5-6-2024

Mesolimbic Dopamine Coding of Social Salience and Valence

Erica Cross

Mesolimbic Dopamine Coding of Social Salience and Valence

by

Erica A Cross

Under the Direction of H. Elliott Albers, PhD

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

in the College of Arts and Sciences

Georgia State University

2024

ABSTRACT

Social interactions can be either rewarding or aversive. In many neuropsychiatric disorders, social pathologies underlie disease etiology. For example, decreased social reward is associated with autism spectrum disorder and social stress is a leading risk factor for mood and anxiety disorders. Social interactions are characterized by their intensity (i.e., salience) and whether they are rewarding or aversive (i.e., valence). The mechanisms underlying the salience and valence of social interactions are not well understood. Social stress is one of the most salient stressors across taxa, but it is unclear how an individual's social status might impact the rewarding or aversive properties of these interactions. If low social rank reduces social reward, it could contribute to the social anhedonia that is characteristic of many social stress-related neuropsychiatric disorders, and if social dominance increases social reward, it could reduce the symptomology of these disorders. The possibility that social stress reduces social reward is supported by evidence that stress can produce long lasting changes in the neural pathways that process reward and reinforcement of behavior, i.e., the mesolimbic dopamine system (MDS), which includes the ventral tegmental area (VTA) and its dopamine (DA) projections to the nucleus accumbens (NAc). Here, we provide evidence to support that DA neurons in the medial VTA (mVTA) that project to the NAc core primarily signal the salience of both rewarding and aversive social interactions. In contrast, DA neurons in the lateral VTA (lVTA) that project to the NAc shell primarily signal valence, with positive valence increasing activation of this subcircuitry. In this dissertation, I tested the hypothesis that dopamine dynamics (DA neuron activity and release patterns) and receptor subtypes within anatomically specific subcircuits of the MDS code, at least in part, the salience and valence of social interactions. Investigating a novel subcircuitry within the MDS that codes for social reward and aversion in males and females seeks to fill the gap in our understanding of

social deficits in neuropsychiatric disorders as well as differences in disease prevalence among men and women.

INDEX WORDS: Nucleus Accumbens, Ventral Tegmental Area, Social reward, Social aversion, Sex differences, Hamster

Mesolimbic Dopamine Coding of Social Salience and Valence

by

Erica A. Cross

Committee Chair: H. Elliott Albers

Committee: Kim Huhman

Kyle Frantz

Scott Russo

Electronic Version Approved:

Office of Graduate Services

College of Arts and Sciences

Georgia State University

May 2024

DEDICATION

For anyone who has struggled with mental health.

ACKNOWLEDGEMENTS

I would like to thank the following people who have been vital to the completion of this research:

- My mentors Dr. Elliott Albers and Dr. Kim Huhman, who taught me not only how to be a good scientist but also how to tell a story with my research. Committee members, Dr. Kyle Frantz and Dr. Scott Russo, whose insight has been vital to this work.

- Albers and Huhman lab members past and present, notably Emma Shaughnessy, Susan Lee, Zachary Grieb, and Johnathan Borland, who I am thankful to have had by my side.

- My parents, Joe and Sue Williams, to whom I would like to express my deepest gratitude for their love and support.

- My husband, Clay, who has been a constant source of love, laughs, and perspective while providing unwavering encouragement throughout the journey toward my PhD.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA: analysis of variance

AP: anterior-posterior

BLA: basolateral amygdala

C: Celsius

CD: conditioned defeat

CFM: carbon fiber microelectrode

CPP: conditioned place preference

DA: dopamine

D1R: D1-like dopamine receptor

D2R: D2-like dopamine receptor

DV: dorsal-ventral

IHC: immunohistochemistry

ip: intraperitoneal

ir: immunoreactivity

MDS: mesolimbic dopamine system

ML: medial-lateral

mPFC: medial prefrontal cortex

MSN: medium spiny neuron

NAc: nucleus accumbens

NAI: non-aggressive intruder

NDS: normal donkey serum

OSP: operant social preference

PBS: phosphate-buffered saline

RA: resident aggressor

SPS: social preference score

TH: tyrosine hydroxylase

VTA: ventral tegmental area

1 INTRODUCTION

1.1 Social reward, social stress, and their relationship to the etiologies and symptomologies of various neuropsychiatric disorders

The reinforcing properties of social interactions, referred to as social reward, are vital to the expression of adaptive social behaviors, as well as to the development and maintenance of relationships among individuals. Despite social reward's importance in biological and psychological health, relatively little is known about the neural mechanisms signaling the rewarding properties of social interactions (Trezza, Campolongo, & Vanderschuren, 2011). Additionally, dysfunctions in social reward processing and social aversion are pervasive across various neuropsychiatric disorders, including autism spectrum disorder, schizophrenia, depression, and anxiety and bipolar disorders (Dichter, Damiano, & Allen, 2012). Oftentimes, social stimuli that typically have a positive valence become less rewarding or even aversive in the case of these disorders.

Social stress is a potent aversive stimulus that can lead to a variety of behavioral effects, including social avoidance, anhedonia, motivational deficits, and an increased propensity to abuse drugs and alcohol in humans and other animals (Huhman, 2006). Furthermore, social stress oftentimes underlies, or exacerbates symptoms, of many neuropsychiatric disorders, while positive social interactions can be protective against the deleterious effects of these disorders on mood and behavior (Trainor, 2011). There is a critical gap in our knowledge of the neural circuits and molecular mechanisms mediating social reward and aversion and how this signaling leads to social deficits in various neuropsychological disorders, which are often comorbid. An improved understanding of how social stress and deficits in social reward contribute to behavioral symptoms that are observed in a variety of human neuropsychological disorders may reveal novel therapeutic

strategies to address the associated dysfunction. Furthermore, examining potential sex differences in these processes and stress is vital to understanding the differences in propensity of each sex to be diagnosed with certain disorders and to allowing for improved therapeutic interventions to address the underlying causes. There is an urgent need to improve treatments for these disorders because current therapeutics are ineffective in a large portion of the patient population (Thase & Denko, 2008), and these disorders carry a huge social and economic burden, in addition to the to the challenges these individuals face (Greenberg et al., 2021; Simon, 2003).

1.2 Social status, stress, and DA signaling are interrelated

Social hierarchies are important to an animals' social behavior and evolutionary success (Sapolsky, 2005). Dominant individuals have priority access to resources like food, territory, and mates. It comes as no surprise then, that winning agonistic encounters is rewarding (Aleyasin et al., 2018; Gil, Nguyen, Mcdonald, & Albers, 2013). Conversely, perception of a loss or low social status has a profound impact on behavior (Huhman, 2006). Social defeat, and associated subordinate status, is associated with higher levels of social stress, impacting physiological and psychological health via activation of the hypothalamic-pituitary-adrenocortical (HPA) axis (Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993). Social stress has also been associated with changes in ingestive behavior (Foster, Solomon, Huhman, & Bartness, 2006; Solomon, Foster, Bartness, & Huhman, 2007) and sleep (Meerlo, Sgoifo, & Turek, 2002), as well as diminished anticipation of, and consumption of, rewarding stimuli (Von Frijtag et al., 2000). Furthermore, defeat is associated with increased anxiety-like behavior such as reduced exploration of open arms in elevated plus maze (Berton, Durand, Aguerre, Mormède, & Chaouloff, 1999) and enhanced acoustic startle (Pulliam, Dawaghreh, Alema-Mensah, & Plotsky, 2010). Social rank is also associated with how an individual responds to a stressor, such that low ranking individuals

are more susceptible to the adverse effects of stress (LeClair & Russo, 2021). For example, dominant hamsters exhibit fewer submissive and defensive postures following social defeat compared to subordinates when subsequently exposed to a social stimulus (Morrison et al., 2014). Given the pervasive impact of social rank on behavior, **Chapter 2** tested the hypothesis that social stress induced by low status or defeat reduces the rewarding properties of social interactions and that social dominance enhances the rewarding properties of these interactions.

1.3 A bidirectional relationship exists between stress and the mesolimbic dopamine system (MDS)

The MDS, which consists of dopaminergic (DA) projections from the VTA to the NAc, is the canonical reward pathway that mediates the reinforcing properties of natural rewards, such as food and sex, as well as of drugs of abuse (Olds & Milner, 1954). While MDS signaling of drug reward has been extensively explored, much less is known about how the brain encodes natural or endogenous rewards. The role of the MDS is broader than just serving as a reward pathway, however, and can be affected by social stress. DA neurons in the VTA process emotionally salient stimuli of both positive *and* negative valence, therefore, this heterogeneous region plays a role in signaling reward as well as aversion (Gunaydin et al., 2014; Lammel, Ion, Roeper, & Malenka, 2011). As a result, the MDS is implicated in stress-related psychopathologies in which individuals experience deficits in motivated behavior and reward processing, such as anhedonia, social withdrawal, and vulnerability to substance abuse (Conway, Compton, Stinson, & Grant, 2006; Venniro et al., 2018). In fact, 20% of individuals diagnosed with mood or anxiety disorders meet the criteria for addiction and 30-40% of individuals with addiction have a comorbid mood or anxiety disorder (Venniro et al., 2018). These considerations suggest a large degree of overlap

among brain regions affected in depression, anxiety disorders, and motivated behavior in the clinic (Russo & Nestler, 2013).

Stressors robustly impact DA signaling in the MDS, in part via corticotropin releasing factor receptors (CRFRs) on dopaminergic neurons in the VTA (Cao et al., 2010; Holly, Debold, & Miczek, 2015). Further, depending on the duration and intensity of the stressor, VTA DA neuronal activity can be increased (Chaudhury et al., 2013; Lucas et al., 2004) or decreased (Tye et al., 2013). For instance, while chronic social defeat stress increases phasic firing of DA neurons, acute social defeat stress decreases phasic firing. In the NAc, changes in synaptic and structural plasticity are also implicated in depression symptomology (Francis et al., 2015). Specifically, chronic stress alters medium spiny neuron (MSN) activity by changing excitatory input and intrinsic excitability of DA receptors which, in turn, impacts depression-like behavioral outcomes in response to stress (Francis & Lobo, 2017). Therefore, it is evident that stress has a substantial impact on MDS function. The NAc is also implicated in the response to social stressors because it integrates VTA DA signals to promote memory formation and appropriate behavioral response to significant events. Pharmacologic inhibition of the NAc with the $GABA_A$ agonist, muscimol, blocks the expression of conditioned defeat (CD). CD is a well characterized response to social stress in which defeated hamsters no longer display territorial aggression, but instead exhibit heightened levels of avoidance and submission (Potegal, Huhman, Moore, & Meyerhoff, 1993). Interestingly, dopamine receptors in the NAc are required for the acquisition, as well as expression, of CD (Gray, Norvelle, Larkin, & Huhman, 2015). Therefore, MDS signaling is necessary for the response to at least some social stressors. While it is clear that extended amygdala circuitry is integral to stress responses, the MDS serves as a strong candidate for encoding, at least in part, social salience as well as positive and negative social valence. Furthermore, the amygdala is

innervated by VTA DA neurons, therefore it does not function independently of MDS input (Bromberg-Martin, Matsumoto, & Hikosaka, 2010). Cumulatively, these data indicate a bidirectional relationship between stress and MDS function. Consequently, the objective of this project is to improve our understanding of how the MDS processes both rewarding and aversive social stimuli.

1.4 Does heterogeneity in VTA and its distinct projections to NAc subregions encode reward and aversion in the MDS?

DA neurons in the VTA show high levels of anatomical and functional heterogeneity, with subregions of the VTA projecting to distinct targets. Prior studies have shown that DA neurons in the medial VTA project primarily to the NAc core. It has been proposed that DA release in different targets (e.g. mPFC) will differentially impact behavior and that distinct VTA circuits generate reward and aversion (Lammel, Lim, & Malenka, 2014; Lammel et al., 2012). It is also possible that some of these projections likely mediate a valence-free coding of salience, signaling intense and behaviorally relevant stimuli. (Bromberg-Martin et al., 2010). Conversely, DA neurons in the lateral VTA primarily project to the lateral NAc shell and activation of this pathway has been associated with rewarding stimuli (Bromberg-Martin et al., 2010; Lammel et al., 2014). These are the more "conventional" DA neurons which likely signal valence, the subjective value assigned to stimuli. The lVTA to NAc shell pathway is not only activated by stimuli with a positive valence (reward), but it is inactive or inhibited, by stimuli with a negative valence (aversion) (Bromberg-Martin et al., 2010; Ungless, Magill, & Bolam, 2004). These anatomically and functionally distinct DA subcircuits allow the MDS to process a wide range of behaviorally relevant stimuli.

DA projections are also electrophysiologically distinct. Axonal projections from the VTA into the NAc core release dopamine in a phasic manner, with high-amplitude transient DA release

that results from a cell-burst firing, referred to as "DA transients." The NAc core receives transients of DA during both rewarding stimuli (Day, Roitman, Wightman, & Carelli, 2007) and aversive stimuli (Anstrom, Miczek, & Budygin, 2009). Phasic DA is proposed to be the signal that mediates rapid behaviorally relevant activation of the MDS. Recent data suggests that DA transients in the core function as a salience cue, orienting attention towards important information associated with highly salient or intense stimuli. This idea proposes that DA transients in the NAc core serve a key function in learning associations so as to appropriately respond to future stimuli of any valence and fits data regarding DA release patterns in response to aversive stimuli (Kutlu, Zachry, Melugin, Cajigas, Chevee, Kelly, et al., 2021). Therefore, it is not surprising that the NAc core responds to threat and is necessary for expression of conditioned fear (Dutta, Beaver, Halcomb, & Jasnow, 2021; Ray, Moaddab, & McDannald, 2022). By contrast, the release of DA in the NAc shell occurs in a tonic manner, with slow changes occurring over a longer time scale than is observed in the NAc core. Tonic DA release in the NAc shell is thought to encode pleasure or the hedonic aspect of rewarding behaviors. For example, tonic DA release in the NAc shell increases during rewarding events and decreases during aversive or noxious events (Yuan, Dou, & Sun, 2019). Furthermore, DA release in the NAc shell is consistent with encoding the valence of stimuli. (Cacciapaglia, Mark Wightman, & Carelli, 2011; Saddoris, Cacciapaglia, Wightman, & Carelli, 2015). The MDS is understood to encode reward and aversion in order to allow individuals maximize exposure to stimuli that are advantageous and avoid stimuli that are disadvantageous. In summary, data collected over decades in the field of reward and reinforcement as well as fear learning reveal that MDS signaling is complex and we yet to have a clear picture of how this circuitry serves to code reward, aversion, and the saliency of many different types of stimuli (e.g., sex, drugs of abuse, etc.).

Here, we propose a specific hypothesis of how subcircuits of the MDS code the nature of **social stimuli**. As can be seen in Figure 1, we propose that DA projections from the mVTA to NAc core code for the salience of social stimuli whereas projections from the lVTA to NAc shell code for the valence of social stimuli. Therefore, in this project we examined DA release in response to social stimuli with a positive and negative valence in males and females in order to critically test our hypothesis on the specific dopaminergic subcircuits in the NAc that mediate salience and valence of social interactions. **Chapter 2 & 3** examined activity and connectivity of the proposed subcircuitry in order to understand the neural mechanisms underlying social reward as well as social aversion, and to determine whether common mechanisms are involved in both. **Chapter 2** examined cFos expression in MDS subregions to test the hypothesis that neural activity of the lateral VTA and the NAc shell code valence, with increased activity coding reward and reduced activity coding aversion. **Chapter 2** also tested the hypothesis that the medial VTA and the NAc core code salience, and that both rewarding and aversive social stimuli are highly salient. **Chapter 3** replicated and extended these findings in the VTA, utilizing co-labeling of TH and cFos in order to investigate the hypothesized roles of DA specific neurons within the mVTA signaling salience and lVTA signaling valence, respectively. Additionally, **Chapter 3** examined DA efflux in the NAc during winning and losing in order to test the hypothesis that rewarding social interactions increase tonic and phasic DA release while aversive social interactions increase phasic DA release but decrease tonic DA. Furthermore, **Chapter 3** utilized retrograde tracers to

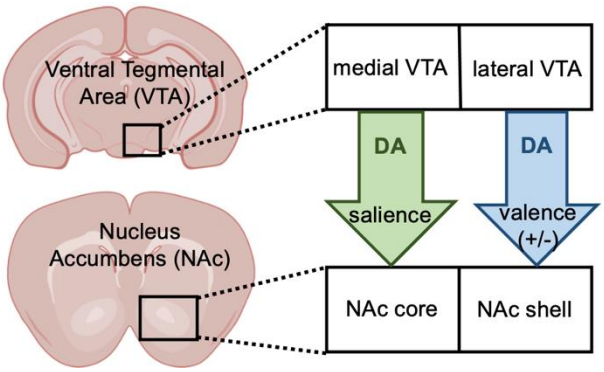

Figure 1: Proposed MDS subcircuitry

test that the mVTA projects primarily to the NAc core and the lVTA projects primarily to the NAc shell.

1.5 Dopamine receptor subtypes influence social status as well as differentially modulating reward and aversion

Dopamine released from the VTA acts on GABAergic Medium Spiny Neurons (MSNs) in the NAc that differentially express DA receptors. Investigation of DA receptors have elucidated distinct roles for the two primary receptor subtypes, D1-like receptors (D1R), which include D1 and D5, and D2-like receptors (D2R), which include D2, D3, and D4. DA receptors are G-protein coupled receptors (GPCRs) and D1Rs are excitatory (G_s coupled) while D2Rs are inhibitory (G_i coupled). D2Rs have a much higher affinity for DA than D1Rs and serve as autoreceptors on the presynaptic terminal. As a result, tonic levels of DA typically activate a larger proportion of D2Rs while phasic DA transients activates more D1Rs. It is thought that these receptor subtypes work in opposition to produce balanced behavioral output (Soares-Cunha, Coimbra, Sousa, & Rodrigues, 2016).

DA receptor expression is correlated with social status in non-human primates (Nader, 2012). D2R antagonism attenuates social dominance in high-ranking rodents and primates but has differential effects on lower ranking individuals (Yamaguchi, Lee, Kato, Jas, & Goto, 2017). While the directionality is less clear, D1R activity is implicated in determining social hierarchy in rodents and primates, as well (Yamaguchi, Lee, Kato, & Goto, 2017). Furthermore, chemogenetic manipulations in the prefrontal cortex (PFC), which bidirectionally signals to the MDS, have associated higher D1R neuron excitability with dominance and higher D2R neuron excitability with being subordinate. In fact, simultaneous chemogenetic inhibition of D1R activity in dominant mice and inhibition of D2R stimulation in subordinate mice reverses social rank, such that the

dominant becomes subordinate, and the subordinate becomes dominant (Xing, Mack, Zhang, McEachern, & Gao, 2022).

D1R and D2R function has also been implicated in signaling the rewarding and aversive properties of stimuli. Increased D1R MSN activity has been associated reward and approach behaviors, while increased D2R MSN activity has been associated with aversion and avoidance behaviors (Calipari et al., 2016; Kravitz, Tye, & Kreitzer, 2012; Lobo et al., 2011). Studies on the mechanisms underlying reward supports the role of D1R in mediating reinforcement. Optogenetic excitation of D1Rs on MSNs is reinforcing enough to induce intracranial self-stimulation and conditioned place preference (CPP), as well as enhancing drug induced CPP (Kravitz et al., 2012; Natsubori et al., 2017). Additionally, pharmacologic stimulation of D1Rs promotes motivation to pursue drug and food rewards (Schmidt, Anderson, & Pierce, 2006; Wakabayashi, Fields, & Nicola, 2004). Alternatively, excitation of D2Rs on MSNs decreases reward in CPP and has been associated with a negative valence and avoidance behavior. Another study revealed that D2R excitation in the NAc is also capable of shifting motivational valence from positive to neutral, or even from positive to negative, depending on the context (Cole, Robinson, & Berridge, 2018). These studies suggest that D2 NAc roles in motivation may be ambivalent or plastic in valence, whereas D1 roles remain more distinctly positive. How D1Rs and D2Rs modulate the valence of social stimuli, however, remains poorly understood. **Chapter 4** tested the hypothesis that DA receptors in the NAc modulate the rewarding properties of social interactions. We tested the prediction that D1R antagonism would decrease social reward and D2R antagonism would increase social reward.

1.6 Sex differences in MDS signaling in response to social stimuli may contribute to divergence between men and women in clinical populations

There are sex differences in the MDS that underlie encoding of reward and motivated behavior (Becker, 2009; Becker & Chartoff, 2019; Walker, Rooney, Wightman, & Kuhn, 1999). For example, DA neurons in the VTA are subject to modulation via sexually dimorphic androgen receptors and MSNs in the NAc have increased dendritic spine density in females compared to males (Kritzer & Creutz, 2008; Wissman, May, & Woolley, 2011). The National Institute of Mental Health (NIMH) recognizes that there are substantial differences between men and women in both the prevalence and clinical course of neuropsychological disorders, however, current treatments are typically identical across the sexes and oftentimes show relatively low clinical efficacy (Bangasser & Valentino, 2014). Our lab has shown that there are distinct sex differences in the rewarding properties of social interactions and in the responses to social defeat. For example, females find same-sex interactions to be more rewarding in both conditioned place preference (CPP) and operant social preference (OSP) than males (Borland, Aiani, et al., 2019; Borland, Rilling, Frantz, & Albers, 2019). Furthermore, males display more defensive and submissive behaviors in response to social defeat than do females, and the behavioral effects typically last longer in males. Conversely, social defeat in females does not lead to the same long-term behavioral changes seen in males (Huhman et al., 2003). Therefore, this project will also examine sex differences in MDS activity in response to rewarding and aversive social stimuli to elucidate the underlying mechanisms mediating sex differences in behavioral responses.

