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Glucocorticoid Regulation of Striatal Dopamine Transmission

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By

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Abstract

Chronic stress is a significant risk factor for the development of numerous psychiatric disorders, including major depressive disorder (MDD). Individuals with MDD exhibit impairments in reward processing and decreased motivation to engage in previously-rewarding activities. Importantly, there is a large sex disparity in MDD diagnosis, such that it affects nearly twice as many women as men. However, it is still unknown how chronic stress contributes to motivational impairments in either men or women with MDD. Chronic stress significantly impairs activity of the hypothalamic-pituitary-adrenal (HPA) axis in the body, leading to elevated levels of the 'stress hormone,' cortisol (corticosterone in rodents; 'CORT'). In a subset of MDD patients, CORT is elevated during the rest period. It has remained unclear if, and how, dysregulation of CORT during the rest period affects motivation to attain rewards in either sex.

In this dissertation, I examined how chronic dysregulation of CORT affected motivation to attain rewards in male and female mice. Using subcutaneous pellet implants, I found that the HPA axes of male and female mice respond differently to the same dose of exogenous CORT, consistent with documented sex differences in HPA axis function. I further went on to show that chronic CORT dysregulation impaired motivation to attain rewards in an operant task in both sexes, but this behavioral deficit was associated with sex-specific alterations in dopaminergic transmission in a part of the brain called the dorsomedial striatum (DMS). In females, CORT dysregulation decreased the amount of dopamine within the DMS. In males, CORT dysregulation impaired activity of the dopamine transporter (DAT) in the DMS. The results of this study indicate there are latent sex differences in how CORT dysregulation impairs DMS dopaminergic

transmission to drive deficits in reward-guided motivation. This work provides the scaffolding for future experiments disentangling the molecular bases for sex-specific effects of glucocorticoids on dopaminergic transmission.

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List of Abbreviations

ACSF – artificial cerebrospinal fluid

AMPAR – AMPA receptor

ANOVA – analysis of variance

A/P – anterior/posterior

AUC – area under the curve

BCA – bicinchoninic acid

BSA – bovine serum albumin

CBG – corticosteroid-binding globulin

CMOS – complementary metal oxide semiconductor

CORT – corticosterone/cortisol

DAT – dopamine transporter

DATi – DAT inhibitor

DMS – dorsomedial striatum

D/V – dorsal/ventral

FR – fixed ratio

EDTA – ethylenediaminetetraacetic acid

ELISA – enzyme-linked immunosorbent assay

$GABA_AR$ – GABA-a receptor

$GABA_BR$ – GABA-b receptor

HPA – hypothalamic-pituitary-adrenal

HPLC-ECD – high performance liquid chromatography-electrochemical detection

LED – light-emitting diode

M/L – medial/lateral

NA – numerical aperture

NAcc – nucleus accumbens core

nAChR – nicotinic acetylcholine receptor

NFM – non-fat milk

NMDAR – NMDA receptor

NMDG – n-methyl-d-glucamine

OCT3 – organic cation transporter 3

OCTi – organic cation transporter 3 inhibitor

pDAT – phosphorylated DAT

PSTH – peri-stimulus time histogram

PVDF – polyvinylidene difluoride

SEM – standard error of the mean

TBS – tris-buffered saline

TBS-T – tris-buffered saline with tween-20

TCA – trifluoroacetic acid

VG – viral genomes

ZT – zeitgeber time

To Mom and Dad

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Chapter 1: Introduction

1.1 Stress and major depressive disorder

Major depressive disorder (MDD) is a significant public health problem, affecting five percent of adults worldwide, with nearly twice as many women diagnosed with the disorder compared to men (Kuehner, 2016; NIMH, 2022; World Health Organization, 2021). Individuals with MDD often present with debilitating symptoms that significantly decrease their quality of life. Notably, individuals with MDD exhibit deficits in positive valence behaviors, such as a loss of pleasure (anhedonia) and decreased motivation to engage in previously rewarding activities (American Psychiatric Association, 2022). However, it remains unclear why individuals with MDD exhibit deficits in positive valence behaviors.

Both genetic and environmental risk factors contribute to the development of MDD (Klengel & Binder, 2013; Lohoff, 2010). Chronic stress, especially, has been shown to be a significant risk factor for MDD (Hammen et al., 2009; Kessler, 1997; McGonagle & Kessler, 1990). Thus, to understand how deficits in positive valence behaviors arise in individuals with MDD, many preclinical researchers have used chronic stress paradigms in rodents to infer the neurobiological and behavioral effects of chronic stress in humans (Cabeza et al., 2021; Dias-Ferreira et al., 2009; Dieterich et al., 2021; Friedman et al., 2017; Lopez & Bagot, 2021).

1.2 How does the body respond to stress?

To understand the effects of stress on reward-related behaviors, we must understand what stress is, and how the body responds to stress. “Stress” is the physical and psychological response to challenging (often uncontrollable) situations, resulting in

feelings of distress. Acute stressors, such as public speaking or being chased by a lion, are adaptive. To deal with acute stressors, the body enacts a transient physiological response that affects all biological systems to cope with the challenge, and then returns to homeostasis (McEwen, 1998, 2000). However, chronic activation of the stress response— as occurs in individuals who experience chronic stress—can be harmful to biological systems, leading to physical illness and decreased psychological well-being (McEwen, 1998, 2000, 2019; McEwen & Akil, 2020).

The hypothalamic-pituitary-adrenal (HPA) axis governs the body's stress response and is active in both a circadian rhythm and during times of stress (Russell & Lightman, 2019; Figure 1-1). Upon the perception of a stressor, activation of the HPA axis begins. First, corticotropin-releasing factor (CRF) is released from the paraventricular nucleus of the hypothalamus, and then travels through the hypophyseal portal system and activates CRF receptors in the anterior pituitary. CRF acts in the anterior pituitary to cause release of adrenocorticotropic hormone (ACTH), which then enters the bloodstream and eventually binds to ACTH receptors in the adrenal glands, above the kidneys. Activation of ACTH receptors in the adrenal glands causes synthesis and release of the "stress hormone," cortisol (corticosterone in rodents; 'CORT'), which enters the bloodstream for widespread circulation. CORT acts on target tissues throughout the body and brain by binding to two types of corticosteroid receptors: the high-affinity mineralocorticoid receptor and low-affinity glucocorticoid receptor (McEwen et al., 1986; Reul & Kloet, 1985). While mineralocorticoid receptors are activated by low levels of circulating CORT, activation of glucocorticoid receptors is only achieved when CORT levels are significantly elevated, such as during times of stress, and when

endogenous circadian CORT is at its peak (Reul & De Kloet, 1986; Reul & Kloet, 1985). Activation of glucocorticoid receptors mobilizes energy stores and alters neuronal functioning in numerous brain regions so that an organism can respond to a change in its environment (McEwen et al., 1986). Critically, CORT binds to glucocorticoid receptors in the hypothalamus and pituitary to inhibit further HPA axis activation, a process termed “negative feedback” (Figure 1-1A). Negative feedback of the HPA axis ensures that CORT release ceases after an organism experiences an acute stressor, thus allowing the body to return to homeostasis. Chronic stress, however, can dysregulate negative feedback of the HPA axis, resulting in chronically elevated levels of CORT (Gómez et al., 2008; Makino et al., 1995; McEwen, 1998; Sze & Brunton, 2020). Furthermore, stressors can alter pharmacodynamics of CORT by decreasing the amount of corticosteroid binding globulin (CBG) in the blood (Fleshner et al., 1995; Spencer et al., 1996). Approximately 90-95% of CORT in the blood is bound by CBG and albumin (another blood protein), leaving it inaccessible for activity in target tissues (Spencer & Deak, 2017). The other 5-10% of CORT is not bound by protein (“free” CORT), and can freely bind to mineralocorticoid and glucocorticoid receptors throughout the body and brain. Thus, when stressors decrease CBG levels in the blood, they indirectly elevate levels of free CORT, which can act on corticosteroid receptors in target tissues.

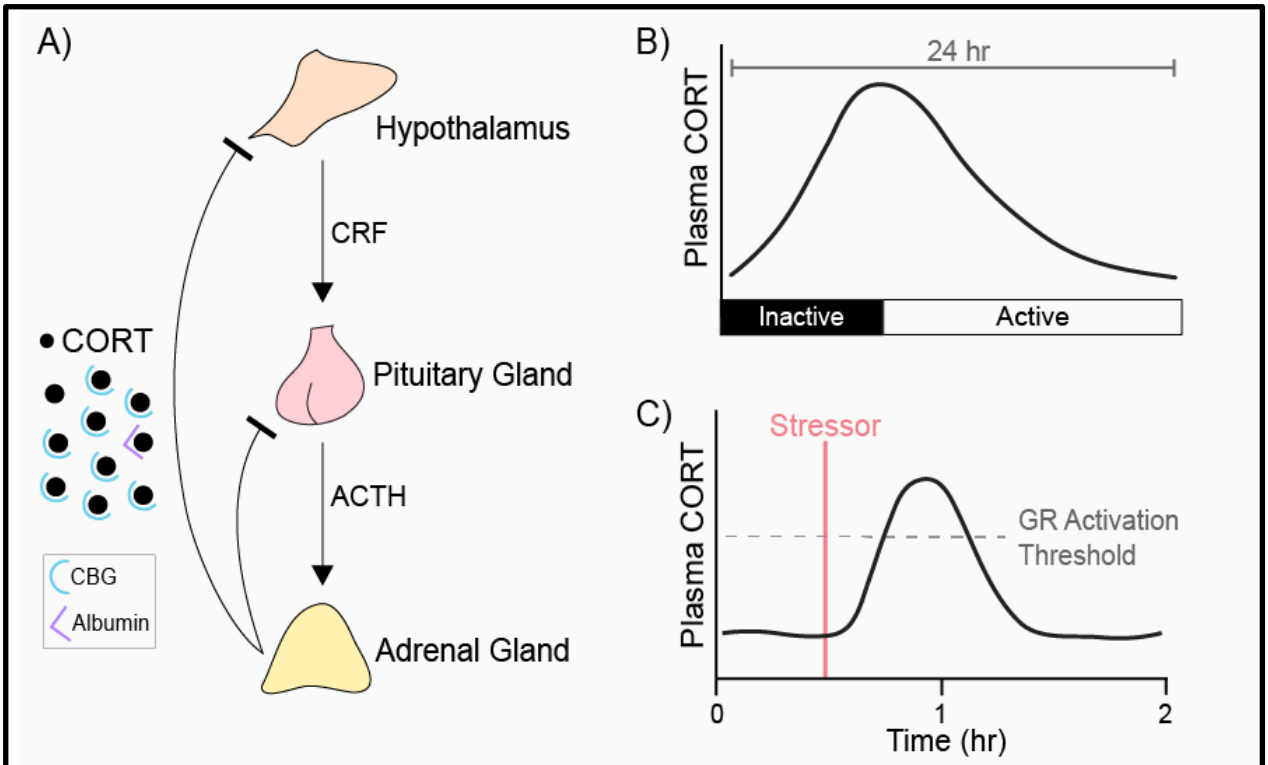

Figure 1-1: The Hypothalamic-Pituitary-Adrenal (HPA) Axis. A) The HPA axis, its hormonal effectors, and relevant blood proteins. CRF= corticotropin-releasing factor, ACTH= adrenocorticotropic hormone, CORT= corticosterone/cortisol, CBG= corticosteroid-binding globulin. B) Circadian rhythm of CORT. C) CORT release in response to a stressor.

Importantly, there are numerous sex differences in HPA axis function, encompassing sensitivity to negative feedback, magnitude of CORT released by stressors, and rates of CORT clearance from the blood (an exceptional review of sex differences in HPA axis function can be found here: Kokras et al., 2019). For these reasons, it is possible that similar stressors, or similar levels of exogenously-administered CORT, can have different effects on HPA axis function, neurobiology, and behavior of males and females. However, very few studies have examined the effects of exogenous CORT on males and females simultaneously.

1.3 Modeling CORT dysregulation in rodents

In the 1990s, researchers formulated the corticosteroid receptor hypothesis of depression, which posited that dysregulation of HPA axis function was a key mechanism for the development of MDD (Holsboer, 2000). Indeed, numerous studies have reported increased levels of circulating CORT in patients with MDD, especially during the evening hours (aka, 'rest period') when CORT levels are normally low (Deuschle et al., 1997; Jarcho et al., 2013; Lamers et al., 2013; Linkowski et al., 1985; Sachar et al., 1973; Wong et al., 2000). Elevated CORT during the rest period in individuals with MDD causes a "flattening" of the circadian CORT rhythm by decreasing the difference between circadian peak and nadir (trough) levels of CORT. However, it has remained unclear if, and how, CORT dysregulation during the rest period contributes to symptoms of MDD, such as decreased motivation to engage in previously-rewarding activities.

To understand how CORT dysregulation affects behavior, researchers directly manipulate plasma CORT levels in rodents by administering exogenous CORT via drinking water, injection, or pellet implantation. Previous studies have shown that CORT dysregulation decreases reward-guided motivation in male rodents, but no such study has reported the effect of CORT dysregulation on reward-guided motivation in females (Dieterich et al., 2019; Gourley et al., 2012). Few studies have examined the effect of CORT dysregulation on reward-guided behaviors in females due to reports that CORT dysregulation has little or no effect on anxiety-like behavior of female mice, but significantly impairs anxiety-like and reward-guided behaviors in males (Dieterich et al., 2019; Mekiri et al., 2017; Yohn et al., 2019). However, it is critical to recognize that not

all behavioral tests are cross-predictive for each other: even if female anxiety-like behavior is unaffected by CORT dysregulation, it does not mean that reward-guided motivation will be similarly unaffected by CORT dysregulation in females. Furthermore, mechanisms for CORT-induced impairments in reward-seeking have not been elucidated in either sex. Altogether, two important questions remain open regarding the effects of CORT dysregulation on reward-guided behavior: 1) does CORT dysregulation similarly affect reward-guided motivation in both sexes; and 2) how does CORT dysregulation affect the brain to induce changes in motivation to attain rewards in either sex?

