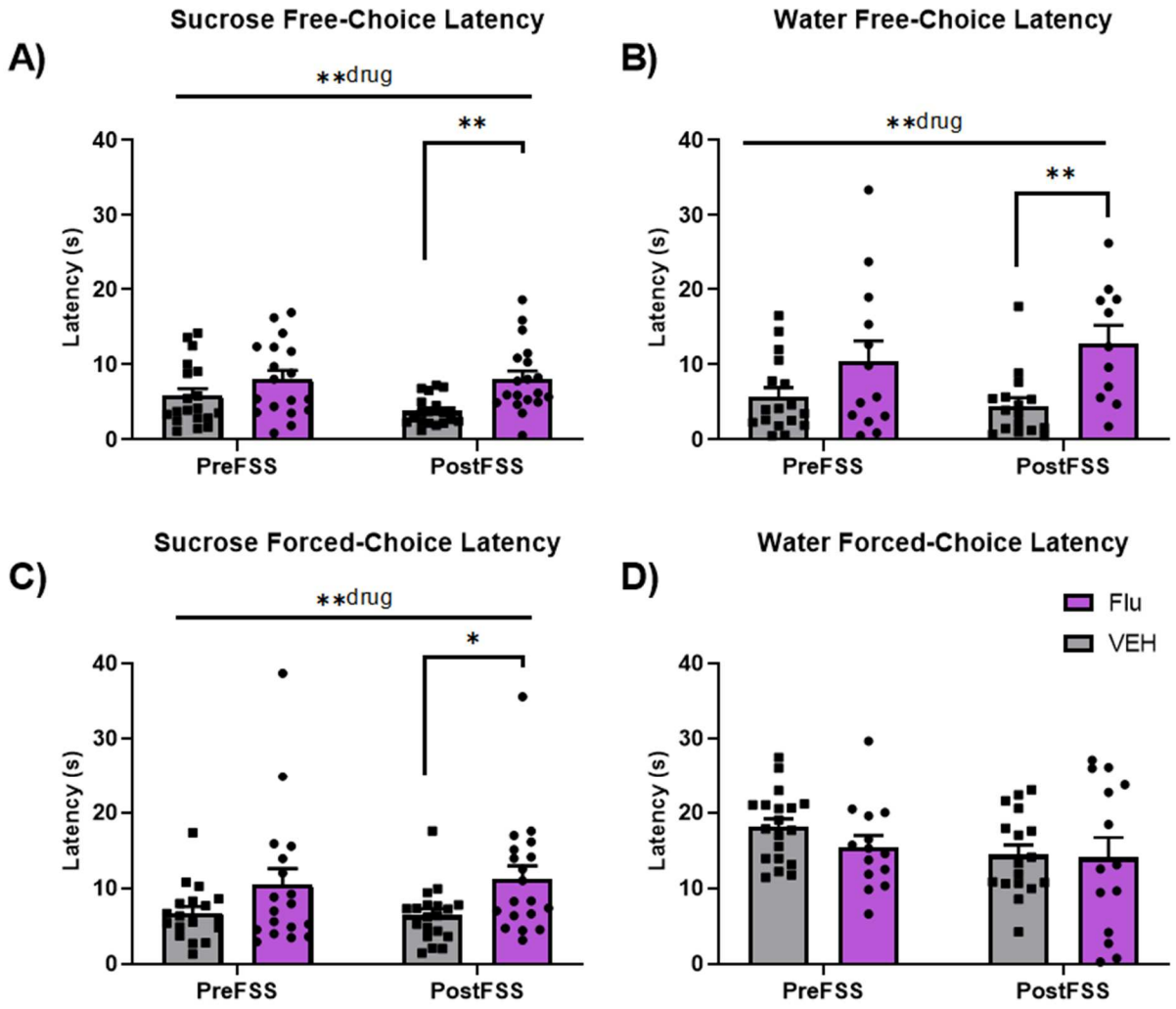
Supplementary Figure 1. Histological verification of injection site. Injection sites for either the cohort that received DA antagonism (right) or CRF antagonism (left). Numbers beside each section indicate the anterior distance in mm from bregma. Atlas adapted from Paxinos and Franklin (2008).



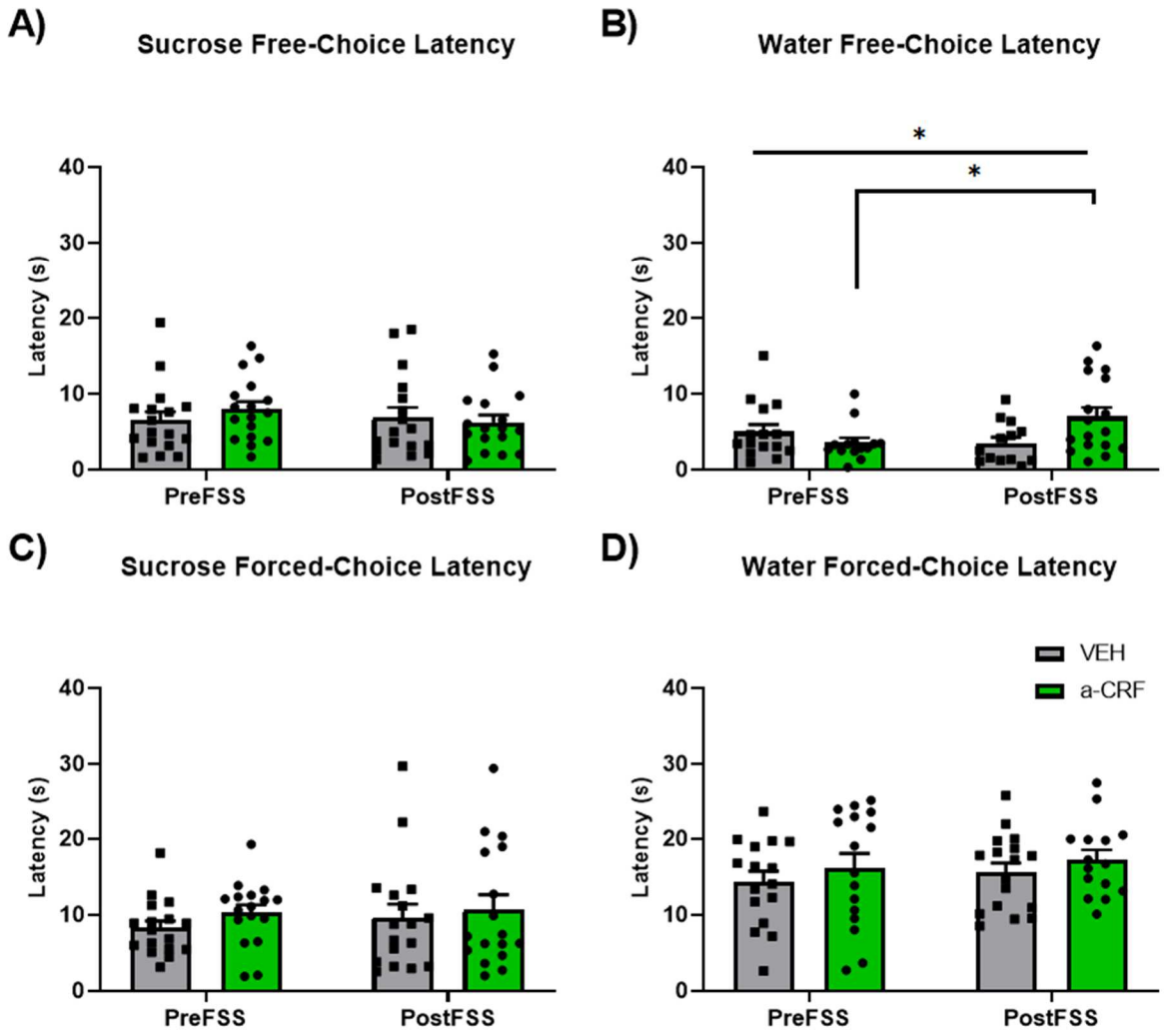
Supplementary Figure 2. Stress-naïve state comparison of male and female latencies to choose. The data here represent the latency from the time of cue onset to the time the animal made a choice during each type of trial for either male (n=10) or female (n=10) mice. **A and B)** Male mice in a stress-naïve state seemed to deliberate longer during free-choice trials for either water or sucrose. **C and D)** Males and females did not differ in the latency to respond during forced-choice trials. Error bars are (±SEM). *P<0.05, **P<0.01



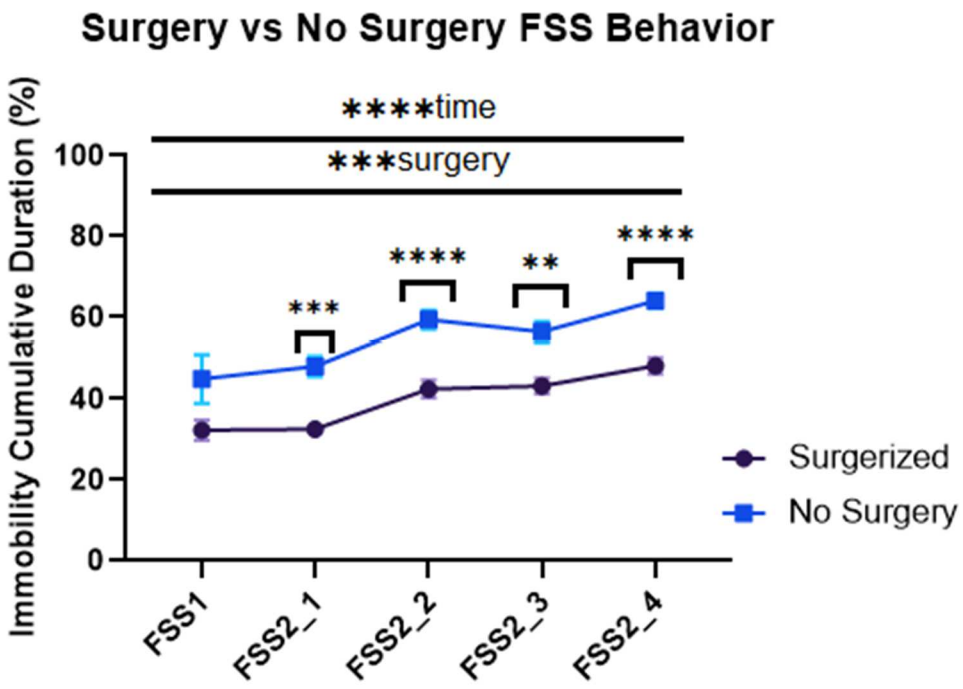
Supplementary Figure 3. Influence of stress on latencies during free- and forced-choice trials. The data here represent the latency from the time of cue onset to the time the animal made a choice during each type of trial for either stressed (n=10) or stress-naive (n=10) mice. **A and B)** Stress did not impact latencies to choose during free-choice trials for either water or sucrose. **C and D)** Stress did not impact latencies to respond during forced-choice trials. Week 1 represents the average latencies before the rFSS (or rest days), and Week 2 is the average of latencies following the stress manipulation. Error bars are (±SEM).



