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Manipulation of Ventral Tegmental Area Dopaminergic Neurons in Stressed Female Mice and its Effect on Behaviors

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Manipulation of Ventral Tegmental Area Dopaminergic Neurons in Stressed Female Mice and its Effect on Behaviors

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Mary Alarcon

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Professor Ashley Juavinett

2021

The thesis of Mary Alarcon is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2021

DEDICATION

I would like to dedicate my thesis to my mother for always believing in my potential, and for her emotional support.

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LIST OF ABBREVIATIONS

ChR2	Channelrhodopsin-2
CMS	Chronic mild stress
COVID-19	Coronavirus disease 2019
DAT-Cre	Dopamine transporter Cre-recombinase
DNA	Deoxyribonucleic acid
EPM	Elevated plus maze
FSS	Forced swim stress
GABA	Gamma-aminobutyric acid
HPA-axis	Hypothalamic pituitary adrenal axis
IACUC	Institutional Animal Care and Use Committee
ICSS	Intracranial self-stimulation
KS	Kolmogorov–Smirnov
LDT	Laterodorsal tegmentum
LHb	Lateral habenula
mPFC	Medial prefrontal cortex
NAc	Nucleus accumbens
NpHR	Halorhodopsin
OFT	Open field test
PBS	Phosphate-buffered saline
PFA	Paraformaldehyde
PPT	Pedunculopontine nucleus
SEM	Standard error of the mean

SDS	Social defeat stress
TH-Cre	Tyrosine hydroxylase Cre-recombinase
VTA	Ventral tegmental area

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VITA

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Major Field: Neurobiology

ABSTRACT OF THE THESIS

Manipulation of Ventral Tegmental Area Dopaminergic Neurons in Stressed Female Mice and its Effect on Behaviors

by

Mary Alarcon

Master of Science in Biology

University of California San Diego, 2021

Professor Kay Tye, Chair
Professor Cory Root, Co-Chair

Stress is a known risk factor for the development of neuropsychiatric disorders. While the ventral tegmental area (VTA) dopaminergic pathway is best known for its involvement in reward and motivation, recent research has suggested that this system could also play another role that is related to stress. Previous studies have shown that females have a higher sensitivity to stress. Despite these observations, research has tended to focus on the effects of stress on males. Therefore, the aim of this study was to investigate whether a non-social stressor impacts the VTA dopaminergic system in DAT-Cre female mice. We also explored whether optogenetic

manipulation of VTA dopaminergic neurons has effects on behavior. Finally, we examined how the timing of stress affects behavior. Our results were not able to demonstrate that VTA dopaminergic manipulations influence behavior, possibly due to the small sample size used for this experiment. Continuing to research the role the VTA dopaminergic system plays in stress in females and males will allow us to uncover any potential therapeutic treatments for stress-related disorders as well as provide a greater benefit to people that have been traditionally excluded from research meant to improve public health.

Introduction

In humans, stress is defined as an inner state that originates from physical demands on the body or environmental and social situations that are perceived as harmful or uncontrollable (Morgan et al., 1986; Mah et al., 2016). An uncontrollable situation humans recently faced was the Coronavirus disease 2019 (COVID-19) pandemic (Seyed et al., 2020). Stress levels increased by 67% in the United States as a result of the COVID-19 pandemic (American Psychological Association, 2021). Given the increase in stress levels, developing better treatment strategies for stress is of great importance. Understanding how stress impacts different brain structures will allow us to develop these treatments. Among research on the impact stress has on the brain, the Hypothalamic Pituitary Adrenal axis (HPA axis), which involves the amygdala, hypothalamus, and the pituitary gland has been the most extensively studied (Stephen & Wand, 2012). Recent studies shed light on the role of another brain structure that is associated with stress called the ventral tegmental area (VTA) (Trainor, 2011).

The VTA is a heterogeneous midbrain region containing different subpopulations of neurons including dopaminergic, glutamatergic, and gamma-aminobutyric acid (GABA)ergic (Lammel et al., 2012; Lammel et al., 2014; Gunaydin et al., 2014; Morales & Margolis, 2017). VTA dopaminergic neurons are also heterogeneous, receiving afferent and efferent projections from different regions of the brain (Morales & Margolis, 2017). Combinatorial neurons are also found in the VTA, which co-release dopamine and GABA, dopamine and glutamate, or glutamate and GABA (Morales & Margolis, 2017). This heterogeneity observed in the VTA might be responsible for the various roles that are associated with this brain region such as reward, aversion, addiction, sociability, and susceptibility (Lammel et al., 2012; Morales & Margolis, 2017). While the VTA dopaminergic system is best known for its involvement in

reward, previous studies suggest that this system could also play another role that is related to stress (Han et al., 2017; Trainor, 2011). Therefore, it is of interest to thoroughly explore the effects of stress in the VTA dopaminergic system to uncover any potential therapeutic treatments for stress and to gain a better understanding of our physiology.

For the purpose of this study, we focused on VTA dopaminergic neurons. To study the dopaminergic system, the dopamine transporter (DAT)-Cre or tyrosine hydroxylase (TH)-Cre transgenic mouse lines that express Cre-recombinase are used to manipulate dopaminergic neurons through optogenetics. Both, tyrosine hydroxylase and the dopamine transporter play crucial roles in the biosynthesis and regulation of dopamine (Daubner *et al.*, 2011; Salatino-Oliveira *et al.,* 2018). Optogenetics is a technique that uses light-sensitive proteins known as opsins to temporally control cellular activity via light delivery (Fenno et al., 2011; Chohan et al., 2020). Based on research done by Lammel and Malenka (2015), DAT-Cre mice have been preferred in dopaminergic studies because this transgenic line expresses high dopaminergic specificity within and around the VTA when compared to TH-Cre mice, which shows low dopamine specific expression (Lammel et al., 2015). For this reason, optogenetic manipulation of the VTA dopaminergic system could reveal insightful information about behavior based on rodent studies.

VTA dopaminergic neurons have low and high frequency firing patterns that are referred to as tonic and phasic firing, respectively (Grace et al., 2007). A low frequency range of 3 to 8 Hz is considered tonic firing (Grace & Bunney, 1984) while a frequency above 14 Hz is considered to be phasic firing (Grace, 2000; Tsai et al., 2009). Interestingly, phasic firing is known to encode reward (Tsai et al., 2009; Chaudhury et al., 2013; Wickham et al., 2013).

To further investigate the role of the VTA dopaminergic neurons' phasic firing in reward, Lammel and associates (2012) aimed to study the inputs to the VTA that have been associated with reward and aversion. This study focused on the projections from the laterodorsal tegmentum (LDT) and lateral habenula (LHb) neurons to the VTA dopaminergic neurons. They used optogenetics to activate LDT neurons that synapse onto the VTA dopaminergic neurons projecting to the nucleus accumbens (NAc) in male TH-Cre mice during a conditioned place preference assay and observed that only phasic, but not tonic activation of the VTA dopaminergic neurons produced a preference response. On the other hand, optogenetic activation of the LHb neurons that synapse onto the VTA dopaminergic neurons projecting to the medial prefrontal cortex (mPFC) resulted in avoidance (Lammel et al., 2012). This study shows how different projections to and from the VTA result in distinct responses like motivated behavior or aversion.

VTA dopaminergic neurons have also been associated with social behavior. In an effort to understand the impact the VTA has on social behavior, Gunaydin and colleagues (2014) studied the projections that motivate or discourage social interaction. In their study, they used optogenetics to manipulate the VTA dopaminergic neurons in TH-Cre female mice during a social interaction behavioral assay. Phasic activation of VTA dopaminergic neurons resulted in an increase in social interaction while inhibition resulted in a decrease. In addition, they also investigated the projections from the VTA dopaminergic neurons to the NAc and mPFC. Activation of the VTA dopaminergic neurons to the NAc resulted in an increase in social interaction but no effect on social interaction was observed when activating the VTA dopaminergic neuron to mPFC pathway. Their findings demonstrated that activation of VTA

dopaminergic neurons that project to the NAc had a stronger effect on social interaction than activation of VTA dopaminergic cell bodies alone (Gunaydin et al., 2014).

The VTA dopaminergic system has also been implicated in addiction. Omelchenko and Sesack (2006) studied cholinergic neurons derived from the pedunculopontine (PPT) nucleus and LDT that synapse onto the VTA dopaminergic and GABAergic neurons, as well as their projections to the NAc and mPFC, in the hopes of understanding the role addictive drugs like nicotine play in this circuit. Nicotine is a nicotinic and muscarinic receptor agonist, and when it binds to these acetylcholine receptors, it increases dopamine and GABA release on the synaptic cleft (Yin & French, 2000). Omelchenko and Sesack's (2006) research found that cholinergic neurons synapsing on VTA dopaminergic and GABAergic neurons also project to the NAc and mPFC, but there are more projections from cholinergic neurons to the VTA-dopamine-NAc pathway than the VTA-dopamine-mPFC pathway or the cholinergic-GABA pathway. Interestingly, the morphology of cholinergic-dopaminergic neurons was asymmetrical, which has been associated with excitatory neurons (Hong et al., 2013), while the cholinergic projections to GABAergic neurons showed more symmetry, which is associated with inhibitory neurons (Panzanelli et al., 2004). When studying the addictive and rewarding properties of drugs like nicotine, it is of great importance to acknowledge the vast heterogeneity of the VTA dopaminergic system.