1.4 Using operant conditioning to measure reward-guided behaviors in rodents

While psychologists and psychiatrists use questionnaires to solicit self-reported measures of motivation in patients, researchers must use behavioral tests to assess motivation in rodents. One common method for measuring reward-guided motivation in rodents is operant conditioning. Since the work of B.F. Skinner, researchers have found that animals will complete an action to receive a reward in the form of food, sucrose, or water (among others) – this is the basis of operant conditioning (Skinner, 1948). There are multiple psychological processes under scrutiny in operant conditioning, including reward learning and motivation. Reward learning in reward-based operant conditioning is assessed by gauging the ability of an organism to learn which action results in delivery of a reward in an operant session (*Reward Learning*, n.d.). If an animal can learn the action-outcome contingency in an operant task, then the animal will repeat the specific action necessary to receive a desired outcome (Yin & Knowlton, 2006). Another important cognitive process under examination during reward-based operant

conditioning is reward-guided motivation. For the purposes of this dissertation, I will refer to motivation as the psychological process underlying the allocation of effort to attain a reward. Importantly, in the context of reward-guided operant conditioning, motivation is apparent in two phases of the task. Motivation in the instrumental phase of the task is reflected in the rate of actions that a subject will perform in order to cause delivery of a reward, while motivation in the consummatory phase of the task is reflected in the rate of actions that a subject will perform to directly interact with the reward stimulus. Thus, motivation during the instrumental phase is regarded as a measure of reward-seeking, and motivation during the consummatory phase is regarded as a measure of hedonic response to the reward stimulus (Salamone & Correa, 2012). When analyzing behavioral outcomes of operant conditioning, it is important to precisely delineate when reward learning is affected vs when reward-guided motivation is affected, as the two processes are thought to be governed by different neurobiological processes, but impairments in either may result in similar behavioral deficits (i.e., decreased operant responding).

1.5 Dopaminergic regulation of reward-guided operant conditioning

Since Skinner's introduction of the concept of operant conditioning to the field of psychology, researchers have dissected the neural mechanisms underlying operant conditioning for rewards, with particular focus on the role of a part of the basal ganglia called the striatum. Due to its combination of cortical and limbic afferents, the striatum is perfectly poised to integrate information about an animal's external environment with its internal state and translate these inputs into a motor output that satisfies its needs (Balleine et al., 2009; Redgrave et al., 2010; Yin & Knowlton, 2006). Based on

functional connectivity, the striatum is subdivided into the dorsal striatum and nucleus accumbens (analogous the human ventral striatum) (Collins & Saunders, 2020; Voorn et al., 2004). The dorsal striatum can be further subdivided into the dorsomedial and dorsolateral subregions (analogous to the human caudate and putamen, respectively), while the nucleus accumbens is subdivided into core and shell subregions (Collins & Saunders, 2020; Lerner et al., 2015). The dorsomedial striatum (DMS) and nucleus accumbens core (NAcc) are particularly crucial for the acquisition and maintenance of operant responding (Corbit et al., 2001; Yin, Knowlton, et al., 2005; Yin, Ostlund, et al., 2005). Critically, the NAcc and DMS receive dense dopaminergic innervation from the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), respectively (Fallon & Moore, 1978). Due to its role as a neuromodulator, dopamine in the NAcc and DMS shapes plasticity of the connections between afferents to striatal subregions and the principal neurons in the striatum, D1- and D2-receptor-expressing medium spiny neurons (Lahiri & Bevan, 2020; Surmeier et al., 2007). Thus, it is no surprise that lesioning dopaminergic projections to the NAcc or DMS significantly impairs operant conditioning. Specifically, lesioning dopaminergic projections to the NAcc impairs effortful responding for rewards, while lesioning dopaminergic projections to DMS impairs action-outcome learning (Lex & Hauber, 2010a; Sokolowski & Salamone, 1998). Importantly, dopaminergic transmission in the striatum occurs in two modes: tonic and phasic (Floresco et al., 2003; Lodge & Grace, 2006). Tonic dopamine release results from the regular, low-frequency firing of dopamine neurons, and is thought to encode motivation (Cagniard et al., 2006; Niv et al., 2007; Sokolowski et al., 1998). Phasic dopamine release results from burst firing of dopamine neurons, occurring in discrete

bouts on top of tonic dopamine (Floresco et al., 2003; Zweifel et al., 2009). Phasic dopamine release is crucial for reward learning, as it mediates the pairing of rewards to their antecedent predictive stimuli or actions (Brown et al., 2011; Saunders et al., 2018; Seiler et al., 2022; Tsai et al., 2009; Willuhn et al., 2012). The magnitude of tonic and phasic dopamine release in the striatum is directly controlled by activity of the dopamine transporter ('DAT'; Ferris et al., 2014; Jones et al., 1998). DAT functions at dopamine axon terminals to reuptake dopamine, which is then repackaged into synaptic vesicles for release. When DAT activity is high, large amounts of dopamine are taken back up into axon terminals and repackaged for release, resulting in large dopamine transients upon depolarization of dopamine neuron axons, and vice versa for when DAT activity is low (Ferris et al., 2014; Jones et al., 1998). Furthermore, DAT activity itself is regulated by post-translational modifications, particularly phosphorylation (Foster & Vaughan, 2017). Numerous studies have demonstrated that phosphorylation of DAT at threonine-53 regulates reuptake capacity of the transporter, with high levels of phosphorylation at threonine-53 being associated with faster rates of reuptake, and vice versa (Alonso et al., 2021; Calipari et al., 2017; Challasivakanaka et al., 2017). Thus, dopamine neuron firing, as well as DAT activity and phosphorylation status, directly affect dopamine release in striatal subregions, such as the NAc and DMS, which can then influence operant responding for rewards.

With the experiments in this dissertation, I sought to understand if, and how, dysregulation of CORT affected reward-guided operant conditioning in male and female mice. Equipped with the knowledge that dopamine release in the NAcc and DMS is critical for the acquisition and maintenance of operant responding for rewards, I

questioned if CORT dysregulation impacted reward-guided operant conditioning in either sex by altering tonic or phasic dopaminergic transmission in the NAcc and/or DMS. The results presented here have significant implications for our understanding of how stress hormones affect reward-guided behavior and dopamine transmission in each sex.

Chapter 2: Chronically dysregulated corticosterone impairs dopaminergic transmission via sex-specific mechanisms

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2.1 Introduction

Major depressive disorder (MDD) is a leading cause of disability worldwide, affecting an estimated 5% of adults (World Health Organization, 2021). Individuals with MDD exhibit decreased motivation and deficits in reward processing (American Psychiatric Association, 2022; Pizzagalli et al., 2019). One important factor that precipitates and exacerbates MDD is stress (McGonagle & Kessler, 1990). CORT (corticosterone in rodents, cortisol in humans) is the body's primary stress hormone, released by the adrenal gland both in a regular circadian rhythm and in response to stressful events. In a subset of individuals with MDD, the circadian regulation of CORT is altered, with chronically elevated levels observed during the rest period (i.e., evening and night; Jarcho et al., 2013; Wong et al., 2000). Increased resting period CORT is particularly evident in psychotic and melancholic depression, and is associated with symptoms of anhedonia and general distress (Deuschle et al., 1997; Doane et al., 2013; Lamers et al., 2013; Linkowski et al., 1985; Nandam et al., 2020; Sachar et al., 1973; Veen et al., 2011). However, it remains unclear how this CORT dysregulation contributes to MDD symptomology.

In rodent preclinical models, chronic elevation of circulating CORT impairs operant responding for rewards, suggesting that elevated CORT may cause impaired reward

processing in humans (Dieterich et al., 2019; Gourley et al., 2012). However, rodent studies have only been carried out in males, leaving open the question of sex differences in the effects of dysregulated CORT. Since MDD is twice as common in women vs men, sex differences in biological responses to dysregulated CORT are important to assess. Furthermore, the biological mechanisms underlying CORT-induced impairments in operant responding in either sex remain unclear.

We hypothesized that CORT dysregulation impacts operant responding by altering dopaminergic transmission. Dopaminergic transmission in the striatum regulates reward processing, motivation, and associative learning (Collins & Saunders, 2020; Lex & Hauber, 2010b, 2010a; Seiler et al., 2022; Sokolowski & Salamone, 1998). Dopaminergic transmission within the striatum occurs in two modes: tonic and phasic (Floresco et al., 2003; Lodge & Grace, 2006). Tonic dopamine is the sustained level of extracellular dopamine in the striatum. It arises from the tonic firing activity of dopamine neurons and is also tightly regulated by dopamine reuptake into terminals by the dopamine transporter, DAT (Ferris et al., 2014; Jones et al., 1998). Tonic dopamine is hypothesized to govern motivation (Niv et al., 2007; Sokolowski et al., 1998). Phasic dopamine transmission occurs when dopamine neurons fire bursts of action potentials in discrete epochs on top of tonic dopamine. Phasic dopamine transients facilitate associative learning about cues and actions that precede rewards (Brown et al., 2011; Saunders et al., 2018; Tsai et al., 2009; Willuhn et al., 2012). We examined whether impaired operant responding for rewards following chronic CORT treatment was associated with impaired tonic and phasic dopaminergic transmission in two striatal

subregions critical for effortful operant responding: the nucleus accumbens core (NAcc) and the dorsomedial striatum (DMS).

2.2 Results

2.2.1 Chronic corticosterone (CORT) treatment increases total plasma CORT in male mice and decreases plasma corticosteroid binding globulin (CBG) levels in both sexes

To chronically elevate plasma CORT levels during the rest period, we implanted male and female mice with subcutaneous slow-release CORT pellets (35 mg, 60-day release); control groups received Placebo pellets of the same size. Slow-release pellets were used to increase circulating CORT levels during the rest period (the light phase for mice), thereby disrupting circadian rhythms of CORT (Leitch et al., 2003; Sarabdjitsingh et al., 2010) as observed in some individuals with MDD (Jarcho et al., 2013; Wong et al., 2000). This approach differs from another commonly used approach, CORT administration via drinking water, which preferentially increases circulating CORT levels during the active phase, when mice drink more often (Godynyuk et al., 2019). To test if slow-release CORT pellet treatment chronically elevated plasma CORT levels during the rest period, we collected blood from Placebo- and CORT-treated mice at zeitgeber time 4-6 (ZT4-6, 4-6 hours after lights on) four weeks after implantation and used an enzyme-linked immunosorbent assay (ELISA) to quantify total plasma CORT (Figure 2-1). There was a significant effect of treatment (Two-way ANOVA, $F_{(1,37)}$=41.18, p<0.0001), a significant effect of sex ($F_{(1,37)}$=11.78, p<0.01), and a significant interaction between treatment and sex ($F_{(1,37)}$=17.25, p<0.001). Notably, we found that CORT pellet implant increased total plasma CORT in male mice only and resulted in higher levels of resting CORT in male vs female mice (Placebo Male vs CORT Male, Tukey's multiple comparisons p<0.0001; CORT Male vs CORT Female, p<0.0001; Figure 2-1B). This sex difference in total plasma CORT four weeks after pellet implantation is consistent

with previous studies in rats (Kott et al., 2016), and likely occurs due to sex differences in hypothalamic-pituitary-adrenal (HPA) axis responsivity (Kokras et al., 2019). However, a limitation of measuring total plasma CORT is that it includes both free and protein-bound CORT. Free CORT can cross the blood-brain barrier, while protein-bound CORT cannot (Breuner & Orchinik, 2002; Lewis et al., 2005; Qian et al., 2011). Corticosteroid binding globulin (CBG) is the primary blood protein that binds CORT. Thus, we questioned if chronic CORT treatment decreased CBG, which could augment circulating levels of free CORT, even in the absence of changes in total levels. Chronic CORT treatment decreased CBG levels with no evidence of sex difference (Figure 2-1C; Two-way ANOVA, significant effect of treatment, $F_{(1,30)}$=16.30, p<0.001; no sex x treatment interaction). Treatment did not affect estrous cyclicity of females (Figure 2-2). We concluded that circulating levels of free CORT are likely elevated in both male and female mice after treatment with subcutaneous slow-release CORT pellets but to differing degrees of severity. Due to the significant sex difference in plasma CORT levels after CORT treatment, we separated the sexes for analysis in all following experiments.

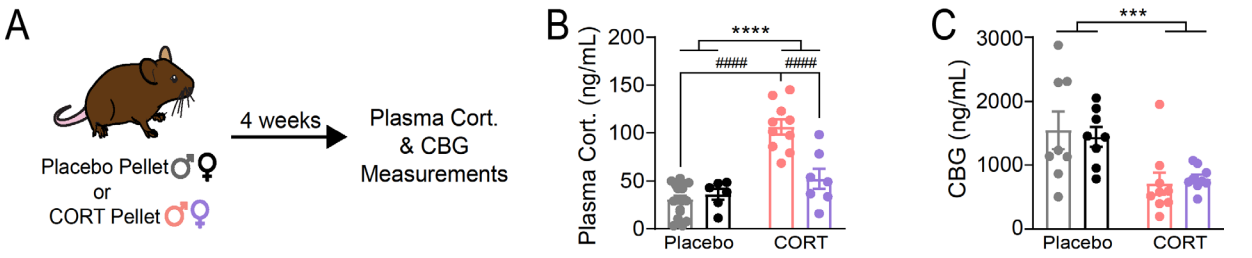

Figure 2-1. Chronic corticosterone (CORT) pellet treatment significantly increases plasma CORT in male mice and decreases plasma corticosteroid-binding globulin (CBG) in both sexes.

A) Experimental timeline for pellet implantation and plasma CORT and CBG measurements.

B) Plasma corticosterone (ng/mL) in male and female mice implanted with a placebo or corticosterone (35 mg; CORT) pellet. Two-way ANOVA, main effect of treatment ****$p<0.0001$, main effect of sex $p<0.01$, main effect of treatment x sex interaction $p<0.001$, multiple comparisons ####$p<0.0001$.

C) Plasma CBG (ng/mL) in male and female mice implanted with a placebo or corticosterone (35 mg; CORT) pellet. Two-way ANOVA, main effect of treatment ***$p<0.001$.