Supplementary Figure 4. Influence of DA antagonism on free- or forced-choice latencies. A and B) Flupenthixol (20µg/0.5µL; n=19) or vehicle (physiological saline; n=19) administered animals differed in their latencies to choose for sucrose and water during free-choice trials and the increase in latency for animals that received flupenthixol was stronger following stress (postFSS). **C)** DA antagonism increased the latency to respond during sucrose forced choice trials, especially following stress. **D)** Latencies were not impacted by drug administration during water-forced choice trials. Error bars are (±SEM). *P<0.05, **P<0.01



Supplementary Figure 5. Influence of CRF antagonism on free- and forced-choice latencies. A) Animals receiving either α-helical CRF $_{(9\text{-}41)}$ (500ng/200nL; Tocris Bioscience; n=17) or its vehicle (0.01% acetic acid in lactated ringer's solution; n=17) did not differ in their latency to choose during free-choice trials. **B)** There existed an overall stress x drug interaction during water free-choice trials and animals that received CRF antagonist after stress had higher latencies than receiving the drug before stress. **C and D)** CRF antagonism did not affect sucrose and water forced-choice latencies to respond to the cue. Error bars are (±SEM). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001



Supplementary Figure 6. Effect of implant surgery on immobility cumulative duration percentages during forced-swim stress. Animals that received bilateral cannulation implants had lower immobility times than animals that did not receive surgery. Data were calculated in percentages to reflect the amount of time spent immobile. FSS1 was 15 minutes. FSS2 were 6-minute bouts separated by 6 minutes. Error bars are (±SEM). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

CHAPTER 3

Dissociable Encoding of Expected Benefits but Not Effort Costs by Mesolimbic Dopamine Persists Through Overtraining in Stable Environments.

Rapheal G. Williams, Nick G. Hollon, Monica M. Arnold, Stefan G. Sandberg, Mark E. Walton, Paul E.M. Phillips

ABSTRACT

Dopamine transmission in the nucleus accumbens has been shown to encode reward prediction errors and influence effortful reward-seeking behavior. We have shown that expected reward magnitude, but not effortful costs, are coded by dopaminergic reward prediction errors. Specifically, we observed greater cue-evoked dopamine release to options that have a high reward magnitude even when an animal prefers an option with a lower reward value. This demonstrates that dopaminergic cached values are not the proprietary basis of choice behavior in a decision-making task that has daily, changing contingencies. Therefore, it remains unclear how dopamine transmission encodes options in an overtraining task, where the options in both reward- and effort-based tasks remain in the same position throughout the experiment and the animal can reliably anticipate effort costs. In the current study, we used fast-scan cyclic voltammetry to record phasic dopamine release in the nucleus accumbens to observe how dopamine reflects the cached-value between two effort and reward options during flexible and inflexible decision-making. We predicted that dopamine release should reflect effort-discounted action values only after an extensive training regimen with unchanging contingencies in a stable environment that leads to inflexible behavior since this effect was not seen with a more flexible regimen. We observed inflexible behavior in animals following overtraining in a reversal task compared to their flexible counterparts. Dopamine release did not reflect utility of either high- or low-effort options, regardless of training regimen, but it did accurately encode reward magnitude

for either a high- or low-reward option even when the animal's subjective preference was inflexible. These results further give evidence to dopamine encoding reward magnitude, but not anticipated effort costs during economic decision-making.

INTRODUCTION

State-action values can be stored by organisms to guide future choices (Daw & Doya, 2006; Morris et al., 2006; Rangel et al., 2008; Lau et al., 2008; Kable & Glimcher, 2009; Da Silva et al., 2018). Dopamine (DA) transmission in the nucleus accumbens is particularly important for the invigoration of effortful reward-seeking behavior and enabling responding to reward-predictive cues (Montague et al., 1996; Schultz et al., 1992, 1997; Bayer & Glimcher, 2005; Steinberg et al., 2013; Hart et al., 2014; Diederen et al., 2017). Moreover, cue-evoked dopamine is thought to encode the subjective value, or expected utility, of actions (Fiorillo et al., 2003, 2013; Tobler et al., 2005; Day et al., 2010; Gan et al., 2010; Nasrallah et al., 2011; Sugam et al., 2012; Pasquereau & Turner, 2013; Lak et al., 2014; Hamid et al., 2015) Mesoaccumbal DA reflects reward magnitude, with greater phasic bursts following rewards of larger magnitudes and their associated discrete cues, leading to the idea that dopamine serves as a stored, or cached-value, signal (Gan et al., 2010). What is unclear though is how phasic dopamine release is associated with effort. Despite this prominent role in effortful reward seeking, there have been inconsistent findings regarding the extent to which effortful response costs are incorporated into the dopaminergic prediction-error signals evoked by reward-predictive cues (Ravel & Richmond, 2006; Wanat et al., 2010; Gan et al., 2010; Day et al., 2010; Pasquereau and Turner, 2013; Hollon et al., 2014; Hamid et al., 2015).

We and others have observed dissociable encoding of expected benefits but not response costs by cue-evoked dopamine transmission (Ravel and Richmond, 2006; Gan et al., 2010;

Wanat et al., 2010; Pasquereau & Turner, 2013; Hollon et al., 2014), demonstrating circumstances in which there are mismatches between dopamine release and subjective value revealed by animals' choices (Gan et al., 2010; Hollon et al., 2014). However, there also have been reports of circumstances in which cue-evoked dopamine transmission more reliably reflects anticipated effort costs (Day et al., 2010; Varazzani et al., 2015). Wanat and colleagues (2010) show that either in a fixed ratio or progressive ratio (escalating costs over each trial) that DA release in the nucleus accumbens (NAc), measured with fast-scan cyclic voltammetry (FSCV), positively correlates with reward delivery, but not cue presentation, following a larger effort expenditure. In another study, when experimenters alter the amount of lever presses for each mg of sucrose to linearly fix price to reward, they found that DA release was negatively correlated with the amount of lever presses the animal had to make (Schelp et al., 2017). This is supported by another rodent study that found an increase in phasic DA release to immediate or low effort options in the task, but in this study, animals were overtrained to their reward and effort contingencies (Day et al., 2010). In monkeys, effort and expected reward value, in relation to the effort required to obtain the reward, show similar patterns of DA release to the rodent studies above, but also give evidence of norepinergic coding of single-dimensions, scaling effort and reward options (Pasquereau & Turner, 2013; Varazzani et al., 2015).

What is unclear though is when two concurrent choices are offered in an effort and reward task structure in the same study, how does the cached-value signal of DA lead to action selection? One study did include manipulating multiple dimensions of value-based decision making and found that DA accurately reflected animals' subjective value and action selection (Lak et al., 2014). We have shown that in a mixed-contingency task that DA cached value does not reflect an animal's choice behavior (Hollon et al., 2014). Instead, phasic DA release in the

NAc coded for reward magnitude, and in the case of choosing for reward, DA reflected the preferred option as seen in other works (Fiorillo et al., 2003; Tobler et al., 2005; Nasrallah et al., 2011; Sugam et al., 2012; Pasquereau & Turner, 2013; Lak et al., 2014). Because the animals' contingencies change from a right or left lever every session in our previous, we tested if this remains to be true even when an animal is overtrained (non-changing contingencies; Day et al., 2010) or forced to switch every test day on choosing low vs. high effort contingencies or low vs. high reward options (Hollon et al., 2014). Unlike in our previous study using a mixed-contingency task, cohorts were run in parallel to each other so that reward and effort could be manipulated separately. We have asked 2 questions: 1) How does either flexible or inflexible decision-making during both reward- and effort-based tasks influence phasic release of mesolimbic dopamine to discrete cues associated with each contingency? 2) How are DA dynamics influenced by a reversal task following flexible and habitual decision-making. We hypothesize thst DA release reflects effort-discounted action values only after an extensive training regimen with unchanging contingencies in a stable environment (Day et al., 2010) that result in inflexible behavior. Accordingly, we hypothesize that DA release does not incorporate effort costs to the extent that animals' preferences would be influenced by a flexible, effort-discounting paradigm with daily, changing contingencies.

METHODOLOGY

Subjects and Surgery

A total of 34 male Sprague-Dawley rats (Charles River Laboratories) that weighed between 250-300 g upon arrival were used for this study. Fourteen rats in the effort cohort and 14 in the reward cohort completed recording sessions included in this study. Two animals were excluded because of electrode misplacement, 3 for failure of electrodes to satisfy criteria for

dopamine detection, and 1 because of postsurgical complications (e.g., headcap loss). Rats were maintained on a 12-h light/dark cycle (lights on at 0700; lights off at 1900), with all behavioral testing occurring during the light phase. Rats were pair-housed until surgery, after which they were housed individually. Rats were anesthetized with isoflurane for bilateral implantation of carbon-fiber microelectrodes (Clark et al., 2010) targeting the nucleus accumbens core (1.3 mm anterior, 1.3 mm lateral, 6.8–7.0 mm ventral to bregma) and a Ag/AgCl reference electrode was implanted posterior to bregma to just below the dura mater. After at least 1 week of recovery post-surgery, rats were food-restricted to 90% of their ad libitum body weight; for all subsequent behavioral procedures, each rat received a total of ~15 g of food consisting of pellets earned as reward during behavioral sessions plus standard laboratory chow after these sessions. Water was available ad libitum in the animals' home cages. All procedures were approved by the University of Washington Institutional Animal Care and Use Committee (IACUC).

Initial Behavioral Training

In their homecages, prior to the first session of training, the food-restricted rats were exposed to the 45-mg food pellets (Bio-Serv dustless precision pellets) that served as rewards for the remainder of the experiment. All training sessions were conducted in one of four standard operant chambers (Med Associates, VT). Each chamber was equipped with a central food magazine and magazine light, a retractable lever on either side of the magazine (6 cm above the grid floor), a cue light above each lever, a house light at the top back left corner of the chamber, and a ventilation fan on the back wall of the sound-attenuating cabinet around the operant chamber to further attenuate external noises (e.g., other behavioral chambers or experimenter noise).

Rats underwent one session of magazine training (free pellets) in which a total of 60 food pellets were delivered non-contingently, one at a time with a variable time interval (60 ± 20 s) before transitioning to autoshaping sessions. One of the two cue lights was illuminated (left or right side counterbalanced between rats), the corresponding lever was extended continuously for the duration of the session, and each press was reinforced on a fixed ratio (FR) 1 continuous reinforcement schedule until rats received 100 pellets in a two-hour session. If rats did not press the lever after 15-20 min of the lever being extended, a pellet was placed behind the lever to encourage the rat to interact with the lever. In the next session, the other cue light was illuminated, and the lever extended for another 100-pellet session reinforced on a continuous FR-1 schedule.

All subsequent sessions consisted of training on discrete trials, such that after completing the response requirement, the cue light turned off, the lever retracted, a pellet was delivered into the central food magazine, and the magazine light was illuminated for six seconds, followed by a variable inter-trial interval (ITI). At this stage of initial training, only one lever was available on each trial (all the trials were "Forced" operant conditioning trials), and each session consisted of 80 total trials (40 for each lever) to mimic the number used in test sessions. Each trial began at the end of the ITI, and the cue light preceded the lever extension by 5s. Rats completed one session of FR-1 with a 20 ± 5 s ITI and unlimited time to initiate responding on each trial which was decreased to 1 min, 30s, and finally 10s to initiate the trial. Animals had 1 minute to complete the FR requirement. For all subsequent behavioral sessions, rats were connected to a head-stage containing a voltammetric amplifier to habituate them to the equipment used for eventual recording sessions. While tethered, subsequent training sessions consisted of one session each of FR-1 with a 20 ± 5 s ITI, FR-4 with a 30 ± 10 s ITI, FR-8 and FR-16 with a 45 ±

15 s ITI for all sessions thereafter. Starting in the FR-16 session, failure to initiate responding within 10 s of lever presentation resulted in an omission of the trial. Training on FR-16 sessions continued until rats completed over 90% of the trials in a session.