Phasic firing of VTA dopaminergic neurons might also be responsible for the susceptible phenotype observed in mice after exposure to social stress (Krishnan et al., 2007; Chaudhury et al., 2013). The social defeat stress (SDS) paradigm exposes the experimental animal to social defeat resulting in physiological stress and social avoidance, a measure of stress (Carnevali et al., 2020). Chaudhury and colleagues (2013) investigated the mechanism that induces susceptibility

in mice using optogenetics to manipulate the VTA dopaminergic system in TH-Cre male mice. Susceptibility was characterized by a decrease in social interaction and sucrose consumption. A decrease in sucrose consumption is a measure of anhedonia, a core symptom of depression (Liu et al., 2018; Muschamp, 2011). They first studied the effect SDS had on VTA dopaminergic neurons. Mice that underwent the SDS paradigm that did not demonstrate the susceptible phenotype were considered resilient. Resilient mice showed normal VTA dopaminergic firing while susceptible mice showed an increase in firing. Additionally, phasic activation of VTA dopaminergic neurons projecting to the NAc during a social interaction assay in mice that were considered resilient resulted in susceptibility, an effect not observed with tonic activation. Optogenetic inhibition of this pathway decreased social avoidance and increased sucrose consumption. This research reveals the significance of the projection from VTA dopaminergic neurons to the NAc on social interaction.

Stress is a risk factor for the development of neuropsychiatric disorders like anxiety and depression (Iñiguez et al., 2014; Solomon, 2017). While a considerable amount of research has been done to study the effect of stress on male rodents (Krishnan et al., 2007; Lammel et al., 2012; Chaudhury et al., 2013), little research has been conducted on the effect of stress on females, even though sex is a factor that affects how an organism responds to stress (Badrinarayan et al., 2012; Trainor, 2011). Females are also at higher risk of developing these disorders (Solomon, 2017). Studies in female rodents have also demonstrated that females have a higher sensitivity to stress than males (Trainor et al., 2011; Rincón-Cortés & Grace, 2017; Solomon, 2017). Rincón-Cortés and Grace (2017) used in vivo electrophysiology to demonstrate that adult female Sprague-Dawley rats are more susceptible to VTA dopaminergic downregulation after stress exposure than males. They recorded from VTA dopaminergic

neurons before and after exposing the rats to forced swim stress (FSS) or chronic mild stress (CMS). CMS is an animal model for mood disorders that affects the mesolimbic dopaminergic system (Willner et al., 2005) and FSS is regarded as an uncontrollable acute stressor (Rincón-Cortés and Grace, 2017). Female rats that underwent either the CMS paradigm or FSS exhibited significantly lower activity of spontaneous VTA dopaminergic neurons compared to males, as well as a longer immobility period during the forced swim stressor. A previous study had shown the antidepressant effects of ketamine by reversing the downregulation of VTA dopaminergic neurons in Wistar-Kyoto rats after inducing acute depression through uncontrollable and inescapable foot shock stress exposure (Belujon & Grace, 2014). Rincon-Cortés and Grace (2017) validated that ketamine administration returns the activity of spontaneous VTA dopaminergic neurons back to baseline in both female and male rats. Ketamine administration also decreased FSS immobility in both sexes.

A distinctive feature of neuropsychiatric disorders is a decrease in social interaction (Trainer et al., 2011; Krishnan et al., 2007; Chaudhury et al., 2013). In social behavioral studies, mice are subjected to the social defeat stress (SDS) paradigm because it results in physiological stress and social avoidance (Carnevali et al., 2020). Trainer and associates (2011) investigated the effects of social stress in both female and male California mice. This species of mice was chosen because both sexes show aggression when defending their territory (Ribble & Salvioni, 1990). The findings of Trainer et al., (2011) study showed that SDS only affected female California mice and not male using a decrease in social interaction as a measure of stress sensitivity. Interestingly, this decrease in social interaction was observed during all estrous phases. When testing for exploratory behaviors in both sexes, there was no observable decrease in either sex, this suggest that SDS is context specific. Despite these observations, the underlying

mechanism resulting in the observed differences in sociability in female mice groups remains unclear.

Therefore, inspired by the research done by Gunaydin et al., (2014), which focused on the effect VTA dopaminergic activation had on social interaction in non-stressed female mice, Wichmann and colleagues (2017) focused on the effect VTA dopaminergic manipulations had on social behavior in stressed female mice. Previous research has shown that in non-stressed mice, activation of the VTA dopaminergic neurons results in an increase in sociability (Gunaydin et al., 2014). In contrast, inhibition of the VTA dopaminergic neurons results in social avoidance in non-stressed mice (Gunaydin et al., 2014). This led Wichmann et al., (2017) to explore whether a non-social stressor could produce similar results when looking at sociability using optogenetics to manipulate the VTA dopaminergic system in stressed female TH-Cre mice. They also aimed to examine the long-term effect of stress by comparing mice receiving the stressor during adolescence (remote stress) versus adulthood (recent stress). Inhibition of the VTA dopaminergic neurons in non-stressed female mice decreased social interaction but did not affect sociability in the stressed groups. Phasic activation resulted in a decrease in social interaction in the stressed groups but no effect on the non-stressed group. Inhibition or activation did not alter locomotion, anxiety, nor novelty seeking in the non-stressed and recent stressed groups, but exploration of the novel object did increase during phasic activation in the remote stress group. To study how stress impacted the VTA dopaminergic reinforcement circuit, mice underwent intracranial self-stimulation, and all experimental mice showed an increase in nose poking during 8 pulses, 30 Hz, 5 ms pulse stimulation but the remote group's nosepokes decreased during the 90-pulse stimulation (Wichmann et al., BioRxiv 2017).

In an effort to contribute to Wichmann and associates (2017) findings, the aim of this experiment was to replicate the study in DAT-Cre female mice instead of TH-Cre female mice to verify that the effects observed in the Wichmann 2017 study were actually due to dopamine manipulation. I investigated whether a non-social stressor impacts the ventral tegmental area dopaminergic neurotransmission on social-related behaviors in female mice. We used DAT-Cre transgenic mice to optogenetically activate or inhibit VTA dopaminergic neurons to investigate the effects VTA manipulation has on social-related behavior in stressed female mice. The forced swim stress model was used to induce non-social stress to ensure that social behavior was not impacted by a social stressor. Mice were subjected to other behavioral assays to explore whether manipulation of VTA dopaminergic neurons also impacts other behaviors. Finally, we examined the long-term effect of stress by comparing recent versus remote stress. Based on Wichmann et al (2017) results, we hypothesized that optogenetic activation of VTA dopaminergic neurons would reduce social interaction in both stressed groups while increasing social interaction in the non-stressed group. We also expected inhibition of VTA dopaminergic neurons to not affect social interaction of both stressed groups while decreasing social interaction of the non-stressed group. Understanding the effect stress has on female mice will allow us to not only comprehend how the VTA dopaminergic pathway is involved in a females' biological response to stress in a social context but will also provide a greater benefit for people that have been traditionally excluded from research meant to improve public health.

Results

To assay social behavior, mice were tested on a 2-day social interaction paradigm. Here, an unfamiliar young female (3-4 weeks old) was introduced into the home cage of the experimental mouse and VTA dopaminergic neuron activity was activated or inhibited in the experimental mouse during one testing session (counterbalanced for order). There was no significant interaction of VTA dopaminergic neuron light activation and social interaction time's automated scoring done by AlphaTracker-2 (Two-way ANOVA, main effect of photostimulation: $F_{1,10} = 0.02080$, $P = 0.8882$; main effect of stress exposure group: $F_{2,10} = 1.266$ $P = 0.3236$; photostimulation-by-stress exposure group interaction: $F_{2,10} = 0.1856$, $P = 0.8334$) (Figure 1A), nor by the manual scoring by ODLog (Two-way ANOVA, main effect of photostimulation: $F_{1,10} = 0.02870$, $P = 0.8688$; main effect of stress exposure group: $F_{2,10} = 1.663$, $P = 0.2380$; photostimulation-by-stress exposure group interaction: $F_{2,10} = 0.2186$, $P = 0.8074$) (Figure 1B). Effect of photostimulation for the non-stress group for AlphaTracker-2 scoring (Kolmogorov–Smirnov (KS) $p = 0.8730$); recent stress group (KS $p = 0.8730$); remote stress group (KS $p = 0.7301$). Effect of photostimulation for the non-stress group for ODLog scoring (KS $p = 1.000$); recent stress group (KS $p = 0.9307$); remote stress group (KS $p = 0.9895$).