1.7 Syrian hamsters as a model species

Syrian hamsters were used in these studies because they are an excellent model species for investigating sex differences in social behavior and the preclinical study of behaviors that underlie

psychiatric health and illness (Terranova et al., 2016). Syrian hamsters are particularly well-suited to study the neurobiological mechanisms underlying social behavior with both positive and negative valence because they are quick to engage in agonistic behavior, demonstrate stereotyped offensive and defensive behaviors, and quickly form a stable dominance hierarchy (McCann & Huhman, 2012; Payne & Swanson, 1970; Potegal, Huhman, Moore, & Meyerhoff, 1993b). Moreover, when paired with a same sex conspecific, both males and females develop highly robust dominant-subordinate relationships (Drickamer, Vandenbergh, & Colby, 1973; Drickamer & Vandenbergh, 1973), unlike other common laboratory rodent species where males are more aggressive than females or additional manipulations are required to induce aggression in females (Blanchard, Flannelly, & Blanchard, 1988; Payne & Swanson, 1970). Thus, any sex differences in the rewarding or aversive properties of social interactions are likely due to differences in the neural mechanisms encoding social salience and valence, and not due to sex differences in the types or quality of social behavior. The ritualized nature of hamster agonistic behavior offers the distinct advantage in that brief encounters rarely result in any physical injury. Additionally, physical contact is not necessary for Syrian hamsters to maintain dominance status and thus, like in primates, social rank in hamsters is an ethologically relevant psychosocial stressor (Huhman, Moore, Mougey, & Meyerhoff, 1992; Meyer & Hamel, 2014). Furthermore, Syrian hamsters have been successfully employed in prior studies of social behaviors with positive and negative valence (Ferris, Axelson, Shinto, & Albers, 1987; Ferris et al., 1997; Gil et al., 2013; Gray et al., 2015; Huhman et al., 1992; Huhman et al., 2003; Meisel & Joppa, 1994; Morrison et al., 2014; Solomon et al., 2007; Song, Borland, Larkin, Malley, & Albers, 2016). In conclusion, Syrian hamsters are an excellent model species to study the neural mechanisms underlying social salience and valence

in both sexes and may offer preclinical data that is more translatability to clinical outcomes in humans.

2 SEX DIFFERENCES IN THE IMPACT OF SOCIAL STATUS ON SOCIAL REWARD AND ASSOCIATED MESOLIMBIC ACTIVATION

Previously published in Physiology & Behavior, https://doi.org/10.1016/j.physbeh.2023.114410, PMID: 37977252

2.1 Abstract

Social stress plays an important role in the etiology of many neuropsychiatric disorders and can lead to a variety of behavioral deficits such as social withdrawal. One way that social stress may contribute to psychiatric disorders is by reducing social motivation and the rewarding properties of social interactions. We investigated the impact of social stress on social reward in the context of winning versus losing agonistic encounters in Syrian hamsters (*Mesocricetus auratus*). First, we tested the hypothesis that social stress resulting from either stable low, or subordinate, social status or from social defeat reduces the rewarding properties of social interactions. Using an Operant Social Preference (OSP) task to measure social reward, we found that both subordinate and socially defeated males made significantly fewer entries into chambers containing novel, same-sex conspecifics compared to males who were dominant (i.e., stably won the agonistic encounters). In females, however, there were no differences in social entries between winners and losers. In a second experiment, we found more activation of the mesolimbic dopamine system (MDS) as assessed with cFos immunohistochemistry in the lateral ventral tegmental area (lVTA) and the nucleus accumbens (NAc) shell of male winners compared to losers. In females, however, there were no differences in activation in the lVTA between winners and losers. Surprisingly, however, winning females displayed significantly more activation in the NAc shell as compared to losing females, despite the lack of behavioral differences. Thus, behavioral and histological data

suggest that there are sex differences in the impact of social status on social reward and associated mesolimbic activation.

2.2 Introduction

Social reward is vital to the expression of adaptive social behaviors as well as to the development and maintenance of relationships among individuals (Trezza et al., 2011). Despite the importance of the rewarding nature of social interactions in biological and psychological health, relatively little is known about how social stimuli that generally have a positive valence can become less rewarding or even aversive in neuropsychiatric disorders. A better understanding of these processes is important for understanding the dysfunctions in social reward processing that are pervasive across disorders such as autism spectrum disorder, schizophrenia, depression, anxiety and bipolar disorders (Dichter et al., 2012).

One factor that may contribute to the dampening of the rewarding properties of social interactions is exposure to social defeat stress or perceptions of low social status. Although social stress can lead to a variety of adverse behavioral symptoms, including social avoidance, anhedonia, and motivational deficits (Blanchard et al., 1993; Huhman, 2006), the extent to which social stress reduces the rewarding properties of social interactions is not known. It is also important to investigate the effects of social stress on social reward in both males and females because existing data suggests that there are sex differences in the consequences of social stress and in the rewarding properties of social interactions (Borland, Frantz, et al., 2018; Borland, Rilling, et al., 2019; Huhman et al., 2003). An improved understanding of the effects of social stress on social reward in males and females will likely contribute to better understanding of the behavioral symptoms observed in a variety of human neuropsychological disorders and the sex differences frequently observed in their occurrence.

Social stress can be produced by a variety of factors including social defeat and low social status. While social defeat and low social status have common features in that both are induced by defeat, there are also important differences. Social defeat is typically induced by one or more attacks from highly aggressive conspecifics that do not have a prior relationship with the subject. In contrast, social subordination (as well as social dominance) develops when two equally matched subjects interact over several days and form a stable relationship in which one subject consistently wins (i.e., becomes dominant) and the other loses (i. e., becomes subordinate).

We first examined whether social stress induced by either social defeat stress or stress produced by stable social subordination reduces the rewarding properties of social interactions, and, conversely whether stable social dominance increases the rewarding properties of social interactions. Next, we examined the neural circuitry mediating social reward. Although the mesolimbic dopamine system (MDS), which includes dopaminergic (DA) projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is the canonical reward pathway that mediates the reinforcing properties of stimuli such as food, sex, and drugs of abuse, its role in mediating social reward is less clear (Lammel et al., 2011). Recent data, however, suggests that pathways from the VTA to NAc can encode key features of social interactions and that manipulations of this projection can modulate social behaviors (Gunaydin et al., 2014). This pathway has also been implicated in social motivation and in the processing of rewarding and aversive stimuli (Lammel et al., 2014; Pignatelli & Bonci, 2015; Schultz, 2016; Watabe-Uchida, Eshel, & Uchida, 2017). It has also been suggested that VTA to NAc projections are central to the neural regulation of social dominance (Ghosal, Sandi, & van der Kooij, 2019). For example, in hamsters, higher numbers of cFos-immunoreactive cells were found in the VTA in individuals that displayed more social dominance behaviors (Gil et al., 2013) and, in rats, social competition

increases c-Fos activation in NAc neurons (Van Der Kooij et al., 2018). There is also evidence that dopamine in the NAc modulates the behavioral changes induced by social defeat stress (Gray et al., 2015). Taken together, these data suggest that VTA to NAc projections may be critical for the rewarding properties of social interactions as well as the site where social status and social stress may act to alter social reward. The VTA is characterized by pronounced anatomical and functional heterogeneity, with different subregions of the VTA projecting to different neural targets (Cacciapaglia et al., 2011; Dutta et al., 2021; Kutlu, Zachry, Melugin, Cajigas, Chevee, Kelly, et al., 2021; Lammel et al., 2014, 2012; Ray et al., 2022; Saddoris et al., 2015; Yuan et al., 2019). For example, the lateral VTA (lVTA) sends substantial projections to the lateral NAc shell and the medial VTA (mVTA) projects to the NAc core (Lammel et al., 2014).

In the second experiment, we examined the neural activation in the lVTA and the NAc shell as well as the medial VTA (mVTA) and the NAc core following social interactions in dominant, subordinate and socially defeated male and female hamsters. These data support the hypothesis that the lVTA and the NAc shell and not the medial VTA (mVTA) and the NAc core mediate social reward in males but that the activity in these subregions in response to social reward is more complex in females.

2.3 Methods

2.3.1 Subjects

Adult male and female Syrian hamsters were bred in the Georgia State University vivarium and maintained on a 14:10 light:dark cycle (lights out at 10:00 am). At two months of age, animals weighed approximately 110–135 g and were individually housed in 24 cm X 33 cm X 20 cm polycarbonate cages filled with corncob bedding and nesting material for two weeks before behavioral testing was initiated (see below). It is important to note that we have demonstrated that

individual housing in hamsters is not stressful (Ross et al., 2017). Food and water were available ad libitum. Prior to behavioral testing, females' estrous cycles were monitored for eight days to confirm regular cyclicity. Males were handled similarly to the females each day. In females, behavioral tests were run on diestrus 1, diestrus 2, and proestrus, with a pause on estrus in order for lordosis to not interfere with social behavior. Behavior tests resumed on the following diestrus 1, and all females were tested on the same day of their cycle for each day of social pairing and behavioral testing. Males were yoked to this schedule. Data collected during the OSP task and for c-Fos quantification were from females on diestrus 2 of their cycle. In addition to the male and female test subjects, male and female non-aggressive intruders (NAIs) were also used during operant social preference training (see below). NAIs were younger (~2months), smaller (100–110 g), and housed four to a cage. The Resident Aggressors (RA) used for defeat training were older (>6 months), singly housed same-sex opponents weighing between 160 and 180 g. All procedures and protocols were performed in accordance with the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Georgia State University.

2.3.2 Establishing Dominant, Subordinate, or Defeat Status or No-status Controls

Subjects were randomized into a group where dominant/subordinate dyads were formed or into a group where the subjects were defeated. To establish dominance and subordinance, hamsters were weight- and sex- matched and paired with the same conspecific for five-minute sessions daily for five days. These pairings occurred in the home cage of one of the hamsters, which was randomly selected as the resident. Residency did not account for dominance, with only ~50 % of residents winning their agonistic encounter and establishing status as dominant. Furthermore, residency did not account for social entries, latency to social entry, or social preference score in

operant social preference. The dominant/subordinate dyads were stable across all 5 days of pairing. Defeated subjects were paired with a sex-matched RA for five-minute sessions over five days. Defeated hamsters were always the intruder in the home cage of a different RA each day. Agonistic encounters were monitored for potential injuries. In the case of an aggressor breaking the skin of a subject, that subject was be excluded to avoid the confound of pain and the inflammation associated with tissue damage. The purpose of including two groups who both lost agonistic encounters was to manipulate the "dose" of social stress, with a stable dominant/subordinate dyad as a moderate stressor in comparison to the more severe, unstable relationship experienced by defeated hamsters which were paired with novel RAs each day. Hamsters in the no-status control group were transported to behavioral testing suite daily where they were handled by the experimenter but underwent no social interactions in their home cage. All subjects were housed singly for two weeks prior to the experiment.

2.3.3 Behavioral Analysis

All social pairings were digitally recorded, and social behaviors were analyzed and quantified by an observer blind to the experimental condition using Noldus Observer (11.5, Leesburg, VA) system and a hamster ethogram (Albers, Huhman, & Meisel, 2002). The second observer scored a random subset of these videos. Inter-rater reliability (i.e., percent agreement) between the two observers was above 90 %. The total duration of four classes of behavior were measured during the social sessions: (1) social behavior (stretch, approach, sniff, nose touching, and flank marking); (2) non-social behavior (locomotion, exploration, grooming, nesting, feeding, and sleeping); (3) submissive/defensive behavior (flight, avoidance, tail up, upright, side defense, full submissive posture, stretch attend, head flag, attempted escape from cage); and (4) aggressive behavior (upright and side offense, chase and attack, including bites) (Albers et al., 2002).

2.3.4 Experiment 1

Subjects underwent social pairings to establish status as described above. Following establishment of status, subjects were trained and tested on the OSP task, as detailed in *Figure 2*.

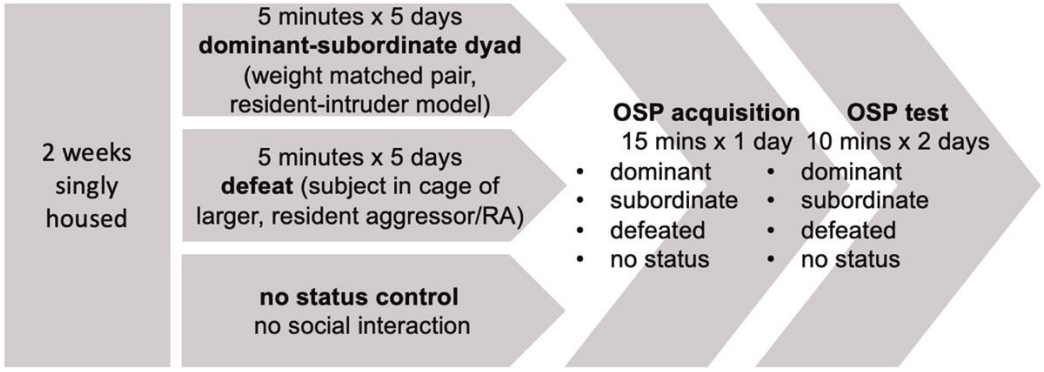

Figure 2: Timeline for Experiment 1

2.3.5 Operant Social Preference (OSP) Task

Operant conditioning sessions began with hamsters placed in the main chamber of the OSP apparatus *(Figure 3)*, equidistant from both smaller stimulus chambers. A NAI was confined to one stimulus chamber, termed the "social chamber" and the other chamber was empty, i.e., the "nonsocial chamber." Assignment of the stimulus hamsters to the right or left chamber was counter-balanced across experimental groups. There was an equal number of subjects in each group encountering social stimuli in the right chamber each of the 3 sessions as those with social stimuli on the left side all 3 sessions. Subjects were allowed to move throughout the apparatus, while stimulus hamsters were confined to one of the small chambers. Twenty seconds after entry into either small chamber, the subject was returned to center of the main chamber. On the first day of conditioning, the subject was given a 15-minute session to acclimate to the apparatus and become accustomed to pushing through the vertical swing doors. On following two days the sessions were 10 min. Our lab has previously utilized the OSP task in order to determine whether

social interactions are rewarding based on their ability to reinforce entries into chambers where social interactions occur. This is assessed by quantifying entries into the social chamber (social entries), latency to social entry, entries into the nonsocial chamber (nonsocial entries), and by calculating a social preference score (SPS), which is the difference between social and nonsocial entries. Further descriptions and validations of the apparatus and task can be found in, Borland, 2017 (Borland et al., 2017; Borland, Grantham, Aiani, Frantz, & Albers, 2018; Borland, Rilling, et al., 2019).

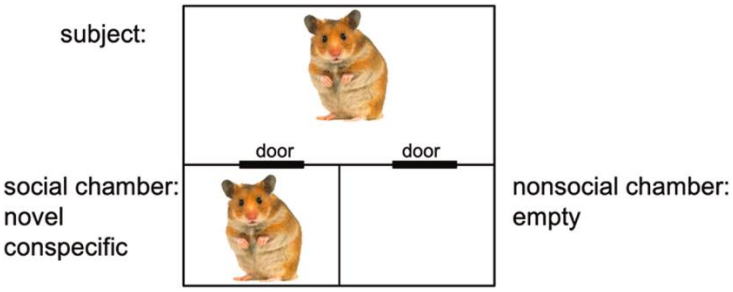

Figure 3: OSP Apparatus

The OSP apparatus was constructed of clear acrylic The apparatus consisted of three chambers: a main chamber (50.8 × 33 × 30.5 cm, l × w × h) and two smaller adjacent chambers (25.4 × 17.8 × 30.5 cm, l × w ×h). Each small chamber is separated from the main chamber by a one-way vertical-swing door (10.2 × 7.6 cm, l × h); smaller chambers can only be accessed from main chamber. Chamber doors were brushed with steel wool to achieve coarse texture, distinct from the rest of apparatus, and doors were perforated with circular holes to allow airflow. Operant conditioning sessions began with hamsters placed in a designated drop zone (10.2 × 7.6 cm, l × h) against the far wall of the main chamber in the apparatus, equidistant from both small chambers. A smaller (100–120 g) non-aggressive (group housed), same- sex stimulus hamster was confined to either the left or right smaller chamber. Assignment of the stimulus hamsters to the right or left chamber was counter-balanced across experimental subjects. Subjects never interacted with the same stimulus hamster across testing days: a new stimulus hamster was provided for each subject on each test day.

2.3.6 Experiment 2

Subjects underwent social pairings to establish status as described above. Ninety minutes following the last social pairing to establish status as dominant, subordinate, or defeated,

perfusions were performed in order to prepare the brains for immunohistochemistry. The timeline for experiment 2 is illustrated in *Figure 4*.

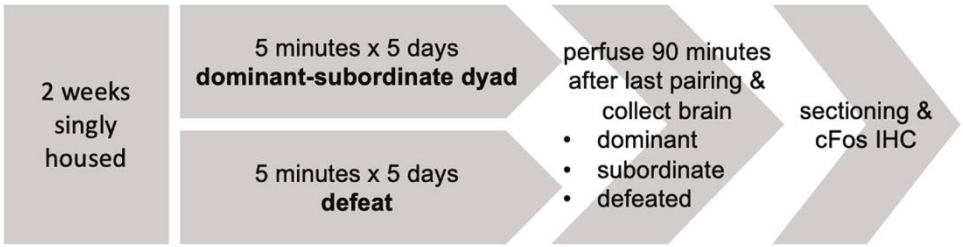

Figure 4: Timeline for Experiment 2

2.3.7 Transcardial Perfusions

Animals were deeply anesthetized with sodium pentobarbital as determined by a lack of response to a toe pinch. An incision was then made into the chest to expose the heart. A needle connected to surgical tubing was inserted into the left ventricle and the right atrium was pierced with surgical scissors. The tubing was run through a pump set to 15 ml/minute. Animals were first perfused with 100 ml cold phosphate buffered saline (PBS) and then with 100 ml cold 4 % paraformaldehyde in PBS (PFA). Brains were then extracted and stored in 4 % PFA for 24 h and then cryoprotected in 30 % sucrose in PBS until they were sectioned.

2.3.8 cFos Immunohistochemistry

Brains were sectioned on a cryostat at 40 μm thickness and stored in cryoprotectant in the − 20 ˚C freezer until the IHC was performed. Three to four sections from each animal were isolated and rinsed five times with PBS to remove excess cryoprotectant. Sections were blocked in a 10 % normal donkey serum in PBS with 1 % Triton-X for 1 hour. Then sections were incubated in primary antibody (ab208942 mouse anti- cFos, 1:500 dilution, Abcam, Boston, MA) diluted in 5 % normal donkey serum diluted in PBS with 1 % Triton-X overnight on a shaker either at room

temperature or at 4 ˚C. Sections were rinsed three times for five minutes in PBS. Sections were then incubated in secondary antibody (Alexa Fluor 594 conjugated donkey anti-mouse IgG, 1:250 dilution, Jackson ImmunoResearch Laboratories, West Grove, PA) for two hours on a shaker at room temperature. Sections were then mounted onto Super Frost Plus slides, dried, and coverslipped with Vectashield Hard Set Mounting Medium for Fluorescence with DAPI (H1500, Vector Laboratories, Burlingame, CA). Using the cell counter plugin on FIJI, cFos positive and DAPI positive cells were quantified in appropriate regions as defined in Morin & Wood, 2001 (Morin & Wood, 2001). The entire brain regions (NAc core, NAc shell, mVTA, and lVTA) were quantified in three sections of tissue and averaged for each subject. A second observer counted a random subset of these microscope images. Inter-rater reliability (i.e., percent agreement) between the two observers was above 90%.

2.3.9 Statistical Analysis

Data were analyzed using Prism 9 (GraphPad). A between subject analysis of variance (ANOVA) was run with sex and status as the factors. Factorial designs of 2 × 2 (*Fig. 4*), 2 × 4 (*Fig. 5*), and 2 × 3 (*Fig. 7*) were used to account for the differing status levels across various analyses. In cases of statistical significance, Tukey's post-hoc tests were used to examine group differences. Statistical significance was conferred at $p < 0.05$.

2.4 Results

2.4.1 Quantification of Aggression During Social Conflict

Both subordinate and defeated hamsters were the target of aggression, so we first compared the duration of aggression received by subordinate hamsters with the duration of aggression received by defeated hamsters during behavioral manipulations in order to determine if there was a dose-response relationship between amount of aggression received and the behavioral responses

observed in the first experiment. As can be seen in *Figure 5*, subordinate hamsters, surprisingly, were the recipient of a significantly longer duration of aggression than were hamsters defeated by an unfamiliar RA (F(1, 38) = 34.60, $p < 0.0001$). There were no sex differences in aggression received by males and females.

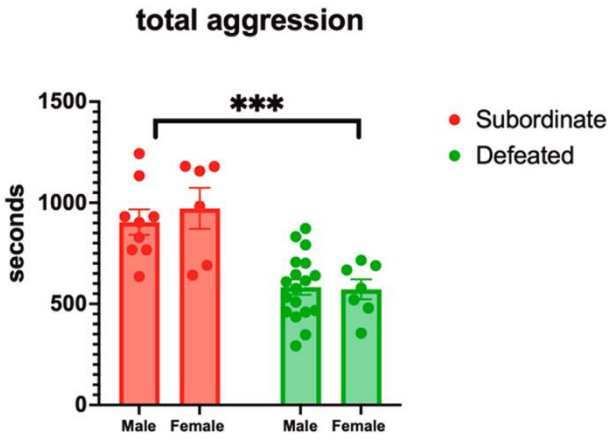

Figure 5: Total Aggression

*The total aggression received during establishment of social status. Males and females who were subordinate experienced the same total duration of aggression during establishment of social status. Similarly, males and females that were socially defeated experienced the same total duration of aggression. Overall, subordinate subjects experienced more total aggression than did those who were defeated. Indicates ***p < 0.0001.*

2.4.2 Effects of Sex and Social Status on Operant Social Preference

There was a significant interaction between sex and social status on the number of entries into the chambers containing another hamster (i.e., social entries) (F (3, 96) = 5.141, $p = 0.0024$, *Fig. 6A*). Furthermore, there was a significant effect of status on latency to enter the social chamber (F (3, 96) = 4.734, $p = 0.0040$, *Fig. 6B*). There was also a significant interaction between sex and status on social preference score (SPS) (F (3, 96) = 5.316, $p = 0.0020$, *Fig. 6C*). **In males**, dominant hamsters made more social entries, had a higher SPS, and had a shorter latency to enter the social chamber than did subordinate or defeated hamsters (*Fig. 6A, B, C*). No significant differences in the number of social entries, latency to enter the social chamber, or SPS was observed between

the no-status control group and the dominant, subordinate, or defeated groups. **In females**, there were no statistically significant differences in number of social entries between dominant, subordinate or defeated hamsters (*Fig. 6A*). In contrast, however, there were significant differences in females in the number of social entries and SPS between the no-status control group and the dominant, subordinate, and defeated groups ($p < 0.05$, *Fig. 6A, C*). There was also a significantly longer latency to enter the social chamber in the defeated group compared to the no-status control group ($p = 0.0242$, *Fig. 6B*). There were, however, no significant differences in latency to enter the social chamber across the dominant, subordinate, and defeated groups *($p > 0.05$, Fig. 6B)*. There were no differences in nonsocial entries across all groups (*Fig. 6D*). Furthermore, there were no differences in the proportion of time subjects spent in the social chamber engaged in social investigation, aggression, or nonsocial behavior *(Fig. 7)*. Lastly, the majority of the time in the social chamber was spent on social investigation across all groups *(Fig. 7)*.