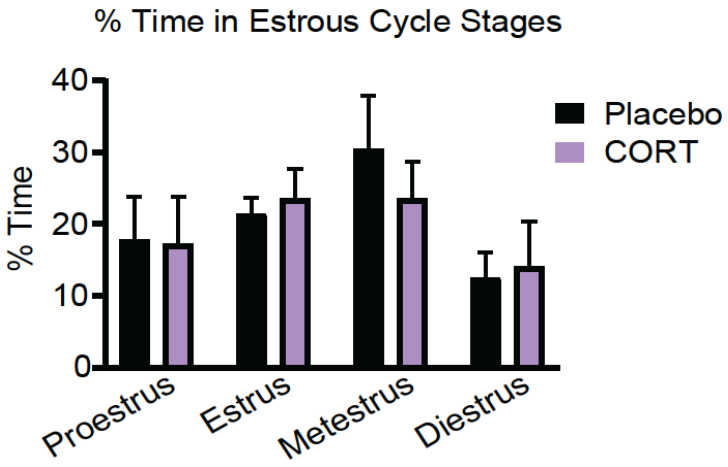

Figure 2-2. Chronic corticosterone treatment does not affect estrous cyclicity of female mice.
Percent of time spent in each phase of the estrous cycle over the course of eight days of sampling. Placebo N=7, CORT N=8.

2.2.2 Chronic CORT treatment impairs motivated reward-seeking in male and female mice

Chronic CORT treatment has previously been shown to impair reward-seeking behaviors in male mice (Dieterich et al., 2019; Gourley et al., 2012). However, it was unclear what effect chronic CORT treatment would have on female mice. To assess reward-seeking behaviors in both sexes, we used operant training. Four weeks after Placebo or CORT pellet implantation, mice began training on a fixed ratio-1 (FR-1) schedule, then advanced to FR-3 and FR-5 (Figure 2-3A). We found that CORT treatment significantly increased the number of days it took both sexes to reach criterion on the FR-1 task (Figure 2-3B,C; Unpaired two-tailed t-test, $p<0.05$ male, $p<0.0001$ female). However, CORT-treated mice readily learned the association between the active nosepoke and reward. CORT- and Placebo-treated mice similarly discriminated between the active and inactive nosepokes during the initial days of FR-1 training (Figure 2-3D,E), but CORT-treated mice were slower to use this associative knowledge to reach the criterion of obtaining 30 rewards per session. This finding suggests that CORT-treated mice have intact reward learning but are less motivated to attain rewards than Placebo-treated mice. After FR-1 criterion was met, CORT-treated mice exhibited decreased rates of nosepoking across FR-3 and FR-5 sessions (Figure 2-3F,G; males: significant effects of treatment [Two-way ANOVA $F_{(1,15)}=5.554$, $p<0.05$], day of training [$F_{(2.513,37.70)}=37.70$, $p<0.0001$], and an interaction between treatment and day of training [$F_{(5,75)}=4.749$, $p<0.001$]; females: significant effects of treatment [Two-way ANOVA $F_{(1,12)}=5.098$, $p<0.05$], day of training [$F_{(3.267,39.20)}=87.47$, $p<0.0001$], and an interaction between treatment and day of training [Two-way ANOVA $F_{(5,60)}=7.294$, $p<0.0001$]).

Again, no deficit in active nosepoke discrimination was observed (in fact, CORT-treated males made significantly fewer inactive nosepokes than Placebo-treated males, Figure 2-3H,I; males: Two-way ANOVA, significant effect of treatment [$F_{(1,15)}$=6.123, p<0.05]; females: no significant effect of treatment). Therefore, as for initial FR1 training, decreased rates of active nosepoking in CORT-treated mice do not stem from impaired learning, but likely arise due to decreased motivation. Motivational deficits in CORT-treated mice are further supported by their impaired rate of rewards earned, particularly on the final days of FR-3 and FR-5 (Figure 2-3J,K; males: Two-way ANOVA, significant effects of day of training [$F_{(3.422,51.34)}$=7.968, p<0.0001], and an interaction between day of training and treatment [$F_{(5,75)}$=3.320, p<0.01); females: Two-way ANOVA, significant effects of treatment [$F_{(1,12)}$=16.15, p<0.01], day of training [$F_{(3.275,39.30)}$=5.835, p<0.01], and an interaction between day of training and treatment [$F_{(5,60)}$=4.427, p<0.01]). While CORT-treated males earned the same number of rewards as Placebo-treated males across operant training (Figure 2-4A), they took longer to earn those rewards, especially on the final days of FR-3 and FR-5 (Figure 2-3L; Two-way ANOVA, significant effect of an interaction between treatment and day of training, p<0.01). CORT-treated females also took longer to earn rewards (Figure 2-3M; Two-way ANOVA, significant effects of treatment [$F_{(1,12)}$=19.20, p<0.001], day of training [$F_{(3.360,40.32)}$=3.978, p<0.05], and an interaction between treatment and day of training [$F_{(5,60)}$=2.648, p<0.05]) and earned significantly fewer total rewards (Figure 2-4B) than Placebo-treated females. These results suggest continued motivational impairments throughout training. In female mice only, CORT treatment significantly decreased reward port entry rates (i.e., actions to

retrieve earned rewards, Figure 2-4C,D), suggesting that CORT treatment may also induce anhedonia in females.

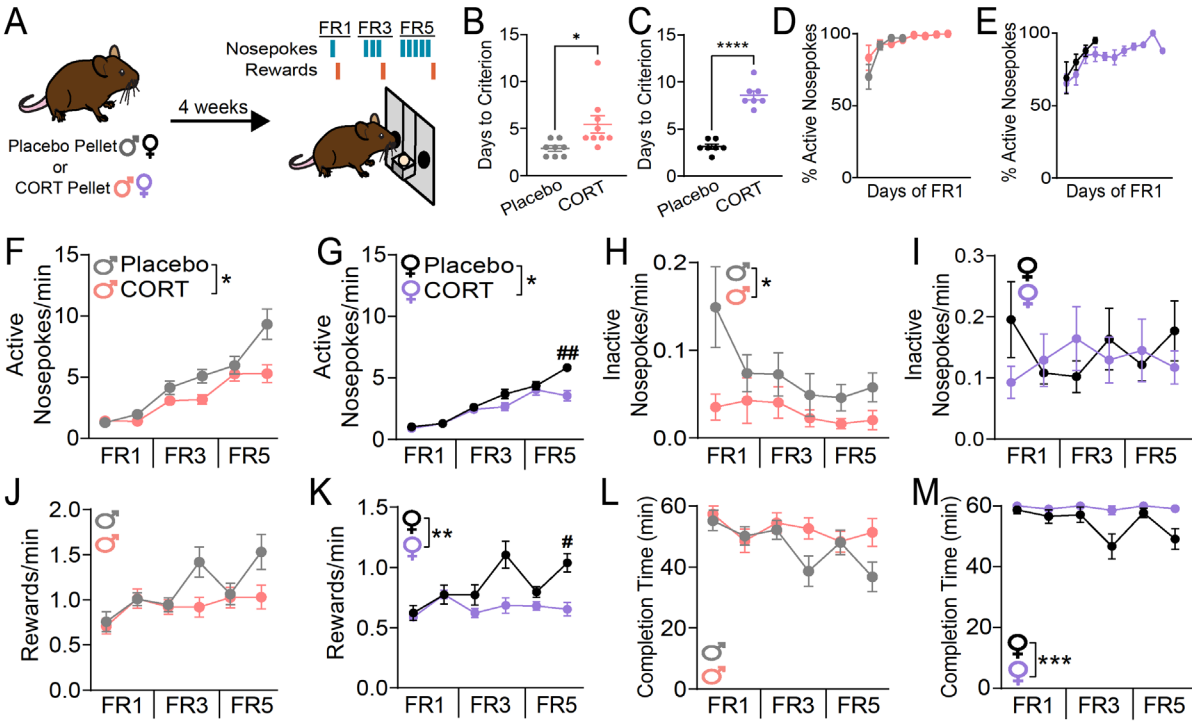

Figure 2-3. Chronic corticosterone treatment impairs motivation in both sexes.
A) Experimental timeline for pellet implantation and operant behavior paradigms with schematic of fixed ratio (FR) paradigms.
B) Days to reach criterion for FR1 in male mice. Unpaired two-tailed t-test *p<0.05. Each point represents an individual.
C) Days to reach criterion for FR1 in female mice. Unpaired two-tailed t-test ****p<0.0001. Each point represents an individual.
D) Percent active nosepokes over days of FR1 until criterion was met in Placebo- (grey) and CORT-treated (pink) male mice. Each point represents mean ± SEM percent active nosepokes for a given day of FR1 training.
E) Percent active nosepokes over days of FR1 until criterion was met in Placebo- (black) and CORT-treated (purple) female mice. Each point represents mean ± SEM percent active nosepokes for a given day of FR1 training.
F) Active nosepoking rates of Placebo- (N=8) and CORT- (N=9) treated male mice across operant behavior paradigms. Two-way ANOVA, main effect of treatment *p<0.05, main effect of treatment x paradigm interaction p<0.001.
G) Active nosepoking rates of Placebo- (N=7) and CORT- (N=7) treated female mice across operant behavior paradigms. Two-way ANOVA, main effect of treatment *p<0.05, main effect of treatment x paradigm interaction p<0.001.
H) Inactive nosepoking rates of Placebo- and CORT-treated male mice across operant behavior paradigms. Two-way ANOVA, main effect of treatment *p<0.05.
I) Inactive nosepoking rates of Placebo- and CORT-treated female mice across operant

behavior paradigms.
J) Reward rates of Placebo- and CORT-treated male mice across operant behavior paradigms.
K) Reward rates of Placebo- and CORT-treated female mice across operant behavior paradigms Two-way ANOVA, main effect of treatment **$p<0.01$, main effect of treatment x paradigm interaction $p<0.01$, multiple comparisons #$p<0.05$.
L) Time to completion of operant session (in minutes) for Placebo- and CORT-treated male mice across operant behavior paradigms. Two-way ANOVA, main effect of treatment x paradigm interaction $p<0.05$.
M) Time to completion of operant sessions (in minutes) for Placebo- and CORT-treated female mice across operant behavior paradigms. Two-way ANOVA, main effect of treatment ***$p<0.001$, main effect of treatment x paradigm interaction $p<0.05$.
Data presented as mean ± SEM.

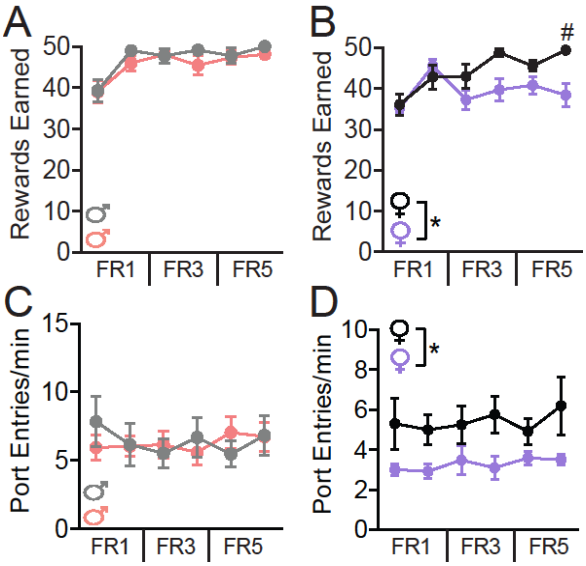

Figure 2-4. Chronic corticosterone treatment decreases number of rewards earned and port entry rate in female mice.
A) Number of rewards earned by Placebo- (grey) and CORT-treated (pink) male mice across days of operant training.
B) Number of rewards earned by Placebo- (black) and CORT-treated (purple) female mice across days of operant training. Two-way ANOVA, main effect of treatment *p<0.05, multiple comparisons #p<0.05.
C) Port entry rate of Placebo- (grey) and CORT-treated (pink) male mice across days of operant training.
D) Port entry rate of Placebo- (black) and CORT-treated (purple) female mice across days of operant training. Two-way ANOVA, main effect of treatment *p<0.05.
Data presented as mean ± SEM.

2.2.3 Chronic CORT treatment does not impair phasic dopamine transmission during reward-seeking

After observing an effect of CORT treatment on reward-seeking in both sexes, we questioned if CORT treatment was impairing phasic dopamine transmission in the striatum. To examine phasic dopamine transients in Placebo- and CORT-treated mice, we injected a virus encoding the fluorescent dopamine sensor, dLight1.3b (AAV9-CAG-dLight1.3b), into the NAcc and DMS. We implanted a fiber optic over each injection site and recorded dLight1.3b transients during operant training using fiber photometry. We found that CORT treatment did not affect phasic dLight1.3b transients in the NAcc or DMS (Figure 2-5), thus we pursued measures of tonic dopamine activity.

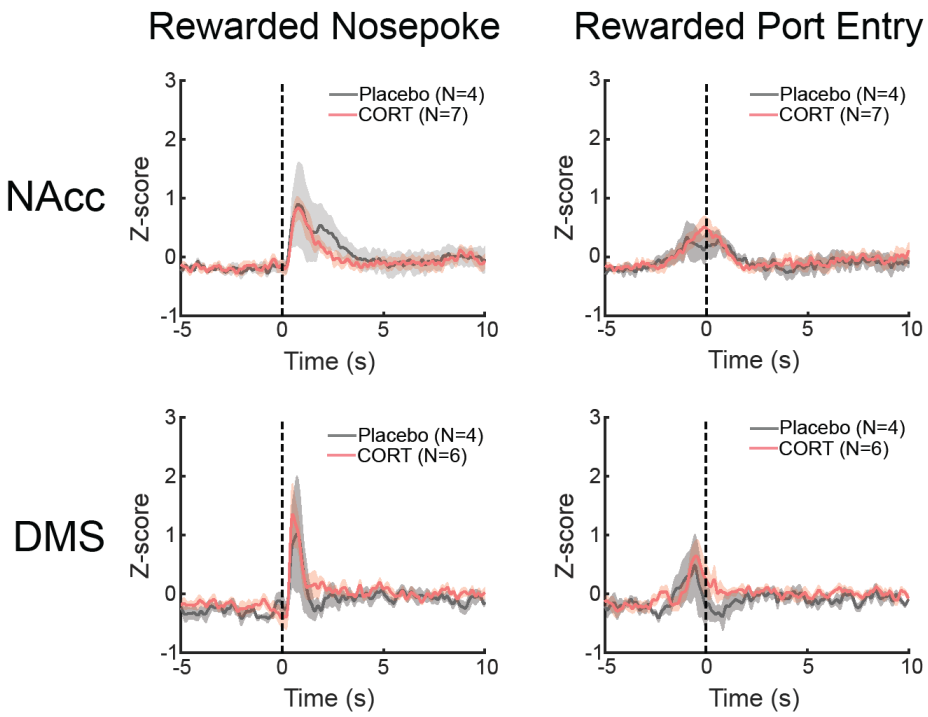

Figure 2-5. Chronic corticosterone treatment does not affect phasic dopamine transmission during operant training.
Peri-stimulus time histograms of dLight1.3b fluorescence recorded from nucleus accumbens core (NAcc; top) and dorsomedial striatum (DMS; bottom) aligned to the time of a rewarded nosepoke (left) and rewarded port entry (right) during FR-5 training. Data from male mice only. Data presented as mean ± SEM.