There was no significant interaction of VTA dopaminergic neuron light inhibition and social interaction time's automated scoring done by AlphaTracker-2 (Two-way ANOVA, main effect of light inhibition: $F_{1,8} = 0.2886$, $P = 0.6057$; main effect of stress exposure group: $F_{2,8} = 2.093$, $P = 0.1857$; light inhibition-by-stress exposure group interaction: $F_{2,8} = 2.207$, $P = 0.1724$) (Figure 1C), nor the manual scoring by ODLog (Two-way ANOVA, main effect of light inhibition: $F_{1,8} = 0.9179$, $P = 0.3661$; main effect of stress exposure group: $F_{2,8} = 2.415$, $P = 0.1512$; light inhibition-by-stress exposure group interaction: $F_{2,8} = 3.259$, $P = 0.0922$) (Figure

1D). Effect of photoinhibition for the non-stress group for AlphaTracker-2 scoring (Kolmogorov–Smirnov (KS) p = 1.000); recent stress group (KS p = 0.600); remote stress group (KS p = 0.7714). Effect of photoinhibition for the non-stress group for ODLog scoring (Kolmogorov–Smirnov (KS) p = 1.000); recent stress group (KS p = 0.099); remote stress group (KS p = 0.7714).

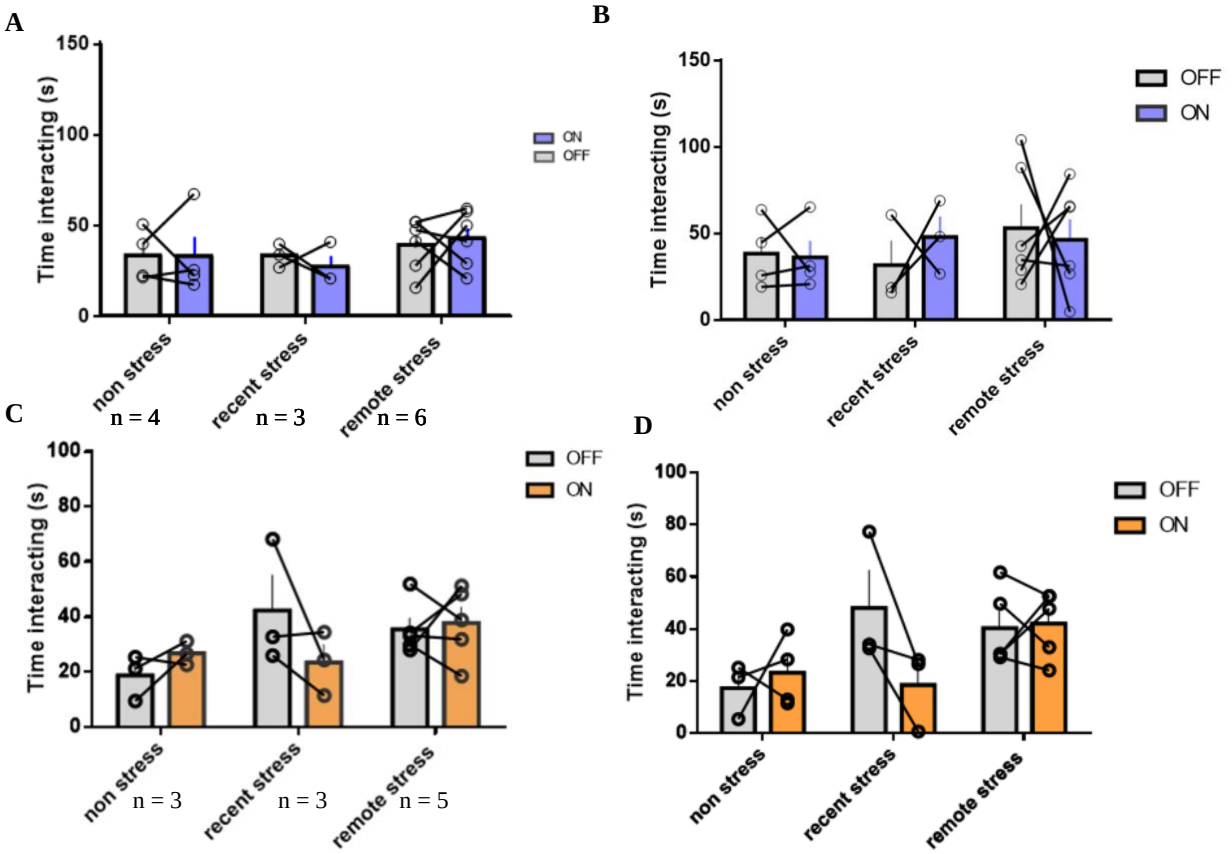

Figure 1. Comparison of Alphatracker-2's automated scoring (A&C) to manual scoring (B&D) while observing the effects of VTA dopaminergic neuron activation or inhibition on social behavior. ON represents the time when the exposure group received photostimulation (blue) or photoinhibition (orange). OFF shown in grey represents no light stimulation. Each circle represents an individual mouse. Error bars represent standard error of the mean (SEM) based on normal distribution.

The effects of VTA dopaminergic neuron light activation or inhibition on social interaction did not differ between the stress exposure groups (One-way ANOVA: $F_{2,10} = 1.354$, P = 0.3018 (Figure 2A); One-way ANOVA: $F_{2,8} = 0.04081$, P = 0.9602 (Figure 2B), respectively).

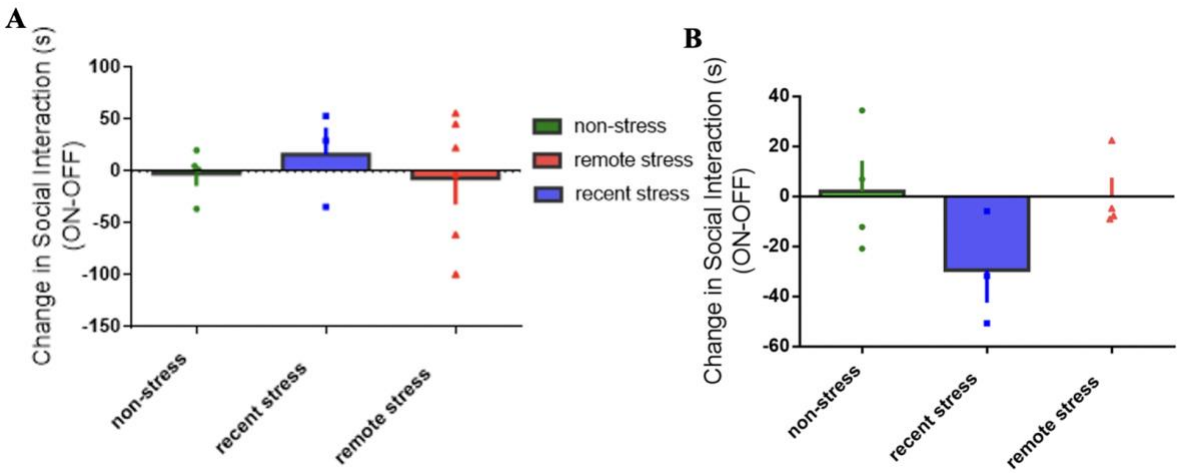

Figure 2. Difference in social interaction during photostimulation (A) or photoinhibition (B). The non-stress group is shown in green circles, the remote stress in red triangles, and the recent stress in blue squares. Error bars represent standard error of the mean (SEM).

To test the anxiogenic effects of photostimulation and photoinhibition on VTA dopaminergic neurons, each exposure group underwent two behavioral assays, Open Field Test (OFT) and Elevated Plus Maze (EPM). There was no significant interaction of photostimulation and stress exposure groups on time in the center of the OFT (Two-way ANOVA, main effect of stimulation: $F_{2,20} = 2.046$, $P = 0.1554$; main effect of photostimulation and stress exposure group: $F_{4,20} = 0.7930$, $P = 0.5435$; main effect of stress exposure group: $F_{2,10} = 0.6466$, $P = 0.5444$) (Figure 3A). There was no significant interaction of photostimulation and stress exposure groups on time in the open arm of the EPM (Two-way ANOVA, main effect of photostimulation: $F_{2,20} = 0.07713$, $P = 0.6153$; main effect of stress exposure group: $F_{2,10} = 0.2560$, $P = 0.7791$; photostimulation-by-stress exposure group interaction: $F_{4,20} = 0.4741$, $P = 0.7542$) (Figure 3B). There was no significant difference between the effect of photoinhibition and stress exposure group on time in the center of the OFT (Two-way ANOVA: main effect of stress exposure group: $F_{2,8} = 1.685$, $P = 0.2451$; photoinhibition-by-stress exposure group interaction: $F_{4,16} = 0.3666$, $P = 0.8289$, but there was a significance in the main effect of photoinhibition: $F_{2,16} =$

3.713, P = 0.0473) (Figure 3C) nor on the time in the open arm of the EPM (Two-way ANOVA: main effect of stress exposure group: $F_{2,8} = 0.4690$, P = 0.6418; photoinhibition-by-stress exposure group interaction: $F_{4,16} = 1.487$, P = 0.2526; main effect of photoinhibition: $F_{2,16} = 0.1093$, P = 0.8971) (Figure 3D). Further anxiety-related testing was done on the Halorhodopsin (NpHR) cohort via the marble burying assay. No significant difference was observed between photoinhibition and exposure group during marble burying (Two-way ANOVA, main effect of photoinhibition: $F_{1,8} = 1.106$, P = 0.3236; main effect of stress exposure group: $F_{2,8} = 1.838$, P = 0.2204; photoinhibition-by-stress exposure group interaction: $F_{2,8} = 0.7603$, P = 0.4985) (Figure 4). These results support previous findings that optogenetic manipulation of VTA dopaminergic neurons does not influence anxiety-related behavior or locomotion (Tsai et al., 2009; Lammel et al., 2012; Gunaydin et al., 2014; Wichmann et al., 2017).