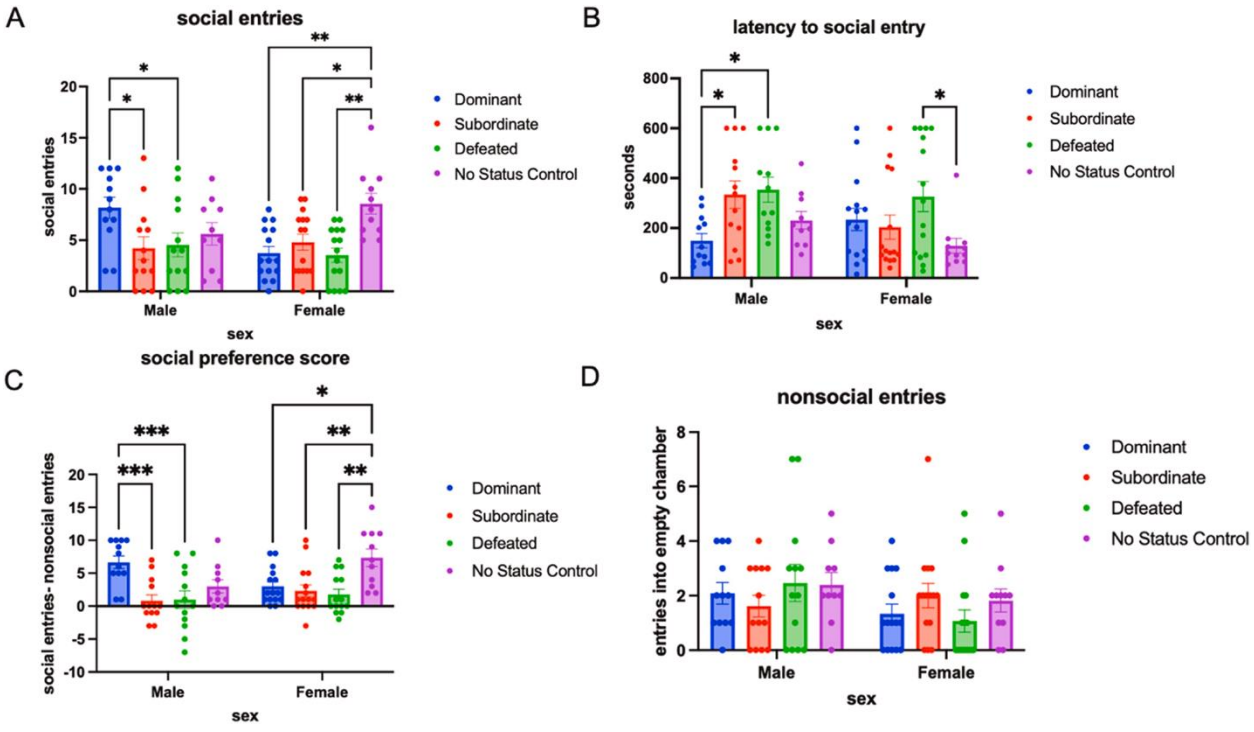

Figure 6: Operant Social Preference

Operant Social Preferences in dominant, subordinate, defeated or no status control hamsters. ***A.*** *Defeated and subordinate males made fewer entries into the social chamber when compared to dominant males. Females' social entries were unaffected by social status; however, no-status control females made more entries than dominant, subordinate, and defeated females.* ***B.*** *Defeated and subordinate males had a longer latency to enter the social chamber when compared to dominant males. Females' latencies were unaffected by social status; however, no-status control females had shorter latencies to social entry compared to defeated.* ***C.*** *Defeated and subordinate males had lower social preference scores (SPS) compared to dominant males. This was calculated as the difference between social entries and nonsocial entries. Females' SPS were unaffected by social status; however, no-status control females had higher SPS than dominant, subordinate, and defeated females. D. There were no differences in nonsocial entries based on sex or status * Indicates $p < 0.05$, ** $p < 0.01$, (2-way ANOVA).*

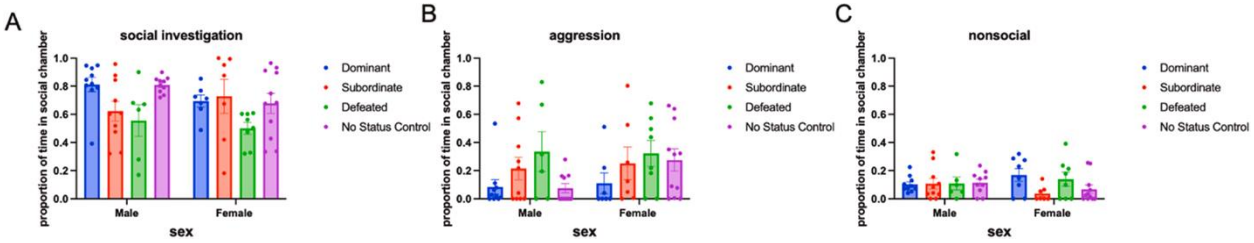

***Figure 7*: Behavior in Social Chamber of OSP**

Behaviors observed while subjects were in the social chamber during OSP A. Proportion of time in social chamber that subject engaged in social investigation B. Proportion of time in social chamber that subject engaged in aggression C. Proportion of time subject was nonsocial.

2.4.3 cFos Immunohistochemistry

Based on the behavioral data obtained in Experiment 3.1, we expected that dominant **males** would express more cFos immunoreactivity than would subordinate or defeated males in the VTA and the NAc. In contrast, **in females** where there were no differences in social reward across groups, we expected no between group differences in cFos immunoreactivity in the VTA or the NAc. We also investigated differences in the medial and lateral VTA as well as NAc core and shell due to functional differences in subdivisions (Lammel et al., 2011, 2014). Unexpectedly, a significant main effect of social status on cFos expression was observed in the NAc shell ($F(2, 36) = 37.94$, $p < 0.0001$, *Fig. 8B*) in **females as well as males**. In contrast, but consistent with our predictions based on the behavioral data from Experiment 3.1, there were significant differences

in cFos immunoreactivity between the dominant males and the defeated males in the lVTA (F (2, 36) = 4.556, p = 0.0034, *Fig. 8D*). No significant differences were observed in cFos immunoreactivity in the lVTA between dominant, subordinate and defeated females. No differences were observed in cFos immunoreactivity in the NAc core *(Fig. 8A)* or the mVTA *(Fig. 8C)* across dominant, subordinate or defeated males or females (*Fig. 8*).

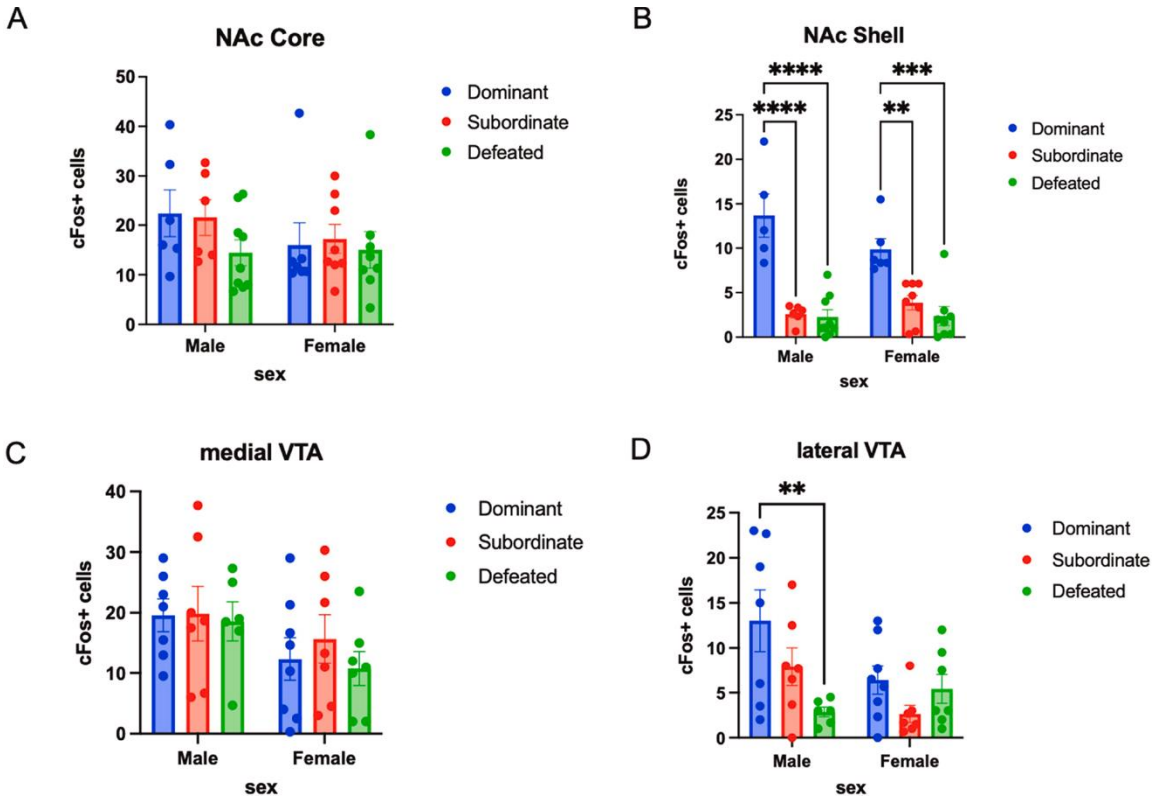

Figure 8: cFos Immunohistochemistry

Activation in of neurons in the nucleus accumbens (NAc) core and shell and the lateral ventral tegmental area (lVTA) and the medial ventral tegmental area (mVTA) measured with cFos immunohistochemistry. ***A.*** *In males and females, the NAc core was equally active during winning and losing* ***B.*** *Conversely, the NAc shell was less active in losers.* ***C.*** *In the lVTA, defeated males had fewer cFos+ cells than did dominant males.* ***D.*** *There were no differences in cFos expression in the medial VTA in either sex. * Indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.*

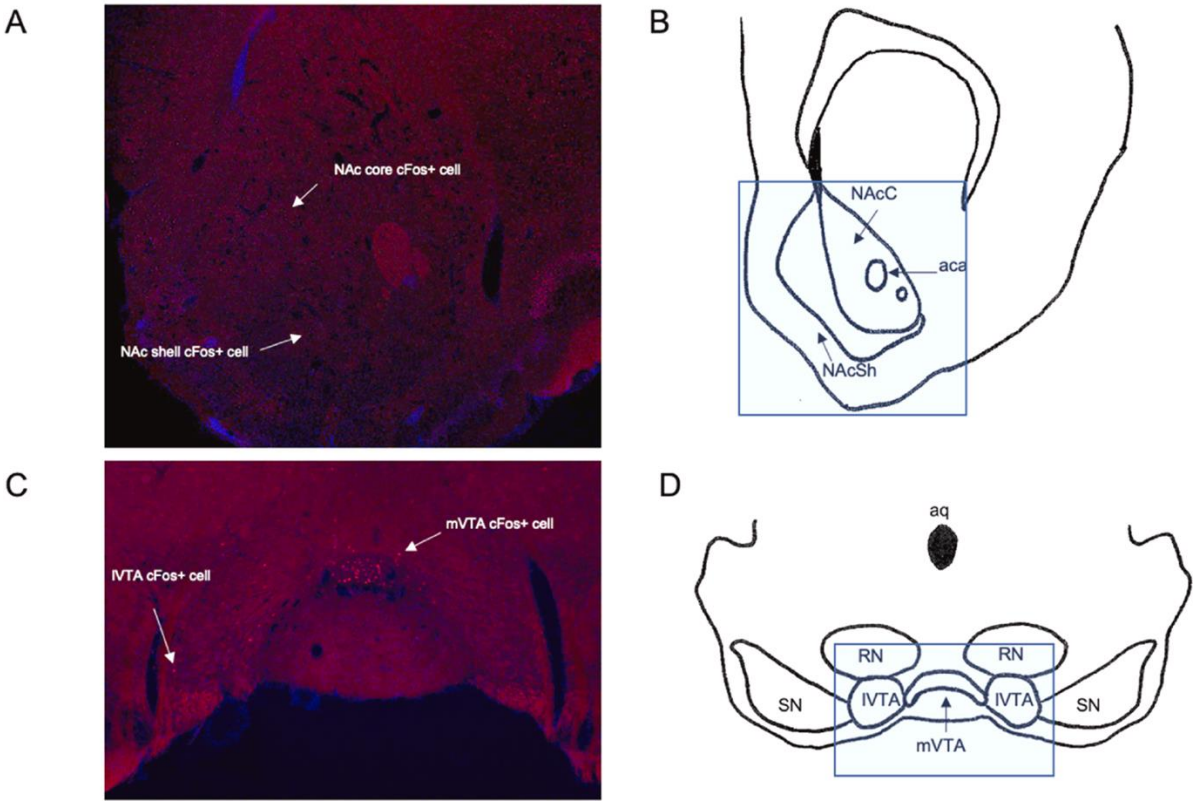

Figure 9: Representative Photomicrographs

Representative Images of cFos immunohistochemistry in the nucleus accumbens (NAc) and ventral tegmental area (VTA). ***A.*** *cFos (red) and DAPI (blue) merged for NAc* ***B.*** *Diagram of region corresponding to microscope image- micrograph is inset of highlighted box (NAc core: NAcC, NAc shell: NAcSh, aca: anterior commissure), Bregma − 2.4 mm* ***C.*** *Representative image of cFos (red) and DAPI (blue) merged for VTA (medial: mVTA, lateral: lVTA)* ***D.*** *Diagram of region corresponding to microscope image- micrograph is inset of highlighted box (medial VTA: mVTA, lateral VTA: lVTA, aq: aqueduct, substantia nigra: SN, red nucleus: RN), Bregma − 4.0 mm.*

2.5 Discussion

In this study, we tested the hypothesis that social stress induced by low status or by defeat reduces the rewarding properties of social interactions and that social dominance enhances the rewarding properties of these interactions. The results in **males** supported this hypothesis; males that were socially defeated or that had stable subordinate status made significantly fewer social entries, had a lower SPS, and had higher latencies to enter social chambers compared to dominant

males. Thus, these data suggest that, **in males**, social stress decreases social reward and the motivation to seek out social interactions. In **females**, however, there were no differences in the number of social entries, SPS, or the latency to enter social chambers among defeated, subordinate and dominant hamsters. There were, however, significantly fewer entries into social chambers by defeated, subordinate and dominant hamsters compared to hamsters in the no-status control group.

These data indicate that the rewarding properties of social interactions were higher in dominant males than in subordinate or defeated males. At present, however, it is hard to know if dominance increases social reward or if defeat/subordinance reduces social reward because no significant differences were observed in social entries between these groups and the no-status control group. In contrast, in females, it appears that social experience regardless of whether it results in dominance, subordinance, or defeat reduces the rewarding properties of social interactions because no-status controls made more social entries than did any of the female groups with social experience. The factors responsible for the sex differences in social reward for dominant males and females are not clear.

One factor that may contribute to the observed sex difference, is that social experience modulates the rewarding properties of social interactions. For example, social interactions are less rewarding for animals housed in social groups than they are for animals that are socially isolated (Douglas, Varlinskaya, & Spear, 2004). There is also evidence for a sex difference in the relationship between dose of social interaction (e.g., number of interactions) and how rewarding those interactions are. It has been proposed that there is an inverted “U” relationship between the dose of social inter- action (e.g., number of interactions) and their rewarding properties and that the U is shifted to the left in females compared to males (Borland, Aiani, et al., 2019; Borland, Rilling, et al., 2019). As a result, the multiple social interactions that occurred before testing may

have modulated the rewarding properties of the social interactions such that the interactions in dominant females were less rewarding than they were in dominant males.

The present study found little evidence of sex differences in the response to social stress. Social stress produced by subordinate status or social defeat produced very low levels of social entries in males and females. Of course, the lack of sex differences in social stress in this experiment could have been a result of a "floor effect" given the very low levels of social entries. Other studies in hamsters have found a sex difference in response to social defeat. Male, but not female, hamsters display a persistent reduction in territorial aggression and an increase in the expression of submissive and defensive behavior (Huhman et al., 2003). The study of sex differences in the response to social stress has been significantly understudied because of the difficulty of inducing social defeat stress in female rodents (Steinman & Trainor, 2017). Interestingly, in another rodent species where females are susceptible to social defeat stress, the California mouse, social defeat stress reduces social interaction responses in females but not males (Trainor et al., 2011). Given that women are twice as likely to be diagnosed with depression than men understanding the underlying sex differences in the etiology of depression remains extremely important.

Another interesting aspect of the study to note is that the dose manipulation ultimately failed. The historically accepted dogma has been that the purpose of submissive behavioral displays is to reduce costly aggression, thereby ultimately minimizing attacks and wounding within social groups (Reddon, Ruberto, & Reader, 2021; Sapolsky, 2005). Thus, we anticipated that the stable dominant/subordinate dyad would be a more moderate stressor than would its more severe counterpart, the unstable relationship in which five novel RAs defeated a single subject. To our surprise, however, there were no differences between these two losing groups in terms of behavior

in the OSP or cellular activation in the MDS. Perhaps even more surprisingly, subordinate hamsters received more overall aggression than did defeated hamsters. Future studies should seek to form a more stable dominant/subordinate relationship in which aggression is lower than that observed in novel pairings. These agonistic encounters used in this study were relatively short (5 min in duration over 5 consecutive days) so pairings of longer durations each day, more days of pairing, or even housing the dyad together could lead to a more stability and thus reduce aggression in the dominant-subordinate pairings for future studies.

We also found support for the hypothesis that neural activation in the lVTA and the NAc shell but not the mVTA and NAc core mediates social reward. Support for this hypothesis was provided in males. No differences in the number of cFos-containing neurons in the mVTA or NAc core were observed between dominant, subordinate or defeated males.

In contrast, in dominant males, which displayed more social entries than did subordinate or defeated males, there were significantly higher numbers of cFos-containing cells in the lVTA and NAc shell. In females, wherein no differences were observed in the number of social entries between dominant, subordinate, and defeated hamsters, there were also no differences in the number of cFos containing cells in the lVTA, mVTA or NAc core among groups. Surprisingly, however, the number of cFos- containing cells in the NAc shell were significantly greater in dominant females than in subordinate or defeated females. These data suggest that, at least in females, the greater activation of NAc shell neurons in dominant females compared to subordinate and defeated females is not the result of differences in social reward between the groups. Given the heterogeneity of cell types within the VTA and NAc it will be important to determine the phenotypes of the activated cells within these regions in future experiments.

In summary, the data in males is consistent with the hypothesis that neural activation in the lVTA and the NAc shell mediates social reward. In females, the pattern of neuronal activation in the lVTA is also consistent with the hypothesis that neuronal activation in the lVTA but not the NAc shell mediates social reward. One possibility is that there is a sex difference in the factors regulating neuronal activation in the NAc shell such that neuronal activation in the NAc shell mediates reward in males, but other factors mediate the pattern of neuronal activation in females. Alternatively, the greater neuronal activation in the NAc shell seen in dominant male and female hamsters compared to subordinate or defeated hamsters may not contribute to differences in social reward but may instead be mediated by other factors such as social status. Using the immediate early gene product cFos as a marker of activation gives us useful information about the response of the cells in NAc core and shell to these social contexts. It is also important to point out that there were differences in the social context between the cFos and OSP experiments.

The cFos data was collected 90 min after the last social interaction between dominant and subordinate hamsters or following social defeat. As a result it is possible that dominant females found winning over their subordinate partner to be rewarding whereas dominant females in the OSP experiment did not find the interactions with a non-aggressive intruder to be rewarding. It will be important to clarify the role of the NAc shell and the lVTA in social reward in females.

To our knowledge, this is one of the first studies to differentiate activity of subregions of the MDS in the social context of winning and losing agonistic encounters. The data revealed that males and females are similar in terms of cellular activation in the NAc shell following establishment of status as dominant, subordinate, or defeated, but there were sex differences in social reward and in cellular activation of the lVTA.

3 A SUBCIRCUIT HYPOTHESIS OF MESOLIMBIC DOPAMINE CODING OF SOCIAL SALIENCE AND VALENCE

3.1 Abstract

The mesolimbic dopamine system (MDS) is comprised of projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). The anatomical and functional heterogeneity of these dopamine (DA) containing projections may explain the ability of this system to respond to a diverse group of rewarding and aversive stimuli ranging from food to sex and drugs of abuse. Surprisingly, however comparatively little is known about the role of the MDS in social reward and social aversion. Here we test the novel hypothesis that one DA-containing subcircuit in the MDS mediates the salience (i.e., the importance of social interactions) and another subcircuit mediates the valence (i.e., whether social interactions are rewarding or aversive. Data obtained using retrograde tracing, immunohistochemistry, dopamine amperometry, and behavioral testing in male and female hamsters supported the hypothesis that DA in the medial VTA (mVTA) to NAc core pathway codes for the salience of social stimuli, while DA in the lateral VTA (lVTA) to NAc shell codes for the valence of social stimuli. Identification of the subcircuitry within the MDS that detects and distinguishes between social saliency, social reward and social aversion contributes to our understanding of the mechanisms underlying adaptive social behavior as well as the deficits in these behaviors that are associated with a diverse group of neuropsychiatric disorders.