Reward and Effort Decision-Making Tasks

Rats, after being assigned to one of four groups in a 2 x 2 design, then performed 10 behavioral decision-making sessions that included 10 pseudorandom blocks of four single-option forced-choice and four dual-option free-choice trials, during which either the reward magnitude or effort requirement differed between the two options (Gan et al., 2010). In free-choice trials, the unchosen lever retracted, and the cue light turned off once rats made an initial press on the chosen lever. A 45 ± 15 s variable inter-trial interval separated each discrete trial, with 80 trials per session. As in prior training the lever(s) extended 5 s after the onset of the cue light(s). For the reward manipulation sessions (n=14), each option required 16 lever presses, with one lever yielding four pellets (HR; high reward option) and the other yielding one pellet (LR; low effort option). For the effort manipulation sessions (n=14), each option yielded one food pellet, with one requiring one lever press (LE; low effort option) and the other requiring 16 presses (HE; high effort option). The flexible or inflexible contingencies assigned to each lever side were either reversed (n=7 per cohort) between each session or contingency remained the same (n=7 per cohort). Each rat performed daily sessions within either the reward or effort manipulation groups until it reached criterion (75% choice in a sliding window of 12 free-choice trials) in fewer than 80 trials for both lever side assignments. After reaching criteria in either manipulation, rats then advanced to the reward and effort decision-making sessions for 20 sessions. After day 20 of the behavioral sessions in our paradigm, behavioral flexibility was

probed in 3 test sessions in which the lever side contingencies were reversed from the contingency assigned on day 20. For the animals assigned in the flexible group, this reversal test was a continuation of the daily, changing contingencies.

Fast-Scan Cyclic Voltammetry Recording Sessions

Voltammetry recording sessions took place on day 10, day 20, reversal day 1, and reversal day 3. The chronically implanted carbon-fiber microelectrodes in the NAc core were connected to a head-mounted voltammetric amplifier for dopamine detection by fast-scan cyclic voltammetry as previously described (Clark et al., 2010). A potential of -0.4 V (versus the Ag/AgCl reference) was applied to the carbon fiber and ramped to +1.3 V and back at a rate of 400 V/s. This voltammetric scan was applied at a frequency of 60 Hz for ~40 min prior to recording the behavioral sessions to prime the electrodes and then at 10 Hz for ~20 min prior to and throughout the recording session. To confirm that electrodes could detect chemically verified dopamine, a series of unexpected food pellets were delivered before and after each recording session. The voltammetry data from a recording session were included in the analysis only if the pre- and post-session pellet deliveries elicited dopamine release whose cyclic voltammogram (electrochemical signature) achieved a high correlation ($r2 \geq 0.75$ by linear regression) with that of a dopamine standard. The current analyses consist of behavior from choice trials and cue-evoked dopamine transmission from forced trials during blocks 3-10 of these recorded sessions.

Statistical Analysis

Post-criterion choice proportions were compared to indifference using two-tailed, one-sample t-tests in Prism 9 (Graphpad). Voltammetry data analysis was carried out using software

written in LabView and Matlab, and area under the curve (AUC) and peak amplitudes were compared with two-tailed, unpaired t-tests in Prism 9 (Graphpad). Following 2000-Hz low-pass filtering, dopamine was isolated from the background-subtracted (one second prior to cue onset) voltammetric signal using chemometric analysis (Heien et al., 2005) using a standard training set based on stimulated dopamine release detected by chronically implanted electrodes (Clark et al., 2010). Dopamine concentration was estimated based on the average post-implantation electrode sensitivity. Noise spikes >1.5 nA versus the immediately preceding and following time points were removed (Gan et al., 2010), and the data were smoothed using a 0.5-s moving average.

Histological Verification

Animals were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg), and the recording site was marked by passing a current (~70 μA) through the carbon-fiber microelectrode for 20 s to make a small electrolytic lesion. Animals were perfused transcardially with physiological saline and then with four-percent paraformaldehyde in phosphate-buffered saline, in which brains also were post-fixed following removal from the skull. Brains were sunk in 15% sucrose solution in PBS for 24 hours, 30% sucrose for at least 72 hours, flash frozen in dry ice, sectioned coronally (30-60 μm) on a cryostat, mounted on slides, and stained with a 0.5% cresyl violet solution.

RESULTS

Following initial training to get animals to lever press for food pellets, food-restricted male Sprague-Dawley rats were divided into two cohorts: one cohort made decisions in an operant effort-based choice task, and the second cohort made decisions in a reward-based task. Within each training session, there were 80 trials separated into 10 blocks of 8 trials: the pseudo-

randomly assigned trials consisted of 4 forced-choice trials which signaled the availability of only one option and 4 free-choice trials in which both options were available. Animals followed discrete visual cues for the left or right (or both for free-choice trials) lever/reward contingency associated with that lever, and contingencies were counterbalanced between animals. After rats made their response, each trial ended with a 45 ± 15 s variable inter-trial interval (ITI). Trials began with cue light presentation for one or both options and 5 s later, the associated levers were extended. In the effort group, animals had to press the lever 1 (low effort, LE) or 16 (high effort, HE) times for 1 pellet. In the reward task, rats had to press the lever 16 times for either 1 (low reward, LR) or 4 (high reward, HR) pellets. During the training period, animals had to reach a criterion of choosing the option with the highest utility in either group (high reward choice or the low effort) 75 percent of all free choices before making decisions for 20 sessions of interest. Furthermore, animals in either the effort or reward cohorts were divided into groups that underwent either inflexible training (left- or right-side contingencies remained the same throughout the sessions) or flexible training (contingencies switched between left and right side daily). To test the habitual nature of responding flexibly or inflexibly, a reversal test was given to animals in which the contingencies were reversed from the final day of training for 3 days (Figure 1). Voltammetric recordings, via FSCV carbon-fiber microelectrodes, were done on days 10 and 20, and then on reversal test days 1 and 3.

Effort-based decision-making was compared between flexible and inflexible groups. In our original decision-making paradigm to test whether phasic dopamine encoded subjective preferences for reward and effort contingencies, animals made decisions in a mixed reward and effort decision-making task in which contingencies were reversed from the left or right lever daily (Hollon et al., 2014). Those results pointed out that cue-evoked phasic DA release coded

for the reward magnitude, but not effort costs, regardless of subjective preference. Specifically, animals preferred to exert less effort for a lower reward, but a large DA release was reliably for high reward in both a medium effort and high effort group. In a study by Day and colleagues (2010), phasic DA seemed to encode for anticipated effort costs following a long period of overtraining to induce inflexible behavior. Therefore, unlike in our previous study in which contingencies changed daily (Hollon et al., 2014), we aimed to test whether the flexibility in decision-making training, within each behavioral paradigm, could impact how DA encodes effort costs. This was done by assigning animals to undergo flexible or inflexible training. We took 4 snapshots of both post-criterion choice behavior and DA transmission during early training, extended training, reversal test day 1, and reversal test day 3.

As mentioned before, animals in the flexible effort-based decision-making task had to choose between a LE or HE option during free-choice trials and the lever that was assigned to LE or HE was switched to the opposite lever daily. Rats assigned to the flexible group demonstrated significant preferences (Figure 2a) for the low-effort option during early training (t_6= 4.027, P = 0.0069), extended training (t_6 = 4.701, P = 0.0033), reversal day 1 (t_6 = 4.325, P = 0.005), and reversal day 3 (t_6 = 5.49, P = 0.0015). Within each session, cue-evoked dopamine release (Figure 2b) was indistinguishable for the low-effort vs. high-effort options. Specifically, the calculated area under the curve (AUC; Figure 2c) nor the peak amplitudes (Figure 2d) for voltametric signals were not different during early training (t_8 = 1.26, P = 0.2433; t_8 = 0.4634, P = 0.6554), extended training (t_7 = 0.4840, P = 0.6432; t_7 = 1.27, P = 0.2447), reversal day 1 (t_7 = 1.287, P = 0.2389; t_7 = 0.3348, P = 0.7476), and reversal day 3 (t_7 = 1.602, P = 0.1532; t_7 = 0.109, P = 0.9162). This was not surprising, considering the task structure and the evidence of a lack of DA-encoded effort costs found previously (Hollon et al., 2014).

Overtraining, or inflexible training, led to more habitual behavior. Rats demonstrated significant preferences (Figure 3a) for the low-effort option throughout fixed training in both the early ($t_6 = 7.898$, $P = 0.0002$) and extended stages ($t_5 = 45.87$, $P < 0.0001$), but they did not update their behavior during the reversal test session days 1 ($t_5 = 2.076$, $P = 0.0925$) and 3 ($t_5 = 0.9205$, $P = 0.3995$). Interestingly, within each session, cue-evoked dopamine release (Figure 3b) was indistinguishable for the low-effort vs. high-effort options as evidenced by the AUC (Figure 3c) and peak amplitudes (Figure 3d) of the DA signals in early ($t_7 = 1.22$, $P = 0.262$; $t_7 = 0.9125$, $P = 0.3919$) and extended training ($t_5 = 0.338$, $P = 0.7491$; $t_5 = 0.4414$, $P = 0.6774$), but also during the first ($t_5 = 0.102$, $P = 0.9227$; $t_5 = 0.2759$, $P = 0.7937$) and third days ($t_3 = 1.259$, $P = 0.297$; $t_3 = 0.1012$, $P = 0.9258$) of reversal testing. Dissimilar to results found by Day and colleagues (2010), our overtrained/inflexible animals' DA did not encode anticipated effort. Therefore, regardless of training regimen, DA does not encode subjective value or effort costs, but we wanted to see how this effect of training impacted reward-based behaviors and the associated phasic DA release in the NAc.

We predicted DA to encode reward magnitude based on previous findings in ours and associated laboratories (Gan et al., 2010; Nasrallah et al., 2011, Hollon et al., 2014), but it was still unclear how the flexibility of the training could have an impact. Animals assigned to undergo flexible training demonstrated significant preferences (Figure 4a) for the large-reward option throughout flexible training in both the early ($t_6 = 3.051$, $P = 0.0225$) and late stages ($t_6 = 12.72$, $P < 0.0001$), but also in the first ($t_6 = 4.656$, $P = 0.0035$) and third ($t_6 = 20.24$, $P < 0.0001$) reversal test sessions. Cue-evoked dopamine release (Figure 4b) was greater for the large-reward vs. small-reward option, encoding the expected reward magnitude in all sessions. The AUC (Figure 4c) and peak amplitudes (Figure 4d) were larger for the HR option during early training

($t_9 = 5.599$, P = 0.0003; $t_8 = 3.474$, P = 0.0084), late training ($t_9 = 4.964$, P = 0.0008; $t_8 = 4.926$, P = 0.0012), reversal test 1 ($t_9 = 3.962$, P = 0.0033; $t_8 = 3.794$, P = 0.0053), and reversal test 3 ($t_9 = 2.358$, P = 0.0427; $t_8 = 2.485$, P = 0.0378).