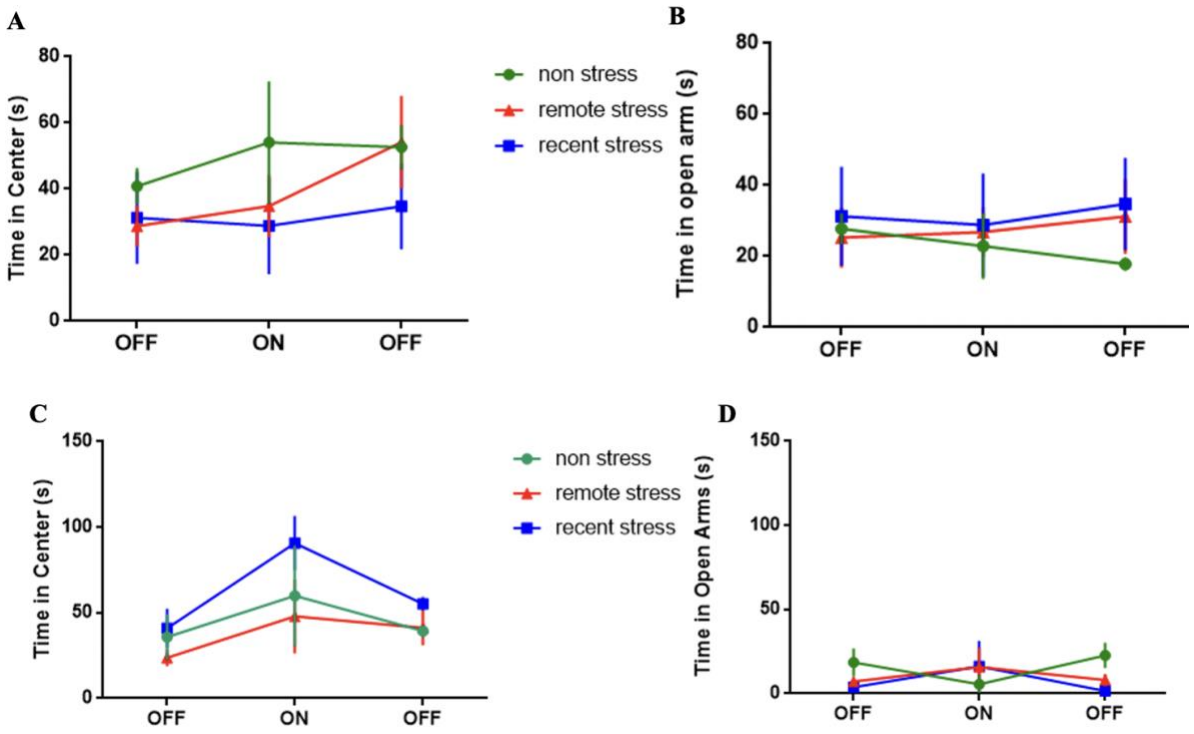

Figure 3. Effect of VTA DA neuron activation (A&B) or inhibition (C&D) on anxiogenic behavior. Error bars represent standard error of the mean (SEM).

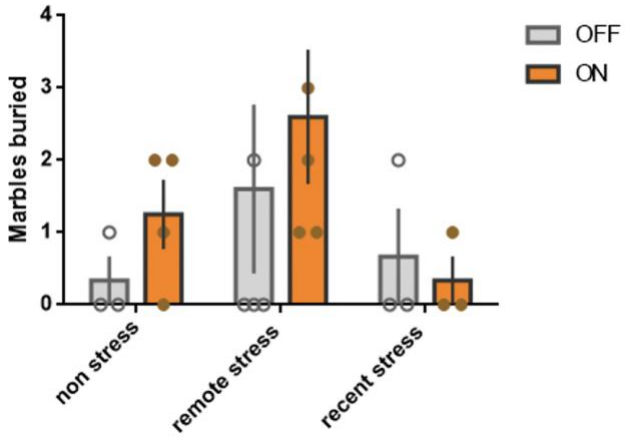

Figure 4. Effect of VTA dopaminergic neuron inhibition on marble burying behavior. The number of marbles buried by each exposure group when laser stimulation was ON is shown in closed orange circles and open circles when OFF. Error bars represent standard error of the mean (SEM).

To determine whether photostimulation or photoinhibition of VTA dopaminergic neurons impacted locomotion, mice were tested in the OFT assay. There were no differences detected between stress exposure and photostimulation (Figure 5A) (Bartlett's test p = 0.4199). There was a significant difference between stress exposure group and photoinhibition (Figure 5B) (Brown-Forsythe test p = 0.004).

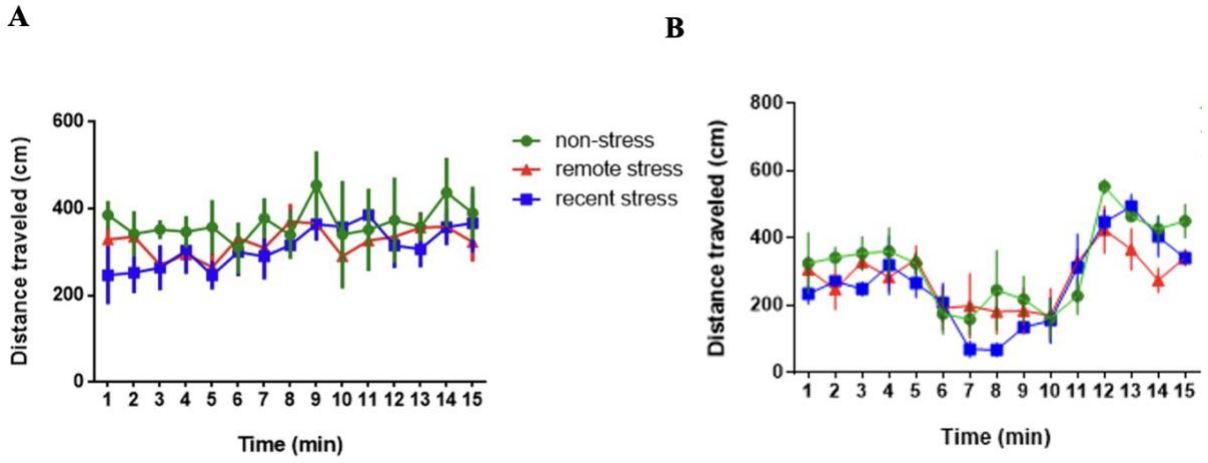

Figure 5. Effect of VTA dopaminergic neuron activation (A) and inhibition (B) on locomotion. OFT was used to measure locomotion. Error bars represent standard error of the mean (SEM).

To examine how stress-induced alterations in dopamine signaling impact the ability of VTA dopaminergic neuron photostimulation to serve as a primary reinforcer, the effects of optically stimulated dopamine release on response rate to intracranial self-stimulation (ICSS) of VTA dopaminergic neurons were assessed. There were no differences detected between stress exposure and optical stimulation of 8-pulses,30 Hz, 5 ms pulse duration, 473 nm (Kruskal-Wallis test $p = 0.3138$), or the stimulation of 90-pulses,30 Hz, 5 ms pulse duration, 473 nm (Kruskal-Wallis test $p = 0.8512$). All groups showed robust intracranial self-stimulation for higher intensity photostimulation (90-pulses,30 Hz, 5 ms pulse duration, 473 nm).