3.2 Introduction

The mesolimbic dopamine system (MDS) is the canonical “reward” pathway that mediates the reinforcing properties of stimuli such as food, sex, and drugs of abuse. The MDS contains dopaminergic (DA) projections from the ventral tegmental area (VTA) to the nucleus accumbens

(NAc). The VTA is characterized by pronounced anatomical and functional heterogeneity with different subregions of the VTA projecting to different neural targets (Cacciapaglia et al., 2011; Dutta et al., 2021; Kutlu, Zachry, Melugin, Cajigas, Chevee, Kelley, et al., 2021; Lammel et al., 2014, 2012; Ray et al., 2022; Saddoris et al., 2015; Yuan et al., 2019). For example in rats and mice, DA neurons in the medial VTA (mVTA) project primarily to NAc core, whereas DA neurons in the lateral VTA (lVTA) project primarily to the lateral NAc shell (Lammel et al., 2014).

Axonal projections from the VTA to the NAc core release dopamine in a phasic manner. This rapid phasic patterns of release of DA, often labelled "transients" were traditionally thought of as learning cues, signaling a reward prediction error (RPE) to encode the difference between anticipated and actual reward (Schultz, 2016; Wise, 2004). While this theoretical framework fits some models of reinforcement, such as drug reward and associated cues, this model does not appear to apply in all reward situations and does not explain DA release in aversive contexts (Kutlu, Zachry, Melugin, Cajigas, Chevee, Kelley, et al., 2021). More recent data collected across different learning paradigms suggests that DA contributes to learning about various contingencies by signaling the perceived saliency of stimuli. This possibility is consistent with the idea that DA release in the NAc core serves a key function in valence-free learning, thus representing a better fit with DA release patterns in response to aversive stimuli than RPE (Bromberg-Martin et al., 2010; Kutlu, Zachry, Melugin, Cajigas, Chevee, Kelley, et al., 2021).

Release of DA in the NAc shell occurs in a tonic manner, with slow changes occurring over a longer time scale than in the core. Tonic DA release in the NAc shell is thought to encode pleasure or the hedonic aspect of rewarding behaviors. Furthermore, DA release in the NAc shell is consistent with encoding the value or valence of stimuli, either positive or negative (Cacciapaglia et al., 2011; Saddoris et al., 2015). This is thought to drive approach toward

rewarding stimuli and avoidance of aversive stimuli, respectively. For example, shell DA increases during rewarding stimuli (i.e. sucrose or drug delivery) which have a positive valence and decreases during aversive stimuli (i.e. bitter taste presentation or noxious foot shock) which have a negative valence (Yuan, Dou, & Sun, 2021).

Although there is considerable information on MDS connectivity and function in certain rewarding and aversive contexts, the role of the MDS in mediating the salience and valence of social stimuli remains almost entirely unknown. Because social stimuli and in particular their salience and valence have a profound impact on social and emotional processes, understanding their neurobiological mechanisms will provide a critical translational link to disorders associated with diminished social reward such as autism spectrum disorder as well as mood and anxiety disorders (Borland, Grantham, et al., 2018; Borland, Rilling, et al., 2019; Gray et al., 2015; Huhman, 2006; Huhman et al., 2003; Luckett, Norvelle, & Huhman, 2012; Song et al., 2016)

Here we test the overarching hypothesis that anatomically distinct subcircuits within the MDS mediate the salience and valence of social interactions. Specifically we propose that DA projections from the mVTA to NAc core code for the salience of social stimuli whereas projections from the lVTA to NAc shell code for the valence of social stimuli (*Figure 1)*. First, we investigated MDS subcircuit connectivity using retrograde tracing studies to determine if the mVTA projects to the NAc core and the lVTA projects to the NAc shell in our model organism, the Syrian hamster (*Mesocricetus auratus*). Next, we tested the hypothesis that phasically released DA in the NAc core encodes the salience of social stimuli whereas tonically released DA in the NAc shell encodes the valence of social stimuli. To test the predictions of this hypothesis, we measured DA efflux in the NAc during winning vs. losing agonistic encounters in male and female hamsters. We predicted that if salience is mediated by the phasic release of DA in the core, then the phasic release of DA

should increase during agonistic encounters whether the subject won or lost the encounter. On the other hand, if tonic release of DA in the NAc shell mediates the valence of social stimuli, then winners (positive valence) should exhibit significantly higher levels of tonic DA release than losers (negative valence).

Next we examined whether anatomically distinct subcircuits within the MDS mediate the salience and valence of social interactions by comparing the neuronal activation in DA-containing neurons in the VTA during winning and losing agonistic encounters. Activation of DA-containing neurons was determined by the colocalization of cFos and tyrosine hydroxylase(TH) immunoreactivity. We predicted that if salience is mediated by projections from the mVTA to the NAc core, then DA containing neurons in the mVTA should display similar levels of activation in winners and losers but significantly more activation than in behavioral controls. Further, if valence of social interactions is mediated by projections from the lVTA to the NAc shell, then DA neurons in the lVTA should display significantly more activation in winners than in losers or controls.

3.3 Methods

3.3.1 Subjects

Adult Syrian hamsters (120-140g, post-natal day 60) were singly housed in a 14:10 reverse light-dark cycle. All animals are given *ad libitum* access to food and water. Resident aggressors (RAs) were older (>6 months old), larger hamsters (160 to 180 g) who were more aggressive and territorial as the result of extended single housing. Non-aggressive intruders (NAIs) were younger (~2 months old), smaller (100-110g) and less aggressive as the result of group housing (i.e., 4 to a cage). All experimental animals were singly housed for two weeks prior to experiments. The estrous cycle of all females were monitored daily by vaginal discharge, and males were also be handled daily as a control for the handling of the females. Male

behavioral testing was also yoked to the females' cycles so that the same number of males and females were tested each day. Because the hamster estrous cycle is 4 days in length, the females' cycles were tracked for two full cycles (i.e. 8 days) before the beginning behavioral experiments. Females began social pairing, behavioral testing or amperometry on Diestrus 1 (D1) in order to avoid testing on the day of estrus. In addition to the male and female experimental subjects, male and female non-aggressive intruders (NAIs) were used during operant social preference training (see below). All procedures and protocols were performed in accordance with the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Georgia State University.

3.3.2 Behavioral Analysis

All social pairings were digitally recorded using Noldus Observer (11.5, Leesburg, VA) system and a hamster ethogram (Albers et al., 2002). All social behaviors were quantified by observers blind to the experimental condition. Inter-rater reliability (i.e., percent agreement) was determined by a second observer scoring a randomly selected subset of the recordings. The agreement of the two observers was above 90%. The total duration of four classes of behavior were measured during the test session: (1) social behavior (stretch, approach, sniff, nose touching, and flank marking); (2) non-social behavior (locomotion, exploration, grooming, nesting, feeding, and sleeping); (3) submissive/defensive behavior (flight, avoidance, tail up, upright, side defense, full submissive posture, stretch attend, head flag, attempted escape from cage); and (4) aggressive behavior (upright and side offense, chase and attack, including bites).

Experiment 1: Tracing projections from VTA to NAc

3.3.3 Retrobead Injection Surgeries

Lumafluor fluorescent latex microspheres or "retrobeads" were utilized as a retrograde neuronal tracer. In order to inject the retrobeads, animals were anesthetized with 5% isoflurane and anesthesia was maintained during surgery with 2-4% isoflurane. The surgical area was clipped, animals placed in ear bars, and the surgical site was cleaned with betadine and ethanol. An incision was made along the midline of the head and the muscle cleared from the skull. Bregma was identified and measured. From bregma, the NAc core (anteroposterior (AP) +3.40 mm; mediolateral (ML) ±2.30 mm; dorsoventral (DV) -6.0 mm) or shell (AP +3.40 mm; ML ±1.80 mm; DV -6.7 mm) was located at a 10^{o} angle towards the midline. A small hole was drilled above the NAc and a 1ml Hamilton syringe was lowered down to the NAc core or shell. 50nl of retrobeads were injected and the needle was held in place for 1 minute to ensure bead delivery and diffusion from the syringe. The incision was closed with a wound clip, and the animal was allowed to recover consciousness in a clean cage placed partially on a heating pad. All hamsters were injected subcutaneously with the anti-inflammatory agent ketofen (5mg/kg) and were monitored closely following surgery. Post injection survival time was 5 days. At this point, hamsters underwent transcardial perfusions (as described below) to fix the tissue and prepare for processing. Brains were sectioned on a cryostat at 40μm thickness, mounted onto SuperFrost Plus slides, dried, and cover slipped with Fluoromount with DAPI (Invitrogen, Carlsbad, CA). Injection into the NAc core or shell was verified. Using the cell counter plugin on FIJI, projections were quantified in mVTA and lVTA as defined in Morin & Wood, 2001. The entire brain regions were quantified in three sections of tissue and averaged for each subject. A second observer counted a random subset

of these microscope images. Inter-rater reliability (i.e., percent agreement) between the two observers was above 90%.

Experiment 2: Amperometry in the NAc

3.3.4 Surgery for Amperometric Recordings

To prepare for intracerebral carbon fiber microelectrode (CFM) guide placement, hamsters were anesthetized with isoflurane (induced at 5% and maintained at 2-4%). A reference electrode (#7065-C Ag/AgCl reference electrode, Pinnacle Technology, Inc) was then implanted in the superficial cortex contralateral to the implanted cannula (#7030 BASi rat guide cannula, Pinnacle Technology, Inc) that was aimed at the NAc (from bregma: anteroposterior (AP) +3.40 mm; mediolateral (ML) ±2.20 mm;. dorsoventral (DV) -5.50 mm; 10^{o} angle). A head mount was also fixed to the top of the skull for CFM surgeries to anchor electrode ports. CFM guide cannulas were secured to the skull with screws and dental adhesive. Dummy caps are inserted to prevent clogging. All hamsters were injected subcutaneously with the anti-inflammatory agent ketofen (5mg/kg) and allowed to recover for at least 1 week prior to behavioral testing. After animals had been tested, hamsters were given a lethal dose of sodium pentobarbital and the damage tract from the CFM cannula was be used to verify placement of the CFM. “Hits” versus “misses” in the NAc were assessed as well as whether the CFM was in the core or shell.

3.3.5 Amperometric Recordings

Prior to carbon fiber microelectrode (CFM: #7002 Pinnacle Technology, Inc) insertion into guide shaft, hamsters were lightly anesthetized by isoflurane. The CFM extended 1.2mm beyond the guide, reaching a final DV of 6.7mm. After CFM insertion, hamsters were placed back into their home cage and both the CFM and reference electrode are connected to the potentiostat (#8407

rat preamplifier, Pinnacle Technology, Inc) by customized connectors. The potentiostat contained an electrically shielded cable and an electrical swivel (#8409 rat commutator/swivel, Pinnacle Technology, Inc) mounted on top of an EEG/EMG stand (#9009, Pinnacle Technology, Inc). Screwing a pin attachment onto the head mount stabilized the sensor connection. Reinforcement of the connection was done using lab film. The sensor was then allowed to equilibrate in the brain (30 min) before experimental testing. The electrical potential of the CFM was set at 600 mV, the peak oxidation potential for DA (monoamines). Current was scanned every second (1Hz). Just before test sessions recording of amperometric signal and time-locked video were initiated (#9056, Box camera and #9056-LENS, 4 mm lens). After sensor equilibration, the following recordings were taken; a 5 min baseline recording (hamsters awake and freely-moving), followed by a 10 min social interaction test recording (a sex matched stimulus hamster introduced into experimental subject's home cage), then a 5 min post social interaction test recording. Data was acquired using a data conditioning and acquisition system (#8401, Pinnacle Technology, Inc) and Sirenia Acquisition + Video Synchronization (version 1.3.3).

3.3.6 Social Interaction

For the social interaction tests, subjects were randomized into the "win" or "lose" groups. "Winners" were then paired with a same sex NAI and deemed a winner once they displayed dominance and the NAI submitted to them (for more information on behavioral analysis see above). "Losers" were paired with same sex RAs and were deemed losers once they submitted to the RA when the RA displayed aggression. All agonistic encounters occurred in the subjects home cage to avoid the confound of residency status. These interactions occurred for 10 minutes.

3.3.7 Histology

Following the amperometric recording, hamsters were euthanized with a lethal dose of sodium pentobarbital (0.25 ml, i.p., Henry Schein Animal Health, Dublin, OH). Brains were extracted and submerged in 10% formalin for at least 24 hours at 4°C. Brains were sectioned at 40μm with a cryostat and mounted on SuperFrost Plus slides. The site of recording was considered to be accurate if damage from the carbon fiber microelectrode track was seen to end within sub-regions of the nucleus accumbens (Morin and Wood, 2001). Data from three animals with tracks of the carbon fiber microelectrodes outside of the NAc core and NAc shell and were excluded from the dataset.

3.3.8 Amperometric Data Analysis

The raw amperometric data and behavioral annotations were acquired during experimental recording and imported from the Pinnacle system to create an Excel sheet of current, plotted as a function of time and an Excel sheet of behavioral annotations, as a function of time. Excel sheets were imported into Python and graphed. For analysis of dopamine transients (phasic changes) during the social interaction test, peaks on the graph were located using a peak threshold value of greater than 2 standard deviations from the mean of the signal for each recording. Furthermore, transients within subjects were compared between the baseline and during social sessions. For analysis of prolonged changes in dopamine efflux (tonic changes), a percent change from baseline was calculated to determine how much the signal changed from the mean of the initial 5 minute baseline recording to the end of the social session. These calculations were chosen to best represent phasic changes in the core and tonic changes in the shell, possibly as a result of winning and losing agonistic encounters. Recordings were organized based on the location of the carbon fiber

electrode. NAc core and NAc shell were analyzed separately for effects of social interaction on tonic and phasic signaling, respectively.

Experiment 3: TH and cFos IHC in the VTA

3.3.9 Social Interaction

For the social interaction tests, subjects were randomized into "win," "lose," of "no aggression control" groups. "Winners" were paired with a same sex NAI and deemed a winner once they displayed dominance and the NAI submitted to them (for more information on behavioral analysis see above). "Losers" were paired with same sex RAs and were deemed losers once they submitted to the RA when the RA displayed aggression. "No aggression control" subjects were paired with an NAI that was constrained within a plastic mesh cage (13.5 × 13.5 × 7 cm) which allowed subjects to see, hear, and smell the NAI, but not to physically contact each other. All social encounters were 10 minutes and occurred in the subjects home cage to avoid issues related to residency status.

3.3.10 Transcardial Perfusions

Ninety minutes following social interaction tests, animals were deeply anesthetized with sodium pentobarbital. Once the animals no longer responded to a toe-pinch and were insensate, an incision was made in the chest to expose the heart. A needle connected to surgical tubing was inserted into the left ventricle and the right atrium was pierced with surgical scissors. The tubing was run through a pump set to 15 ml/minute. Animals were first perfused with 100 ml cold phosphate buffered saline (PBS) followed by 100ml cold 4% paraformaldehyde in PBS (PFA). Brains were then extracted and stored in 4% PFA for 24 hours and then cryoprotected in 30% sucrose in PBS until they were sectioned.

3.3.11 Immunohistochemistry

Brains were sectioned on a cryostat at 40 μm thickness and stored in cryoprotectant in the -20°C freezer until the IHC was performed. Three to four sections from each animal were isolated and rinsed 5 times with PBS to remove excess cryoprotectant. Sections were blocked in a 10% normal donkey serum in PBS with 1% Triton-X for 1 hour. Then sections were incubated in primary antibodies for cFos (ab208942 mouse anti-cFos, 1:500 dilution, Abcam, Boston, MA) and TH (ab208942 rabbit anti-TH, 1:5000, Abcam, Boston, MA) diluted in 5% normal donkey serum diluted in PBS with 1% Triton-X overnight on a shaker at room temperature. Sections were rinsed 3 times for 5 minutes in PBS. Sections were then incubated in appropriate secondary antibodies (Alexa Fluor 594 conjugated donkey anti-mouse IgG, 1:250 dilution, Jackson ImmunoResearch Laboratories, West Grove, PA) and (Alexa Fluor 488 conjugated-donkey anti-rabbit IgG, 1:250, Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 hours on a shaker at room temperature. Sections were rinsed 3 times for 5 minutes in PBS. Sections were then mounted onto SuperFrost Plus slides, dried, and cover slipped with Vectashield Hard Set Mounting Medium for Fluorescence with DAPI (H1500, Vector Laboratories, Burlingame, CA). Using the cell counter plugin on FIJI, cFos positive and TH positive cells were quantified in appropriate regions as defined in Morin & Wood, 2001. The entire brain regions (mVTA and lVTA) were quantified in three sections of tissue and averaged for each subject. A second observer counted a random subset of these microscope images. Inter-rater reliability (i.e., percent agreement) between the two observers was above 90%.

3.3.12 Statistical Analysis

Data were analyzed using Prism 9 (GraphPad) utilizing between subject analysis of variance (ANOVA). In cases of statistical significance, Tukey's post-hoc tests were used to

examine group differences (or appropriate non-parametric tests if the data do not meet the assumptions of parametric tests). Statistical significance was conferred at $p < 0.05$.

3.4 Results

3.4.1 Subcircuits of the MDS

We investigated the projections from the VTA to the NAc in Syrian hamsters using Lumaflour fluorescent beads as a retrograde tracer. Injections of the beads were centered on the NAc core or the NAc shell *(Figure 10)*. Injections into the NAc core led to almost exclusive expression of retrobeads within the mVTA. Indeed, significantly more retrobeads were found in the mVTA than in the lVTA in males and females, $F(2,30)= 51.30$, $p<0.001$, *Figure 10A.* In contrast, when retrobeads were injected into the NAc shell, there were significantly more beads found in the lVTA than in the mVTA, $F(2,30)= 98.00$, $p<0.001$, *Figure 10B.* Notably, there was a small degree of overlap between these subcircuits. Analysis of the retrograde tracing data revealed that 75.6% percent of cells labeled with retrobeads projecting to the NAc core originated in the mVTA and 86.6% percent of cells labeled with retrobeads projecting to the NAc shell originated in the lVTA.

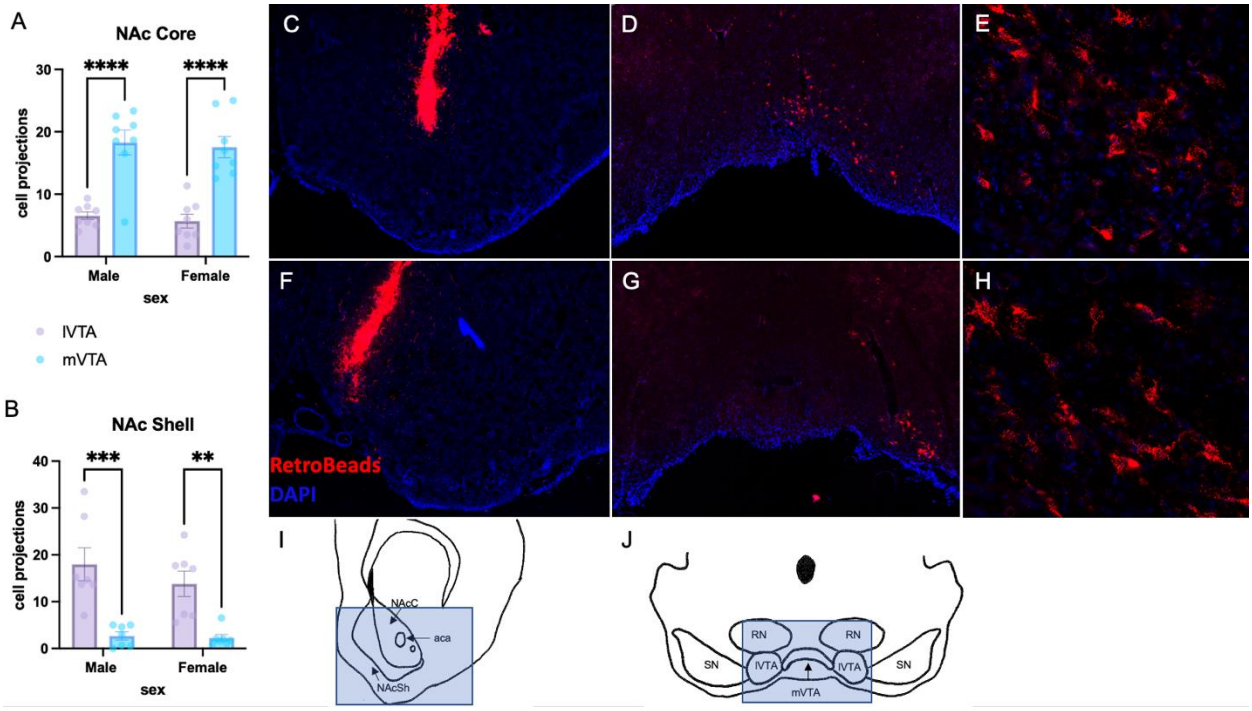

Figure 10: Retrograde Tracing of Projections from VTA to NAc

A*. Number of cells projecting from the lateral VTA (lVTA) and medial VTA (mVTA) to the NAc core in males and females.* ***B.*** *Number of cells projecting from the lateral VTA (lVTA) and medial VTA (mVTA) to the NAc shell in males and females.* ***C.*** *Representative photomicrograph of Retrobead (red) injection into the NAc core (blue-DAPI) at 4x magnification.* ***D.*** *Representative photomicrograph of Retrobead tracers (red) originating from the NAc core, primarily in the mVTA at 4x magnification.* ***E.*** *Retrobeads in the mVTA at 20x magnification.* F. *Representative photomicrograph of Retrobead (red) injection into the NAc shell (blue-DAPI) at 4x magnification.* ***G.*** *Representative photomicrograph of Retrobead tracers (red) originating from the NAc shell, primarily in the lVTA at 4x magnification.* ***H.*** *Retrobeads in the mVTA at 20x magnification.* I. *Highlighted box representing region of tissue section represented in NAc representative photomicrographs.* ***J.*** *Highlighted box representing region of tissue section represented in VTA representative photomicrographs. ** Indicates $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.*

3.4.2 Salience and valence of social interactions

In order to investigate the salience and valence of social interactions we randomly assigned male and female hamsters to “win” or “lose” groups. “Winners” were paired with a same sex intruder that was not aggressive whereas “losers” were paired with same sex resident aggressor. Both winners and losers engaged in intense social interactions reflecting the salience of these pairings. There were, however, significant differences in the social behavior expressed by winners

and losers as indicated by a main effect of outcome (winning versus losing). Males and females that won agonistic encounters exhibited significantly more aggressive behaviors (pin, bite, chase) than those who lost, $F(1,56)= 76.82$, $p<0.001$, *Figure 11B*. There was also an interaction of sex x outcome (win/loss) with female winners expressing more aggression than male winners $F(1,56)=10$, $p<0.001$. Males and females who lost exhibited significantly more submissive behaviors (tail lift, flee) than those who won, $F(1,56)= 114.3$, $p<0.001$, *Figure 11C*. There were no differences in social investigation or nonsocial behavior, *Figure 11D, E*.