With a fixed regimen to choose for 1 or 4 pellets (even with 16 presses), rats demonstrated significant post-criterion preferences (Figure 5a) for the large-reward option throughout fixed training during early ($t_6 = 794.9$, P < 0.0001) and extended training ($t_6 = 795$, P < 0.0001), but they did not update their behavior during the reversal test sessions 1 ($t_5 = 5.364$, P = 0.003) and 3 ($t_5 = 1.01$, P = 0.3588). They still chose the lever that had been previously paired with receiving HR even though they only received LR – an effect that seemed to dwindle by the third reversal session. Cue-evoked dopamine release (Figure 5b) encoded the expected reward magnitude in all sessions, even in the reversal test session when rats had not yet updated their behavioral choices. The AUC (Figure 5c) and peak amplitudes (Figure 5d) were larger in most cases during early ($t_{12} = 5.030$, P = 0.0003; $t_7 = 1.33$, P = 0.2254) and late training ($t_{12} = 3.022$, P = 0.0106; $t_7 = 0.5219$, P = 0.6178), but also during the first ($t_{10} = 3.459$, P = 0.0061; $t_7 = 1.351$, P = 0.2186) and third reversal sessions ($t_{10} = 2.015$, P = 0.0716; $t_7 = 1.717$, P = 0.1297). It is unclear why peak amplitudes were not significant in this group of animals, but it is possible that training regimen influences the peak amplitude of dopamine dynamics without changing subjective preference to the high reward option.

DISCUSSION

Extensive training with fixed contingencies led to inflexible behavior, indicated by rats' inability to update their choices following a contingency reversal. It is said that a large portion of our daily behaviors have already become habitual or ritualistic (Toffler, 1970). Habits are a result of repetitive actions that become routine-like, but this has been difficult to model in humans

(Graybiel, 2008; Watson & de Wit, 2018; de Wit et al., 2018; Linnebank et al., 2018). Overtraining in our mixed-contingency decision-making paradigm led to inflexible behavior, as evidenced by the persistence to choose a high-effort option on the same lever that a low-effort option was originally presented on, an effect not seen in animals trained on a contingency that was reversed daily. Overtraining has long been documented to be resistant to reversal learning (Gabriel et al., 1981; Mauk et al., 1983), outcome devalutation (Iguchi et al., 2017), and extinction (Dickinson et al., 1995), and it has been shown to have a birdirectional relationship between dopaminergic plasticity and reflexive and habitual learning, especially during instrumental conditioning (Shan et al., 2015; Radke et al., 2019). The relative insensitivity of mesolimbic dopamine to anticipated effort persisted despite this overtraining in stable, unchanging environments.

The reliable encoding of expected benefits was observed with either training history, and it preceded changes in animals' behavioral preferences. The replication of the same effect of how dopamine does not reflect a preferred option, but rather a cached value of reward magnitude, was observed (Hollon et al., 2014). This contests the idea of DA encoding a utility signal but continues to support the findings that dopamine reflects a learning signal of expected value of high or low reward (Gan et al., 2010). Animals still selected the most beneficial option, especially under food restricted options, so there must be neural correlates of anticipated effort, since in our paradigm, animals still occasionally explored a high-effort or low-reward option in forced-choice trials. Maybe the animals 'regretted' making those selections (Steiner & Redish, 2014; Sweis et al., 2018[a, b]).

Across either training regimen, mesolimbic dopamine transmission reflects expected benefits but does not incorporate effortful response costs to the extent that they influence animals'

subjective behavioral preferences. One study that supports this idea is one done by Lak and colleagues (2014) who altered subjective value in head-fixed mice to use a steering wheel to choose a left or right option when the stimulus (a grating pattern on a screen) is presented. Stimuli for either side represented rewards (water drops) of differing magnitudes led the mouse to move the steering wheel to the center to receive reward, and to manipulate cognitive effort, investigators change the contrast of the stimuli to the screen. They found that DA accurately reflected animals' subjective value and action selection. Another study found that in either a concurrent effort task (number of lever presses or duration of holding a lever for reward) and a concurrent value task (sucrose pellets vs sucrose solutions), effortful responding, but not value responding, was diminished by the dopamine receptor 2 antagonist, haloperidol. Although dopaminergic neurons in the striatum have been shown to encode flexible and fixed value memories, the work analyzed the caudate tail and head regions (Kim et al., 2014, 2015; Jiang & Kim, 2018).

Our results give evidence for anticipated effort during habitual and goal-directed behaviors to be represented elsewhere. Work by Yin and colleagues has given extensive evidence that habitual and goal-directed instrumental conditioning is largely regulated by structures within basal ganglia, namely the dorsolateral and dorsomedial striatum (Yin & Knowlton, 2006; Yin et al., 2004, 2005, 2006, and 2008; O'Hare et al., 2016). It is said that these systems, especially in the context of dopaminergic relevance, may compete with one another to guide action selection (Balliene & O' Doherty, 2010; Dezfouli & Balliene, 2013; Sommer et al., 2014). Competing systems are also commonly observed in tasks that have a risk or probabilistic component, where striatal activation is usually associated with the magnitude or expected value of one option and frontal cortices (PFC or OFC) being associated with probability (Tobler et al., 2007; Knutson et

al., 2005, 2008). The mPFC has been shown to have a negative correlation with paying unreasonable prices for a reward (Knutson et al., 2007), which gives evidence to the neural representation of costs. Cognitive flexibility, as well as anticipated reward stability, has been largely though to be influenced by the OFC (Riceberg & Shapiro, 2012 Gremel & Costa, 2013). Additionally, the anterior cingulate cortex has been shown to represent reward-state spaces, but this may not be applicable to our simple task when comparing it to complex human or primate behavior when analyzing either model-free or model-based decisions (Akam et al., 2021). It is reported that human goal-directed actions can be represented in the medial prefrontal cortex and posterior cingulate cortex, while habitual actions notably activate the insula, dorsal caudate, and the precentral gyrus. To make matters a bit more complex, the brain regions involved also depend on the learning stage of the task involved frontoparietal cortical structures and the dorsal striatum (Erymilaz et al., 2017).

Trying to make the correct decision is important, but that is not all there is to it. In most novel situations, one cannot know what the best decision is going to be. What is important are the decision one makes afterwards, and the effort put in to make that decision correct.

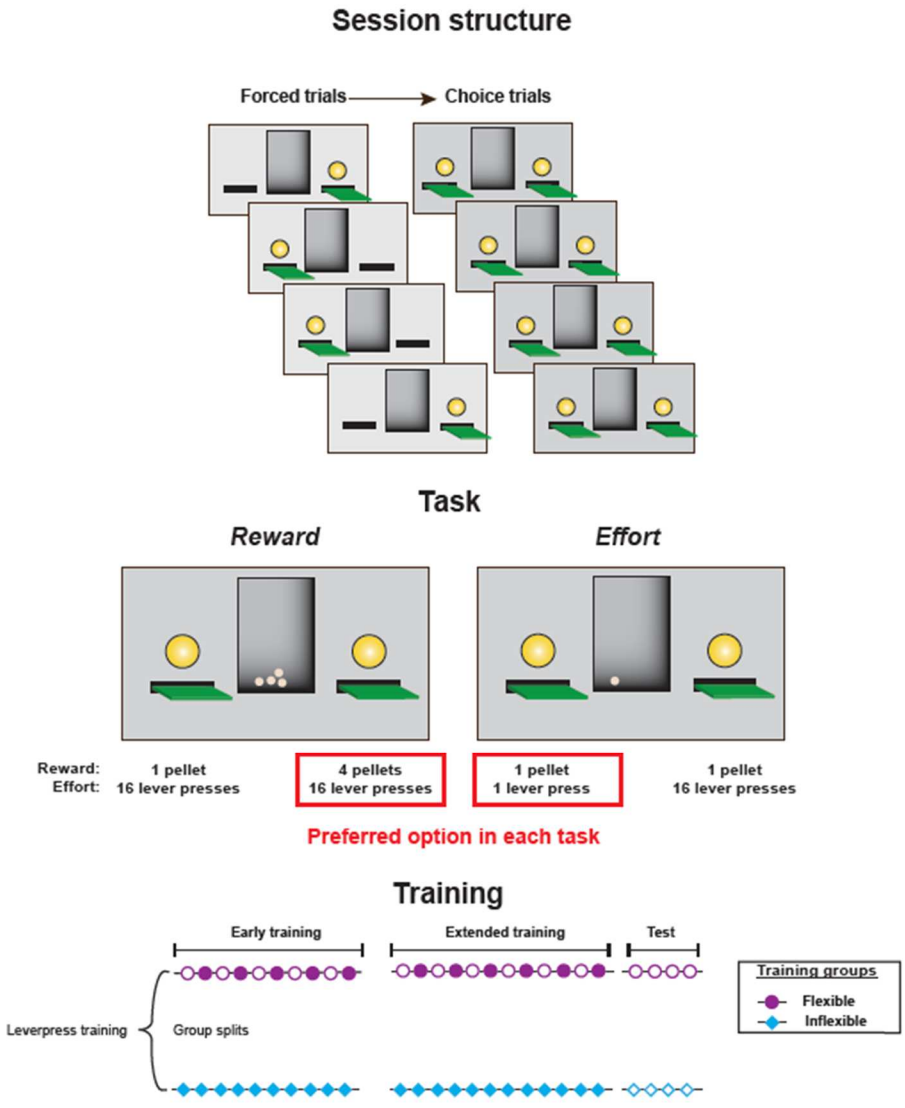


Figure 1. Task structure for effort- and reward-based decision-making. Following initial training and after reaching a criterion of over 75% choice for the preferred options (HR or LE; highlighted in red), rats made choices for food in a pseudo-randomly organized session consisting of 80 trials. The trials were broken down into 10 blocks: each block consisted of 4 forced-choice trials, and 4 free-choice trials. Within the task, animals in the reward cohort had to press a lever 16 times for either 1 or 4 pellets. Animals in the effort cohort had to press the lever 1 or 16 times to receive 1 food pellet. Animals were also split into two groups following auto-shaping and magazine training. The animals assigned to undergo flexible decision-making (purple) had their lever/reward contingencies changed daily from right or left (in a counterbalanced fashion). Rats in the overtraining, or inflexible group (blue), had contingencies that did not change throughout all 20 sessions of recorded behavior. All animals had to perform in a reversal test for 4 sessions with unchanging lever/reward contingencies to gauge how 'sticky' their behavior was following training.