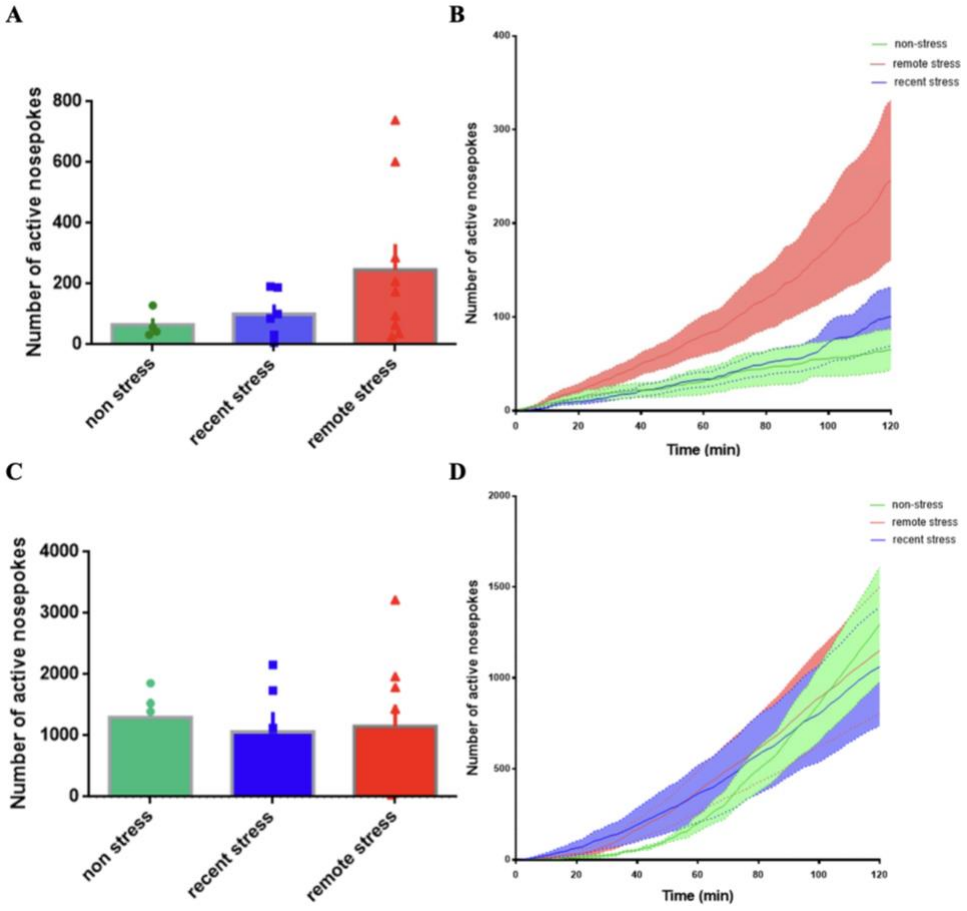

Figure 6. Effect of VTA dopaminergic neuron activation on intracranial self-stimulation (ICSS). A 120 min ICSS session was administered where responses at the active nosepoke port were reinforced with optical stimulation (either 8 or 90 pulses, 30 Hz, 5 ms pulse duration, 473 nm). Active nosepokes during the 8-pulse stimulation (A&B). Active nosepokes during the 90-pulse stimulation (C&D). Error bars represent standard error of the mean (SEM). The non-stress group is shown in green, the remote stress in red, and the recent stress in blue.

To assay explorative behavior, mice were tested on a 2-day novel object assay. A novel figurine was introduced into the cage of the experimental mouse for both days, but light inhibition was only done on one testing session (counterbalanced for order). There was no significant interaction of photoinhibition and exposure group in the novel object assay (Two-way ANOVA, main effect of photoinhibition stimulation: $F_{1,8} = 1.151$, $P = 0.3146$; main effect of stress exposure group: $F_{2,8} = 0.1268$, $P = 0.8826$; photoinhibition-by-stress exposure group interaction: $F_{2,8} = 0.3724$, $P = 0.7004$) (Figure 7A). The effects of VTA dopaminergic neuron light inhibition on novel object exploration did not differ between the stress exposure groups (one-way ANOVA, $F = 0.3635$, $p = 0.7041$).

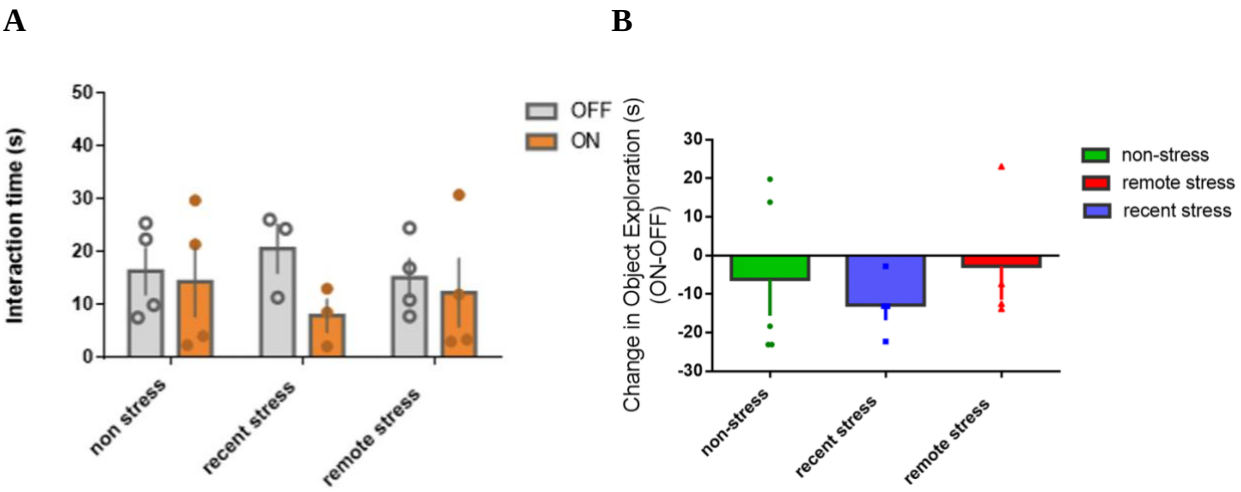

Figure 7. Effects of VTA dopaminergic neuron inhibition on explorative behavior. (A) ON represents the time when the exposure group received photoinhibition (orange). OFF shown in grey represents no light stimulation. Each circle represents an individual mouse. Error bars represent standard error of the mean (SEM). (B) Difference in object exploration during photoinhibition. The non-stress group is shown in green circles, the remote stress in red triangles, and the recent stress in blue squares. Error bars represent standard error of the mean (SEM).

Discussion

Stress has been associated with the development of psychiatric disorders (Iñiguez et al., 2014; Solomon, 2017). Females have been shown to be more susceptible to stress and the development of these disorders when compared to males (Solomon, 2017). Despite the importance of the relationship between stress exposure and the development of mental health disorders, few researchers have studied the effects of stress on females (Trainor et al., 2011; Rincón-Cortés & Grace, 2017; Wichmann et al., 2017). In an effort to diminish this discrepancy, this study investigated whether a non-social stressor impacts the VTA dopaminergic neurotransmission on social-related behaviors in female mice. We also explored whether manipulation of VTA dopaminergic neurons impacted other behaviors. Finally, we examined the long-term effect of stress by comparing recent versus remote stress.

The results of our experiment failed to support the hypothesis that optogenetic activation of VTA dopaminergic neurons reduces social interaction in the groups exposed to the non-social stressor (Wichmann et al., 2017), while increasing the social interaction in the control group that did not receive the stressor (Gunaydin et al., 2014) (Fig. 1A-B & 2A). We were also not able to support the hypothesis that VTA dopaminergic neuron inhibition decreases social interaction in non-stressed mice (Gunaydin et al., 2014) while not affecting the stressed groups (Wichmann et al., 2017) (Fig. 1C-D & 2B). The time when the stressor was given, whether it was during adolescence or adulthood had no effects on behavior (Fig. 1 & 2).

In the Wichmann et al., (2017) study, TH-Cre female mice were used as the main animal model, with a very small subset of DAT-Cre mice. Our study only used DAT-Cre female mice, and it is a possibility that this transgenic mouse line behaves differently under the same experimental conditions. Gunaydin *et al.*, (2014) also used TH-Cre female mice to investigate

the role VTA dopaminergic neurons have on social behavior in non-stressed mice. This possibility can be examined by simultaneously running the same experiments on both transgenic mouse lines. If the TH-Cre mouse line produces the same results as prior studies and the DAT-Cre line behaves as it did in this experiment, that might support the hypothesis of transgenic mouse line behavioral differences. However, if the TH-Cre line and DAT-Cre line both produce similar behavioral responses as the DAT-Cre line in the present study, then further investigation is necessary.

Although the DAT-Cre transgenic mouse line has been shown to express a higher dopaminergic specificity in the VTA when compared to the TH-Cre mouse lines (Lammel et al., 2015), it may be a possibility that the TH-Cre line might be better suited for examining the effects of VTA dopaminergic optogenetic manipulation on behavior. In spite of this possibility, it is important to note that both, tyrosine hydroxylase and the dopamine transporter play crucial roles in the biosynthesis and regulation of dopamine (Daubner *et al*., 2011; Salatino-Oliveira *et al.*, 2018). Another possibility for this null result could be that the sample size of my project compared to Wichmann et al., (2017) study was considerably smaller. This present study did not perform a power analysis to determine the correct sample size, therefore, it is possible that this contributed to the null results (Serdar et al., 2021).