2.2.4 Chronic CORT treatment decreases tissue dopamine content of the dorsomedial striatum (DMS) in female mice

To investigate whether chronic CORT treatment influenced dopamine content of the striatum, we analyzed tissue samples from the NAcc and DMS of Placebo- and CORT-treated mice using high-performance liquid chromatography and electrochemical detection (HPLC-ECD) of dopamine. CORT treatment did not affect NAcc dopamine content in either sex (Figure 2-6B), but significantly decreased DMS dopamine content in female mice (Figure 2-6C; Unpaired two-tailed t-test, $p<0.01$). Therefore, although acute CORT has effects on NAcc dopamine (Wheeler et al., 2017), DMS dopamine is more sensitive to chronic CORT treatment in females.

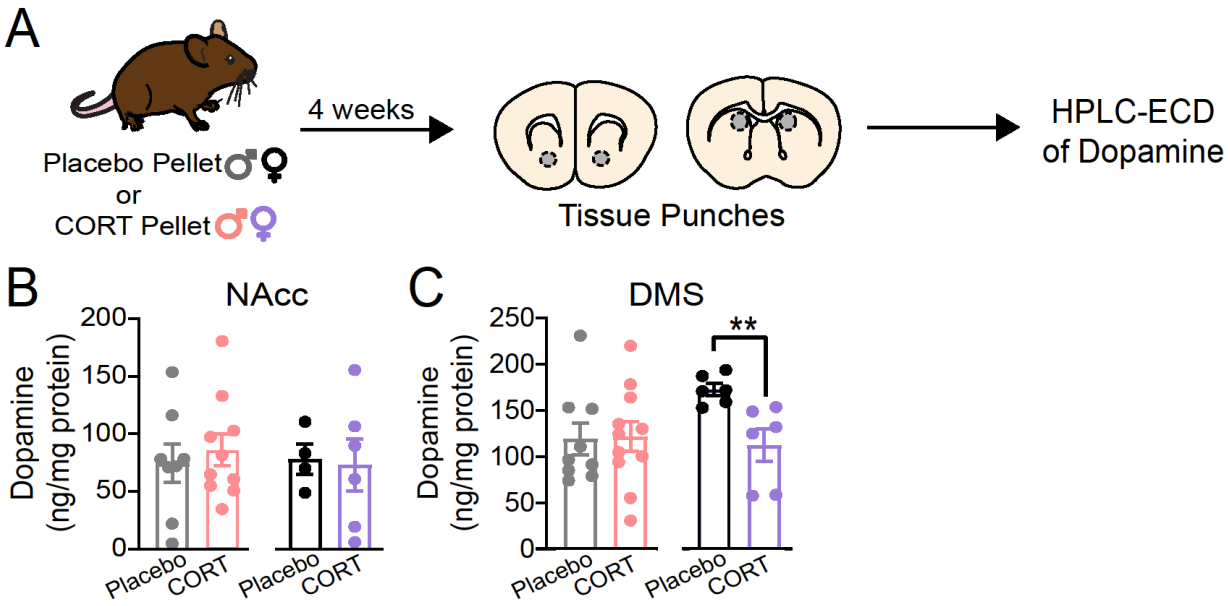

Figure 2-6. Chronic corticosterone treatment significantly decreases tissue dopamine content of the dorsomedial striatum (DMS) in female mice.
A) Experimental timeline for pellet implantation, tissue punches, and HPLC-ECD of dopamine.
B) Tissue dopamine content of the nucleus accumbens core (NAcc) of placebo- and CORT-treated male and female mice.
C) Tissue dopamine content of the DMS of placebo- and CORT-treated male and female mice. Unpaired two-tailed t-test, **p<0.01.
Each point represents an individual. Data presented as mean ± SEM.

2.2.5 Chronic CORT treatment impairs *ex vivo* dopamine transporter (DAT) function in the DMS of male mice

One mechanism that regulates levels of tonic dopamine in the striatum is modulation of dopamine transporter (DAT) function (Jones et al., 1998). By altering decay rates of phasic dopamine transients, changes in DAT function can alter the timescale for integration of phasic dopamine signals, allowing or disallowing the buildup of tonic levels when dopamine neurons are active. Chronic DAT impairment can also cause compensation in the dopamine system, altering the rate of synthesis of new dopamine (Jones et al., 1998). To investigate DAT function in mice chronically treated with CORT, we assayed dopamine dynamics in an *ex vivo* slice preparation. We injected a virus encoding the fluorescent dopamine sensor, dLight1.3b (AAV9-CAG-dLight1.3b), into the NAcc and DMS, and implanted Placebo or CORT pellets during the same surgery. Four weeks later, we prepared striatal tissue sections and electrically evoked dopamine release while imaging dLight1.3b fluorescence (Figure 2-7A; Figure 2-8). To mimic tonic and phasic dopamine neuron firing, we used a single stimulation pulse or a burst of 5 pulses at 20 Hz, respectively. We quantified the decay of evoked dLight1.3b transients by calculating a ‘tau-off’ value and used it as a measure of the speed of extracellular dopamine clearance (Patriarchi et al., 2018; Salinas et al., 2022). CORT treatment did not significantly increase tau-off in male (Figure 2-7B,L) or female (Figure 2-7G,Q) mice in response to either one or five pulses at baseline in the DMS or NAcc (Figure 2-9). However, the lack of change could be due to compensation for chronic DAT impairment. To elucidate how DAT activity was contributing to tau-off, we washed a DAT inhibitor, GBR12909 (1μM, ‘DATi’), onto the slice. We also tested the contribution of another monoamine transporter, Organic Cation Transporter 3 (OCT3) (Graf et al., 2013), to

dopamine clearance by washing on an OCT3 inhibitor, normetanephrine (50μM; 'OCTi'). OCT3 is a low-affinity, high-capacity non-specific monoamine transporter (Gasser, 2019). Although OCT3 does not regulate synaptic dopamine levels as effectively as DAT, CORT binds OCT3 directly and inhibits reuptake, making it important to examine in our studies (Gasser & Lowry, 2018; Graf et al., 2013; Holleran et al., 2020; McReynolds et al., 2017). In the NAcc, we did not observe any significant effect of CORT treatment on tau-off after DAT and OCT3 inhibition in either sex (Figure 2-9). Using one stimulation pulse in the DMS, we did not observe significant differences in tau-off between Placebo- and CORT-treated mice after DAT and OCT3 inhibition (Figure 2-7D-F,I-K), although there was a trending effect of CORT treatment in male mice (Figure 2-7D, Two-way ANOVA, $F_{(1,16)}$=3.636, p=0.07). In response to five pulses in the DMS, DAT inhibition slowed dopamine clearance in Placebo-treated males, but had no effect in CORT-treated males, indicating DAT function is impaired in the DMS of CORT-treated males (Figure 2-7N-P; Two-way ANOVA, significant effect of treatment, $F_{(1,14)}$=8.566, p<0.05). In females, CORT treatment did not impair DAT or OCT3 function in the DMS (Figure 2-7S-U). CORT treatment did not affect the amplitude of dLight1.3b fluorescence elicited by one or five stimulation pulses in the DMS of either sex (Figure 2-10).

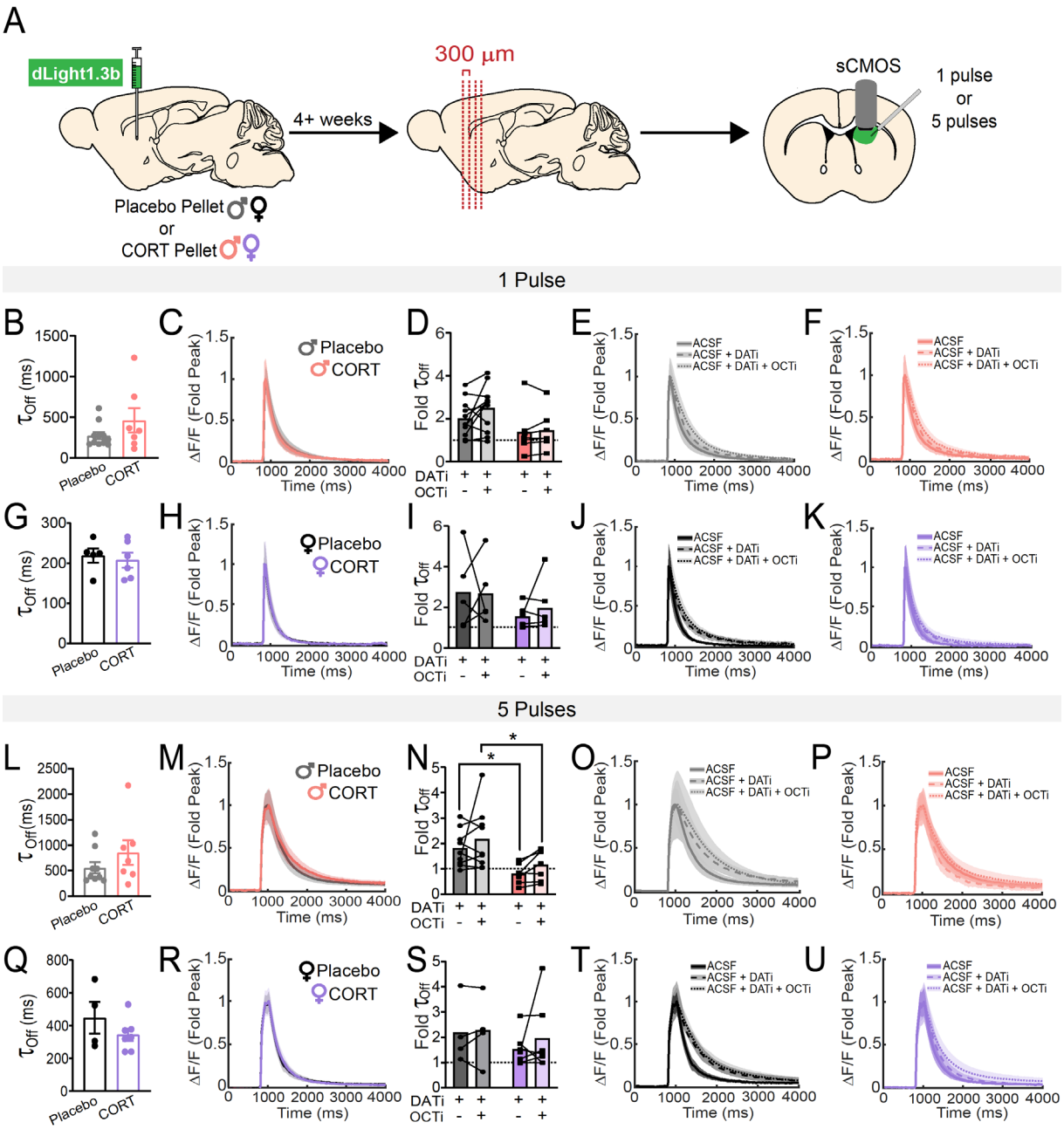

Figure 2-7. Chronic corticosterone treatment impairs *ex vivo* dopamine transporter (DAT) function in the dorsomedial striatum (DMS) of male mice.
A) Experimental timeline for viral injection, pellet implantation, and slice imaging experiments.
B) dLight1.3b fluorescence tau-off values after a single electrical stimulation of the DMS in acute tissue slices from male mice.
C) Average dLight1.3b fluorescence traces, normalized to the peak of dLight1.3b fluorescence. after a single electrical stimulation of the DMS in acute tissue slices from male mice.