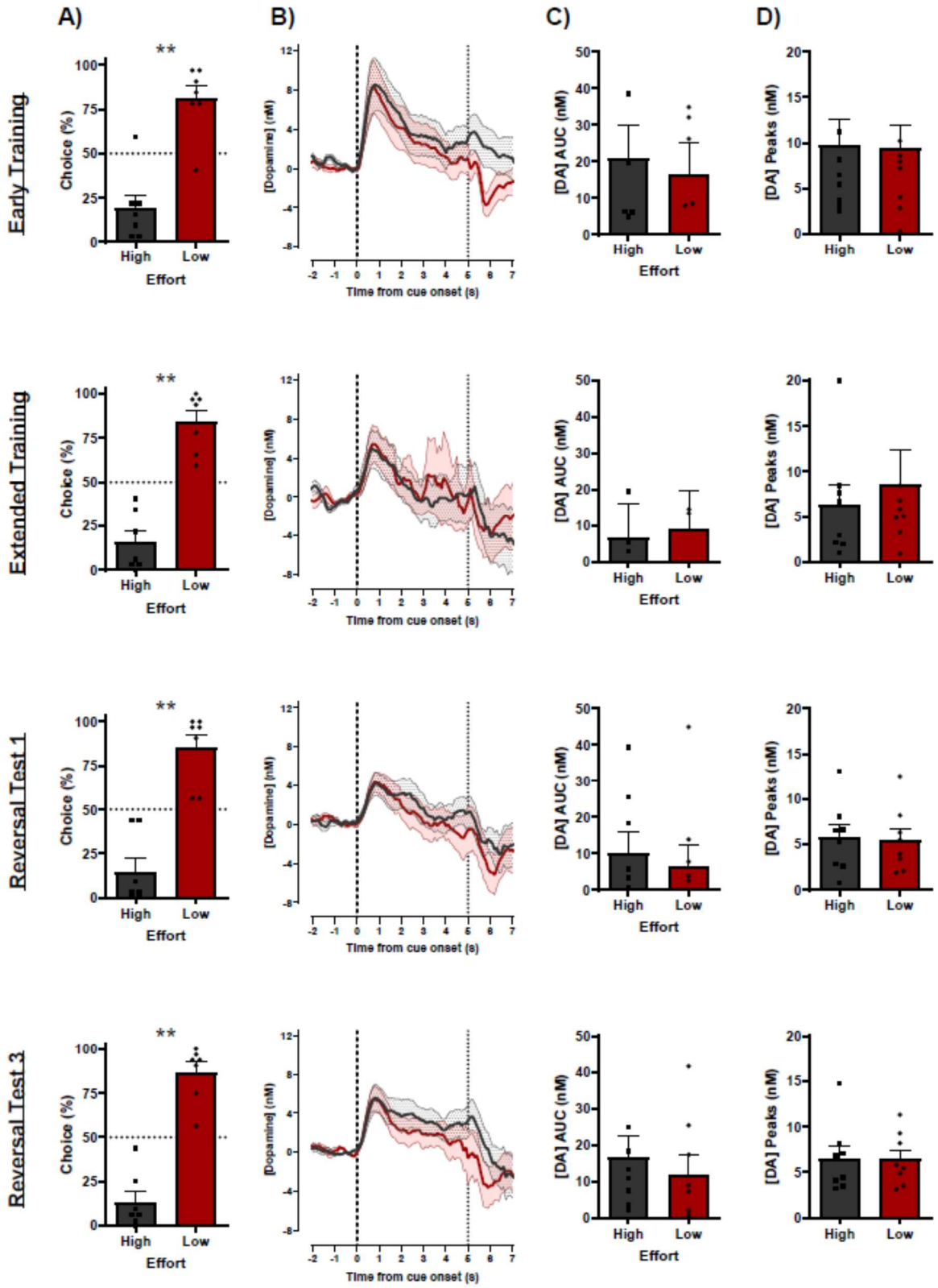

A)
B)
C)
D)
Early Training
Extended Training
Reversal Test 1
Reversal Test 3
Choice (%)
High
Low
Effort
**
[Dopamine] (nM)
Time from cue onset (s)
[DA] AUC (nM)
[DA] Peaks (nM)

Figure 2. Effects of performing effort-based decision-making task with a flexible design on choice and cue-evoked DA. Rats (n=7; 9 recording sites) had to choose between pressing the lever 16 times (black/grey color scheme) or 1 time (red color scheme) for 1 food pellet, and the associated lever which was assigned to 16 or 1 press(es) changed daily throughout the training until the reversal test. Each row represents a different time within the chronology of the experiment following initial behavioral training: early training data was taken from day 10, extended training data was taken from day 20, reversal tests 1 and 3 were the first and third days of reversal testing in which the lever/reward contingency was the opposite of the final training day. **A)** Choice percentage data was the percentage of the chosen option during free-choice trials for the LE or HE lever. **B)** Representative traces of recorded cue-evoked dopamine (nM) via FSCV averaged for the whole session during forced-choice trials for either the LE or HE. The cue (dashed line) was presented 5 seconds before the lever extension (dotted line). **C)** Calculated definite integral between each time point (100ms) to get area under the curve (nM) for the whole trace. **D)** Recorded peak amplitudes of dopamine released to cue for small or large anticipated effort cost. Error bars are (±SEM). **P<0.01

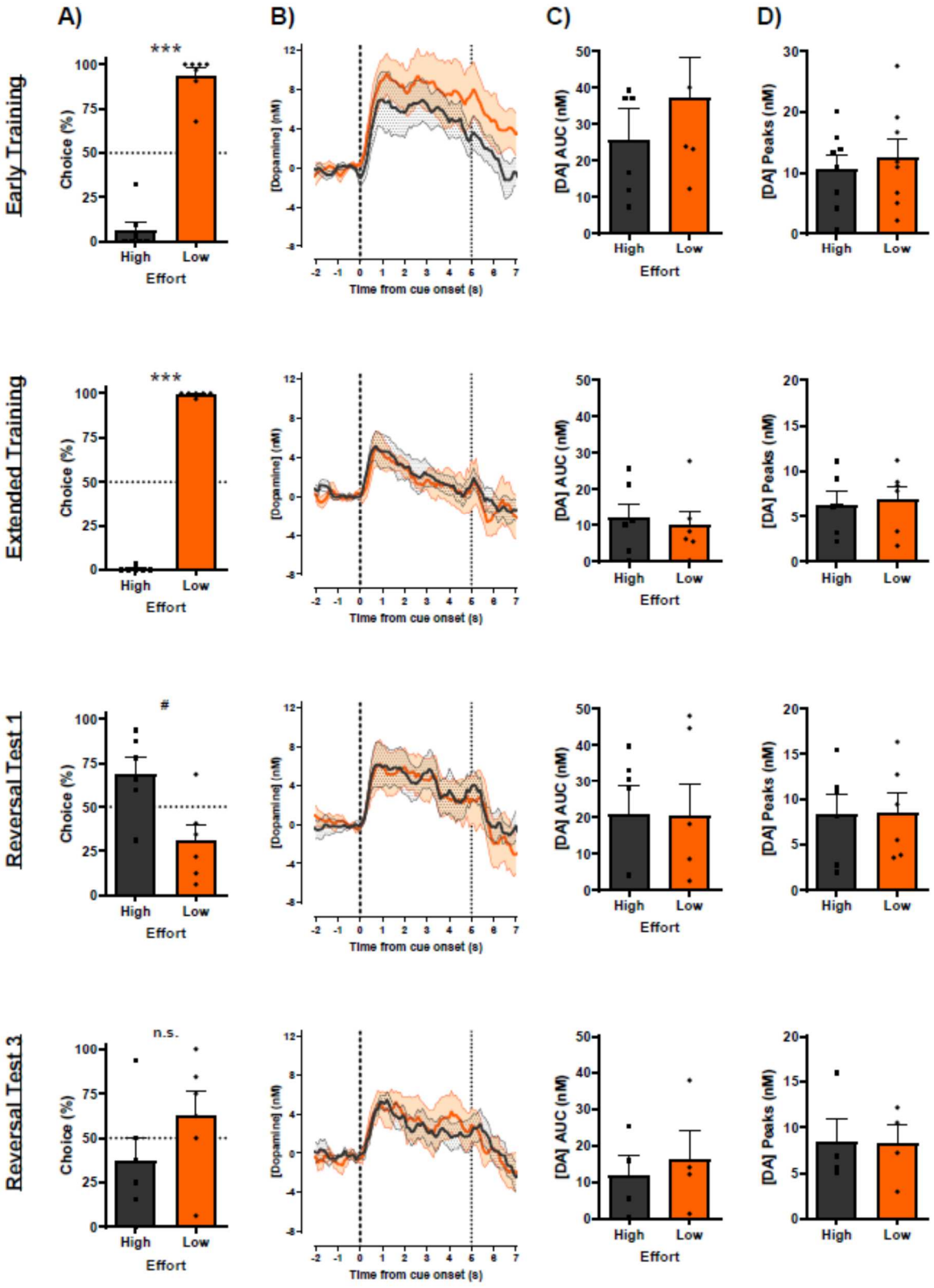


Figure 3. Effects of performing effort-based decision-making task with an inflexible design on choice and cue-evoked DA. Rats (n=7; 8 recording sites) had to choose between pressing the

lever 16 times (black/grey color scheme) or 1 time (orange color scheme) for 1 food pellet, and the associated lever which was assigned to 16 or 1 press(es) did not change throughout the training until the reversal test. Each row represents a different time within the chronology of the experiment following initial behavioral training: early training data was taken from day 10, extended training data was taken from day 20, reversal tests 1 and 3 were the first and third days of reversal testing in which the lever/reward contingency was the opposite of the training days. **A)** Choice percentage data was the percentage of the chosen option during free-choice trials for the LE or HE lever. **B)** Representative traces of recorded cue-evoked dopamine (nM) via FSCV averaged for the whole session during forced-choice trials for either the LE or HE. The cue (dashed line) was presented 5 seconds before the lever extension (dotted line). **C)** Calculated definite integral between each time point (100ms) to get area under the curve (nM) for the whole trace. **D)** Recorded peak amplitudes of dopamine released to cue for small or large anticipated effort cost. Error bars are (±SEM). *P<0.05, ***P<0.001, #P=0.0925

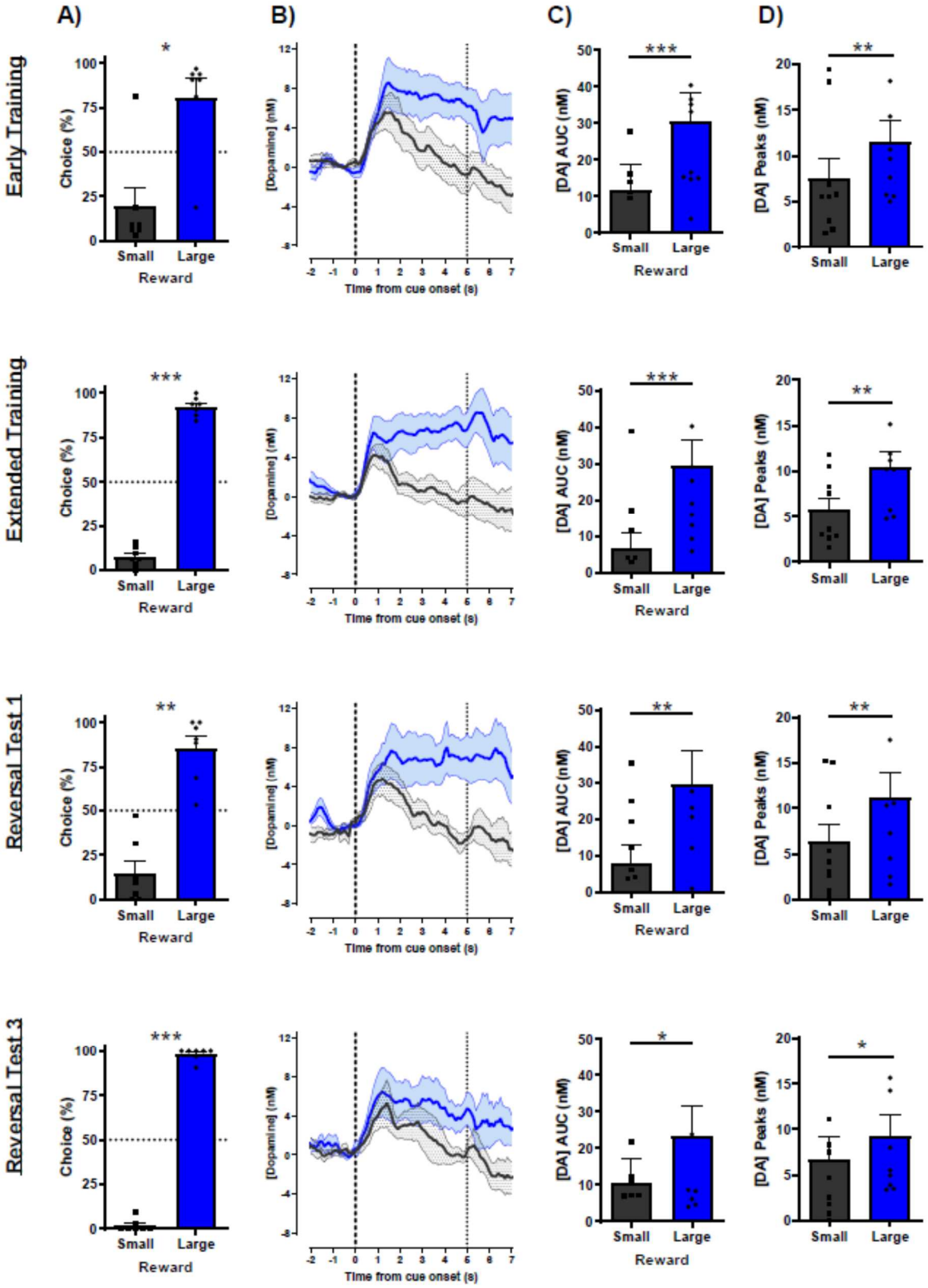

A)
B)
C)
D)
Early Training
Extended Training
Reversal Test 1
Reversal Test 3
Choice (%)
Small
Large
Reward
[Dopamine] (nM)
Time from cue onset (s)
[DA] AUC (nM)
[DA] Peaks (nM)