Early life stress is a risk factor for neuropsychiatric disorders (Klinger *et al*., 2019). Surprisingly, Wichmann *et al.*, (2017) and this present study showed no significant difference in behavior when comparing the long-term effects of stress (Fig. 1 & 2). A possible explanation could be that the period of stress exposure when female mice are more susceptible to early life stress might have passed (Klinger *et al*., 2019). In future studies, this possibility can be investigated by exposing adolescent female mice to the stressor before ~P28 and comparing

whether exposure to a stressor at an earlier age result in differences in behavior when comparing early life stress to adult stress. Another possibility could be that the effects of early life stress might not be apparent when comparing behavior but instead, comparing neural activity of the VTA dopaminergic system might produce observable differences between early life stress and adult stress. Previous studies have demonstrated a downregulation of dopaminergic neurons in the VTA after stress exposure in female and male rodents (Tye et al., 2013; Rincón-Cortés and Grace 2017). Future direction experiments should perform an in vivo electrophysiological study under the same conditions as this present experiment to explore whether early life stress results in a larger downregulation of VTA dopaminergic neurons when compared to adult stress.

VTA dopaminergic manipulation did not influence social behavior across exposure groups. Previous studies have shown that activating the VTA dopaminergic neurons in non-stressed mice increases social interaction (Gunaydin *et al.*, 2014). A possibility for this observation might be that this experiment did not take into account the heterogeneity of the VTA (Lammel *et al*., 2012 & 2014; Gunaydin *et al*., 2014; Morales and Margolis, 2017). This present study only targeted VTA dopaminergic cell bodies with no specificity and did not investigate the VTA dopaminergic neuron projections to the nucleus accumbens (NAc), a pathway that has been associated with motivated behavior (Lammel et al., 2012; Gunaydin et al., 2014; Omelchenko and Sesack, 2006). Gunaydin et al., (2014) found that the projection from VTA dopaminergic neurons to the NAc resulted in more calcium activity, a measure of neural activity (Fosque *et al.*, 2015) during social interactions than when just measuring neural activity of VTA dopaminergic neurons alone. Future studies should investigate the VTA dopaminergic projections to the NAc to explore whether optogenetic manipulation of this projection influences social behavior after non-stress exposure.

Continued research on the dynamics occurring in the VTA dopaminergic system as a result of stress exposure is necessary in order to develop more effective treatment options for mental health disorders that impact social behavior.

Material and Methods

Animals:

Female heterozygous dopamine transporter (DAT)-Cre transgenic mice were used for all experiments. At ~P21 all mice were weaned and kept housed on a reverse 12-hour light/dark cycle with food and water *ad libitum* for the rest of the experimental timeline. All mice were group-housed in pairs of 2-5. Mice were randomly assigned to an exposure group (non-stress, recent stress, or remote stress) and mice housed together were always subjected to the same exposure. The recent group was exposed to FSS 7 days before the behavioral assays data collection, while the remote group received the FSS 60 days prior. Remote stress was performed between P28 and P32 and recent stress between P86 and P90 (Figure 8). The non-stress mice groups did not undergo FSS to serve as the control. Behavioral testing occurred around P97. All mice were naive before any experimental procedure. No animals were reused from other studies. The channelrhodopsin (ChR2) cohort consisted of 4 non-stress, 9 remote stress, and 6 recent stress mice. The halorhodopsin cohort consisted of 4 non-stress, 4 remote stress, and 3 recent stress mice. All experimental protocols were approved by Salk's IACUC.

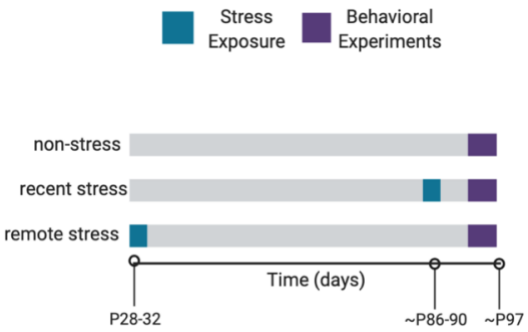

Figure 8. Experimental timeline of each exposure group. The groups are composed of a non-stress group, a recent stress group, and a remote stress group. The exposure stress groups underwent the forced swim test paradigm for five days, represented in blue. The remote stress group received the stressor during adolescence (~P28-32). The recent stress group received the stressor during adulthood (~P86-90). The non-stress group served as the control. All exposure groups underwent the behavioral experiments on day ~P97 represented in purple. P represents days postnatal.

Stereotaxic virus injection and optic fiber implantation

Mice (~8-9 weeks of age) were anesthetized with isoflurane (5% for induction, 2% after) and placed in a stereotaxic frame on a heat pad. A 10μL Nanofil syringe with a 33-gauge beveled microinjection needle was used to infuse the virus with a microsyringe pump and its controller. Virus was infused at a rate of 100 nL per min. Following infusion, the needle was raised 50 μm and then kept in place for an additional 10 min before being slowly withdrawn. All stereotaxic coordinates are relative to bregma. For photoactivation, mice were unilaterally injected at two sites in the VTA (-3.35 mm anteriorposterior (AP); 0.35 mm mediolateral (ML); -4.25 and -4.1 mm dorsoventral (DV)) with a total of 1.4 μL of virus (AAV5-EF1a-DIO-ChR2(H134R)-eYFP; UNC Viral Core; Chapel Hill, NC). An optic fiber (300 μm core, 0.37 numerical aperture [NA], Thorlabs, Newton, NJ, USA) was unilaterally implanted over the VTA (-3.35 mm AP; 0.35 mm ML and -3.7 mm DV) and secured to the skull using a base layer of adhesive dental cement (C&B Metabond; Parkell, Edgewood, NY) followed by a second layer of cranioplastic cement (Ortho-Jet; Lang Dental, Wheeling, IL). For photoinhibition experiments, the same amount of virus (AAV5-EF1a-DIO-eNpHR3.0-eYFP; UNC Viral Core; Chapel Hill, NC) was injected at two sites in the VTA (-3.35 mm AP; 0.35 mm ML and -4.25 and -4.1 mm DV). The optical fiber was positioned between the 2 hemispheres medially above the VTA (-3.35 mm AP; 0.0 mm ML and -3.2 mm DV) and secured in the same way as above. The incision was closed with sutures and mice were given a subcutaneous injection of Meloxicam (1.5 mg/kg) and saline (~1 mL) prior to recovery on a heat pad; 0.03 mg/kg Buprenorphine Sustained-Release (BUP-SR) was administered prior to the start of the surgery. All behavioral experiments were conducted 4 weeks after surgery.

Swim stress

Mice in the recent and remote stress group were subjected to 5-day swim stress in which they were exposed to 15 min swim sessions on day 1, 3, and 5 and four swim sessions of 6 min each separated by 6 min of rest on day 2 and 4. Water temperature was maintained at 26 ± 1 ºC. Mice's weights were recorded prior to and after the swim stress. After removal from water, mice were returned to their home cage and allowed to recover under a heating pad for 30 min. After the 30 minutes on the heating pad, mice were returned to the animal facility.

Behavioral assays

All behavioral tests were performed at least 4 weeks following viral injection to allow sufficient time for transgene expression. Mice were tested during the dark phase and allowed to acclimate to the behavioral testing room for at least 45 min prior to testing. Mice were handled and connected to an optical patch cable for 5 days before being subjected to any behavioral assay. All behavioral tests were recorded by a video camera located directly above the respective arena. The EthoVision XT video tracking system (Noldus, Wageningen Netherlands) was used to track mouse location, velocity, and movement of head, body, and tail. All measurements displayed are relative to the center of the mouse body.

Social interaction assay:

Social interaction was performed in the mice's home cage. All cage mates were temporarily moved to a holding cage and the experimental mouse was allowed to explore its home cage freely for 1 min (habituation). A novel young (3-5 weeks of age) female was introduced into the cage and the two mice were then allowed to interact freely for 3 min (test

session). Each experimental mouse underwent two social interaction tests separated by 24 hours, with one intruder paired with optical stimulation and a different one with no stimulation. Groups were counterbalanced for order of light stimulation. All behaviors were video recorded and analyzed using AlphaTracker version 2 software and a custom MATLAB script that scores social interaction as defined by any period of time in which the experimental mouse was actively investigating the intruder, including behaviors such as face or body sniffing, anogenital sniffing, direct contact, and close following (<1 cm). The videos were also manually scored by the experimenter using ODLog software (Macropod software) in order to compare it to the Alphatracker algorithm.

Novel object exploration:

The novel object test was performed exactly like the social interaction assay. Instead of a young intruder, a figurine was introduced into the mouse's home cage and the total time spent investigating the object over 3 minutes was quantified. Objects were thoroughly cleaned with acetic acid (0.03%) in between tests. Each experimental mouse underwent two novel object investigation tests separated by 24 hours, with one trial paired with optical stimulation and one with no stimulation, counterbalanced for order of light stimulation and object. All behaviors were video recorded and analyzed using AlphaTracker version 2 software and a custom MATLAB script that scores novel object exploration as defined by any period of time in which the experimental mouse was actively investigating the object.