D) Fold change of tau-off values of dLight1.3b fluorescence in the presence of inhibitors for the dopamine transporter (DATi) and organic cation transporter 3 (OCTi), normalized to tau-off values of dLight1.3b fluorescence in the absence of any transporter inhibitors, after a single electrical stimulation of the DMS in acute tissue slices from male mice. Two-Way ANOVA, trending effect of treatment $p=0.07$.
E, F) Average dLight1.3b fluorescence traces, normalized to the peak of dLight1.3b fluorescence, after a single electrical stimulation of the DMS in acute slices from Placebo- (E) and CORT- (F) treated male mice, in the presence and absence of DATi and OCTi.
G) dLight1.3b fluorescence tau-off values after a single electrical stimulation of the DMS in acute tissue slices from female mice.
H) Average dLight1.3b fluorescence traces, normalized to the peak of dLight1.3b fluorescence, after a single electrical stimulation of the DMS in acute tissue slices from female mice.
I) Fold change of tau-off values of dLight1.3b fluorescence in the presence of DATi and OCTi, normalized to tau-off values of dLight1.3b fluorescence in the absence of any transporter inhibitors, after a single electrical stimulation of the DMS in acute tissue slices from female mice.
J, K) Average dLight1.3b fluorescence traces, normalized to the peak of dLight1.3b fluorescence, after a single electrical stimulation of the DMS in acute tissue slices from Placebo- (J) and CORT- (K) treated female mice, in the presence and absence of DATi and OCTi.
L) dLight1.3b fluorescence tau-off values after a 20 Hz, 5 pulse electrical stimulation of the DMS in acute tissue slices from male mice.
M) Average dLight1.3b fluorescence traces, normalized to the peak of dLight1.3b fluorescence, after a 20 Hz, 5 pulse electrical stimulation of the DMS in acute tissue slices from male mice.
N) Fold change of tau-off values of dLight1.3b fluorescence in the presence of DATi and OCTi, normalized to tau-off values of dLight1.3b fluorescence in the absence of any transporter inhibitors, after a 20 Hz, 5 pulse electrical stimulation of the DMS in acute tissue slices from male mice. Two-Way ANOVA, main effect of treatment $p<0.05$, multiple comparisons $*p<0.05$.
O, P) Average dLight1.3b fluorescence traces, normalized to the peak of dLight1.3b fluorescence, after a 20 Hz, 5 pulse electrical stimulation of the DMS in acute tissue slices from Placebo- (O) and CORT- (P) treated male mice, in the presence and absence of DATi and OCTi.
Q) dLight1.3b fluorescence tau-off values after a 20 Hz, 5 pulse electrical stimulation of the DMS in acute tissue slices from female mice.
R) Average dLight1.3b fluorescence traces, normalized to the peak of dLight1.3b fluorescence, after a 20 Hz, 5 pulse electrical stimulation of the DMS in acute tissue slices from female mice.
S) Fold change of tau-off values of dLight1.3b fluorescence in the presence of DATi and OCTi, normalized to tau-off values of dLight1.3b fluorescence in the absence of any transporter inhibitors, after a 20 Hz, 5 pulse electrical stimulation of the DMS in acute tissue slices from female mice.

T, U) Average dLight1.3b fluorescence traces, normalized to the peak of dLight1.3b fluorescence, after a 20 Hz, 5 pulse electrical stimulation of the DMS in acute tissue slices from Placebo- (T) and CORT- (U) treated female mice, in the presence and absence of DATi and OCTi.
Points represent the average of 2-3 sweeps from a single individual. Data presented as mean ± SEM.

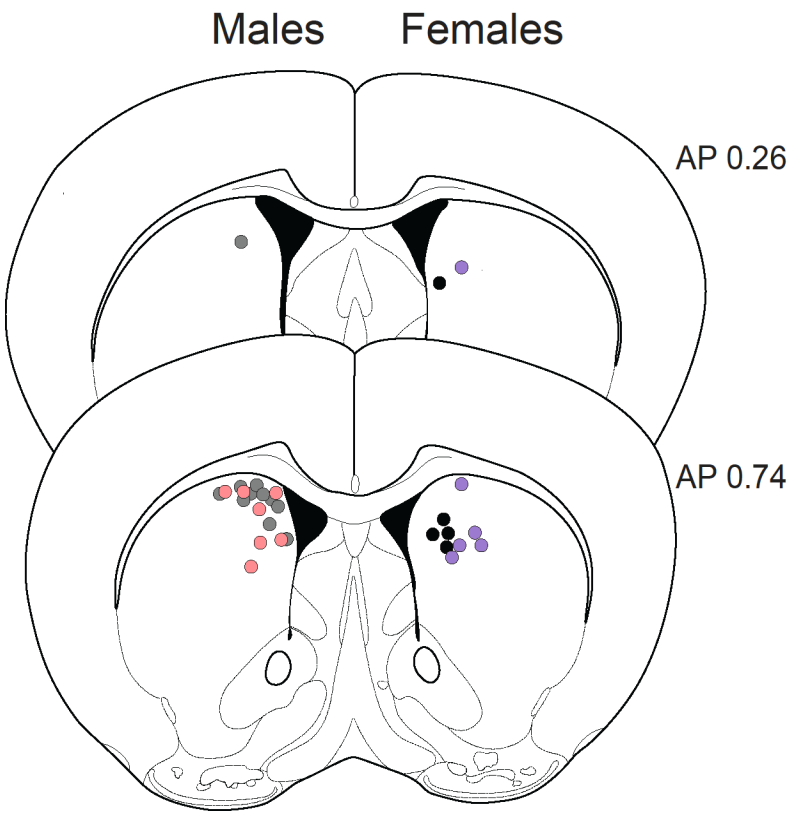

Figure 2-8. *Ex vivo* dLight1.3b recording sites in the dorsomedial striatum (DMS). Recording sites from Placebo-treated males (gray), CORT-treated males (pink), Placebo-treated females (black), and CORT-treated females (purple). AP coordinate indicates slice position relative to bregma.

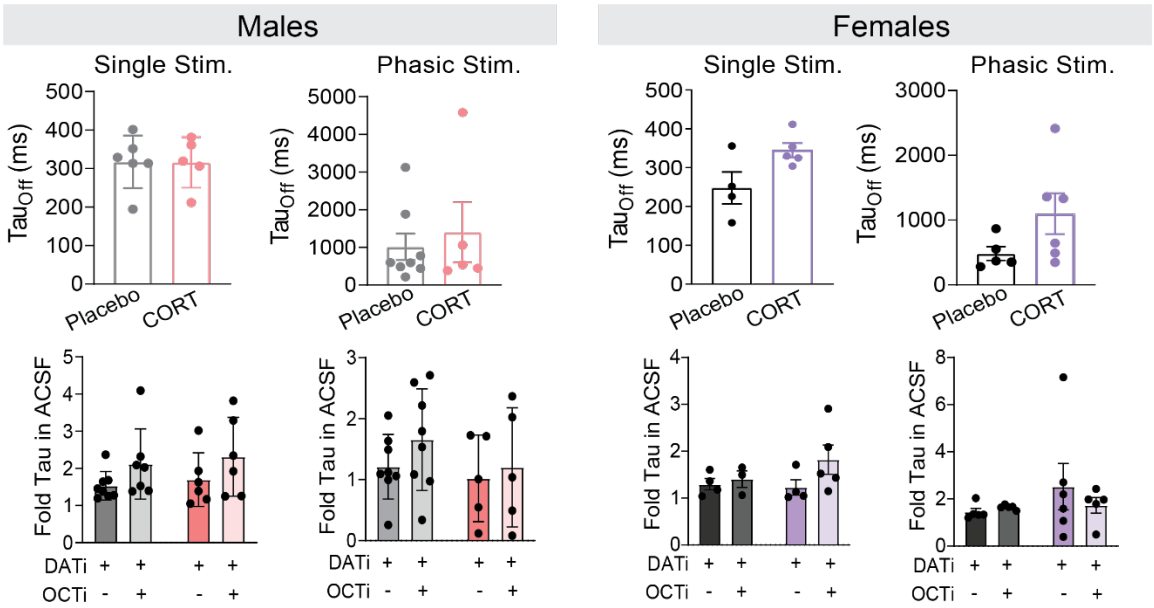

Figure 2-9. Chronic corticosterone treatment does not affect *ex vivo* dopamine transporter (DAT) function in the nucleus accumbens core (NAcc) of either male or female mice.
Top: tau-off of dLight1.3b signal in the NAcc after a single or phasic stimulation, in males (left) and females (right). Bottom: fold change of tau-off values of dLight1.3b fluorescence in the presence of DATi and OCTi, normalized to tau-off values of dLight1.3b fluorescence in the absence of any transporter inhibitors, after a single or phasic stimulation in the NAcc of males (left) and females (right).

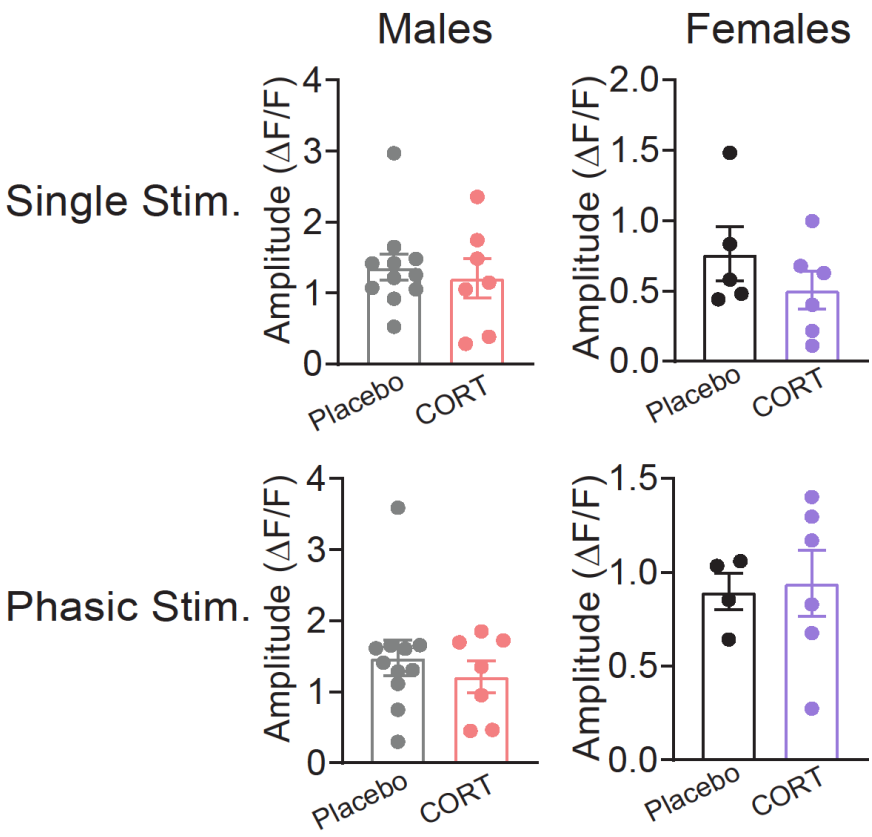

Figure 2-10. Chronic corticosterone treatment does not affect amplitude of electrically-evoked dLight1.3b fluorescence in the dorsomedial striatum (DMS) of male or female mice.
Amplitudes of electrically-evoked dLight1.3b events, normalized to baseline fluorescence, in the DMS of male (left) and female (right) mice, in response to a single electrical stimulation (top) or phasic electrical stimulation (bottom).

2.2.6 Chronic CORT treatment impairs *in vivo* dopamine transporter (DAT) function in the DMS of male mice

To verify our *ex vivo* results, we designed an experiment to examine DAT function *in vivo* using fiber photometry. We injected a virus encoding dLight1.3b (AAV9-CAG-dLight1.3b) into the DMS of male and female mice and implanted a fiber optic in DMS for *in vivo* recording during behavior (Figure 2-12). Four weeks later, mice were placed in an open field to collect baseline locomotor and dLight1.3b fluorescence data (Figure 2-11A). After ten minutes of baseline data collection, mice were injected with the DAT inhibitor, GBR12909 (20 mg/kg, i.p.), and returned to the open field for forty minutes. In mice with high DAT activity, injection of a DAT inhibitor should increase locomotion, with a concordant increase in extracellular dopamine in DMS (measured as a change in the dLight1.3b fluorescence area-under-the-curve (AUC)). After DAT inhibition, locomotion of CORT-treated male mice was blunted relative to Placebo-treated male mice (Figure 2-11D; Two-way ANOVA, significant effect of treatment [$F_{(1,17)}$=6.776, p<0.05], trending effect of time [$F_{(2.344,39.86)}$=2.835, p=0.06]). Furthermore, CORT-treated male mice exhibited significant blunting of dLight1.3b AUC after DAT inhibition compared to Placebo-treated male mice (Figure 2-11E; Two-way ANOVA, significant effects of treatment [$F_{(1,9)}$=5.418, p<0.05], time [$F_{(2.439,21.95)}$=7.083, p<0.01], and the interaction between treatment and time [$F_{(49,441)}$=2.979, p<0.0001]).

DAT inhibition could increase dLight1.3b AUC by increasing the decay constant of dLight1.3b transients, leading to a larger AUC per transient. Longer dopamine clearance times (indicated by higher decay constants) could then slowly increase baseline dLight1.3b fluorescence, reflecting a slow buildup of tonic dopamine. Such an increase in baseline fluorescence would also contribute to an increase in dLight1.3b

AUC following DAT inhibition. To differentiate between these two effects, we analyzed the decay constants of dLight1.3b transients recorded during open field behavior before and after DAT inhibition. We found that DAT inhibition increased both the decay time constants of *in vivo* dLight1.3b transients and led to a buildup of baseline dLight1.3b fluorescence (Figure 2-13). We observed non-significant trends in which both of these effects were greater in Placebo-treated males than CORT-treated males (Figure 2-13; Two-way ANOVA, effect of treatment p=0.06 for decay constants, p=0.08 for baseline fluorescence). Thus, we concluded that the significant difference in DMS dLight1.3b AUC between Placebo- and CORT-treated males after DAT inhibition (Figure 2-11E) is the result of a *combined* effect on the decay of individual dLight1.3b transients and an increase in the baseline dLight1.3b fluorescence due to integration of slowly decaying transients.

In female mice, we did not observe differences between treatment conditions (Figure 2-11F-H). We observed a significant effect of time on locomotion (Figure 2-11G; Two-way ANOVA, $F_{(2.062,24.75)}$=9.222, p<0.001) and on dLight1.3b AUC (Figure 2-11H; Two-way ANOVA, $F_{(2.642,23.78)}$=7.124, p<0.01) after DAT inhibition in both Placebo- and CORT-treated groups. We concluded that CORT treatment impairs DAT function in the DMS of male, but not female, mice. However, from these data it was unclear *how* CORT treatment impaired DAT function.