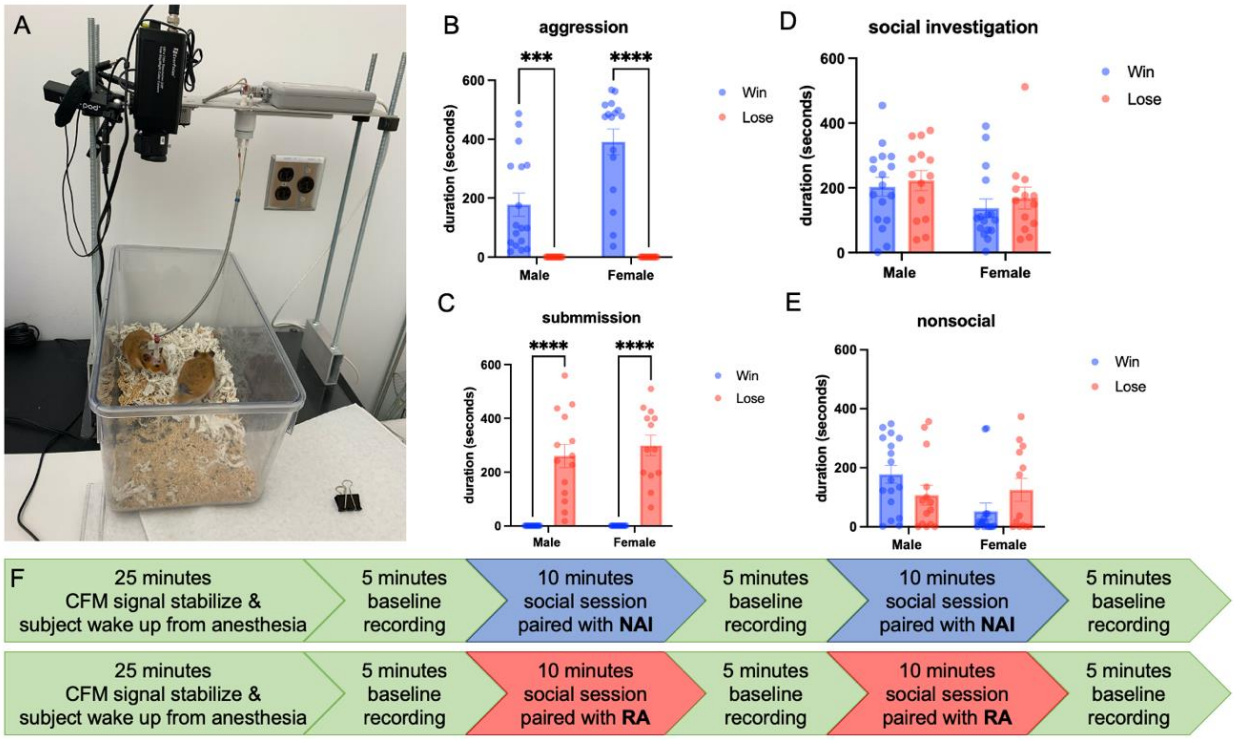

Figure 11: Experimental Setup and Social Behavior

***A**. Photo of amperometric recording equipment and camera used to record behavior in the subjects home cage.* ***B.** Total duration (seconds) of aggressive behavior exhibited by male and female subjects who won or lost.* ***C.** Total duration (seconds) of submissive behavior exhibited by male and female subjects who won or lost.* ***D.** Total duration (seconds) of social investigation exhibited by male and female subjects who won or lost.* ***E**. Total duration (seconds) of nonsocial behavior exhibited by male and female subjects who won or lost.* ***F.** Timeline of experimental procedures. *** Indicates $p < 0.005$, **** $p < 0.001$ (2-way ANOVA).*

3.4.3 Phasic release of DA in the NAc core encodes the salience of social interactions

To test the hypothesis that phasic release of DA in the NAc core mediates the salience of social stimuli, transient release patterns of DA (i.e. DA transients) were quantified during amperometric recordings in males and females during winning and losing agonistic encounters. There were no differences in the number of DA transients in the NAc core between winners and losers during the first social session (S1), *Figure 12A*, or the second social session (S2), *Figure 12B.* Furthermore, there were no differences in the number of DA transients during S1 compared to S2 in winners or losers, *Figure 12C*. There was, however, a main effect of sex on DA transients such that females (regardless of winning or losing) had more transients than males, $F(1,26)=5.447$, $p=0.0276$. Importantly, there was a significant increase in DA transients during the first social session (S1) compared to baseline in both winners and losers, $F(1,55)= 130.0$, $p<0.001$, *Figure 12D-H*. There were no differences in the phasic release of dopamine in the NAc core during a win or a loss. There was, however, a robust increase in the phasic release of dopamine during all agonistic encounters compared to baseline DA release recorded when subjects were alone in their home cages. DA transients corresponded to aggressive behaviors in winners and submissive behaviors in losers. A representative example of a simultaneous change in DA corresponding to aggression and submission are shown in in *Figure 12I* and *Figure 12J*, respectively. Amperometric recordings analyzed in this section included electrodes exclusively located in the NAc core as determined by histological analysis, *Figure 12K*.

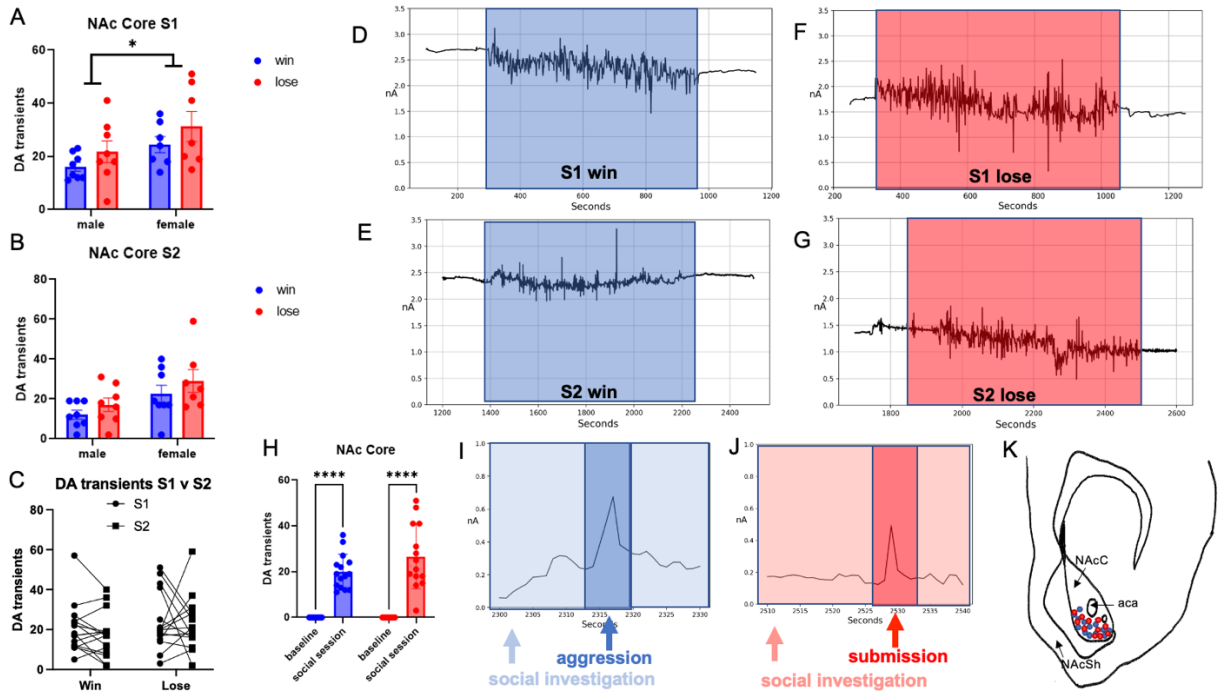

Figure 12: Amperometry Recordings in the NAc Core

A. *DA transients recorded during the first session of social interaction (S1) for male and female winners and losers.* ***B.*** *DA transients recorded during the second session of social interaction (S2) for male and female winners and losers.* ***C.*** *Comparison of DA transients recorded during S1 and S2 in males and females that won and lost (collapsed across sex because of the absence of sex differences in DA transients between winners and losers).* ***D.*** *Representative trace of DA release in the NAc core of a female during a win in S1 (highlighted in blue) flanked by baselines recorded before and after the social interaction.* ***E.*** *Representative trace of DA release in the NAc core of a male during a win in S2 (highlighted in blue) flanked by baselines recorded before and after.* ***F.*** *Representative trace of DA release in the NAc core of a male during a loss in S1 (highlighted in red) flanked by baselines recorded before and after (male).* ***G.*** *Representative trace of DA release in the NAc core of a female during a loss in S2 (highlighted in blue) flanked by baselines recorded before and after (female).* ***H.*** *DA transients recorded at baseline compared to first social session in winners and losers (collapsed across sex).* ***I.*** *Representative 30 second interval of DA release corresponding to aggressive behavior (highlighted in darker blue).* ***J.*** *Representative 30 second interval of DA release corresponding to submissive behavior (highlighted in darker red).* ***K.*** *Carbon fiber microelectrode (CFM) placement within the NAc core, Bregma -2.4 mm. **** Indicates $p < 0.001$ (2-way ANOVA).*

3.4.4 Tonic release of DA in the NAc Shell encodes the valence of social interactions

To test the hypothesis that the tonic release of DA in the NAc shell mediates the valence of social stimuli, the percent change in DA from baseline was quantified during amperometric recordings in males and females while winning and losing agonistic encounters. There was a main

effect of wining/losing in the NAc shell during the first social session (S1) $F(1,26)= 26.46$, $p<0.0001$, *Figure 13A*, and during the second social session (S2), $F(1,26)= 14.05$, $p=0.0010$, *Figure 13B.* These data are represented in a percent change from baseline to capture tonic changes from the beginning to the end of the social session. The winners had a positive change from baseline, i.e. an increase in tonic DA, while the losers had a negative change from baseline, i.e. a decrease in tonic DA. There were no differences in percent change from baseline during S1 compared to S2 in winners or losers (sexes pooled), *Figure 13C.* All hamsters who won the first agonistic encounter also won the second encounter, and all hamsters who lost the first encounter also lost the second encounter. There was, however, one exception in which one female won the first social interaction but lost the second social interaction. Consistent with our hypothesis, during the first social encounter when this hamster won, there was an increase in tonic DA, whereas during the second session when the hamster lost, there was a decrease in tonic DA, *Figure 13I.* This serendipitous finding provides important additional support for the robust effect of winning and losing on tonic DA release, in this case was observed within the same animal. Amperometric recordings analyzed in this section included electrodes exclusively located in the NAc core as determined by histological analysis, *Figure 13H*.

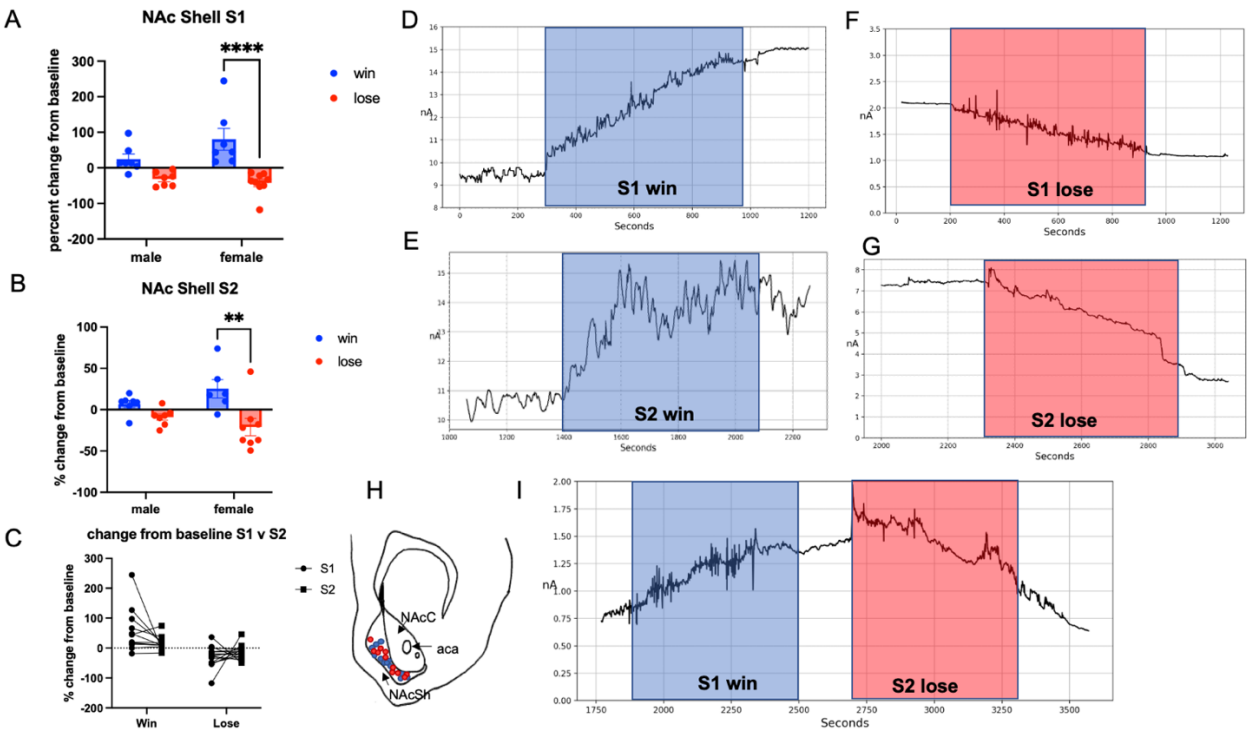

Figure 13: Amperometry Recordings in the NAc Shell

A*. Percent change from baseline in tonic dopamine release during first session of social interaction (S1) in male and female winners and losers.* ***B.*** *Percent change from baseline in tonic dopamine release during the second session of social interaction (S2) in male and female winners and losers.* ***C.*** *Comparison of changes in DA release from baseline recorded during S1 and S2 in male and female winners and losers (collapsed across sex because of the absence of sex differences).* ***D.*** *Representative DA trace in the NAc shell of a male during a win in S1 (highlighted in blue) flanked by baselines recorded before and after.* ***E.*** *Representative DA trace in the NAc shell of a female during a win in S1 (highlighted in blue) flanked by baselines recorded before and after.* ***F****. Representative trace in the NAc shell of a male during a loss in S1 (highlighted in red) flanked by baselines recorded before and after.* ***G.*** *Representative DA trace in the NAc shell of a male during a loss in S2 (highlighted in red) flanked by baselines recorded before and after.* ***H.*** *Carbon fiber microelectrode (CFM) placement within the NAc shell, Bregma -2.4 mm.* ***I.*** *DA traces in the NAc shell of a female during a win in S1 and a loss in S2. ** Indicates $p < 0.01$, **** $p < 0.001$ (2-way ANOVA).*

3.4.5 DA cells in the mVTA are activated by the salience of social stimuli while DA cells in lVTA are activated by valence

Next, we tested the hypotheses that DA cells in the mVTA are activated by the salience of social stimuli whereas that DA cells in the lVTA are activated by the valence of social stimuli.

Double-label immunohistochemistry for TH and cFos was utilized in order to identify active DA cells. The number of TH active cells in the mVTA did not differ between winners and losers (sexes pooled). There was, however, a significant difference between winners and no aggression controls, as well as losers and no aggression controls, such that winners and losers both had more cells co-labeled with TH and cFos than controls, $F(2,30)= 51.30$, $p<0.001$, *Figure 14A.* In the lVTA, significant differences were found in the number of TH activated cells between male or female winners versus losers. There were also differences in the number of TH activated cells between male and female winners versus no aggression controls, $F(2,30)=98.00$, $p<0.001$, *Figure 14B*. Overall, winners had more TH active cells in the lVTA than losers and no aggression controls. No differences were found between male or female losers versus no aggression controls.

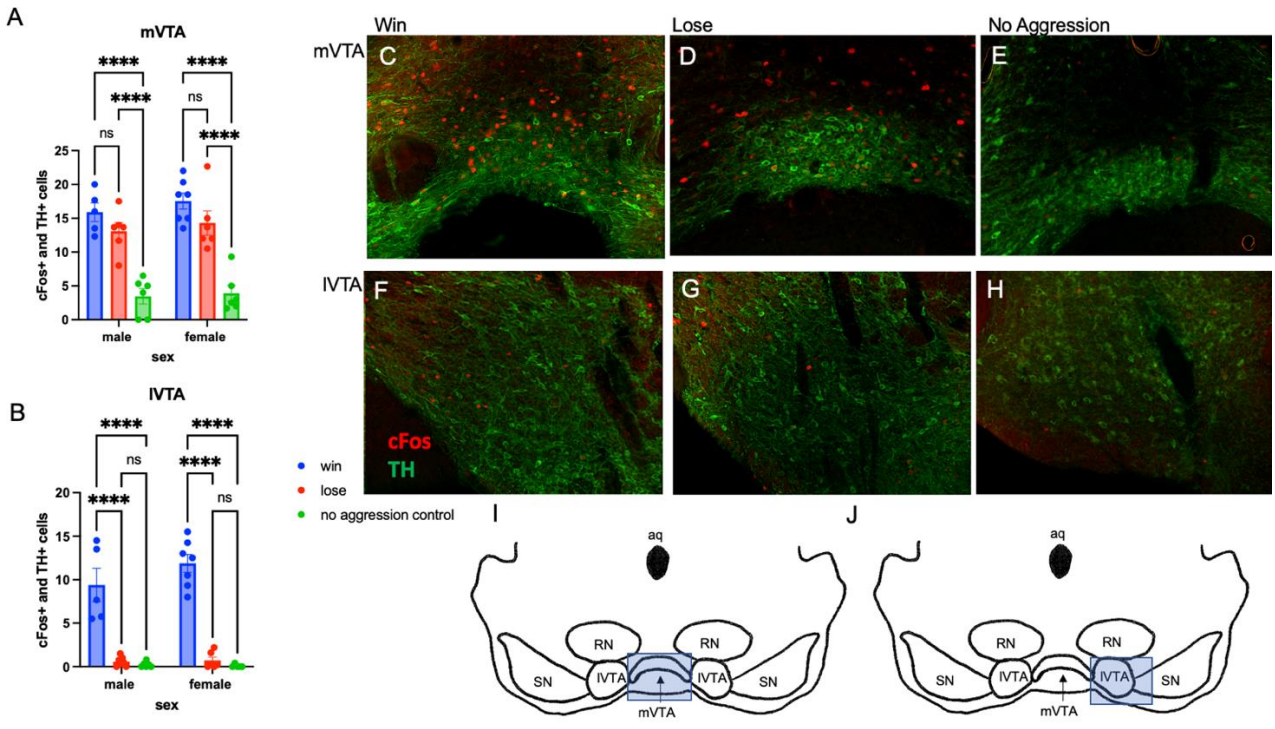

Figure 14: Double Labeling of cFos and TH Immunoreactivity in the VTA

A. *Cells in the medial VTA (mVTA) expressing both cFos and TH in males and females who experienced a win, loss, or a no contact social interaction (no aggression control).* ***B.*** *Cells in the lateral VTA (lVTA) expressing both cFos and TH in males and females who experienced a win, loss, or a no contact social interaction (no aggression control).* ***C.*** *Representative photomicrograph of TH (green) and cFos (red) expression in the mVTA following a win.* ***D.*** *Representative photomicrograph of TH and cFos in the mVTA following a loss.* ***E.*** *Representative photomicrograph of TH (green) and cFos (red) in the mVTA following a no contact social interaction without aggression.* ***F.*** *Representative photomicrograph of TH (green) and cFos (red)expression in the lVTA following a win.* ***G.*** *Representative photomicrograph of TH (green) and cFos (red)in the lVTA following a loss.* ***H.*** *Representative photomicrograph of TH (green) and cFos (red) in the lVTA following a no contact social interaction without aggression.* ***I.*** *Highlighted box representing region of tissue section represented in mVTA representative photomicrographs, Bregma -4.0 mm.* ***J.*** *Highlighted box representing region of tissue section represented in mVTA representative photomicrographs, Bregma -4.0 mm. **** Indicates p < 0.001 (2-way ANOVA).*

3.5 Discussion

These data support the hypothesis that distinct subcircuits within the MDS mediate the salience and valence of social stimuli. The existence of two major subcircuits in the hamster MDS was identified; one that projects from the mVTA to the NAc core and the other that projects from the lVTA to the NAc shell. Similar subcircuits have been reported in other rodent species, suggesting that this organization represents a key property of the MDS. Support for the hypothesis that one of these subcircuits (i.e., mVTA to NAc core) mediates the salience and the other subcircuit (i.e., lVTA to NAc shell) mediates the valence of social stimuli was provided by data from the amperometry studies. Direct comparisons of phasic DA release in the NAc core during winning and losing agonistic encounters revealed a significant increase in transient release of DA compared to baseline levels of release, in both sexes. There were no differences in the magnitude of transient DA release during wins compared to losses. The onset of aggression or submission and the increase in transient DA release appeared to occur simultaneously. Because both winning and losing interactions are highly salient, the absence of any differences in transient DA release between wins and losses supports the hypothesis that DA in the core mediates the salience of these

interactions. Females had more transient release of DA than males which is consistent with previous reports of sex differences in DA content and release in rats (Kritzer & Creutz, 2008; McArthur, McHale, & Gillies, 2007; Walker et al., 1999). It is unknown, however if females find agonistic encounters more salient than males. The present results reinforce previous work suggesting that DA release in the NAc core as a salience signal in the context of nonsocial stimuli with either positive or negative valence (Bromberg-Martin et al., 2010; Kutlu, Zachry, Melugin, Cajigas, Chevee, Kelley, et al., 2021).