Elevated plus maze assay:

The elevated plus maze was made of grey plastic and consisted of two open arms (30 x 5 cm) and two enclosed arms (30 x 5 x 30 cm) extending from a central platform (5 x 5 cm). The maze was elevated 75 cm from the floor. Individual mice were connected to the patch cable and allowed 1 min of exploration before the 15 min session was initiated. Each session was divided into three 5 min epochs with only the second epoch with light stimulation.

Open field test:

Individual mice were connected to the patch cable and placed in the center of the open field (53 x 53 cm) at the start of the session. The open field test consisted of a 15 min session with three 5 min epochs in which the mouse was permitted to freely investigate the chamber. Stimulation was given only during the second epoch.

Marble burying:

A mouse cage was filled halfway with corncob bedding for the marble burying task. 12 black marbles were placed on the surface of the bedding in 4 rows of 3 marbles located 3 cm apart. Experimental mouse was placed in the corner of the cage and underwent two marble burying assays separated by 24 hours, with one paired with optical stimulation and a different one with no stimulation. Groups were counterbalanced for order of light stimulation. Behavior was recorded via video camera positioned directly above the arena using Logitech. Photographs of the behavioral arena before (undisturbed) and after each 10 min session were obtained and marbles that were at least 75% buried were counted. In between subjects, cage bedding was shuffled, and marbles were cleaned with 0.03% acetic acid.

Intracranial self-stimulation:

Immediately before the start of the session, mice were connected to a patch cord and placed in standard Med-Associates (St. Albans, VT, USA) operant chambers equipped with an active and inactive nose-poke directly below two cue lights as well as audio stimulus generators and video cameras. A 2-hour optical self-stimulation session began with the onset of low volume white noise and illumination of both nose pokes. Each active nose poke performed by the mouse resulted in optical stimulation of VTA cell bodies (either 8 or 90 pulses, 30 Hz, 5 ms pulse duration). Concurrently, the cue-light above the respective port was illuminated and a distinct tone was played (1 kHz and 1.5 kHz counterbalanced), providing a visible and auditory cue whenever a nosepoke occurred. Both active and inactive nosepoke time-stamps data were recorded using Med-PC software and analyzed using custom-written MATLAB scripts (Mathworks; Natick, MA).

Tube test:

A transparent Plexiglass tube with a 30 cm length and a 3 cm internal diameter was used to permit the experimental mouse to comfortably pass through without turning around. Mice were individually trained to pass through the tube for 5 days, 4 times on each side of the tube. For testing to determine rank, all mice in each cage were tested in a round-robin design in a randomized order. For each pair, mice were released at opposite ends of the tube simultaneously. The mouse that either itself backed out or was pushed out from the end where it was released was designated as "subordinate" whereas the other mouse was designated as "dominant". Social ranks obtained with the tube test were considered stable when obtaining the same results for 3 or more days in a row. The mouse tube test assay was adapted from Lindzey

and colleagues (Lindzey et al., 1961) and validated by Padilla-Coreano et al (Padilla-Coreano et al., 2020)

Laser Delivery

For optical manipulations during behavioral assays, the laser was first connected to a patch cord with a pair of FC/PC connectors on each end (Doric; Québec, Canada). This patch cord was connected through a fiber-optic rotary joint (Doric; Québec, Canada), which allows free rotation of the fiber, with another patch cord with a side of FC/PC connector and a ferrule connection on the other side that delivers the laser via a chronic optic fiber. Phasic activation of VTA cell bodies consisted of 30 Hz bursts of eight 5 ms pulses of 473 nm light delivered every 5 sec at a light power output of 20 mW of blue light generated by a 100 mW 473 nm DPSS laser (OEM Laser Systems; Draper, UT), delivered via an optical fiber. Inhibition of VTA cell bodies was performed with 593 nm light delivered constantly at a light power output of 5 mW of yellow light, generated by a 593 nm DPSS laser. Laser output was manipulated with a Master-8 pulse stimulator (A.M.P.I.; Jerusalem, Israel). Onset of laser light was determined by behavioral hardware.

Monitoring of estrous cycle

After behavioral testing each day, a vaginal swab was collected using a cotton tipped swab (Puritan Medical Products Company; LLC Guilford, ME) wetted with saline (Byers et al., 2012). The cells were spread on a microscope slide. Slides were examined under a light microscope in order to determine the stage of the estrous cycle phase via vaginal cytology.

Immunohistochemistry and confocal microscopy

All mice were anesthetized with sodium pentobarbital and then transcardially perfused with ice cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS (pH 7.3). Extracted brains were post-fixed in 4% PFA overnight and then transferred to 30% sucrose in PBS until equilibration. 50μm-thick coronal sections were sliced using a sliding microtome (HM430: Thermo Fisher Scientific, Waltham, MA) and stored in PBS at 4°C until processed for immunohistochemistry. Free-floating sections were blocked for 1 hour at room temperature in Triton 0.3%/PBS and 3% normal donkey serum. Primary antibody (chicken anti-TH 1:1000; AB39702, Millipore, Temecula, CA) was incubated for 24 hrs at 4°C in Triton 0.3%/PBS and 3% normal donkey serum. Sections were then washed 4 times for 10 min each with PBS and incubated with secondary antibody (Cy3 or Alexa-647 donkey anti-chicken 1:1000; 703-605-155 Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) and a deoxyribonucleic acid (DNA) specific fluorescent probe (DAPI: 4',6-Diamidino-2-Phenylindole, 1:50,000) for 2 hours at room temperature. Sections were washed again for 4 x 10 min with PBS followed by mounting on microscope slides with PVA-DABCO. Fluorescence images were acquired using an Olympus FV1000 confocal laser scanning microscope using a 10x/0.40 NA oil-immersion objective. Mice without viral expression or mistargeted fiber placements were excluded from further analysis.

Statistics

Statistical analyses were performed using commercial software (GraphPad Prism, GraphPad Software, Inc, La Jolla, CA. Group comparisons were made using Kruskal-Wallis test, Bartlett's test, Brown-Forsythe test, Kolmogorov-Smirnov test, and repeated measures analysis

of variance (ANOVA), including one-way, and two-way ANOVAs as indicated. P-values reported reflect values corrected for the multiple comparisons using these methods. Significance thresholds are noted as $*p \leq 0.05$. All data are shown as mean ± SEM.

References

Badrinarayan, A., Wescott, S. A., Vander Weele, C. M., Saunders, B. T., Couturier, B. E., Maren, S., & Aragona, B. J. (2012). Aversive stimuli differentially modulate real-time dopamine transmission dynamics within the nucleus accumbens core and shell. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *32*(45), 15779–15790.

Belujon, P., & Grace, A. A. (2014). Restoring mood balance in depression: ketamine reverses deficit in dopamine-dependent synaptic plasticity. *Biological psychiatry*, *76*(12), 927–936.

Carnevali, L., Montano, N., Tobaldini, E., Thayer, J. F., & Sgoifo, A. (2020). The contagion of social defeat stress: Insights from rodent studies. *Neuroscience and biobehavioral reviews*, *111*, 12–18.

Chaudhury, D., Walsh, J. J., Friedman, A. K., Juarez, B., Ku, S. M., Koo, J. W., Ferguson, D., Tsai, H. C., Pomeranz, L., Christoffel, D. J., Nectow, A. R., Ekstrand, M., Domingos, A., Mazei-Robison, M. S., Mouzon, E., Lobo, M. K., Neve, R. L., Friedman, J. M., Russo, S. J., Deisseroth, K., … Han, M. H. (2013). Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature*, *493*(7433), 532–536.

Chohan, M. O., Esses, S., Haft, J., Ahmari, S. E., & Veenstra-VanderWeele, J. (2020). Altered baseline and amphetamine-mediated behavioral profiles in dopamine transporter Cre (DAT-Ires-Cre) mice compared to tyrosine hydroxylase Cre (TH-Cre) mice. *Psychopharmacology*, *237*(12), 3553–3568.

Daubner, S. C., Le, T., & Wang, S. (2011). Tyrosine hydroxylase and regulation of dopamine synthesis. *Archives of biochemistry and biophysics*, *508*(1), 1–12.

Fenno, L., Yizhar, O., & Deisseroth, K. (2011). The development and application of optogenetics. *Annual review of neuroscience*, *34*, 389–412.

Fosque, B. F., Sun, Y., Dana, H., Yang, C. T., Ohyama, T., Tadross, M. R., Patel, R., Zlatic, M., Kim, D. S., Ahrens, M. B., Jayaraman, V., Looger, L. L., & Schreiter, E. R. (2015). Neural circuits. Labeling of active neural circuits in vivo with designed calcium integrators. *Science (New York, N.Y.)*, *347*(6223), 755–760.