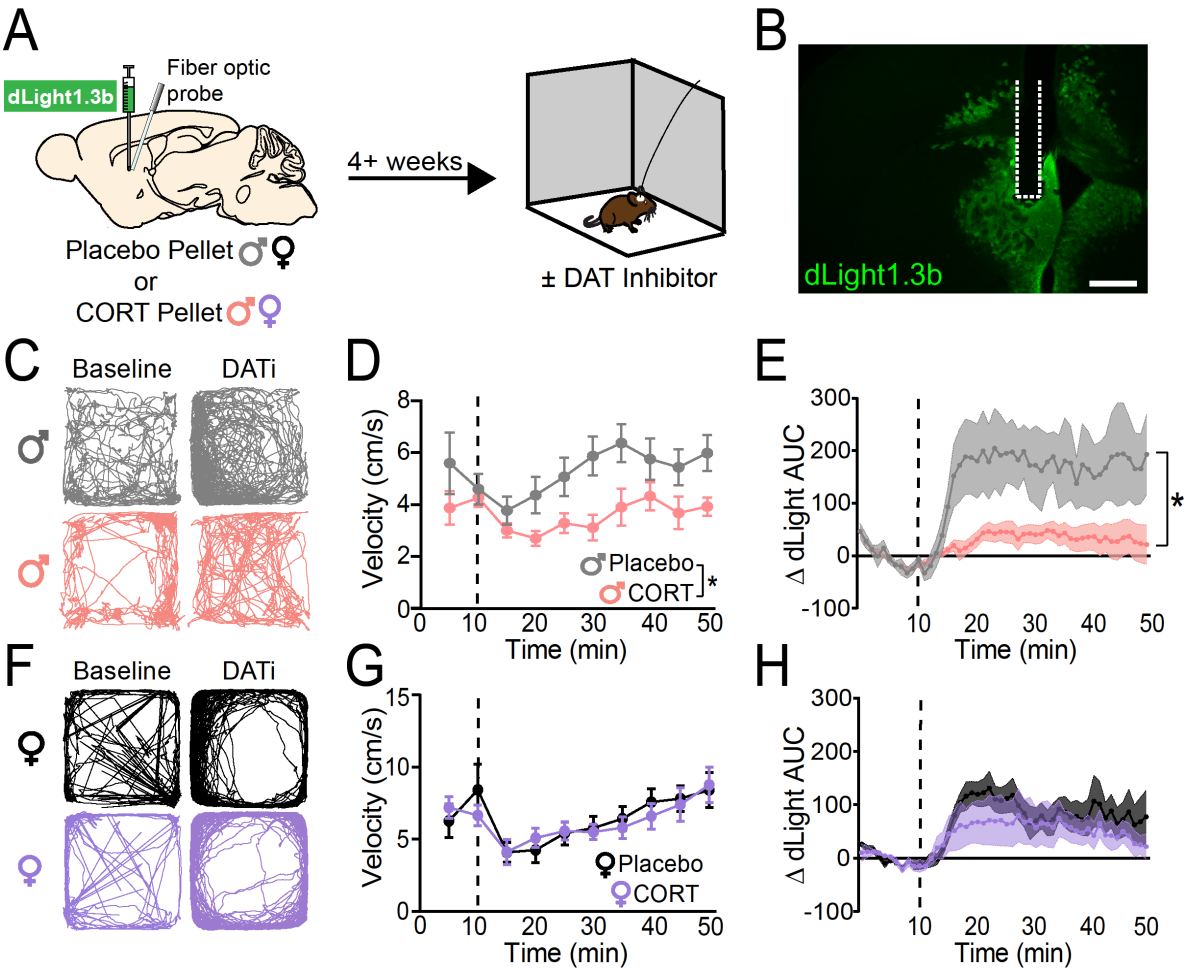

Figure 2-11. Chronic corticosterone treatment impairs *in vivo* dopamine transporter (DAT) function in the dorsomedial striatum (DMS) of male mice.
A) Experimental timeline for viral injection, fiber optic implant, pellet implantation, and open field behavior.
B) Representative image of dLight1.3b viral spread and fiber optic implantation site (outlined in dashed white line). Scale bar equals 500 micrometers.
C) Representative activity traces of male mice (Placebo, top; CORT, bottom) during the ten-minute baseline period ('Baseline') and the last ten minutes of recorded activity after injection of the DAT inhibitor GBR12909 (20 mg/kg, i.p., 'DATi').
D) Velocity of Placebo- (N=10) and CORT- (N=9) treated male mice, in averaged five-minute bins, before and after injection with the DAT inhibitor, GBR12909 (20 mg/kg, i.p.; injection time indicated by vertical dashed line). Two-Way ANOVA, main effect of treatment *p<0.05, trending effect of time p=0.06.
E) Change in dLight1.3b area-under-the-curve (AUC) relative to the minute average of the ten-minute baseline period prior to injection with the DAT inhibitor, GBR12909 (20 mg/kg, i.p.; injection time indicated by vertical dashed line) in male mice. Two-Way ANOVA, main effect of treatment *p<0.05, main effect of time p<0.01, main effect of treatment x time interaction p<0.0001. Placebo N=5, CORT N=6.

F) Representative activity traces of female mice (Placebo, top; CORT, bottom) during the ten-minute baseline period (‘Baseline’) and the last ten minutes of recorded activity after injection of the DAT inhibitor GBR12909 (20 mg/kg, i.p., ‘DATi’).
G) Velocity of Placebo- (N=6) and CORT- (N=8) treated female mice, in averaged five-minute bins, before and after injection with the DAT inhibitor, GBR12909 (20 mg/kg, i.p.; injection time indicated by vertical dashed line). Two-Way ANOVA, main effect of time $p<0.001$.
H) Change in dLight1.3b AUC relative to the average of the ten-minute baseline period prior to injection with the DAT inhibitor, GBR12909 (20 mg/kg, i.p.; injection time indicated by vertical dashed line) in female mice. Two-Way ANOVA, main effect of time $p<0.01$. Placebo N=5, CORT N=6.
Data presented as mean ± SEM.

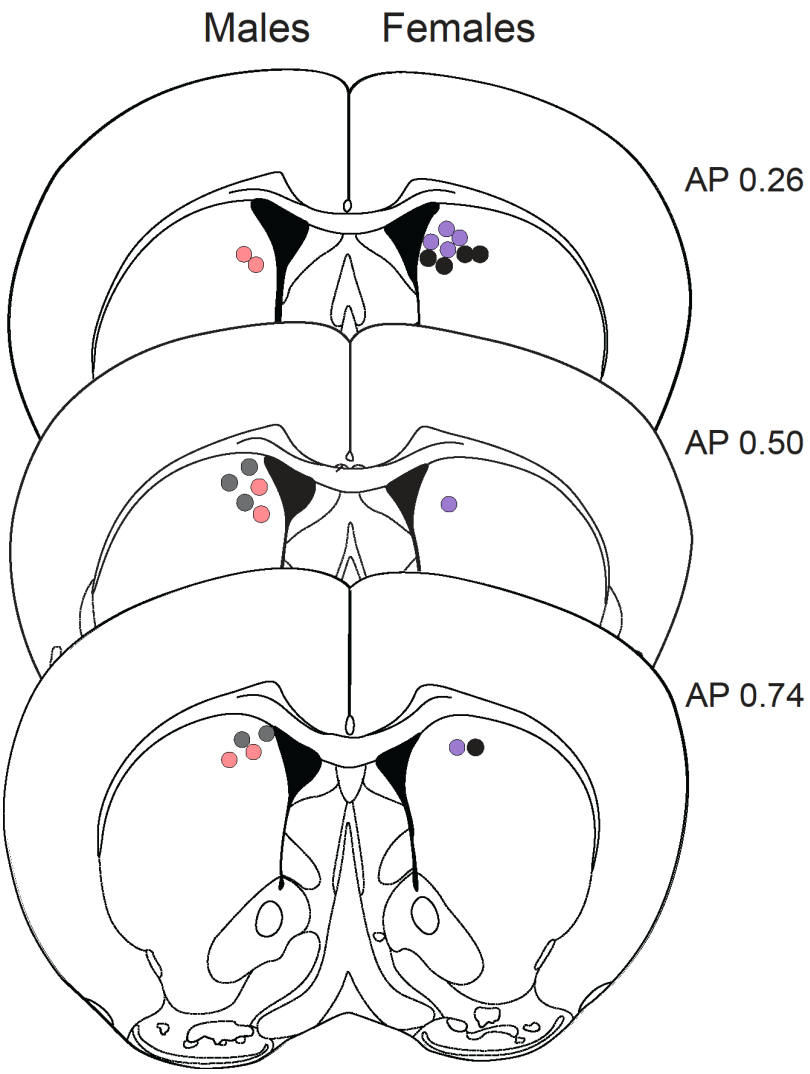

Figure 2-12. Fiber optic probe implant sites.
Terminal sites of fiber optic probes in Placebo-treated males (gray), CORT-treated males (pink), Placebo-treated females (black), and CORT-treated females (purple). Each point represents one individual. AP coordinate indicates slice position relative to bregma.

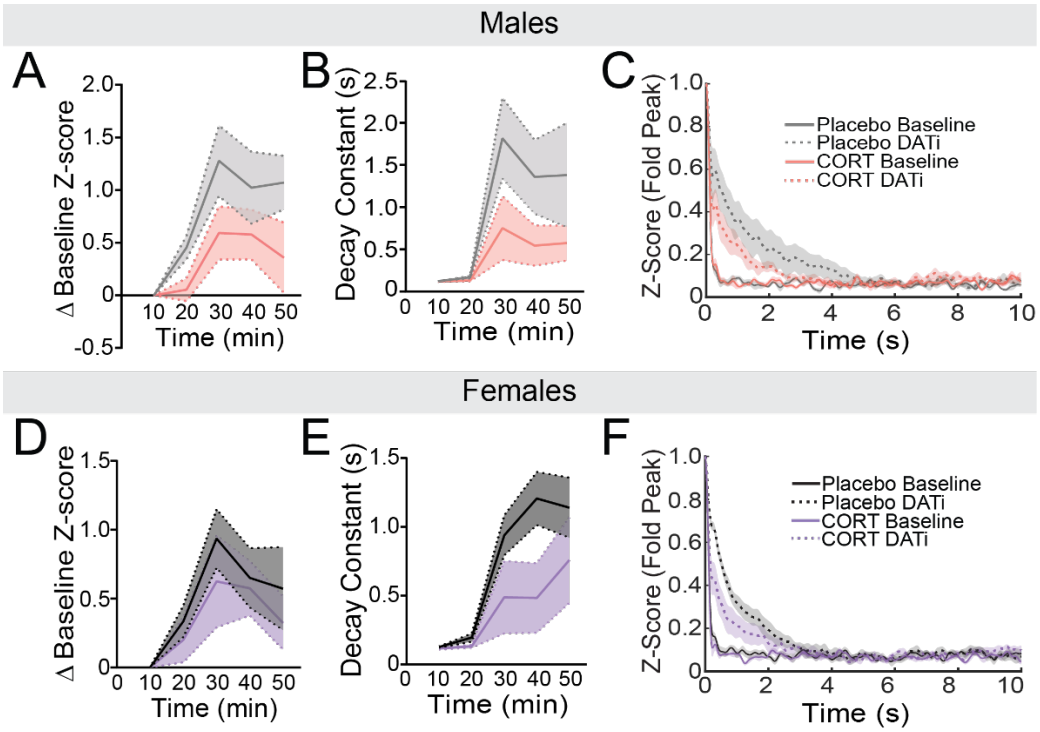

Figure 2-13. Chronic corticosterone treatment tends to blunt increased baseline fluorescence and decay constant of dLight1.3b after DAT inhibition in the dorsomedial striatum (DMS) of male mice.

A) Change in baseline z-score of dLight1.3b fluorescence in the DMS after DAT inhibition in males (Two-Way ANOVA, main effect of time $p<0.01$, trending effect of treatment $p=0.08$). Placebo N=5, CORT N=6.

B) Decay constant of dLight1.3b in the DMS before and after DAT inhibition in males (Two-Way ANOVA, main effect of time $p<0.001$, trending effect of treatment $p=0.06$).

C) Average dLight1.3b decay during the 10 minutes before DAT inhibition ('Baseline') and during the last 10 minutes of recorded dLight1.3b fluorescence after DAT inhibition ('DATi') in the DMS of male mice.

D) Change in baseline z-score of dLight1.3b fluorescence in the DMS after DAT inhibition in females (Two-Way ANOVA, main effect of time $p<0.01$). Placebo N=5, CORT N=6.

E) Decay constant of dLight1.3b in the DMS before and after DAT inhibition in females (Two-Way ANOVA, main effect of time $p<0.001$).

F) Average dLight1.3b decay during the 10 minutes before DAT inhibition ('Baseline') and during the last 10 minutes of recorded dLight1.3b fluorescence after DAT inhibition ('DATi') in the DMS of female mice.

Data presented as mean ± SEM.

2.2.7 Chronic CORT treatment decreases phosphorylation of DAT at threonine-53 in the DMS of males

To assess how CORT treatment impaired DAT function, we examined DAT expression and post-translational modifications of DAT, which regulate reuptake activity (Foster & Vaughan, 2017; Gowrishankar et al., 2018; Jones et al., 1998). Specifically, we examined phosphorylation at threonine-53, a known regulatory site (Alonso et al., 2021; Calipari et al., 2017; Gowrishankar et al., 2018; Stewart et al., 2021). We collected DMS tissue punches from Placebo- and CORT-treated male and female mice and fractionated the tissue homogenate to isolate membrane-bound proteins. We then performed western blots probing for DAT and Thr53 phospho-DAT (pDAT). We found that CORT treatment had no effect on total levels of membrane-bound DAT in males or females (Figure 2-14C,D). However, CORT treatment significantly decreased pDAT in male mice (Unpaired two-tailed t-test, $p<0.05$), but not female mice (Figure 2-14E,F). These results suggest that CORT treatment impairs DMS DAT function in male mice by decreasing phosphorylation of DAT at threonine-53, and further supports the conclusion that DMS DAT is unaffected by CORT treatment in female mice.