Figure 4. Effects of performing reward-based decision-making task with a flexible design on choice and cue-evoked dopamine. Rats (n=7; 10 recording sites) had to choose between 1 (black/grey color scheme) or 4 (blue color scheme) pellets with 16 presses and the associated lever which was assigned to 1 or 4 pellets changed daily from left to right and vice versa throughout the training until the reversal test. Each row represents a different time within the chronology of the experiment following initial behavioral training: early training data was taken from day 10, extended training data was taken from day 20, reversal tests 1 and 3 were the first and third days of reversal testing in which the lever/reward contingency was the opposite of the final training day. **A)** Choice percentage data was the percentage of the chosen option during free-choice trials for the LR or HR lever. **B)** Representative traces of recorded cue-evoked dopamine (nM) via FSCV averaged for the whole session during forced-choice trials for either the LR or HR options. The cue (dashed line) was presented 5 seconds before the lever extension (dotted line). **C)** Calculated definite integral between each time point (100 ms) to get area under the curve (nM) for the whole trace. **D)** Recorded peak amplitudes of dopamine released to either a small or large reward. Error bars are (±SEM). *P<0.05, **P<0.01, ***P<0.001

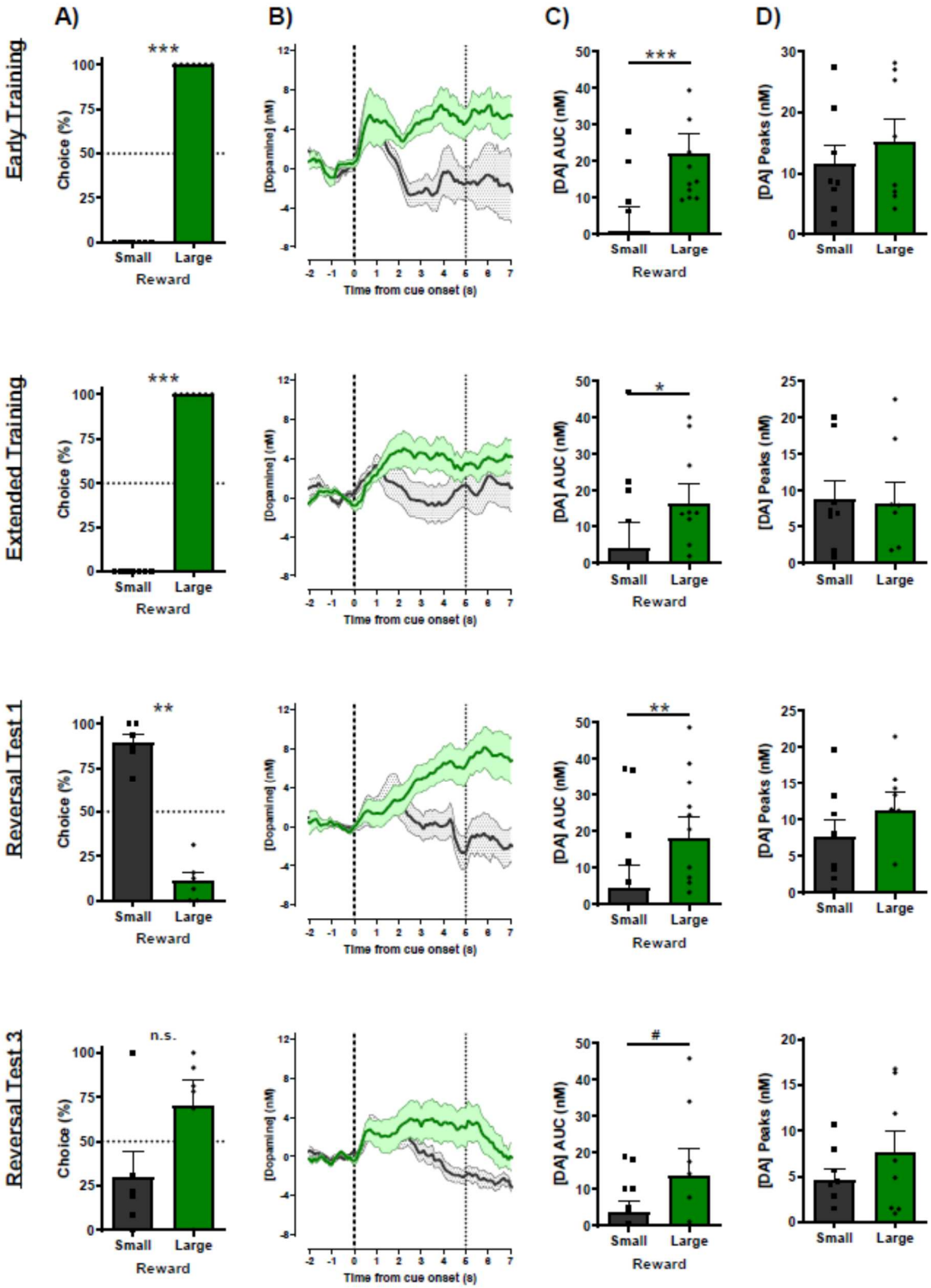
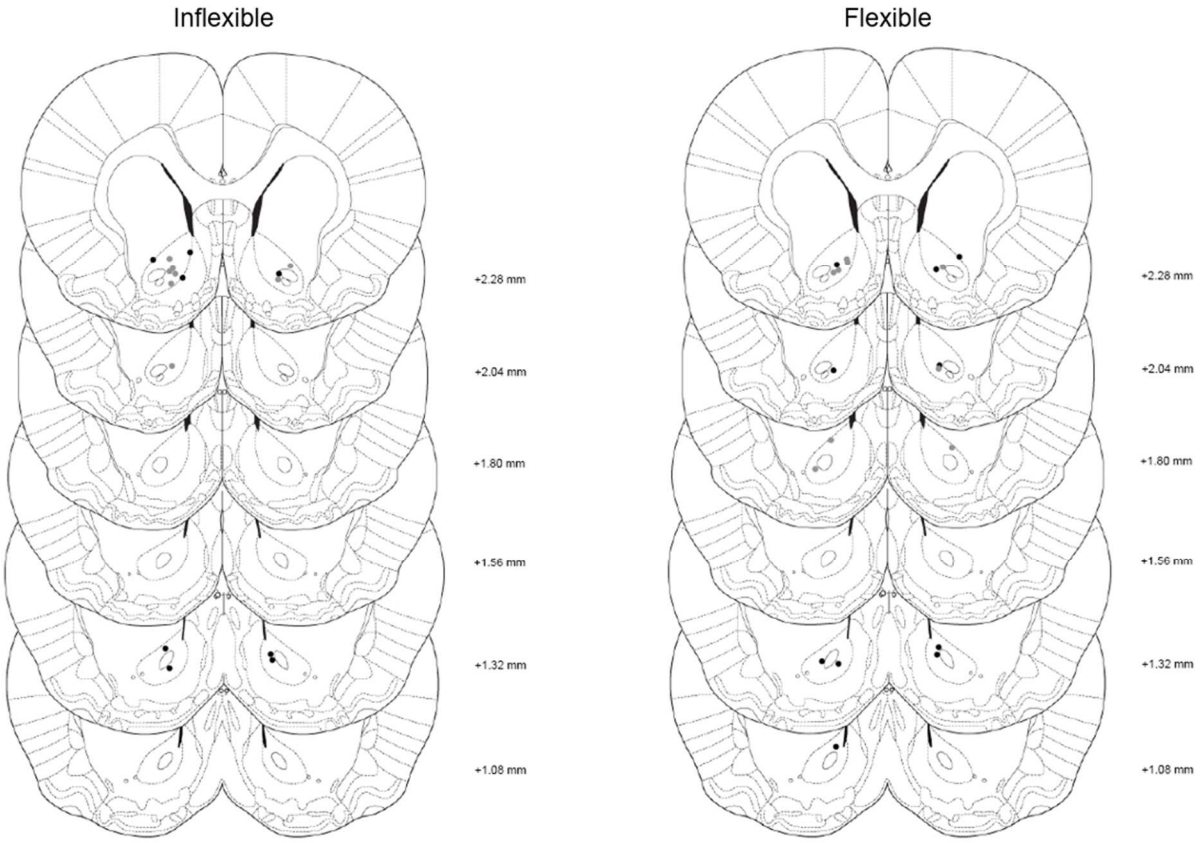

A)
B)
C)
D)
Early Training
Extended Training
Reversal Test 1
Reversal Test 3
Choice (%)
Small
Large
Reward
***
**
n.s.
[Dopamine] (nM)
Time from cue onset (s)
[DA] AUC (nM)
[DA] Peaks (nM)
*
#

Figure 5. Effects of performing reward-based decision-making task with an inflexible design on choice and cue-evoked dopamine. Rats (n=7; 13 recording sites) had to choose between 1 (black/grey color scheme) or 4 (green color scheme) pellets with 16 presses and the associated lever which was assigned to 1 or 4 pellets did not change throughout the training until the reversal test. Each row represents a different time within the chronology of the experiment following initial behavioral training: early training data was taken from day 10, extended training data was taken from day 20, reversal tests 1 and 3 were the first and third days of reversal testing in which the lever/reward contingency was the opposite of the training days. **A)** Choice percentage data was the percentage of the chosen option during free-choice trials for the LR or HR lever. **B)** Representative traces of recorded cue-evoked dopamine (nM) via FSCV averaged for the whole session during forced-choice trials for either the LR or HR. The cue (dashed line) was presented 5 seconds before the lever extension (dotted line). **C)** Calculated definite integral between each time point (100ms) to get area under the curve (nM) for the whole trace. **D)** Recorded peak amplitudes of dopamine released to either a small or large reward. Error bars are (±SEM). *P<0.05, **P<0.01, ***P<0.001, #P=0.0716



Supplementary Figure 1. Histological verification of carbon-fiber microelectrode placement site. Electrode placement sites verified by making an electrolytic lesion for either the cohort that underwent an inflexible (left) or flexible (right) training regimen. Reward cohort represented by grey dots and effort cohort represented by black dots. Numbers beside each section indicate the anterior distance in mm from bregma. Atlas adapted from Paxinos and Watson (2005).