Grace, A. A., & Bunney, B. S. (1984). The control of firing pattern in nigral dopamine neurons: single spike firing. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, *4*(11), 2866–2876.

Grace A. A. (2000). The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. *Addiction (Abingdon, England)*, *95 Suppl 2*, S119–S128.

Grace, A. A., Floresco, S. B., Goto, Y., & Lodge, D. J. (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends in neurosciences*, *30*(5), 220–227.

Gunaydin, L. A., Grosenick, L., Finkelstein, J. C., Kauvar, I. V., Fenno, L. E., Adhikari, A., Lammel, S., Mirzabekov, J. J., Airan, R. D., Zalocusky, K. A., Tye, K. M., Anikeeva, P., Malenka, R. C., & Deisseroth, K. (2014). Natural neural projection dynamics underlying social behavior. *Cell*, *157*(7), 1535–1551.

Han, X., Jing, M. Y., Zhao, T. Y., Wu, N., Song, R., & Li, J. (2017). Role of dopamine projections from ventral tegmental area to nucleus accumbens and medial prefrontal cortex in reinforcement behaviors assessed using optogenetic manipulation. *Metabolic brain disease*, *32*(5), 1491–1502.

Hong, E., Santhakumar, K., Akitake, C. A., Ahn, S. J., Thisse, C., Thisse, B., Wyart, C., Mangin, J. M., & Halpern, M. E. (2013). Cholinergic left-right asymmetry in the habenulo-interpeduncular pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(52), 21171–21176.

Iñiguez, S. D., Riggs, L. M., Nieto, S. J., Dayrit, G., Zamora, N. N., Shawhan, K. L., Cruz, B., & Warren, B. L. (2014). Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. *Stress (Amsterdam, Netherlands)*, *17*(3), 247–255.

Klinger, K., Gomes, F. V., Rincón-Cortés, M., & Grace, A. A. (2019). Female rats are resistant to the long-lasting neurobehavioral changes induced by adolescent stress exposure. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*, *29*(10), 1127–1137.

Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., Laplant, Q., Graham, A., Lutter, M., Lagace, D. C., Ghose, S., Reister, R., Tannous, P., Green, T. A., Neve, R. L., Chakravarty, S., Kumar, A., Eisch, A. J., Self, D. W., Lee, F. S., … Nestler, E. J. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*, *131*(2), 391–404.

Lammel, S., Lim, B. K., Ran, C., Huang, K. W., Betley, M. J., Tye, K. M., Deisseroth, K., & Malenka, R. C. (2012). Input-specific control of reward and aversion in the ventral tegmental area. *Nature*, *491*(7423), 212–217.

Lammel, S., Lim, B. K., & Malenka, R. C. (2014). Reward and aversion in a heterogeneous midbrain dopamine system. *Neuropharmacology*, *76 Pt B*(0 0), 351–359.

Lammel, S., Steinberg, E. E., Földy, C., Wall, N. R., Beier, K., Luo, L., & Malenka, R. C. (2015). Diversity of transgenic mouse models for selective targeting of midbrain dopamine neurons. *Neuron*, *85*(2), 429–438.

Lindzey,G., Winston, H., & Manosevitz, M. (1961). Social dominance in inbred mouse strains. *Nature*, *191*, 474–476.

Liu, M. Y., Yin, C. Y., Zhu, L. J., Zhu, X. H., Xu, C., Luo, C. X., Chen, H., Zhu, D. Y., & Zhou, Q. G. (2018). Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nature protocols*, *13*(7), 1686–1698.

Mah, L., Szabuniewicz, C., & Fiocco, A. J. (2016). Can anxiety damage the brain?. *Current opinion in psychiatry*, *29*(1), 56–63.

Morales, M., & Margolis, E. B. (2017). Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. *Nature reviews. Neuroscience*, *18*(2), 73–85.

Morgan, C. T., King, R. A., Weisz, J. R., & Schopler, J. (1986). *Introduction to psychology*. New York: McGraw-Hill.

Muschamp, J. W., Van't Veer, A., Parsegian, A., Gallo, M. S., Chen, M., Neve, R. L., Meloni, E. G., & Carlezon, W. A., Jr (2011). Activation of CREB in the nucleus accumbens shell produces anhedonia and resistance to extinction of fear in rats. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, *31*(8), 3095–3103.

Omelchenko, N., & Sesack, S. R. (2006). Cholinergic axons in the rat ventral tegmental area synapse preferentially onto mesoaccumbens dopamine neurons. *The Journal of comparative neurology*, *494*(6), 863–875.

Padilla-Coreano, N., Batra, K., Patarino, M., Chen, Z., Rock, R., Zhang, R., Hausmann, S., Weddington, J., Patel, R., Zhang, Y., Fang, H., Keyes, L., Libster, A., Matthews, G., Curley, J., Fiete, I., Lu, C., Tye, K. (2020). A cortical-hypothalamic circuit decodes social rank and promotes dominance behavior. *Research Square*, 1-23.

Panzanelli, P., Homanics, G. E., Ottersen, O. P., Fritschy, J. M., & Sassoè-Pognetto, M. (2004). Pre- and postsynaptic GABA receptors at reciprocal dendrodendritic synapses in the olfactory bulb. *The European journal of neuroscience*, *20*(11), 2945–2952.

Ribble, D.O., Salvioni, M., (1990) Social organization and nest co-occupancy in *Peromyscus californicus*, a monogamous rodent. Behav Ecol Sociobiol 26: 9–15.

Rincón-Cortés, M., & Grace, A. A. (2017). Sex-Dependent Effects of Stress on Immobility Behavior and VTA Dopamine Neuron Activity: Modulation by Ketamine. *The international journal of neuropsychopharmacology*, *20*(10), 823–832.

Salatino-Oliveira, A., Rohde, L. A., & Hutz, M. H. (2018). The dopamine transporter role in psychiatric phenotypes. *American journal of medical genetics. Part B, Neuropsychiatric genetics: the official publication of the International Society of Psychiatric Genetics*, *177*(2), 211–231.

Serdar, C. C., Cihan, M., Yücel, D., & Serdar, M. A. (2021). Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. *Biochemia medica*, *31*(1), 010502.

Seyed Hosseini, E., Riahi Kashani, N., Nikzad, H., Azadbakht, J., Hassani Bafrani, H., & Haddad Kashani, H. (2020). The novel coronavirus Disease-2019 (COVID-19): Mechanism of action, detection and recent therapeutic strategies. *Virology*, *551*, 1–9.

Solomon M. B. (2017). Evaluating social defeat as a model for psychopathology in adult female rodents. *Journal of neuroscience research*, *95*(1-2), 763–776.

Stephens, M. A., & Wand, G. (2012). Stress and the HPA axis: role of glucocorticoids in alcohol dependence. *Alcohol research: current reviews*, *34*(4), 468–483.

Stress in America™ 2020: A National Mental Health Crisis. (2021).

Trainor B. C. (2011). Stress responses and the mesolimbic dopamine system: social contexts and sex differences. *Hormones and behavior*, *60*(5), 457–469.

Trainor, B. C., Pride, M. C., Villalon Landeros, R., 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), e17405.

Tsai, H. C., Zhang, F., Adamantidis, A., Stuber, G. D., Bonci, A., de Lecea, L., & Deisseroth, K. (2009). Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science (New York, N.Y.)*, *324*(5930), 1080–1084.

Tye, K. M., Mirzabekov, J. J., Warden, M. R., Ferenczi, E. A., Tsai, H. C., Finkelstein, J., Kim, S. Y., Adhikari, A., Thompson, K. R., Andalman, A. S., Gunaydin, L. A., Witten, I. B., & Deisseroth, K. (2013). Dopamine neurons modulate neural encoding and expression of depression-related behaviour. *Nature*, *493*(7433), 537–541.

Wichmann, R., Vander Weele, C. M., Yosafat, A. S., Schut, E. H. S., Verharen, J. P. H., Sridharma, S., Siciliano, C. A., Izadmehr, E. M., Farris, K. M., Wildes, C. P., Kimchi, E. Y., Tye, K. M. (2017). Acute stress induces long-lasting alterations in the dopaminergic system of female mice. *BioRxiv preprint*. Manuscript submitted for publication.

Wickham, R., Solecki, W., Rathbun, L., McIntosh, J. M., & Addy, N. A. (2013). Ventral tegmental area α6β2 nicotinic acetylcholine receptors modulate phasic dopamine release in the nucleus accumbens core. *Psychopharmacology*, *229*(1), 73–82.

Willner, P., Hale, A. S., & Argyropoulos, S. (2005). Dopaminergic mechanism of antidepressant action in depressed patients. *Journal of affective disorders*, *86*(1), 37–45.

Yin, R., & French, E. D. (2000). A comparison of the effects of nicotine on dopamine and non-dopamine neurons in the rat ventral tegmental area: an in vitro electrophysiological study. *Brain research bulletin*, *51*(6), 507–514.