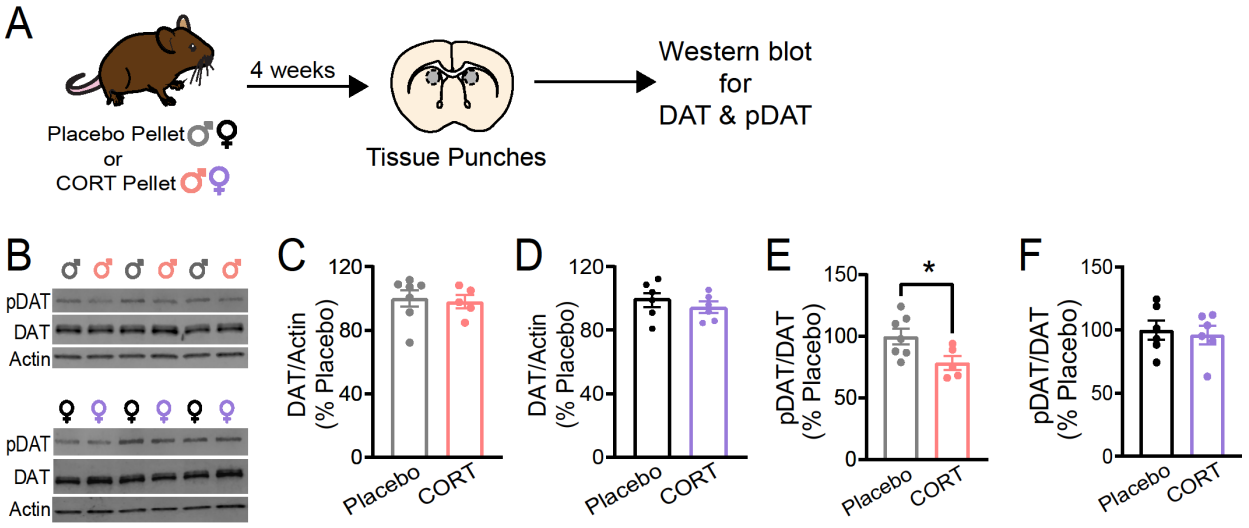

Figure 2-14. Chronic corticosterone treatment decreases phosphorylation of the dopamine transporter (DAT) at threonine-53 in the dorsomedial striatum (DMS) of male mice.

A) Experimental timeline for pellet implantation, tissue punches, and western blot experiments.

B) Representative western blots for phosphorylated DAT at threonine-53 ('pDAT'), DAT, and beta-actin ('Actin') from DMS tissue samples of male mice (top) and female mice (bottom).

C) Membrane-bound DAT expression, normalized to Actin and plotted as a percent of Placebo expression, from DMS tissue samples of male mice.

D) Membrane-bound DAT expression, normalized to Actin and plotted as a percent of Placebo expression, from DMS tissue samples of female mice.

E) pDAT expression, normalized to DAT and plotted as a percent of Placebo expression, from DMS tissue samples of male mice. Unpaired two-tailed t-test *$p<0.05$.

F) pDAT expression, normalized to DAT and plotted as a percent of Placebo expression, from DMS tissue samples of female mice.

Each point represents a single individual. Data presented as mean ± SEM.

2.3 Discussion

Previous studies in rodents have shown that chronic dysregulation of circulating CORT – a condition that also occurs in subsets of human MDD patients – impairs reward processing, but there was little mechanistic insight into *how* CORT dysregulation impairs reward processing. Further, preclinical literature previously reported effects of CORT on operant responding for rewards only in male rodents, yet humans with MDD are majority female. Here, we specifically set out to study both male and female mice and to identify mechanisms by which chronic CORT dysregulation might impact dopaminergic transmission, which is known to underlie operant responding for rewards. We found that chronic CORT treatment impairs motivation to attain rewards in operant paradigms in both male and female mice (Figure 2-3F,G), but by sex-divergent mechanisms. In females, CORT treatment decreases tissue dopamine content in the dorsomedial striatum (DMS; Figure 2-6C). In males, CORT treatment impairs dopamine transporter (DAT) function in DMS (Figure 2-7,11,14). Despite differing mechanisms, both males and females experienced changes in dopaminergic transmission specifically in DMS, tying dopaminergic function in this striatal subregion to the observed deficits in motivation. This discovery is consistent with studies showing that DMS dopamine governs goal-directed operant responding for rewards and tying tonic dopamine to motivation (Grospe et al., 2018; Lex & Hauber, 2010a; Niv et al., 2007; Seiler et al., 2022; Sokolowski et al., 1998). Critically, chronic CORT treatment did not affect dopaminergic transmission in the NAcc, consistent with reports that adrenalectomy and

CORT replacement do not affect NAcc extracellular dopamine levels (Barrot et al., 2000).

Our discovery of a latent sex difference in the mechanism by which DMS dopamine transmission is affected by CORT treatment adds to a growing body of literature indicating that males and females can display different underlying mechanisms to achieve similar functional or behavioral outcomes (De Vries, 2004; Oberlander & Woolley, 2017). Therefore, it is important not to assume that a lack of observed sex differences at a high level of analysis precludes sex differences in mechanism. Indeed, we must continue to probe for sex differences at the molecular level if we are to appropriately translate preclinical discoveries into medicines that act at the molecular level.

Based on our results, we speculate that reward processing deficits observed in MDD patients with dysregulated CORT may similarly be due to impaired DMS dopamine transmission, caused by distinct mechanisms in males and females. Our speculation is consistent with recent studies showing that individuals with MDD exhibit decreased DAT expression and tonic dopamine within the dorsal striatum (Hamilton et al., 2018; Pizzagalli et al., 2019). However, to address the aspect of our hypothesis dealing with sex differences, human data must be analyzed by sex. If the sex-divergent mechanisms by which DMS dopamine transmission is impaired in mice hold true for humans, this would suggest that medications for MDD should be tailored by sex. Further, it may be valuable to distinguish MDD patient populations by phenotyping for CORT dysregulation. Notably, previous failed attempts to translate HPA axis-based therapies from rodent models to humans failed to account for sex differences (Kokras et

al., 2019). They also did not include analyses of CORT status or other aspects of HPA axis function, which could help segregate patient populations most amenable to an HPA axis-focused therapeutic approach.

One caveat of our studies is that the chronic CORT treatment we applied significantly increased total plasma CORT in males only. This finding suggests that CORT elevation drives the behavioral and neurobiological effects observed in males, but it is less clear whether CORT elevation is achieved in females. Free CORT, which crosses the blood-brain barrier, may be increased in females due to lower CBG levels, but this hypothesis is not fully confirmed. The sex difference in total plasma CORT levels in response to CORT pellet implantation is consistent with an extensive literature demonstrating sex differences in feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis, which may be fundamental to consider in a variety of stress studies, not only ours (Bangasser, 2013; Kokras et al., 2019). Further, at least one clinical report associated lower CBG levels with MDD in female patients only, which our CORT treatment model intriguingly recapitulates (Maes et al., 1996). Follow-up studies are necessary to understand how CORT dosage affects total and free plasma CORT levels in males and females. The dose-response effects of CORT on behavior are known to follow an ‘inverted U’ wherein levels of CORT that are either too high or too low are problematic (McEwen & Akil, 2020). While we focused these studies on understanding the effects of elevated CORT, on the downswing of the inverted U, clinical studies have also found that some individuals with MDD exhibit insufficient CORT levels (Bremmer et al., 2007). Therefore, understanding the dose-response relationship between CORT and dopaminergic system function in males and females is important for understanding

the full spectrum of MDD etiology. By testing the effects of a range of CORT doses in males and females, we will better understand potential sex differences (or similarities) in CORT's effects on dopaminergic system function and behavior.

Our findings inspire two related questions regarding CORT's effects on females: 1) how does chronic CORT treatment decrease DMS dopamine content in females, and 2) why are females resistant to changes in DAT function? CORT treatment's lack of effect on DMS DAT function in females is likely not due to an interaction with the estrous cycle, as the estrous cycle modulates dopamine reuptake in the ventral, but not dorsal, striatum (Calipari et al., 2017; Walker et al., 1999). Female resistance to CORT-induced impairments in DAT function could be due to their faster metabolism of CORT (Woodward et al., 1991), which could change how chronic CORT treatment impacts gene expression changes in dopamine neurons (among other cell types) through pharmacodynamic differences in glucocorticoid receptor activation. Future studies are needed to address glucocorticoid receptor occupancy and downstream signaling changes that may underlie the effects of CORT on DAT function in males, and dopamine content in females. Sex differences in feedback inhibition of the HPA axis could also lead to a variety of complex effects. For example, chronic CORT treatment could lead to sex differences in expression and secretion of corticotropin releasing hormone (CRH), a neuropeptide that mediates HPA axis activity and has been shown to modulate dopaminergic transmission and decrease operant responding for rewards (Hupalo et al., 2019; Joëls & Baram, 2009; Kelly & Fudge, 2018; Lemos et al., 2012; Wanat et al., 2013). This possibility is supported by previous observations that depressive-like behaviors in females (e.g., immobility in the forced swim test) are less

directly dependent on CORT levels than the same behaviors in males (Kokras et al., 2021). Future studies are necessary to determine if, and how, levels of CRH or other HPA axis-related signaling molecules are affected by CORT pellet implantation in both sexes.

In sum, our studies suggest that impairment of DMS dopaminergic transmission is a key mechanism underlying stress-induced deficits caused by CORT dysregulation. Our studies lay the groundwork for further dissecting the relationship between CORT signaling and dopaminergic circuit function. It will also be interesting to investigate how chronic CORT treatment affects downstream DMS circuit function and corticostriatal plasticity to sustain the effects of chronic CORT treatment (Surmeier et al., 2007). The more we elucidate these pathways, identifying common mechanisms as well as sex differences, the more we will progress towards new therapeutic approaches for stress-related psychiatric disorders such as MDD.

Chapter 3: Methods

3.1 Animals & housing

Adult (10+ weeks) male and female C57BL/6J mice were group-housed by sex and treatment (2-5 mice per cage) and given ad libitum access to food and water, unless otherwise specified. Mice were housed on a 14:10 hour light/dark cycle, in a temperature- and humidity-controlled environment. All experimental procedures were approved by the Northwestern University Animal Care and Use Committee. All experiments were completed at zeitgeber time 4-6 (4-6 hours after lights-on).

3.2 Subcutaneous pellet implant

At 10+ weeks of age, mice were anesthetized with isoflurane and given analgesics to minimize pain after surgery. Hair was removed from the lateral portion of the neck using Nair, and the skin was swabbed with alcohol and iodine. A small incision was made, and Placebo or Corticosterone (35 mg; 60-day release; Innovative Research of America) slow-release pellets were implanted subcutaneously in the space between the shoulder and neck. The incision was closed with non-absorbable sutures. For *ex vivo* slice imaging and *in vivo* photometry experiments, pellets were implanted during stereotaxic surgeries.

3.3 Tail blood sampling & enzyme-linked immunosorbent assays (ELISAs)

Mice were gently restrained, and 30 µL of blood was sampled from the lateral tail vein. Plasma was separated out of blood by centrifugation at 4°C. Plasma samples were stored at -80°C prior to corticosterone and corticosteroid binding globulin (CBG) quantification. Blood draws occurred between zeitgeber times 4-6 (4-6 hours after lights turned on). Plasma corticosterone was quantified using a Corticosterone ELISA kit from

Enzo Life Sciences (ADI-900-097), according to the manufacturer's instructions. Plasma CBG levels were quantified using a CBG ELISA kit from LifeSpan BioSciences, Inc. (LS-F11038), according to the manufacturer's instructions.

3.4 Estrus cycle tracking

To determine estrus cycle stage, cell samples were collected from the vaginas of female mice using vaginal lavage. Briefly, 100 microliters of ddH20 was aspirated into a 200 microliter pipette tip using a bulb. Mice were gently grasped by the tail and approximately 50 microliters of ddH20 was dispensed into the mouse's vaginal opening (without touching the opening to avoid inducing pseudopregnancy), then aspirated back into the pipette tip; this process was repeated 2-3 times. After the sample was collected, it was dispensed onto a clean glass microscope slide and covered with a glass coverslip. The sample was immediately viewed under a light microscope and estrus cycle stage was determined by cytology (Byers et al., 2012; McLean et al., 2012).

3.5 Stereotaxic surgeries

At 10+ weeks of age, mice were anesthetized with isoflurane and given analgesics to minimize pain. Hair on the skin of the top of the head was removed using Nair, then swabbed with alcohol and iodine. A single incision was made down the midline of the skull, then a hole was drilled above the injection site for the dorsomedial striatum (DMS; +0.8 A/P, 1.5 M/L, -2.8 D/V, relative to bregma) and nucleus accumbens core (NAcc: +1.6 A/P, 0.8 M/L, -4.1 D/V). 500 nL of AAV9-CAG-dLight1.3b ($7x10^{11}$ VG/mL) (Patriarchi et al., 2018) was injected into the DMS and NAcc at a rate of 100 nL/min using a Hamilton syringe. The needle remained in place for five minutes after injection before being slowly retracted. For fiber photometry experiments, a fiber optic (Doric, 400

μm core, 0.66 NA) was implanted over the DMS injection site. The hemispheres of injection sites were counterbalanced across treatment groups and sexes.

3.6 Operant conditioning

Mice were food restricted to 85% of their ad libitum weight and monitored for maintenance of this weight throughout operant training. Operant sessions lasted 60 minutes, or until mice received the maximum number of rewards available (50 rewards). Mice were initially trained to acquire sucrose rewards from the reward port of an operant box (Med Associates) in the absence of any contingency. Mice then advanced to a fixed-ratio (FR) schedule of training during which they had to nosepoke once for one sucrose pellet (FR-1). After earning at least 30 rewards for two consecutive days (criterion for advancement), mice were advanced to FR-3 training, in which they had to nosepoke three times for one sucrose pellet. After reaching criterion for advancement, mice were advanced to FR-5 training.

3.7 Sample preparation for high performance liquid chromatography-electrochemical detection and western blot

After 4+ weeks after pellet implantation, mice were sacrificed by cervical dislocation and brains were flash-frozen in liquid nitrogen. Using a brain matrix, brains were sectioned into 1mm-thick sections and tissue punches of the nucleus accumbens core (NAcc) and dorsomedial striatum (DMS) were removed from tissue sections. Tissue punches were stored at -80ºC. For HPLC-ECD, samples were sent to the Neurochemistry Core at Vanderbilt University for quantification of dopamine and its metabolites. For western blot, tissue punches were membrane fractionated using a Mem-PER Plus Protein Extraction kit from ThermoFisher, according to the manufacturer’s instructions. A BCA

Assay (ThermoFisher) was used to quantify protein content of samples. Samples were combined with Laemmli buffer and heated at 95ºC for five minutes.