Support for the hypothesis that the IVTA to NAc shell subcircuit mediates the valence of social interactions comes from the direct comparisons of tonic DA release in the NAc shell during agonistic encounters with different outcomes. A significantly higher level of tonic DA release was observed in winners compared to losers, with tonic DA release increasing from baseline in winners and decreasing from baseline in losers. The possibility that positive valence (i.e., winning) results in increased tonic DA release and negative valence (i.e., losing) results in reduced tonic DA release was further reinforced by the data from one unusual case in which a female won the first encounter but lost the second encounter. While this relationship between winning and DA elevations was observed in both sexes, post hoc analyses revealed that the data obtained in females were driving this effect. Thus we propose that the outcomes of agonistic encounters have the same valence for males and females but have stronger magnitude in females than males.

The studies using cFos immunoreactivity to examine the activation of TH positive cells in the mVTA versus lVTA following agonistic encounters also provide strong support for our hypothesis. As predicted, mVTA cells were activated similarly in all agonistic encounters, above behavioral control levels, suggesting a role in coding salience of these social interactions. Yet the levels of activation did not differ between winning and losing outcomes, suggesting valence is not

reflected in this subcircuit. In contrast, TH positive cells in the lVTA displayed significantly greater activation in winners than in losers or controls that did not socially interact. Taken together these data provide strong support for the overarching hypothesis that anatomically distinct subcircuits within the MDS mediate the salience and valence of social interactions.

These data are consistent with prior work indicating that distinct DA subpopulations serve different functions and extend this research by demonstrating how DA subpopulations respond differentially to social stimuli in hamsters (Bromberg-Martin et al., 2010; Cross, Huhman, & Albers, 2024; Gil et al., 2013; Lammel et al., 2011, 2012; Matsumoto & Hikosaka, 2009). A striking relationship was displayed between the phasic release of DA in the NAc core and the expression of behaviors related to dominance versus subordinance.

It was not possible, however, to determine if DA release occurred prior to, or after the expression of behavior. Our best estimate of this relationship was that these events occurred simultaneously. The present data do not have the temporal resolution to determine whether DA release induced the behavioral response or whether the behavioral response induced DA release. Of course, it remains possible that these events truly do occur simultaneously. Regardless of the exact timing of the DA release and the expression of dominant/subordinate behavior, it seems clear that DA release in the NAc core potentially provides an orienting signal that indicates the occurrence of social events, regardless of valence.

The results of the present study also suggest that tonic DA release in the NAc shell increases during social interactions with a positive valence and decreases during social interactions with a negative valence. Therefore, DA release within the NAc shell may be involved in assigning motivational value or valence to promote approach to rewarding stimuli and promote avoidance of aversive stimuli. These data suggest that a shift in our conceptual thinking on the mechanisms

underlying reward and aversion may be warranted. Interestingly, the Research Domain Criteria (RDoC) framework set forth by the NIMH has suggested the existence of “Positive valence systems” that are primarily responsible for responses to positive motivational situations and “Negative valence systems” that are primarily responsible for responses to aversive situations. The present data suggest the subcircuit from lVTA to NAc core may be a positive/negative valence system where reward is mediated by increased tonic DA release and aversion is mediated by decreased tonic DA release. Of course, it is likely that the lVTA to NAc core subcircuit is only one element reflecting reward and aversion. In stress and fear learning models, signaling in the amygdala, lateral habenula (LHb), lateral septum (LS) and other pertinent brain regions are integrated in the coding of aversion and avoidance processes. Underscoring the importance of the MDS, however, recent studies show that the LHb and LS affect behavior through GABAergic inhibition of DA neurons in the VTA (Hu, 2016; Li et al., 2022). Specifically, LS inhibition of VTA DA release, in the context of social stress, appears to occlude social reward (Li et al., 2022). Furthermore, amygdalar regions such as the central amygdala and basolateral amygdala respond to more than just aversive stimuli and also interface with the MDS (Janak & Tye, 2015). Therefore, this shift in theoretical framework by observing mesolimbic DA as a mediator of both rewarding and aversive stimuli seeks to integrate prior findings and take a more comprehensive approach to understanding these processes. Examining the how this novel subcircuitry signals the salience and valence of social stimuli is critical to elucidating the mechanisms mediating social behavior and will, ultimately, give us insights into social pathologies associated with various neuropsychiatric and neurodevelopmental disorders.

4 CONTRIBUTIONS OF DOPAMINE RECEPTOR SUBTYPES IN THE NUCLEUS ACCUMBENS TO SOCIAL REWARD

4.1 Abstract

The Nucleus Accumbens (NAc) is a key component of the mesolimbic dopamine system (MDS), which mediates motivated behavior. Here we investigated the role of dopamine receptors in the NAc in the rewarding properties of social interactions. The NAc contains multiple subpopulations of medium spiny neurons (MSNs) with one population expressing D1-type dopamine receptors (D1Rs), another expressing D2-type receptors (D2Rs), and a third expressing both. The canonical view is that D1Rs mediate reward and approach while D2Rs mediate aversion and avoidance. There is a significant gap in our knowledge, however, in the roles of these receptor subtypes in the context of the rewarding properties of social behavior. The roles of D1Rs and D2Rs in mediating social reward were evaluated with the use of the selective D1R antagonist SCH23390 and the D2R antagonist eticlopride injected into the NAc. Our findings demonstrate that D1R antagonism decreases social reward in an operant social preference task while D2R antagonism had no effect. These data suggest that D1Rs are necessary for social reward while D2Rs are not.

4.2 Introduction

There are distinct subpopulations of GABAergic medium spiny neurons (MSNs) in the nucleus accumbens (NAc) which differ in their expression of dopamine (DA) receptors. MSNs can be classified as containing DA D1-like receptors (D1R) or DA D2-like receptors (D2R) or both D1R and D2Rs. These DA receptors are G-protein coupled receptors (GPCRs), that are excitatory as with G_s coupled D1Rs or that are inhibitory as with G_i coupled D2Rs. For decades it has been proposed that these two receptor populations work in opposition to produce balanced motor output, and recently, the same dichotomy has been proposed for valenced behaviors.

Whereas D1R containing MSNs appear to mediate reinforcement and reward, D2R containing MSNs have been associated with punishment and aversion (Soares-Cunha et al., 2016, 2019). Recent data supports the role of D1Rs in mediating reinforcement. Optogenetic excitation of D1R containing MSNs induce intracranial self-stimulation and conditioned place preference (CPP), as well as enhancing drug induced CPP (Kravitz et al., 2012; Natsubori et al., 2017). In addition, stimulation of D1Rs promotes motivation to pursue drug and food rewards (Schmidt et al., 2006; Wakabayashi et al., 2004). In contrast, excitation of MSMs expressing D2Rs results in a reduction of reward in CPP and in some cases can be associated with a negative valence and avoidance behavior. There is also evidence, however, that D2Rs can play a role in positive valence (Soares-Cunha et al., 2016; Steinberg et al., 2014; Trifilieff et al., 2013) although this may be context specific. Activation of D2Rs in the NAc can shift motivational valence from positive to neutral, or even from positive to negative. In summary, the role of D1Rs in mediating positive valence in the NAc is consistent while the role of D2Rs in mediating valence are more ambiguous. (Cole et al., 2018).

Despite decades of work on the functions of D1Rs and D2Rs in the rewarding and aversive properties of many different types of stimuli (e.g., drugs of abuse) little is known about the role of these receptors in the rewarding or aversive properties of social interactions. The present study directly compared the roles of D1Rs and D2Rs in NAc on social reward and aversion. We tested the hypothesis that DA receptors in the NAc modulate the rewarding and aversive properties of social interactions with D1R activation increasing social reward and D2R activation decreasing social reward. We used the Operant Social Preference (OSP) task to determine whether the D1R antagonist, SCH23390, decreases the rewarding properties of social interactions and whether the D2R antagonist, eticlopride, increases the rewarding properties of social interactions. Our findings

indicate that D1R antagonism decreases social reward in the OSP task relative to controls. By comparison, D2R antagonism did not significantly impact social reward suggesting that D2Rs may have a more nuanced contribution to social motivation than D1Rs. Disentangling DA receptor contributions to the rewarding properties of social interaction is crucial to our understanding of how social stimuli with a positive or negative valence are processed and thus has implications for various neuropsychiatric and neurodevelopmental disorders.

4.3 Methods

4.3.1 Subjects

Adult male and female Syrian hamsters were bred in the Georgia State University vivarium and maintained on a 14:10 light:dark cycle (lights out at 10:00 am). At two months of age, animals weighed approximately 110-135g and were individually housed in 24cm X 33cm X 20cm polycarbonate cages filled with corncob bedding and nesting material for two weeks before behavioral testing was initiated (see below). It is important to note that we have previously demonstrated that individual housing in hamsters is not stressful (Ross et al., 2017). Food and water were available *ad libitum*. Prior to behavioral testing, females' estrous cycles were monitored for eight days to confirm regular cyclicity. Males were handled in a manner that was similar to the way females were handled each day. In females, behavioral tests were conducted on diestrus 1, diestrus 2, and proestrus, with a pause on estrus in order for lordosis to not interfere with social behavior. All females were tested on the same day of their cycle for each day of social pairing and behavioral testing. Males were yoked to this schedule. In addition to the male and female test subjects, male and female non-aggressive intruders (NAIs) were also used during operant social preference training (see below). NAIs were younger (~2months), smaller (100-110g), and housed four to a cage. All procedures and protocols were performed in accordance with

the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Georgia State University.

4.3.2 Behavioral Analysis

All social pairings were digitally recorded, and social behaviors were analyzed and quantified by 2 observers blind to the condition using Noldus Observer (11.5, Leesburg, VA) system and a hamster ethogram (Albers et al., 2002). The second observer scored a random subset of these videos. Inter-rater reliability (i.e., percent agreement) between the two observers was above 90%. The total duration of four classes of behavior were measured during the test session: (1) social behavior (stretch, approach, sniff, nose touching, and flank marking); (2) non-social behavior (locomotion, exploration, grooming, nesting, feeding, and sleeping); (3) submissive/defensive behavior (flight, avoidance, tail up, upright, side defense, full submissive posture, stretch attend, head flag, attempted escape from cage); and (4) aggressive behavior (upright and side offense, chase and attack, including bites).

4.3.3 Operant Social Preference (OSP) Task

Operant conditioning sessions began with hamsters placed in the main chamber of the OSP apparatus, equidistant from both smaller stimulus chambers. A complete description and validation of the apparatus and task can be found in, Borland, 2017 (Borland et al., 2017; Borland, Frantz, et al., 2018; Borland, Grantham, et al., 2018). A NAI was confined to one stimulus chamber, termed the "social chamber" and the other chamber was empty, i.e., the "nonsocial chamber." Assignment of the stimulus hamsters to the right or left chamber was counter-balanced across experimental groups such that an equal number of subjects in each group encountered the social stimulus in the right chamber each of the 3 sessions as those who encountered the social stimulus with on the left

side in all 3 sessions. Subjects were allowed to move throughout the apparatus, while stimulus hamsters were confined to one of the small chambers. Twenty seconds after the experimental subject entered one of the small chambers, the subject was returned to center of the main chamber. On the first day of conditioning, the subject was given a 15-minute session to acclimate to the apparatus and become accustomed to pushing through the vertical swing doors. On following two days the sessions were 10 minutes. Our lab has previously used the OSP task in order to determine whether social interactions are rewarding based on their ability to reinforce entries into chambers where social interactions occur. This is assessed by quantifying entries into the social chamber (social entries), latency to social entry, entries into the nonsocial chamber (nonsocial entries), and by calculating a social preference score (SPS), which is the difference between social and nonsocial entries.

4.3.4 Cannulation Surgery

Animals were anesthetized with inhaled 5% isoflourane and kept anesthetized during surgery with 2-4% isofluorane. The fur around the surgical area was clipped, animals placed in ear bars, and the surgical site cleaned with betadine and ethanol. An incision was made along the midline of the head and the muscle was cleared from the skull. Bregma was identified and measured. From bregma, the NAc was located using the following stereotaxic coordinates (anteroposterior (AP) +3.40 mm; mediolateral (ML) ±2.20 mm; dorsoventral (DV) -2.50 mm; at a 10^{o} angle towards the midline. A small hole was drilled above the NAc and the guide cannula was implanted. The guide cannula was secured to the skull using OrthoJet dental cement and a stabilizing head screw was placed in the skull to allow the OrthoJet to remain stable during healing and subsequent testing. A dummy cap was placed in the cannula, the incision was closed with a wound clip, and the animal was allowed to recover consciousness in a clean cage placed partially

on a heating pad. Animals were monitored closely following surgery to ensure that the animal recovered and that the guide cannula stayed in place. All hamsters were injected subcutaneously with the anti-inflammatory agent ketofen (5mg/kg) and allowed to recover for at least 1 week prior to behavioral testing. After animals were tested, hamsters were given a lethal dose of sodium pentobarbital. Ink was injected into each cannula to verify the sites of injection.

4.3.5 Infusions

Prior to behavioral testing and infusions, animals were habituated to handling and having the dummy cap removed and replaced. For infusions, animals were lightly restrained, and a 30 gauge needle placed in the cannula guide. The needle was connected to surgical tubing and a 1 mL Hamilton syringe on a Harvard apparatus. The needle extended 4.2mm beyond the cannula, reaching a final DV of 6.7mm into the NAc. The Harvard apparatus delivered 200nl of drug or vehicle (saline) over 30 seconds at a rate of 400 nl/minute and the needle was held in place for an extra 30 seconds to ensure drug delivery and diffusion from the cannula track. The animal remained in its home cage for 5 minutes after the infusion prior to the start of behavioral testing.

4.3.6 Pharmacological Agents

SCH23390, a D1R antagonist, and eticlopride, a D2R antagonist, were obtained from Sigma, St. Louis, MO. Both are potent and highly selective. SCH23390 has Ki values of 0.2nM and 0.3nM for the D1 and D5 receptor, respectively (Bourne, 2001). Eticlopride has a Ki value of 0.09nM for D2-like receptors compared to 10,000nM for D1-llike (Hall, Köhler, & Gawell, 1985; Seeman & Ulpian, 1988). Both drugs were dissolved in sterile saline and microinjected into the NAc at dose of 3.75μg in150nl of saline (Gray et al., 2015). Controls were injected with sterile saline.

4.3.7 Statistical Analysis

Data were analyzed using Prism 9 (GraphPad) utilizing between subject analysis of variance (ANOVA). In cases of statistical significance, Tukey's post-hoc tests were used to examine group differences (or appropriate non-parametric tests if our data do not meet the assumptions of parametric tests). Statistical significance was conferred at $p < 0.05$.

4.4 Results

There was a significant effect of treatment/drug on social entries (i.e. number of entries into the chamber containing a hamster), $F(2,44)= 14.14$, $p<0.0001$, *Figure 15A*, social preference score (SPS), $F(2,44)= 12.14$, $p<0.001$, *Figure 15B,* and social latency (i.e. latency to enter the social chamber) $F(2,44)= 9.257$, $p=0.0004$, *Figure 15C*. The D1R antagonist SCH23390 decreased social entries and SPS in OSP. Furthermore, D1R antagonism increased the social latency of the first entry into the social chamber. There was also a main effect of sex on SPS $F(2,44)= 5.920$, $p=0.0191$. Post hoc analyses revealed differences between the D1R antagonist and saline treated groups, specifically in females. The data from females drove the main effect on social entries ($p=0.0011$), social preference score ($p=0.0018$), and social latency ($p=0.0020$). No differences were observed in the duration of social investigation, nonsocial behavior, or aggression during the OSP task across treatment groups. There was, however, a main effect of sex on social investigation $F(2,44)=6.564$, $p=0.0143$, *Figure 16A*, and on aggression $F(2,44)=7.724$, $p=0.0083$, *Figure 16C*. Females were more aggressive overall and therefore spent a smaller proportion of their time in the social chamber engaging in social investigation. Additionally, there was no effect of treatment on locomotion $F(2,44)= 2.57$, $p=0.0867$, *Figure 17A.*

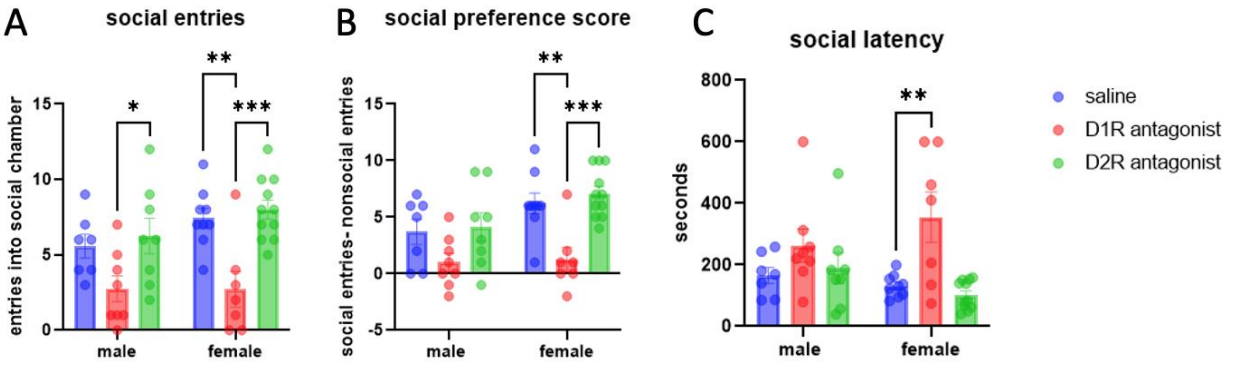

Figure 15: Effect of DA Receptor Antagonism on Operant Social Preference

A. *Females injected with D1R antagonist into the NAc made significantly fewer entries into the social chamber in OSP compared to saline. Both males and females injected with D1R antagonist made fewer social entries than those injected with D2R antagonist.* ***B.*** *Social Preference Score (SPS) was calculated as the difference between social entries and nonsocial entries. Females injected with D1R antagonist had a significantly lower SPS than those injected with saline or D2R antagonist.* ***C.*** *Females injected with D1R antagonist took longer to enter the social chamber, thus had a longer social latency than females injected with saline.*

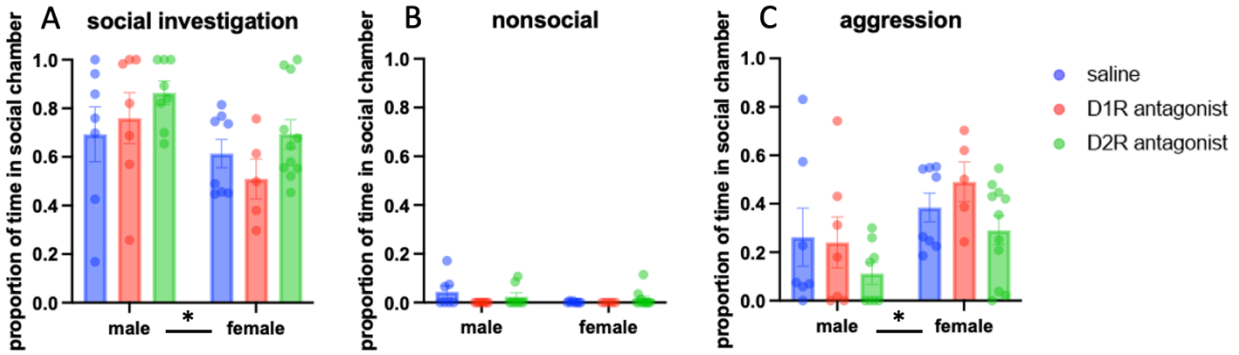

Figure 16: Behavior in Social Chamber of OSP

A. *Proportion of time that the subjects engaged in social investigation in the social chamber, main effect of sex.* ***B.*** *Proportion of time the subjects were nonsocial.* ***C.*** *Proportion of time that subjects engaged in aggression in the social chamber, main effect of sex.*

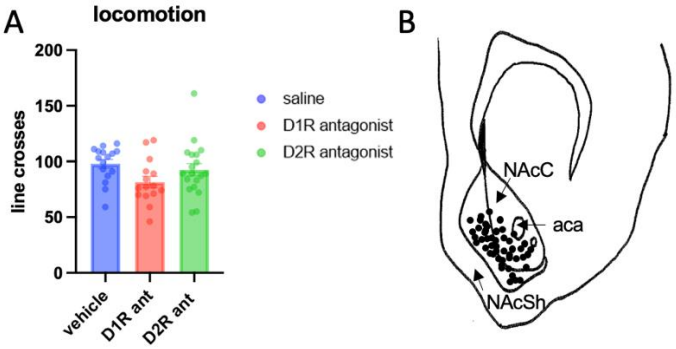

Figure 17: Locomotion & Site Checks

A. *Number of line crosses in the OSP apparatus to measure overall locomotion.* ***B.*** *Sites of injection, Bregma -2.4 mm.*

4.5 Discussion

In this study, we tested the hypothesis that DA receptors in the NAc modulate the rewarding properties of social interactions with D1R activation increasing social reward and D2R activation decreasing social reward. The results support the hypothesis that D1Rs are critical for social reward because D1R antagonism by SCH23390 in the NAc decreased social entries, social preference scores and increased the latency to enter social chamber. Post hoc analyses revealed that this effect was driven primarily by the data obtained in females, however the same relationship existed in males. These data are consistent with other studies indicating that D1R MSNs are important for signaling reward and positively valenced stimuli. Alternatively, however, another possibility is that D1Rs signal the salience of social stimuli regardless of valence (Zachry et al., 2023). This study focused on social stimuli with a positive valence, however, future studies should examine the role of D2Rs in processing social stimuli with a negative valence to appropriately discern whether D1R activation is necessary for social reward versus detection of social salience.