CHAPTER 4

CONCLUSION

The information in this dissertation conveyed lessons from two different stories about quite orthogonal independent manipulations on decision-making behavior. In the first chapter, I gave an extensive background of DA, its involvement in reward-related behaviors, DA implications for neuroeconomic processes, and how stress can influence DA and choice behavior. I hoped to be able to tell a story about how the environment can influence complex behaviors, especially in the context of economic decisions and choice behaviors. The findings in Chapter 2 demonstrate that the story of how DA and CRF interact with stress in the NAc can be much more complicated than originally thought in the preceding study (Lemos et al., 2012). In Chapter 3, I showed that, in conjunction to accumbal, phasic dopamine's function as a cached-value of reward magnitude not being enough to code for action selection or subjective value (Hollon et al., 2014), DA does not code effort-based choices in neither an overtrained animal nor an animal whose reward contingencies change daily.

The Interaction between CRF, DA, and Stress

Corticotropin-releasing factor has had a complicated history in both its discovery and nomenclature, especially since many of the studies analyzing it and its effects call it either corticotrophin-releasing factor or corticotrop(h)in-releasing hormone (CRH). Therefore, there needs to be a standard name for it, at least, as a start before standardization of how investigators should test its effects. Confusion exists in the substance abuse field for the therapeutic effects of CRF which most studies test its effects on addictive behaviors, namely in the reinstatement of

drug-seeking (Zorrilla et al., 2014). The results in CRF to ameliorate reinstatement of either, alcohol-, heroin-, and cocaine-seeking all depended on the role of how investigators induced reinstatement (Shaham et al., 1997; Erb et al., 1998; Shaham et al., 1998; Le et al., 2000; Shalev et al., 2003, 2006). Additionally, the efficacy of antagonists, such as α-helical CRF, have been very controversial, even to the investigators who synthesized the first ones (Rivier & Rivier, 2014; Spierling & Zorrilla, 2017). The effects of CRF also depend on the strain of the rodent used in a given study (Conti et al., 1994). Adding to complexity of the molecular mechanisms, is the existence of the CRF-BP, a membrane-associated protein which binds CRF, dimerizes, and removes CRF from the periphery, but also in the brain to lead to other transcriptional consequences (Ungless et al., 2003).

Moreover, our stressor may have not been enough to elicit a depressed phenotype. A concurrent study in our lab also failed to produce robust behavioral effects of rFSS on behavior (3-Chamber Social Approach, Tail Suspension Test, and Elevated Plus Maze), even when being able to replicate the effects of CRF on phasic DA release *in vitro* (Steger et al., 2020). Even when using a 5-day rFSS stress paradigm, other investigators found no effects on depressive like behaviors in a sucrose preference test, an open field test, and a tail suspension test, but they did find evidence for depressive-like behavior following a social defeat paradigm (which is unfeasible in female mice; Mul et al., 2016). This points to the possibility of immobility time in the rFSS as more of an adaptive behavior, like tonic immobility, instead of a depressed state (Molendijk & de Kloet, 2014).

I have provided novel insight to the function of DA and CRF interactions in the NAc, in contrast to *in vivo* studies done in rats. Previously in our lab, CRF and CRF antagonists were microinjected into the VTA, and found opposing effects in reward sensitivity of CRF in the VTA

compared to results seen when CRF is administered to the NAc after an acute stress of 1 hr. restraint stress (Wanat et al., 2013). This acute stress is comparable to the stressor used by Shafiei and colleagues (2012) in which they saw both a reduced preference and an increased latency to choose in an effort-based decision-making. To support their findings, they used an even shorter time of restraint stress (20 min.) in another study and found no alterations in choice, but an increase in choice latency in the stressed animals that was also brought out by central and intra-VTA injections of CRF. The effects on motivation were reversed with α-helical CRF (Bryce & Floresco, 2016). This highlights the relevance and subjectivity of the acute stressor time, but also to the mode of decision-making (e.g., effort-based decision-making and progressive ratio tasks). Interestingly, in humans it is thought that patients with MDD also have a reduced motivation to work for rewards, which isn't the absence of reward per se (Treadway et al., 2012; Yang et al., 2014). Similar effects on motivation and decision-making accuracy were seen in studies that analyzed depressive human and rat behavior that was rescued by κ-opioid receptor antagonist, concurrently (Beard et al., 2015; Van'T Veer et al., 2012). Although I used mice as a follow up to the Lemos study, my hypothesis was informed by the evidence purported in rats. Alternatively, this experiment could be done with rats, but one would need to replicate the DA profile *in vitro*, and if this study provided valuable information, more genetic experiments could be done in transgenic mice. It would be remarkably interesting to test this interaction in an effort-based decision task, since I believe that CRF's role in the nucleus accumbens must contain some information on the coding of subjectively effortful choice.

Evidence for a new target of CRF in the NAc are the cholinergic interneurons of the striatum, in which muscarinic and nicotinic receptors interact with CRF receptors to influence corticostriatal synapses to mediate DA-dependent plasticity, learning, and depressive behaviors

(Wang et al., 2006; Cheng et al., 2019). Cholinergic interneurons (<1% of cells in striatum) facilitate DA via activation of nicotinic receptors on DA terminals to increase DA release (Yorgason et al., 2017), and these cholinergic interneurons mainly express CRFR1 (Lemos et al., 2019). Collins and colleagues (2016) were able to bidirectionally influence both dopamine release and reward-seeking behaviors in a Pavlovian-to-instrumental transfer task by targeting these cholinergic interneurons. Inhibition of muscarinic receptors or nicotinic receptors suppresses or invigorates, respectively, both behavior and cue-evoked DA release (Collins et al., 2016). These neurons have been implicated in influencing depressive behaviors as well (Warner-Schmidt et al., 2012).

Flexible versus Stable Environments/Contingencies and How Dopamine Transmission Reflects Expected Effort and Reward

"Blessed are the flexible, for they will never be bent out of shape," a quote by Dr. Michael McGriffy, always reminds me to always be ready to adapt, not be stubborn, and be open for change. However, trying to always stay goal-directed despite the energy efficiency of habit formation, proves to be a difficult feat. I will frequently check to see if I locked a door or turned an oven eye off, even if I just did so less than 60 seconds ago. It is said that a large portion of our daily behaviors have already become habitual or ritualistic (Toffler, 1970). Overtraining in our mixed-contingency decision-making paradigm led to inflexible behavior, as evidenced by the persistence to choose a high-effort option on the same lever that a low-effort option was originally presented on, an effect not seen in animals trained on a contingency that was reversed

daily. Additionally, the replication of the same effect of how dopamine does not reflect a preferred option, but rather a cached value of reward magnitude, was observed (Hollon et al., 2014). This contests the idea of DA encoding a utility signal but continues to support the findings that dopamine reflects a learning signal of expected value of high or low reward (Gan et al., 2010). Animals still selected the most beneficial option, especially under food restricted options, so there must be neural correlates of anticipated effort, since in our paradigm, animals still occasionally explored a high-effort or low-reward option in forced-choice trials. Maybe the animals 'regretted' making those selections (Steiner & Redish, 2014; Sweis et al., 2018[a, b]). Nonetheless, overall incentives - nature, size, and timing of rewards - of the task invigorated action selection and led to more of those same actions in free-choice trials. It is still very unclear what the neural substrates of effort are and if DA has anything to do with effortful choices (Walton & Bouret, 2019). One study analyzing the effects of D2R antagonism found that D2R antagonism, via haloperidol i.p injections, in a concurrent effort choice or concurrent value choice task could reduce elicit an effort-averse behavior in mice but not change value-based decisions (Bailey et al., 2020). Of course, the dopaminergic manipulation could have been refined by injecting haloperidol into a DA-rich brain region, rather than having off-target effects of the drug throughout the body. Once again, cholinergic interneurons became of interest when it was found that these neurons were sensitive to anticipated effort and the effort of the action (Nougaret & Ravel, 2015), and the role of serotonin has also been purported to encode some aspects of effort-based decisions (Bailey et al., 2018).

I believe that one of the caveats of this study is underestimating what is considered a flexible option in an operant-conditioning paradigm. Simply switching between two contingencies (left vs right lever) daily, may still become habitual responding. For example, if

the animals only learned those two options over time, one could interpret this as overtraining for two options, so when tested in our reversal task, of course, the animals could switch between these two options reliably. It would be interesting to have the ability to introduce a third (and more) option, to see how DA reflects this an even more variable schedule of reinforcement. One example of a well-studied conditioned behavior that involves more than two options is the 5-choice serial reaction time task (5-CSRTT) which is used to measure impulsive behavior in preclinical settings that can be pharmacologically, chemogenetically, and optogenetically manipulated (Higgins et al., 2017; Carr et al., 2018). In this behavior, an animal is trained to hold a lever for a variable amount of time until the presentation of discrete cues in five different sections of an apparatus and then when pressing then pressing another level (or nosepoke) under the discrete cue, going back to a food receptacle to receive reward. Premature releases of the initial lever before discrete cue presentation, omission of the trial, or an incorrect response shows the impulsivity or attentional ability of the animal. The metrics of this behavior were shown to be DA- and cholinergic-dependent (Balachandran et al., 2018) and relied on the cortical and striatal circuitry valuation systems mentioned in Chapter 1 (Pasansky et al., 2019; de Kloet et al., 2021; Flores-Dourojeanni et al., 2021). It is also well-known that the number of options can parametrically affect choice reaction time in humans and primates, leading to an economic phenomenon called ‘overchoice and the anticipation of regret’ or ‘choice overload’ (Iyengar & Lepper, 2000; Gourville & Soman, 2005; Shah & Wolford, 2007; Scheibehenne et al., 2010; Hadar & Sood, 2014). Although some economic studies purport that choice overload does not occur if the subject has enough time to choose between a large variety (Inbar et al., 2011).

Final Remarks on The Future of Neuroeconomics

Imagine the stress of being at supermarket of any variety and one was required to choose between the multitude of products very quickly, which would not be a problem if the individual thoroughly researched products beforehand or enjoyed gambling with their money and products to bring into their home. Indeed, having to many options can be stressful. There could exist a bidirectional relationship between decision-making and stress, where deciding could be stressful (cognitive fatigue; Mulette-Gillman et al., 2015) and environmentally induced stress levels (e.g., keeping children from breaking or opening anything whilst shopping) could be influence the subjective stress of making that decision. Elucidating the neural substrates of decision-making behavior, I think, is going to be just as complex as trying to understand the neural correlates of conscious behavior (Tononi et al., 2016; Koch et al., 2016), so to that extent there are many questions still unanswered. We cannot rule out the ethical implications of learning more and more about decision-making skills, especially in the largely patriarchal and capitalistic society. For example, studies examining sex differences in economic theory have large implications that extend to advertisements and marketing strategies (Lazzaro et al., 2016). This young and provocative field is providing information on the how humans make financial decisions (Knutson & Bossaerts, 2007), and has relevance for naming neuroeconomics in a different light of neuromarketing, which some could view as unethical (Lee et al., 2007). It is basically using neuroscience's empirical evidence to sell people stuff that they do not need (e.g., soft drinks, sugary foods, and name brand clothing). Neuromarketing relies on neuroimaging and physiological recordings to find areas of the brain that correlate with buying something. The neural response's recorded from fMRI and EEG results can sometimes be different than the

actual answer that individuals would give. Data taken from McClure and colleagues (2004[b]) gives evidence that Coke provides a robust preference over Pepsi, using semi-anonymous taste tasks. When people are given both chemically similar products in a double-blind test, the preference failed to be shown. This supports the fact that brand knowledge and advertising bombardment influences what people prefer to drink, even if they do not need it. The subjects in the test had responses in the hippocampus and dorsolateral prefrontal cortex which are correlated to biased behavior based on affect. The employment of most of our limbic system influences our rational cortex in ways that may lead to compulsive purchasing behavior, which is not beneficial to providing what we need for survival. Although some neuromarketing research can give scholarly insight to what provokes our decisions and then try to alleviate compulsive buying or overconsumption, contemporary neuromarketing and marketing seem to just want to find a 'buy button' in the brain, especially since the main companies that have strong marketing strategies have more money to provide for research to do so. Many companies capitalize on our impulsivity, and therefore neuromarketing should be cautiously approached.