3.8 High performance liquid chromatography and electrochemical detection (HPLC-ECD) of dopamine

Biogenic amines were measured in the Vanderbilt University Neurochemistry Core.

Tissue Extraction: Tissues were kept frozen at -80°C and were held on dry ice prior to the addition of homogenization buffer in order to prevent degradation of biogenic amines. Tissues were homogenized, using a handheld sonic tissue dismembrator, in 100-750 µl of 0.1M TCA containing 0.01M sodium acetate, 0.1mM EDTA, and 10.5 % methanol (pH 3.8). Ten microliters of homogenate was used for the protein assay. The samples were then spun in a microcentrifuge at 10,000 g for 20 minutes. Supernatant was removed for HPLC-ECD analysis. HPLC was performed using a Kinetix 2.6um C18 column (4.6 x 100 mm, Phenomenex, Torrance, CA USA). The same buffer used for tissue homogenization is used as the HPLC mobile phase.

Protein assay: Protein concentration in cell pellets was determined by BCA Protein Assay Kit (Thermo Scientific). Ten microliter tissue homogenate was distributed into 96-well plate and 200 µl of mixed BCA reagent (25 ml of Protein Reagent A is mixed with 500 µl of Protein Reagent B) was added. The plate was incubated at room temperature for two hours for the color development. A BSA standard curve was run at the same time. Absorbance was measured by a plate reader (POLARstar Omega), purchased from BMG LABTECH Company.

3.9 *Ex vivo* dLight1.3b imaging

At least 4 weeks after pellet implantation and stereotaxic surgery, mice were anesthetized with Euthasol (Virbac, 1 mg/kg) and transcardially perfused with ice-cold N-methyl-D-glucamine (NMDG) (Ting et al., 2014) artificial cerebrospinal fluid (ACSF). Coronal tissue sections (300 µm thick) containing the DMS were cut using a vibratome (Leica VT1200) and transferred to NMDG ACSF at 33°C. Slices recovered in HEPES ACSF and holding ACSF, as described previously (Ambrosi & Lerner, 2022; Ting et al., 2014). All solutions were saturated with carbogen (95% Oxygen, 5% Carbon Dioxide) and their pH and osmolarity were adjusted to 7.3-7.4 and 300±5 mOsm, respectively. Slices were transferred to a recording chamber in ACSF, held at 30-32°C. For recording, ACSF contained blockers for AMPARs (NBQX, 5µM), NMDARs (D-AP5, 50µM), nAChRs (DHβE, 1µM), $GABA_A$Rs (Picrotoxin, 50µM), and $GABA_B$Rs (CGP-54626, 2µM). Dopamine release was evoked using a bipolar stimulating electrode (FHC, Inc.) placed ~300 microns from the imaging site. All stimulations were 4V, with a pulse width of 0.5ms. After baseline recordings, the DAT inhibitor GBR-12909 (1µM) was applied to slices, followed by the OCT3 inhibitor Normetanephrine (50µM). dLight1.3b fluorescence was imaged using a scientific CMOS camera (Hamamatsu Orca-Flash 4.0LT), with a sampling rate of 33 Hz. dLight1.3b tau-off values were calculated using a custom MATLAB script.

3.10 *In vivo* dLight1.3b fiber photometry

Fiber photometry experiments occurred at least four weeks after pellet implantation and stereotaxic surgeries. Mice were attached to a fiber optic patch cord (Doric, 400 µm core, 0.66 NA) and gently placed in an open field (28 x 28 cm). After 10 minutes in the

open field, mice were injected with the DAT inhibitor, GBR-12909 (20 mg/kg), and returned to the open field for another 40 minutes. Fiber photometry data was collected throughout the entire time that mice were in the open field. GuPPy, an open-source Python-based photometry data analysis pipeline, was used to determine dLight1.3b transient timepoints (Sherathiya et al., 2021). Locomotor activity was recorded using Noldus Ethovision XT 16.

To calculate dLight1.3b area-under-the-curve (AUC), dLight1.3b fluorescence was normalized by fitting it with the isosbestic fluorescence curve, filtered with a 1 second median filter, downsampled to 1Hz, then dLight1.3b AUC was calculated in one-minute bins for the entire trace, and normalized to the average 1-minute AUC of the drug-free period by subtraction (Moya et al., 2022).

To calculate the baseline change in dLight1.3b fluorescence and the change in dLight1.3b decay after DAT inhibition *in vivo*, we normalized the dLight1.3b fluorescence by fitting it with the isosbestic fluorescence curve, then made peri-stimulus time histograms (PSTHs) of dLight1.3b transients in 10-minute intervals before and after injection with the DAT inhibitor, GBR12909. PSTHs included z-score data from 5 seconds prior to each dLight1.3b transient. We defined the ‘baseline z-score’ as the average z-score of first second of this 5 second pre-transient period. To calculate the decay constant of *in vivo* dLight1.3b transients, we normalized the average binned dLight1.3b decays to their peak z-score, then fit the decay of dLight1.3b transients with a double exponential. The timepoint in the normalized dLight1.3b decay where the z-score decreased to 36.8% of the fitted curve peak was defined as the decay constant.

3.11 Western blotting

An equal amount of protein from each sample was loaded in a Tris-Glycine gel (Invitrogen). Protein was transferred to a PVDF membrane and blocked in either 5% bovine serum albumin (BSA) in Tris-buffered saline + 0.1% Tween-20 (TBS-T) for phospho-DAT, or 5% non-fat milk (NFM) in TBS-T for DAT and Beta-Actin. Membranes were blocked for one hour at room temperature, then incubated in primary antibody in blocking buffer overnight at 4°C. Membranes were washed in TBS-T, then incubated in secondary antibody in blocking buffer for 1-2 hours at room temperature. Membranes were imaged using a Licor Odyssey Fc Imaging System. Densitometric analysis was completed using ImageJ. Protein expression was normalized to the average of the sex-matched Placebo group for statistical analysis.

3.12 Statistical analysis

All statistical analyses were completed using GraphPad Prism. Two-way ANOVAs, with sex and treatment as factors, were used for analysis of plasma CORT and CBG; Tukey's multiple comparisons test was used for analyzing multiple comparisons. For all other datasets, sex was included as a factor, but no significant effects of the interaction between sex and treatment were found. Due to a lack of sex x treatment interaction, and the a priori observation that CORT pellet implantation differently affected plasma CORT levels in males and females, the data in all figures except Figure 2-1 were disaggregated and analyzed by sex. Operant data were analyzed using Two-way ANOVAs, with treatment and training session as factors, and multiple comparisons were completed using Sidak's multiple comparisons test. HPLC-ECD data, *ex vivo* dLight1.3b tau-off values (in the absence of any transporter inhibitors), and western blot data were

analyzed using Two-tailed Unpaired t-tests. *Ex vivo* dLight tau-off values in the presence of transporter inhibitors were analyzed using Two-way ANOVAs, with treatment and inhibitors as factors, and Sidak's multiple comparisons test was used for analyzing multiple comparisons. Locomotor activity and *in vivo* dLight1.3b AUC analyses were completed using Two-way ANOVAs, with treatment and time as factors, and multiple comparisons were completed using Sidak's multiple comparisons test. P-values less than 0.05 were considered statistically significant.

Chapter 4: Conclusions and Future Directions

As with all scientific endeavors, the work presented here has inspired numerous questions that remain to be answered. While primary conclusions from this work were laid out in Chapter 2, they bear repeating and contextualizing here.

4.1 The HPA axes of male and female mice respond differently to exogenous CORT treatment

In this study, subcutaneous CORT pellet implantation significantly increased total plasma CORT levels in male mice only; the same dose of exogenous CORT did not affect total plasma CORT in female mice. The sex difference in total plasma CORT levels after CORT pellet implantation is consistent with previous literature that has shown a similar increase in total plasma CORT levels in males (at the same circadian time point), but no change in plasma CORT levels in females (Kott et al., 2016; Leitch et al., 2003). So, why did the sexes respond differently to the same dose of CORT? One potential reason why females do not exhibit increased CORT levels four weeks after pellet implantation may be due to the fact that females exhibit a higher rate of CORT clearance than males (Colby & Kitay, 1972). Future studies are necessary to understand how the HPA axes of males and females differentially respond to CORT dysregulation, as this will have significant implications for how the field interprets sex differences in stress-induced behavioral and neurobiological deficits.

4.2 Chronic CORT dysregulation impairs reward-guided motivation in both sexes

The primary behavioral finding from my dissertation work is that chronic CORT dysregulation impairs reward-guided motivation in both male and female C57BL/6J mice. While previous studies had reported little or no effect of CORT administration on

anxiety-like behaviors in females, my work showed that chronic CORT administration impaired reward-guided motivation in females (Mekiri et al., 2017; Yohn et al., 2019). So, why did I see an effect of CORT dysregulation on reward-guided motivation in females, while others report little or no effect in anxiety-like behaviors? Again, it's important to recognize that not all behavioral tests are cross-predictive for each other, likely due to different neural circuits underlying distinct behaviors (Morel et al., 2022; Ortiz et al., 2022). Therefore, it's likely that CORT dysregulation in females affects the neural circuits underlying reward-seeking, but not those responsible for anxiety-like behaviors. Another reason I may have observed an effect of CORT treatment on reward-seeking in females is the route of administration of CORT. Previous studies administered CORT in the drinking water (Mekiri et al., 2017; Yohn et al., 2019), a route of administration that may retain the circadian rhythm of plasma CORT levels. In contrast, pellet implantation abolishes circadian (and ultradian) CORT rhythmicity (Leitch et al., 2003; Sarabdjitsingh et al., 2010). Future studies are necessary to understand if abolishing CORT rhythmicity is necessary to observe behavioral effects of chronic CORT administration in females.

4.3 Chronic CORT dysregulation impairs DMS dopamine transmission via sex-divergent mechanisms

Notably, in the work presented here, CORT-induced deficits in reward-guided motivation in males and females were *associated* with sex-divergent impairments in DMS dopaminergic transmission. However, it's unclear if the CORT-induced changes in DMS dopaminergic transmission present in each sex *caused* the motivational deficits observed in operant conditioning. Future studies restoring DAT phosphorylation at

threonine-53 in the DMS of CORT-treated males are necessary to address causality, but currently there are no methods for precisely and exclusively restoring DAT phosphorylation at threonine-53 in specific striatal subregions. Future studies centered on restoring normal dopamine levels exclusively in the DMS of CORT-treated females are needed to address the question of whether or not CORT-induced changes in DMS dopamine content cause deficits in motivation in females. Regardless, the present work adds to a growing body of literature demonstrating sex differences in DAT regulation in the dorsal striatum (Gowrishankar et al., 2018; Stewart et al., 2022).

Another open question is how chronic CORT treatment decreases DMS dopamine content in females. CORT treatment in females could impair vesicular monoamine transporter 2 (VMAT2) function since VMAT2 exhibits greater activity in females than males, and impaired VMAT2 function would be predicted to decrease tonic dopamine (Dluzen & McDermott, 2008). Future studies are necessary to identify the molecular mechanisms responsible for decreased DMS dopamine content in CORT-treated females.

4.4 Future Directions

Dopamine is a key neuromodulator between afferents and D1- and D2-Receptor expressing medium spiny neurons (MSNs) in the striatum. Previous work has shown that chronic stress decreases neuronal density in the DMS (Dias-Ferreira et al., 2009), but it’s unclear how stress affects the plasticity between afferents and MSNs in the dorsal striatum. Since CORT is elevated by stress, and we show that CORT treatment impairs dopamine transmission in the DMS, it will be especially interesting for future studies to examine if, and how, CORT dysregulation affects plasticity between afferents

and D1- and D2R-MSNs within the DMS of males and females to sustain behavioral deficits in reward-seeking.

The observation that CORT dysregulation impairs DMS dopamine transmission in both sexes is a significant advancement for our understanding of how chronic stress may alter reward-guided behaviors. However, it's unclear where CORT is acting to exert its effects on dopamine transmission. Dopamine neurons express glucocorticoid receptors (Harfstrand et al., 1986), so is CORT directly altering dopamine transmission via glucocorticoid receptor signaling in dopamine neurons? Or is CORT perhaps altering dopamine neuron physiology by acting via glucocorticoid receptors on afferents to dopamine neurons? Notably, alterations in CORT levels do not happen in a vacuum. Changes in CORT can alter levels of CRF, another neuropeptide that has been shown to impair operant responding for rewards (Lemos et al., 2012; Wanat et al., 2013). Thus, future studies interrogating the molecular mechanisms underlying CORT-induced dysfunction of DMS dopaminergic transmission are critical for elucidation of novel targets for treatment of stress-induced motivational impairments.

Perhaps the most interesting finding from the work presented here is the latent sex difference in CORT-induced impairments of DMS dopaminergic transmission. Why does CORT dysregulation impair DMS DAT function in males, but not females? Could it be due to the sex difference in total plasma CORT after CORT treatment? Or might it be that CORT dysregulation affects different molecular pathways in dopamine neurons of males and females, thus leading to different effects on DMS dopamine transmission. Future studies focused on the relationship between CORT dose and DMS dopamine

transmission are necessary to clarify if higher levels of plasma CORT would impair DMS DAT function in females, as it does in males. Additionally, future studies examining how CORT dysregulation alters molecular signaling pathways within dopamine neurons of males and females will be illuminating for our understanding of how sex and CORT dysregulation interact to affect dopamine transmission in the brain.

Final Remarks

As a whole, the work presented in this dissertation revealed that CORT dysregulation impairs reward-seeking in both sexes, albeit with sex-divergent effects on DMS dopamine transmission. These findings are critically important for future studies on the behavioral effects of CORT dysregulation because they demonstrate that, contrary to previous belief, CORT dysregulation *can* significantly impair behavior of females, but impairments are task-specific. Moreover, these studies reveal potential mechanisms for CORT-induced reward-seeking deficits in both sexes. Overall, this dissertation provides the basis for future studies to further dissect the latent sex difference in CORT dysregulation's effects on DMS dopaminergic transmission, which may contribute to CORT-induced deficits in reward-seeking in both sexes.

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VITA

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