Additionally, we tested the hypothesis that antagonism of D2Rs in the NAc increases social reward. The results, however, do no support this hypothesis. D2R antagonism by eticlopride had

no statistically significant effect on entries, SPS, or latency in OSP. Therefore, the role of D2R in social reward remains unclear and requires further investigation.

One limitation of our approach is that we were unable to investigate the role of D1Rs and D2Rs within the NAc core versus the shell. Given the proximity of these two regions and the diffusion of the drug from the injection site it was not possible to selectively target the core versus the shell. Understanding the roles of D1Rs and D2Rs in these subregions may be important because there appears to be differential expression of DA receptors in these subregions and recent data suggest that the NAc core and the NAc shell serve different functions in reward processing (Lammel et al., 2011; Yang et al., 2018). MSNs containing D1Rs and MSNs containing D2Rs are homogeneously distributed in the NAc core, however, in the shell D2Rs are more highly expressed in the medial and ventral portions and D1Rs are found in the lateral portion which is deemed "D2R poor zone" (Gangarossa et al., 2013). Therefore, understanding receptor subtype function within NAc subregions may help us better understand the heterogeneity of DA signaling within the NAc.

While SCH23390 and eticlopride are highly effective at targeting D1Rs and D2Rs, respectively, there is always the possibility of crosstalk with other types of receptors. Recent studies have shown that SCH23390 can impact 5HT2C (Millan, Newman-Tancredi, Quentric, & Cussac, 2001) and inhibit G protein-coupled inwardly rectifying potassium (GIRK) channels (Kuzhikandathil & Oxford, 2002). To avoid this, future studies could use genetic techniques, such as CRISPR-mediated deletion of DA receptor subtypes in order to more specifically assess the contributions of D1Rs versus D2Rs.

Another possibility is that the whole of DA receptor subtype contributions may be worth more than the sum of its parts. Studies have shown a cooperative effect of D1 and D2 activation inducing self-administration (Ikemoto, Glazier, Murphy, & McBride, 1997) and in mediating the

reinforcing properties of optogenetic self-stimulation of VTA-NAc projections (Steinberg 2014). More recent studies suggest they work in tandem to provide information regarding specific valence-independent aspects of associative learning (Zachry et al., 2023). Clarifying how D1R and D2Rs receptors may work synergistically to produce behavioral output could provide a better understanding of MDS functions. The capacity of D1R and D2R MSNs to convey specific types of information suggests there may be substantial interplay between these receptors. This interaction likely facilitates the refinement of responses to salient environmental cues. However, it remains important to consider the potential for varying degrees of impact from individual neuronal subpopulations (i.e. D1R MSNS and D2R MSNs) in both appetitive and aversive associative learning. Additionally, we must explore how these subpopulations may contribute differently across distinct contexts, namely those with positive and negative valences. Ultimately, these data will be critical for conceptualizing how two cellular populations signal information about the salience and/or valence of social stimuli.

5 CONCLUSION

The goal of this dissertation was to investigate mesolimbic dopamine signaling of the salience and valence of social stimuli. Specifically, I tested the hypothesis that dopamine dynamics (DA neuronal activity and release patterns) and receptor subtypes within anatomically specific subcircuits of the MDS code, at least in part, the salience and valence of social interactions. I sought to determine whether DA projections from the mVTA to NAc core code for salience while DA projections from the lVTA to the NAc shell code for valence in the context of winning and losing agonistic encounters.

Chapter 2 examined sex differences in the impact of social status on social reward and associated mesolimbic activation. Specifically, **Chapter 2** tested the hypothesis that social stress induced by low status or defeat reduces the rewarding properties of social interactions and that social dominance enhances the rewarding properties of these interactions. Using an Operant Social Preference (OSP) task to measure social reward, we found that both subordinate and socially defeated males made significantly fewer entries into chambers containing novel, same-sex conspecifics compared to males who were dominant (i.e., stably won the agonistic encounters). In females, however, there were no differences in social entries between winners and losers. We also began to assess a subcircuit hypothesis of mesolimbic dopamine coding of social salience and valence. **Chapter 2** examined cFos expression in MDS subregions to test the hypothesis that neural activity of the lateral VTA and the NAc shell code valence, with increased activity coding reward and reduced activity coding aversion. In males, there was more activation in the lateral ventral tegmental area (lVTA) and the nucleus accumbens (NAc) shell in winners compared to losers. In females, however, there were no differences in activation in the lVTA between winners and losers. Surprisingly, winning females displayed significantly more activation in the NAc shell as

compared to losing females, despite the lack of behavioral differences. Thus, behavioral and immunohistochemical data suggest that there are sex differences in the impact of social status on social reward and associated mesolimbic activation.

Chapter 3 used retrograde tracing to test the hypothesis that the mVTA primarily projects to the NAc core whereas the lVTA projects to the NAc shell. This work confirmed that the subcircuit connectivity in our model species corresponded to those that have been previously mapped out in mice and rats. Injections of fluorescent retrograde tracers called "retrobeads" into the NAc core led to virtually exclusive expression of retrobeads within the mVTA. In contrast, when retrobeads were injected into the NAc shell, there were significantly more beads found in the lVTA than in the mVTA. Analysis of the retrograde tracing data revealed that 75.6% percent of cells labeled with retrobeads projecting to the NAc core originated in the mVTA and 86.6% percent of cells labeled with retrobeads projecting to the NAc shell originated in the lVTA.

Additionally, **Chapter 3** examined DA release in the NAc during winning and losing in order to test the hypothesis that rewarding social interactions increase tonic and phasic DA release while aversive social interactions increase phasic DA release but decrease tonic DA. Direct comparisons of phasic DA release in the NAc core during winning and losing agonistic encounters revealed a significant increase in transient release of DA compared to baseline levels of release in both sexes. There were no differences in dopamine transients during wins compared to losses. Both winning and losing agonistic encounters are highly salient social stimuli, therefore increased transient release of DA during these encounters and the absence of any differences between wins and losses supports the hypothesis that phasic DA release in the core mediate salience. Direct comparisons of tonic DA release in the NAc shell during agonistic encounters revealed a significant increase in tonic DA in winners compared to losers. Winning has a positive valence

while losing has a negative valence, therefore, this increase from baseline in winners compared to losers supports the hypothesis that tonic DA mediates the valence of social stimuli.

Chapter 3 tested the hypothesis that DA specific cells in the medial VTA codes salience while DA cells in the lateral VTA code valence. Both winning and losing agonistic encounters are highly salient social stimuli despite having opposite valence. A "no aggression" control group where hamsters were in close proximity but were not allowed to interact was utilized to parse out salience. This group maintained a social context but eliminated physical contact and intense agonistic behaviors. Co-labeling of cFos and TH in the VTA revealed that the mVTA has more active DA cells during salient social stimuli (agonistic encounters) while the lVTA has more active DA cells during social stimuli with a positive valence (winning).

Chapter 4 explored the contributions of dopamine receptor subtypes in the NAc to social reward. **Chapter 4** tested the hypothesis that DA receptors in the NAc modulate the rewarding properties of social interactions with D1R activation increasing social reward and D2R activation decreasing social reward. D1R antagonism in the NAc decreased social entries and social preference score in OSP, while increasing latency to enter social chamber, indicative of decreased social reward. Post hoc analyses revealed that this effect was driven by data collected in females, although the same relationship was found in males. Overall, these data indicate that D1R activity in the NAc are necessary for social reward. It is still unclear, however, whether D1Rs are mediating the positive valence or salience in the case of social reward. D2R antagonism had no effect on social reward in OSP, therefore contributions of D2R activity to social reward remain uncertain and require further investigation.

It is well understood that there are sex differences within the MDS (Becker, 2009; Becker & Chartoff, 2019; Walker et al., 1999), as well as in the incidence of neuropsychiatric disorders

(Cover, Maeng, Lebrón-Milad, & Milad, 2014; Gobinath, Choleris, & Galea, 2017), however, little is known about the underlying causes of these differences. Future work is necessary to elucidate the mechanism underlying the sex difference observed in response to social stress Chapter 2. It also remains unknown why the data from females were driving some of the effects observed in Chapters 3 & 4. Specifically, females had more DA transients in the NAc during agonistic encounters and had a larger impact of D1R receptor antagonism on social reward. Future studies should seek to disentangle the underlying mechanisms of these observed effects and how DA signaling in the NAc may differ in females. Prior work from our lab has shown that there are distinct sex differences in the rewarding properties of social interactions and in the responses to social defeat (Borland, Aiani, et al., 2019; Borland, Rilling, et al., 2019; Cross et al., 2024; Gil et al., 2013; Huhman et al., 2003) Furthermore, it is clear that sex is an important factor in the results of these studies and remains a key consideration moving forward in understanding MDS signaling of social reward and aversion.

The importance of social salience and valence in the expression of adaptive and maladaptive behavior, and in the establishment and maintenance of social relationships, remains to be fully appreciated. There is increasing evidence that dysfunctions in the mechanisms mediating reward (i.e. MDS) plays a substantial role in the expression of a large number of psychiatric disorders, including substance abuse, mood & anxiety disorders, and schizophrenia, as well as neurodevelopmental disorders including autism spectrum disorder and attention deficit hyperactivity disorder. Furthermore, 20% of individuals diagnosed with mood or anxiety disorders meet the criteria for addiction and 30-40% of addicts have a comorbid mood or anxiety disorder (Venniro et al., 2018). Indeed, it has been proposed that dysfunctions in the MDS may be present

in many different psychiatric and neurodevelopmental disorders and represent a common target for their treatment (Dichter et al., 2012, Russo 2013).

Recently, the National Institute of Mental Health has developed the Research Domain Criteria (RDoC) as a "research framework for new approaches to investigating mental disorders" and has defined two of these domains as "Negative Valence Systems" that are primarily responsible for responses to aversive stimuli and "Positive Valence Systems" that are primarily responsible for responses to rewarding stimuli. While there may be two different systems for mediating negative and positive valence our data suggest that both negative and positive valence could be mediated by the same system. This shift in theoretical framework by observing mesolimbic DA as a mediator of both rewarding and aversive stimuli seeks to integrate prior findings and take a more comprehensive approach to these processes. Furthermore, "Social Processes" is another domain of function within the RDoC framework, thereby emphasizing the importance of understanding the mechanisms of typical social function and dysfunction on a behavioral and biological level. Often, social stimuli that normally have a positive valence can become less rewarding or even aversive in the case of various neuropsychiatric and neurodevelopmental disorders. Examining MDS signaling social stimuli with positive and negative valence and how these processes overlap as well as differ gives key insight into mental health and illness.

Ultimately, this work fills a gap in our knowledge of the role of the MDS in social contexts with a positive or negative valence. Assessing MDS processing of rewarding and aversive social stimuli by separating the stimuli into its characteristic components, salience and valence, aims to shift the theoretical approach to understanding social stimuli. In summary, this dissertation has proposed a novel subcircuitry within the MDS and provided evidence to support the proposed

putative roles. By studying social reward and aversion as opposite sides of the same coin processed by one system, rather than completely separate processes, perhaps, we can gain a better understanding of the mechanisms of social function and how it can change to social dysfunction. This shift in our approach has important implications for understanding the etiology of neuropsychiatric disorders with a social pathology component, and ultimately, discovering novel, more effective, therapeutics.

REFERENCES

Albers, H. E., Huhman, K. L., & Meisel, R. L. (2002). Hormonal Basis of Social Conflict and Communication. *Hormones, Brain and Behavior*, 393–433. https://doi.org/10.1016/B978-012532104-4/50008-1

Aleyasin, H., Flanigan, M. E., Golden, S. A., Takahashi, A., Menard, C., Pfau, M. L., … Russo, S. J. (2018). Cell-type-specific role of Δfosb in nucleus accumbens in modulating intermale aggression. *Journal of Neuroscience*, *38*(26), 5913–5924. https://doi.org/10.1523/JNEUROSCI.0296-18.2018

Anstrom, K. K., Miczek, K. A., & Budygin, E. A. (2009). Increased phasic dopamine signaling in the mesolimbic pathway during social defeat in rats. *Neuroscience*, *161*(1), 3–12. https://doi.org/10.1016/j.neuroscience.2009.03.023

Bangasser, D. A., & Valentino, R. J. (2014). Sex differences in stress-related psychiatric disorders: Neurobiological perspectives. *Frontiers in Neuroendocrinology*, *35*(3), 303–319. https://doi.org/10.1016/J.YFRNE.2014.03.008

Becker, J. B. (2009). Sexual differentiation of motivation: A novel mechanism? *Hormones and Behavior*, *55*(5), 646–654. https://doi.org/10.1016/j.yhbeh.2009.03.014

Becker, J. B., & Chartoff, E. (2019). Sex differences in neural mechanisms mediating reward and addiction. *Neuropsychopharmacology*, *44*(1), 166–183. https://doi.org/10.1038/s41386-018-0125-6

Berton, O., Durand, M., Aguerre, S., Mormède, P., & Chaouloff, F. (1999). Behavioral, neuroendocrine and serotonergic consequences of single social defeat and repeated fluoxetine pretreatment in the Lewis rat strain. *Neuroscience*, *92*(1), 327–341. https://doi.org/10.1016/S0306-4522(98)00742-8

Blanchard, D. C., Sakai, R. R., McEwen, B., Weiss, S. M., & Blanchard, R. J. (1993). Subordination stress: Behavioral, brain, and neuroendocrine correlates. *Behavioural Brain Research*, *58*(1–2), 113–121. https://doi.org/10.1016/0166-4328(93)90096-9

Blanchard, R. J., Flannelly, K. J., & Blanchard, D. C. (1988). Life-span studies of dominance and aggression in established colonies of laboratory rats. *Physiology & Behavior*, *43*(1), 1–7. https://doi.org/10.1016/0031-9384(88)90089-3

Borland, J. M., Aiani, L. M., Norvelle, A., Grantham, K. N., O'Laughlin, K., Terranova, J. I., … Albers, H. E. (2019). Sex-dependent regulation of social reward by oxytocin receptors in the ventral tegmental area. *Neuropsychopharmacology*, *44*(4), 785–792. https://doi.org/10.1038/s41386-018-0262-y

Borland, J. M., Frantz, K. J., Aiani, L. M., Grantham, K. N., Song, Z., & Albers, H. E. (2017). A novel operant task to assess social reward and motivation in rodents. *Journal of Neuroscience Methods*. https://doi.org/10.1016/j.jneumeth.2017.06.003

Borland, J. M., Frantz, K. J., O'Laughlin, K., Norvelle, A., Grantham, K. N., Aiani, L. M., … Albers, H. E. (2018). Sex-dependent regulation of social reward by oxytocin receptors in the ventral tegmental area. *Neuropsychopharmacology*, (October), 1–8. https://doi.org/10.1038/s41386-018-0262-y

Borland, J. M., Grantham, K. N., Aiani, L. M., Frantz, K. J., & Albers, H. E. (2018). Role of oxytocin in the ventral tegmental area in social reinforcement. *Psychoneuroendocrinology*. https://doi.org/10.1016/j.psyneuen.2018.05.028

Borland, J. M., Rilling, J. K., Frantz, K. J., & Albers, H. E. (2019). Sex-dependent regulation of social reward by oxytocin: an inverted U hypothesis. *Neuropsychopharmacology*, *44*(1), 97–110. https://doi.org/10.1038/s41386-018-0129-2

Bourne, J. A. (2001). SCH 23390: The First Selective Dopamine D1-Like Receptor Antagonist. *CNS Drug Reviews*, *7*(4), 399–414. https://doi.org/https://doi.org/10.1111/j.1527-3458.2001.tb00207.x

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in Motivational Control: Rewarding, Aversive, and Alerting. *Neuron*, *68*(5), 815–834. https://doi.org/10.1016/j.neuron.2010.11.022

Cacciapaglia, F., Mark Wightman, R., & Carelli, R. M. (2011). Rapid dopamine signaling differentially modulates distinct microcircuits within the nucleus accumbens during sucrose-directed behavior. *Journal of Neuroscience*, *31*(39), 13860–13869. https://doi.org/10.1523/JNEUROSCI.1340-11.2011

Calipari, E. S., Bagot, R. C., Purushothaman, I., Davidson, T. J., Yorgason, J. T., Peña, C. J., … Nestler, E. J. (2016). *In vivo imaging identifies temporal signature of D1 and D2 medium spiny neurons in cocaine reward*. *113*(10), 2726–2731. https://doi.org/10.1073/pnas.1521238113

Cao, J.-L., Covington Iii, H. E., Friedman, A. K., Wilkinson, M. B., Walsh, J. J., Cooper, D. C., … Han, M.-H. (2010). *Mesolimbic Dopamine Neurons in the Brain Reward Circuit Mediate Susceptibility to Social Defeat and Antidepressant Action*. https://doi.org/10.1523/JNEUROSCI.3177-10.2010

Chaudhury, D., Walsh, J. J., Friedman, A. K., Juarez, B., Ku, S. M., Koo, J. W., … Han, M. H. (2013). Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature*, *493*(7433), 532–536. https://doi.org/10.1038/nature11713

Cole, S. L., Robinson, M. J. F., & Berridge, K. C. (2018). Optogenetic self-stimulation in the nucleus accumbens: D1 reward versus D2 ambivalence. *PLoS ONE*, *13*(11), 1–29.

https://doi.org/10.1371/journal.pone.0207694

Conway, K. P., Compton, W., Stinson, F. S., & Grant, B. F. (2006). Lifetime comorbidity of DSM-IV mood and anxiety disorders and specific drug use disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *The Journal of Clinical Psychiatry*, *67*(2), 247–257. https://doi.org/10.4088/jcp.v67n0211

Cover, K. K., Maeng, L. Y., Lebrón-Milad, K., & Milad, M. R. (2014). Mechanisms of estradiol in fear circuitry: Implications for sex differences in psychopathology. *Translational Psychiatry*, *4*(March). https://doi.org/10.1038/tp.2014.67

Cross, E. A., Huhman, K. L., & Albers, H. E. (2024). Sex differences in the impact of social status on social reward and associated mesolimbic activation. *Physiology & Behavior*, *273*, 114410. https://doi.org/10.1016/j.physbeh.2023.114410

Day, J. J., Roitman, M. F., Wightman, R. M., & Carelli, R. M. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nature Neuroscience*, *10*(8), 1020–1028. https://doi.org/10.1038/nn1923

Dichter, G. S., Damiano, C. A., & Allen, J. A. (2012). Reward circuitry dysfunction in psychiatric and neurodevelopmental disorders and genetic syndromes: Animal models and clinical findings. *Journal of Neurodevelopmental Disorders*, *4*(1), 1–43. https://doi.org/10.1186/1866-1955-4-19

Douglas, L. A., Varlinskaya, E. I., & Spear, L. P. (2004). Rewarding properties of social interactions in adolescent and adult male and female rats: Impact of social versus isolate housing of subjects and partners. *Developmental Psychobiology*, *45*(3), 153–162. https://doi.org/10.1002/dev.20025

Drickamer, L. C., Vandenbergh, J. G., & Colby, D. R. (1973). Predictors of dominance in the

male golden hamster (Mesocricetus auratus). *Animal Behaviour*, *21*(3), 557–563. https://doi.org/10.1016/s0003-3472(73)80016-8

Drickamer, L. C., & Vandenbergh, J. G. (1973). Predictors of social dominance in the adult female golden hamster (Mesocricetus auratus). *Animal Behaviour*, *21*(3), 564–570. https://doi.org/10.1016/S0003-3472(73)80017-X

Dutta, S., Beaver, J., Halcomb, C. J., & Jasnow, A. M. (2021). Dissociable roles of the nucleus accumbens core and shell subregions in the expression and extinction of conditioned fear. *Neurobiology of Stress*, *15*, 100365. https://doi.org/10.1016/J.YNSTR.2021.100365

Ferris, C. F., Axelson, J. F., Shinto, L. H., & Albers, H. E. (1987). Scent marking and the maintenance of dominant/subordinate status in male golden hamsters. *Physiology & Behavior*, *40*(5), 661–664. https://doi.org/10.1016/0031-9384(87)90114-4

Ferris, C. F., Melloni, R. H. J., Koppel, G., Perry, K. W., Fuller, R. W., & Delville, Y. (1997). Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(11), 4331–4340. https://doi.org/10.1523/JNEUROSCI.17-11-04331.1997

Foster, M. T., Solomon, M. B., Huhman, K. L., & Bartness, T. J. (2006). Social defeat increases food intake, body mass, and adiposity in Syrian hamsters. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *290*(5), 1284–1293. https://doi.org/10.1152/ajpregu.00437.2005

Francis, T. C., Chandra, R., Friend, D. M., Finkel, E., Dayrit, G., Miranda, J., … Lobo, M. K. (2015). Nucleus accumbens medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. *Biological Psychiatry*, *77*(3), 212–222.