The questions that still need answering in neuroeconomic research lie in both our technological advancements and in how we define the anatomical organization within brain regions like the striatum (Brimblecombe & Cragg, 2017; Castro & Bruchas, 2019). New techniques like refined optogenetic technologies (Rivnay et al., 2017), the dopamine-specific *dLight* (Patriarchi et al., 2018), and sensors for any of the G-coupled protein receptors (Jing et al., 2019), that allow investigators to view cellular activity with astounding temporal resolution will shed new light on previously constrained ideas. There is also the question of the importance and relevance of heteromers between multiple receptors (Carriba et al., 2008). This work should provide information into the elusive, but very frequently documented, function of dopamine in relation to

guiding an organism's decisions – a subject that continues to fuel my passion for neuroscience research.

"The differences in income between the poor world and the rich world are so great that people have to be interested" -Esther Duflo, who won the Nobel Memorial Prize in Economic Sciences

Continuing down an academic research path, I hope to further elucidate the mechanisms of environmental influences on decision-making and reward in the context of how poverty affects economic choice.

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CURRICULUM VITAE

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Education

University of Washington, Seattle. . . . . Seattle, WA
Graduate Program in Neuroscience 2015-present
GPA: 3.52

Georgia State University . . . . . Atlanta, GA
Neuroscience Institute; Neuroscience B.S. 2011-2015
Overall GPA: 3.86; Major GPA: 4.0

Positions and Research Experience

Veterans Affairs Puget Sound Health Care System . . . . . Seattle, WA
Research Associate July, 2021
Advisor: Dr. John F. Neumaier
We will work on mouse and rat behavioral research, maintaining transgenic mouse strains, molecular methods including RNAscope, western blot, and viral vector surgeries to study alcohol withdrawal effects on microglia.

University of Washington, Seattle . . . . . Seattle, WA
Graduate Research Assistant, Neuroscience Graduate Program 2015-present
Advisor: Dr. Paul E.M. Phillips
We study the role of dopamine in decision-making and psychopathologies such as substance abuse using fast-scan cyclic voltammetry. My first project on this lab analyzed how either flexible or habitual decision-making phenotypes affect phasic dopamine release. Currently, I study how chronic stress and its biological correlates interact with phasic dopamine release and reward responsivity in a novel decision-making task in mice.

Oregon Health and Sciences University . . . . . .Portland, OR
Undergraduate Research Assistant, Department of Behavioral Neuroscience Summer 2014
Advisor: Dr. K. Matthew Lattal

We studied the behavioral effects of different stimulus arrays within the animal model of addiction. This laboratory incorporated a learning and memory approach to treating addiction by analyzing the effects of environmental cues on minimizing relapse after food pellet or intravenous cocaine self-administration.

Georgia State University . . . . . Atlanta, GA

Undergraduate Research Assistant, Neuroscience Institute 2013-2015

Advisor: Dr. Kyle J. Frantz

We studied the neurobehavioral aspects of the animal model of addiction in both adolescent and adult male rats. We used intravenous drug self-administration to test hypotheses about age differences in drug-related reward and reinforcement. We tested environmental influences (e.g. housing conditions) on cocaine intake as well. I gained proficiency in performing catheterization surgeries in addition to the skills associated with self-administration.

Georgia State University . . . . . Atlanta, GA

Neuroscience Education and Training Program (NET/work) 2013-2015

Advisors: Drs. Christopher T. Goode and Kyle J. Frantz

Under this NIH-funded Blueprint Program for Enhancing Neuroscience Diversity through Undergraduate Research Education (BP-ENDURE) two-year paid internship program, I gain the knowledge and skills to pursue a career in neuroscience through research experience, oral presentations, and professional development workshops.

Grants, Honors, and Scholarships

NSP Associate & Professional Development Award 2016-2017
NIH Neuroscience Graduate Training Grant 2016-2017
NSF Graduate Research Fellowship Program. 2016-present
ARCS Foundation Scholar Award 2015-present
HOPE Scholarship 2011-2015
PELL Grant 2011-2015
Dean's List, 4 out of 8 semesters. 2011-2015
President's List, 4 out of 8 semesters 2011-2015

Professional Development and Presentations

R.G. Williams, S.S. Sandberg, & P.E.M. Phillips (2017 - 2021) *Influence of Stress on Economic Decision-Making in Mice*

- Oral Presentation. NIH Blueprint Diversity Conference. Virtual. April 8th, 2021. Virtual lightning talk.
- Poster. Society for Neuroeconomics Annual Conference. Dublin, Ireland. October 6th, 2019. This study employed the use of non-selective corticotropin-releasing factor (CRF) and dopamine (DA) antagonists on novel concurrent-choice operant conditioning paradigm before and after chronic stress induction.
- Oral Presentation. Neuroscience Student Symposium. April 18th, 2019. University of Washington, Seattle. Presented update of results of decision-making experiment in mice.
- Poster. Society for Neuroscience Annual Conference: Diversity in Neuroscience. Nov. 7, 2018. Washington D.C. This study used male and female mice to analyze the effects of chronic stress on a novel decision-making task performance in male and female mice.
- Poster. Society for Neuroscience Annual Conference: Diversity in Neuroscience. Nov. 11, 2017. Washington D.C.
- PowerPoint presentation. Behavioral Neuroscience Seminar. April 11th, 2017. University of Washington, Seattle.
- PowerPoint presentation. NIH Blueprint Diversity Conference. April 16th, 2020. Virtual Conference.

L.C. Kruse, A.G. Schindler, **R.G. Williams**, S.J. Weber, & J.J. Clark (2017) *Maladaptive Decision-Making in Adults with a History of Adolescent Alcohol Use, in a Preclinical Model, Is Attributable to the Compromised Assignment of Incentive Value during Stimulus-Reward Learning*

- Publication. Frontiers in Behavioral Neuroscience. July 25th, 2017. University of Washington, Seattle.

R.G. Williams, B.F. Williams, A.C. White, J.P. Duffy, A. Jackson, J.P. Polites & K.J. Frantz (2015 & 2014) *Influence of Environmental Factors on Lever-pressing Behavior in Adolescent and Adult Male Rats*.

- PowerPoint presentation. NET/work Spring Symposium. April 16, 2015. Georgia State University, Atlanta, GA. A more developed version (larger sample size, statistics, and more data collected and analyzed) than the 2014 presentation.
- PowerPoint presentation. NET/work Spring Symposium. March 27, 2014. Georgia State University. Atlanta, GA.

R.G. Williams, L.N. Hitchcock, J.D. Raybuck & K.M. Lattal (2014) *Effects of Discrete Cues on Extinction and Context-Induced Reinstatement after Self-Administration.*

- Poster. 2014 Equity & CURE Interns Poster Presentation. August 8, 2014. Department of Behavioral Neuroscience. Oregon Health and Science University. Portland, OR.

R.G. Williams, A.C. White, J.P. Polites, C. Li, B.F. Williams & K.J. Frantz (2013) *Differential Effects of Simple vs. complex Stimulus Arrays (Discrete Cue vs. Cue + Context) on Reinstatement of Cocaine-Seeking in Adolescent and Adult Male Rats*.

- Poster. Society for Neuroscience Annual Conference: Diversity in Neuroscience & Faculty for Undergraduate Neuroscience (FUN). November 9-10, 2013. San Diego, CA.

R.G. Williams & M. Hoque (2014) *Mutant Chocolate.*

- Poster and teaching station. Atlanta Science Festival: Discovery Day. March 22, 2014. Georgia State University

R.G. Williams, A.C. White, J.P. Polites, C. Li, B.F. Williams & K.J. Frantz (2013) *Differential Effects of Simple vs. complex Stimulus Arrays (Discrete Cue vs. Cue + Context) on Reinstatement of Cocaine-Seeking in Adolescent and Adult Male Rats*.

- Poster. BRAIN summer poster session. August 1, 2013. Georgia State University. Atlanta, GA.

Teaching Experience

University of Washington Seattle, Neurobiology 302 . . . . . Autumn 2016

Role: I served as a teaching assistant for the lab and lecture portion of this systems-level biology undergraduate class under the instruction of Drs. Martha Bosma, Joe Sisneros, and Michael Kennedy.

Professional Affiliations and Community Involvement

NIH Blueprint Diversity Conference . . . . . April 2020 & April 2021

Role: In 2020, my role as a sponsored student for this virtual workshop was to learn ways to effectively advance my career and maintain mental health and positive coping strategies during the COVID-19 pandemic. In 2021, I presented my research from Paul Phillips' laboratory as well as participated in activities that cultured better understanding of leadership, emotional intelligence, conflict management, and science communication.

Society for Advancement of Chicano and Native Americans in Science . . . . . 2018 & 2019

Role: For this diversity-centered conference, my duty was to recruit prospective graduate students for the UW Graduate Program in Neuroscience.

UW Neuroscience Grey Matters Magazine. . . . . November 2018-present

Role: I serve as a graduate student liaison to edit and provide reviewers for this undergraduate magazine.

UW Neuroscience Diversity Committee . . . . . August 2018-present
Role: As the only student member of this committee, I provide student input for our program's diversity initiative.

UW Neuroscience Admission/Recruitment Committee . . . . . .May 2018-2019

UW GO-MAP Outreaching Grads Program 2017-2020
Role: As a part of this inclusive program ran by Dr. Carolyn Jackson, I assist with reaching out to prospective students of color for all graduate programs at the UW and facilitating event coordination for current URM students.

UW Electrochemical Society. 2016-present

Brains On: Children's' Science Podcast. . . . . May 2019
Role: I featured on an episode that explains the largest neurochemical contributors to the feeling of happiness.

UW Neuroscience External Speaker Seminar committee . . . . . .May 2016-May 2018

Art Neureau . . . . . May 2016 - 2020
Role: I am co-host and organizer of this neuroscience-themed art gallery with pieces made by researchers at the UW.

Nu Rho Psi Honor Society . . . . . 2015 - present

Collegiate Neuroscience Society . . . . . 2014 - present

Atlanta Science Festival . . . . . .March 2014 & March 2015
Role: As a part of the Science Education paid internship, I worked with the Georgia State University chapter of the Atlanta Science Festival to coordinate the events on campus. I also volunteered to work at different events throughout the event week and held a teaching station about comparisons between chocolate and genetically modified chocolate.

Alpha Lambda Delta . . . . . 2012 - 2016