Francis, T. C., & Lobo, M. K. (2017). Emerging Role for Nucleus Accumbens Medium Spiny Neuron Subtypes in Depression. *Biological Psychiatry*, *81*(8), 645–653. https://doi.org/10.1016/j.biopsych.2016.09.007

Gangarossa, G., Espallergues, J., d'Exaerde, A. de K., El Mestikawy, S., Gerfen, C. R., Hervé, D., … Valjent, E. (2013). Distribution and compartmental organization of GABAergic medium-sized spiny neurons in the mouse Nucleus Accumbens. *Frontiers in Neural Circuits*, *7*(FEBRUARY 2013), 1–20. https://doi.org/10.3389/fncir.2013.00022

Ghosal, S., Sandi, C., & van der Kooij, M. A. (2019). Neuropharmacology of the mesolimbic system and associated circuits on social hierarchies. *Neuropharmacology*, *159*(January), 107498. https://doi.org/10.1016/j.neuropharm.2019.01.013

Gil, M., Nguyen, N. T., Mcdonald, M., & Albers, H. E. (2013). Social reward: Interactions with social status, social communication, aggression, and associated neural activation in the ventral tegmental area. *European Journal of Neuroscience*, *38*(2), 2308–2318. https://doi.org/10.1111/ejn.12216

Gobinath, A. R., Choleris, E., & Galea, L. A. M. (2017). Sex, hormones, and genotype interact to influence psychiatric disease, treatment, and behavioral research. *Journal of Neuroscience Research*, *95*(1–2), 50–64. https://doi.org/10.1002/jnr.23872

Gray, C. L., Norvelle, A., Larkin, T., & Huhman, K. L. (2015). Dopamine in the nucleus accumbens modulates the memory of social defeat in Syrian hamsters (Mesocricetus auratus). *Behavioural Brain Research*, *286*, 22–28. https://doi.org/10.1016/j.bbr.2015.02.030

Greenberg, P. E., Fournier, A.-A., Sisitsky, T., Simes, M., Berman, R., Koenigsberg, S. H., & Kessler, R. C. (2021). The Economic Burden of Adults with Major Depressive Disorder in

the United States (2010 and 2018). *PharmacoEconomics*, *39*(6), 653–665. https://doi.org/10.1007/s40273-021-01019-4

Gunaydin, L. A., Grosenick, L., Finkelstein, J. C., Kauvar, I. V., Fenno, L. E., Adhikari, A., … Deisseroth, K. (2014). Natural neural projection dynamics underlying social behavior. *Cell*, *157*(7), 1535–1551. https://doi.org/10.1016/j.cell.2014.05.017

Hall, H., Köhler, C., & Gawell, L. (1985). Some in vitro receptor binding properties of [3H]eticlopride, a novel substituted benzamide, selective for dopamine-D2 receptors in the rat brain. *European Journal of Pharmacology*, *111*(2), 191–199. https://doi.org/https://doi.org/10.1016/0014-2999(85)90756-3

Holly, E. N., Debold, J. F., & Miczek, K. A. (n.d.). *Increased mesocorticolimbic dopamine during acute and repeated social defeat stress: modulation by corticotropin releasing factor receptors in the ventral tegmental area*. https://doi.org/10.1007/s00213-015-4082-z

Hu, H. (2016). Reward and Aversion. *Annual Review of Neuroscience*, *39*, 297–324. https://doi.org/10.1146/annurev-neuro-070815-014106

Huhman, K L, Moore, T. O., Mougey, E. H., & Meyerhoff, J. L. (1992). Hormonal responses to fighting in hamsters: separation of physical and psychological causes. *Physiology & Behavior*, *51*(5), 1083–1086. https://doi.org/10.1016/0031-9384(92)90097-l

Huhman, K. L. (2006). Social conflict models: Can they inform us about human psychopathology? *Hormones and Behavior*, *50*(4), 640–646. https://doi.org/10.1016/J.YHBEH.2006.06.022

Huhman, K. L., Solomon, M. B., Janicki, M., Harmon, A. C., Lin, S. M., Israel, J. E., & Jasnow, A. M. (2003). Conditioned defeat in male and female syrian hamsters. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2003.05.001

Ikemoto, S., Glazier, B. S., Murphy, J. M., & McBride, W. J. (1997). Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(21), 8580–8587. https://doi.org/10.1523/JNEUROSCI.17-21-08580.1997

Janak, P. H., & Tye, K. M. (2015). From circuits to behaviour in the amygdala. *Nature*, *517*(7534), 284–292. https://doi.org/10.1038/nature14188

Kravitz, A. V, Tye, L. D., & Kreitzer, A. C. (2012). *Distinct roles for direct and indirect pathway striatal neurons in reinforcement*. *15*(6). https://doi.org/10.1038/nn.3100

Kritzer, M. F., & Creutz, L. M. (2008). Region and sex differences in constituent dopamine neurons and immunoreactivity for intracellular estrogen and androgen receptors in mesocortical projections in rats. *Journal of Neuroscience*, *28*(38), 9525–9535. https://doi.org/10.1523/JNEUROSCI.2637-08.2008

Kutlu, M. G., Zachry, J. E., Melugin, P. R., Cajigas, S. A., Chevee, M. F., Kelley, S. J., … Calipari, E. S. (2021). Dopamine release in the nucleus accumbens core signals perceived saliency. *Current Biology*, 1–14. https://doi.org/10.1016/j.cub.2021.08.052

Kutlu, M. G., Zachry, J. E., Melugin, P. R., Cajigas, S. A., Chevee, M. F., Kelly, S. J., … Calipari, E. S. (2021). Dopamine release in the nucleus accumbens core signals perceived saliency. *Current Biology*, *31*(21), 4748-4761.e8. https://doi.org/10.1016/J.CUB.2021.08.052

Kuzhikandathil, E. V, & Oxford, G. S. (2002). Classic D1 dopamine receptor antagonist R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390) directly inhibits G protein-coupled inwardly rectifying potassium channels. *Molecular Pharmacology*, *62*(1), 119–126. https://doi.org/10.1124/mol.62.1.119

Lammel, S., Ion, D. I., Roeper, J., & Malenka, R. C. (2011). Projection-Specific Modulation of Dopamine Neuron Synapses by Aversive and Rewarding Stimuli. *Neuron*, *70*(5), 855–862. https://doi.org/10.1016/j.neuron.2011.03.025

Lammel, S., Lim, B. K., & Malenka, R. C. (2014). Reward and aversion in a heterogeneous midbrain dopamine system. *Neuropharmacology*, *76*(PART B), 351–359. https://doi.org/10.1016/j.neuropharm.2013.03.019

Lammel, S., Lim, B. K., Ran, C., Huang, K. W., Betley, M. J., Tye, K. M., … Malenka, R. C. (2012). Input-specific control of reward and aversion in the ventral tegmental area. *Nature*. https://doi.org/10.1038/nature11527

Li, L., Durand-de Cuttoli, R., Aubry, A. V, Burnett, C. J., Cathomas, F., Parise, L. F., … Russo, S. J. (2022). Social trauma engages lateral septum circuitry to occlude social reward. *Nature*, (July 2021). https://doi.org/10.1038/s41586-022-05484-5

Lobo, M. K., Iii, H. E. C., Chaudhury, D., Friedman, A. K., Sun, H., Damez-werno, D., … Nestler, E. J. (2011). Cell Type Specific Loss of BDNF Signaling Mimics Optogenetic Control of Cocaine Reward. *October*, *330*(6002), 385–390. https://doi.org/10.1126/science.1188472.Cell

Lucas, L. R., Celen, Z., Tamashiro, K. L. K., Blanchard, R. J., Blanchard, D. C., Markham, C., … Mcewen, B. S. (2004). Repeated exposure to social stress has long-term effects on indirect markers of dopaminergic activity in brain regions associated with motivated behavior. *Neuroscience*, *124*(2), 449–457. https://doi.org/10.1016/j.neuroscience.2003.12.009

Luckett, C., Norvelle, A., & Huhman, K. (2012). The role of the nucleus accumbens in the acquisition and expression of conditioned defeat. *Behavioural Brain Research*, *227*(1), 208–

214. https://doi.org/10.1016/j.bbr.2011.10.012

Matsumoto, M., & Hikosaka, O. (2009). Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature, 459*(7248), 837–841. https://doi.org/10.1038/nature08028

McArthur, S., McHale, E., & Gillies, G. E. (2007). The size and distribution of midbrain dopaminergic populations are permanently altered by perinatal glucocorticoid exposure in a sex- region- and time-specific manner. *Neuropsychopharmacology, 32*(7), 1462–1476. https://doi.org/10.1038/sj.npp.1301277

McCann, K. E., & Huhman, K. L. (2012). The effect of escapable versus inescapable social defeat on conditioned defeat and social recognition in Syrian hamsters. *Physiology & Behavior, 105*(2), 493–497. https://doi.org/10.1016/j.physbeh.2011.09.009

Meerlo, P., Sgoifo, A., & Turek, F. W. (2002). The effects of social defeat and other stressors on the expression of circadian rhythms. *Stress*, *5*(1), 15–22. https://doi.org/10.1080/102538902900012323

Meisel, R. L., & Joppa, M. A. (1994). Conditioned place preference in female hamsters following aggressive or sexual encounters. *Physiology & Behavior*, *56*(5), 1115–1118. https://doi.org/10.1016/0031-9384(94)90352-2

Meyer, J. S., & Hamel, A. F. (2014). Models of stress in nonhuman primates and their relevance for human psychopathology and endocrine dysfunction. *ILAR Journal*, *55*(2), 347–360. https://doi.org/10.1093/ilar/ilu023

Millan, M. J., Newman-Tancredi, A., Quentric, Y., & Cussac, D. (2001). The “selective” dopamine D1 receptor antagonist, SCH23390, is a potent and high efficacy agonist at cloned human serotonin2C receptors. *Psychopharmacology*, *156*(1), 58–62.

https://doi.org/10.1007/s002130100742

Morrison, K. E., Bader, L. R., Clinard, C. T., Gerhard, D. M., Gross, S. E., & Cooper, M. A. (2014). Maintenance of dominance status is necessary for resistance to social defeat stress in Syrian hamsters. *Behavioural Brain Research*, *270*, 277–286. https://doi.org/10.1016/J.BBR.2014.05.041

Nader, M. (2012). SOCIAL DOMINANCE IN FEMALE MONKEYS: DOPAMINE RECEPTOR FUNCTION AND COCAINE REINFORCEMENT. *Biological Psychiatry*, *23*(1), 1–7. https://doi.org/10.1038/jid.2014.371

Natsubori, A., Tsutsui-Kimura, I., Nishida, H., Bouchekioua, Y., Sekiya, H., Uchigashima, M., … Tanaka, K. F. (2017). Ventrolateral striatal medium spiny neurons positively regulate food-incentive, goal-directed behavior independently of D1 and D2 selectivity. *Journal of Neuroscience*, *37*(10), 2723–2733. https://doi.org/10.1523/JNEUROSCI.3377-16.2017

OLDS, J., & MILNER, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative and Physiological Psychology*, *47*(6), 419–427. https://doi.org/10.1037/h0058775

Payne, A. P., & Swanson, H. H. (1970). Agonistic behaviour between pairs of hamsters of the same and opposite sex in a neutral observation area. *Behaviour*, *36*(4), 260–269.

Pignatelli, M., & Bonci, A. (2015). Role of Dopamine Neurons in Reward and Aversion: A Synaptic Plasticity Perspective. *Neuron*, *86*(5), 1145–1157. https://doi.org/10.1016/j.neuron.2015.04.015

Potegal, M., Huhman, K., Moore, T., & Meyerhoff, J. (1993). Conditioned defeat in the Syrian golden hamster (Mesocricetus auratus). *Behavioral and Neural Biology*, *60*(2), 93–102. https://doi.org/10.1016/0163-1047(93)90159-f

Pulliam, J. V. K., Dawaghreh, A. M., Alema-Mensah, E., & Plotsky, P. M. (2010). Social defeat stress produces prolonged alterations in acoustic startle and body weight gain in male Long Evans rats. *Journal of Psychiatric Research*, *44*(2), 106–111. https://doi.org/10.1016/J.JPSYCHIRES.2009.05.005

Ray, M. H., Moaddab, M., & McDannald, M. A. (2022). Threat and Bidirectional Valence Signaling in the Nucleus Accumbens Core. *The Journal of Neuroscience*, *42*(5), 817–833. https://doi.org/10.1523/jneurosci.1107-21.2021

Reddon, A. R., Ruberto, T., & Reader, S. M. (2021). Submission signals in animal groups. *Behaviour*, *159*, 1–20. https://doi.org/10.1163/1568539X-bja10125

Ross, A. P., Norvelle, A., Choi, D. C., Walton, J. C., Albers, H. E., & Huhman, K. L. (2017). Social housing and social isolation: Impact on stress indices and energy balance in male and female Syrian hamsters (Mesocricetus auratus). *Physiology and Behavior*. https://doi.org/10.1016/j.physbeh.2017.05.015

Russo, S. J., & Nestler, E. J. (2013). The brain reward circuitry in mood disorders. *Nature Reviews Neuroscience*, *14*(9), 609–625. https://doi.org/10.1038/nrn3381

Saddoris, M. P., Cacciapaglia, F., Wightman, R. M., & Carelli, R. M. (2015). Differential Dopamine Release Dynamics in the Nucleus Accumbens Core and Shell Reveal Complementary Signals for Error Prediction and Incentive Motivation. *The Journal of Neuroscience*, *35*(33), 11572 LP – 11582. https://doi.org/10.1523/JNEUROSCI.2344-15.2015

Sapolsky, R. M. (2005). The influence of social hierarchy on primate health. *Science*, *308*(5722), 648–652. https://doi.org/10.1126/science.1106477

Schmidt, H. D., Anderson, S. M., & Pierce, R. C. (2006). Stimulation of D1-like or D2 dopamine

receptors in the shell, but not the core, of the nucleus accumbens reinstates cocaine-seeking behaviour in the rat. *European Journal of Neuroscience*, *23*(1), 219–228. https://doi.org/10.1111/j.1460-9568.2005.04524.x

Schultz, W. (2016). Dopamine reward prediction error coding. *Dialogues in Clinical Neuroscience*, *18*(1), 23–32. https://doi.org/10.31887/dcns.2016.18.1/wschultz

Seeman, P., & Ulpian, C. (1988). Dopamine D1 and D2 receptor selectivities of agonists and antagonists. *Advances in Experimental Medicine and Biology*, *235*, 55–63. https://doi.org/10.1007/978-1-4899-2723-1_5

Simon, G. E. (2003). Social and economic burden of mood disorders. *Biological Psychiatry*, *54*(3), 208–215. https://doi.org/10.1016/s0006-3223(03)00420-7

Soares-Cunha, C., Coimbra, B., Sousa, N., & Rodrigues, A. J. (2016). Reappraising striatal D1- and D2-neurons in reward and aversion. *Neuroscience and Biobehavioral Reviews*, *68*, 370–386. https://doi.org/10.1016/j.neubiorev.2016.05.021

Soares-Cunha, C., de Vasconcelos, N. A. P., Coimbra, B., Domingues, A. V., Silva, J. M., Loureiro-Campos, E., … Rodrigues, A. J. (2019). Nucleus accumbens medium spiny neurons subtypes signal both reward and aversion. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-019-0484-3

Solomon, M. B., Foster, M. T., Bartness, T. J., & Huhman, K. L. (2007). Social defeat and footshock increase body mass and adiposity in male Syrian hamsters. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *292*(1), 283–290. https://doi.org/10.1152/ajpregu.00330.2006

Song, Z., Borland, J. M., Larkin, T. E., Malley, M. O., & Elliott, H. (2016). *Activation of oxytocin receptors, but not arginine-vasopressin V1a receptors, in the ventral tegmental*

area of male Syrian hamsters is essential for the reward-like properties of social interactions. 164–172. https://doi.org/10.1016/j.psyneuen.2016.09.001.Activation

Steinberg, E. E., Boivin, J. R., Saunders, B. T., Witten, I. B., Deisseroth, K., & Janak, P. H. (2014). Positive reinforcement mediated by midbrain dopamine neurons requires D1 and D2 receptor activation in the nucleus accumbens. *PLoS ONE*, *9*(4). https://doi.org/10.1371/journal.pone.0094771

Steinman, M. Q., & Trainor, B. C. (2017). Sex differences in the effects of social defeat on brain and behavior in the California mouse: Insights from a monogamous rodent. *Seminars in Cell and Developmental Biology*, *61*, 92–98. https://doi.org/10.1016/j.semcdb.2016.06.021

Terranova, J. I., Song, Z., Larkin, T. E., Hardcastle, N., Norvelle, A., Riaz, A., & Albers, H. E. (2016). Serotonin and arginine–vasopressin mediate sex differences in the regulation of dominance and aggression by the social brain. *Proceedings of the National Academy of Sciences*, *113*(46), 13233–13238. https://doi.org/10.1073/pnas.1610446113

Thase, M. E., & Denko, T. (2008). Pharmacotherapy of Mood Disorders. *Annual Review of Clinical Psychology*, *4*(1), 53–91. https://doi.org/10.1146/annurev.clinpsy.2.022305.095301

Trainor, B. C. (2011). Stress responses and the mesolimbic dopamine system: Social contexts and sex differences. *Hormones and Behavior*, *60*(5), 457–469. https://doi.org/10.1016/j.yhbeh.2011.08.013

Trainor, B. C., Pride, M. C., Landeros, R. V., Knoblauch, N. W., Takahashi, E. Y., Silva, A. L., & Crean, K. K. (2011). Sex differences in social interaction behavior following social defeat stress in the monogamous California mouse (peromyscus californicus). *PLoS ONE*, *6*(2). https://doi.org/10.1371/journal.pone.0017405

Trezza, V., Campolongo, P., & Vanderschuren, L. J. M. J. (2011). Evaluating the rewarding

nature of social interactions in laboratory animals. *Developmental Cognitive Neuroscience*, *1*(4), 444–458. https://doi.org/10.1016/j.dcn.2011.05.007

Trifilieff, P., Feng, B., Urizar, E., Winiger, V., Ward, R. D., Taylor, K. M., … Javitch, J. A. (2013). Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation. *Molecular Psychiatry*, *18*(9), 1025–1033. https://doi.org/10.1038/mp.2013.57

Tye, K. M., Mirzabekov, J. J., Warden, M. R., Ferenczi, E. A., Tsai, H. C., Finkelstein, J., … Deisseroth, K. (2013). Dopamine neurons modulate neural encoding and expression of depression-related behaviour. *Nature*, *493*(7433), 537–541. https://doi.org/10.1038/NATURE11740

Ungless, M. A., Magill, P. J., & Bolam, J. P. (2004). Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science (New York, N.Y.)*, *303*(5666), 2040–2042. https://doi.org/10.1126/science.1093360

Van Der Kooij, M. A., Hollis, F., Lozano, L., Zalachoras, I., Abad, S., Zanoletti, O., … Sandi, C. (2018). Diazepam actions in the VTA enhance social dominance and mitochondrial function in the nucleus accumbens by activation of dopamine D1 receptors. *Molecular Psychiatry*, *23*(3), 569–578. https://doi.org/10.1038/mp.2017.135

Venniro, M., Zhang, M., Caprioli, D., Hoots, J. K., Golden, S. A., Heins, C., … Shaham, Y. (2018). Volitional social interaction prevents drug addiction in rat models. *Nature Neuroscience*, *21*(November). https://doi.org/10.1038/s41593-018-0246-6

Von Frijtag, J. C., Reijmers, L. G. J. E., Van der Harst, J. E., Leus, I. E., Van den Bos, R., & Spruijt, B. M. (2000). Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. *Behavioural Brain Research*, *117*(1–2),

137–146. https://doi.org/10.1016/S0166-4328(00)00300-4

Wakabayashi, K. T., Fields, H. L., & Nicola, S. M. (2004). Dissociation of the role of nucleus accumbens dopamine in responding to reward-predictive cues and waiting for reward. *Behavioural Brain Research*, *154*(1), 19–30. https://doi.org/10.1016/j.bbr.2004.01.013

Walker, Q. D., Rooney, M. B., Wightman, R. M., & Kuhn, C. M. (1999). Dopamine release and uptake are greater in female than male rat striatum as measured by fast cyclic voltammetry. *Neuroscience*, *95*(4), 1061–1070. https://doi.org/10.1016/S0306-4522(99)00500-X

Watabe-Uchida, M., Eshel, N., & Uchida, N. (2017). Neural Circuitry of Reward Prediction Error. *Annual Review of Neuroscience*, *40*, 373–394. https://doi.org/10.1146/annurev-neuro-072116-031109

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience*, *5*(6), 483–494. https://doi.org/10.1038/nrn1406

Wissman, A. M., May, R. M., & Woolley, C. S. (n.d.). *Ultrastructural analysis of sex differences in nucleus accumbens synaptic connectivity*. https://doi.org/10.1007/s00429-011-0353-6

Xing, B., Mack, N. R., Zhang, Y.-X., McEachern, E. P., & Gao, W.-J. (2022). Distinct Roles for Prefrontal Dopamine D1 and D2 Neurons in Social Hierarchy. *The Journal of Neuroscience*, *42*(2), 313–324. https://doi.org/10.1523/jneurosci.0741-21.2021

Yamaguchi, Y., Lee, Y. A., Kato, A., & Goto, Y. (2017). The roles of dopamine D1 receptor on the social hierarchy of rodents and nonhuman primates. *International Journal of Neuropsychopharmacology*, *20*(4), 324–335. https://doi.org/10.1093/ijnp/pyw106

Yamaguchi, Y., Lee, Y. A., Kato, A., Jas, E., & Goto, Y. (2017). The roles of dopamine D2 receptor in the social hierarchy of rodents and primates. *Scientific Reports*, *7*, 2–11. https://doi.org/10.1038/srep43348

Yang, H., de Jong, J. W., Tak, Y. E., Peck, J., Bateup, H. S., & Lammel, S. (2018). Nucleus Accumbens Subnuclei Regulate Motivated Behavior via Direct Inhibition and Disinhibition of VTA Dopamine Subpopulations. *Neuron*, *97*(2), 434-449.e4. https://doi.org/10.1016/j.neuron.2017.12.022

Yuan, L., Dou, Y.-N., & Sun, Y.-G. (2019). Topography of Reward and Aversion Encoding in the Mesolimbic Dopaminergic System. *The Journal of Neuroscience*, *39*(33), 6472 LP – 6481. https://doi.org/10.1523/JNEUROSCI.0271-19.2019

Yuan, L., Dou, Y. N., & Sun, Y. G. (2021). Topography of reward and aversion encoding in the mesolimbic dopaminergic system. *Journal of Neuroscience*, *39*(33), 6472–6481. https://doi.org/10.1523/JNEUROSCI.0271-19.2019

Zachry, J. E., Kutlu, M. G., Yoon, H. J., Leonard, M. Z., Chevée, M., Patel, D. D., … Calipari, E. S. (2023). D1 and D2 medium spiny neurons in the nucleus accumbens core have distinct and valence-independent roles in learning. *Neuron*, 835–849. https://doi.org/10.1016/j.neuron.2023.11.023