Social Transmission of a Stress-Related Neuroadaptation

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Abstract

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Stress is highly pervasive in humans, impacting motivated behaviors with an enormous toll on life quality. Many of the effects of stress are orchestrated by neuropeptides such as corticotropin-releasing factor (CRF). It has previously been shown that in stress-naïve male mice, CRF acts in the core of the nucleus accumbens (NAc) to produce appetitive effects and to increase dopamine release; yet in stress-exposed male mice CRF loses its capacity to modulate NAc dopamine release and is aversive. In the current research we tested whether this effect is comparable in females to males, and whether the neuroadaptation is susceptible to social transmission. We found that, like in males, CRF increased dopamine release in stress-naïve but not stress-exposed female mice. Importantly, this persistent physiological change was not accompanied by overt behavioral changes that would be indicative of a depression- or anxiety-like phenotype. Nonetheless, when these mice were housed for seven days with stress-naïve conspecifics, the cage mates also exhibited a loss of dopamine potentiation by CRF. These data demonstrate asymptomatic, yet pervasive, transmission of stress-related neuroadaptations in the population.

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Glossary

Abbreviation	Word
5-HT	Serotonin
aCSF	Artificial cerebrospinal fluid
ASVG-30	Antisauvagine-30
BNST	Bed nucleus of the stria terminalis
CeA	Central nucleus of the amygdala
CMS	Chronic mild stress
CNO	Clozapine N-oxide
CNS	Central nervous system
FSS	Forced swim stress
CPA	Conditioned place aversion
CPP	Conditioned place preference
CRF	Corticotropin-releasing factor
CRFR1	CRF receptor type 1
CRFR2	CRF receptor type 2
CSF	Cerebrospinal fluid
DREADD	Designer receptor exclusively activated by designer drugs
DRN	Dorsal raphe nucleus
EPM	Elevated plus maze
FSCV	Fast-scan cyclic voltammetry
BLA	Basolateral amygdala
GCaMP	Genetically encoded calcium indicator
HPA axis	Hypothalamic-adrenal-pituitary axis
ICV	Intracerebroventricular
LC	Locus coeruleus
LS	Lateral septum
NAc	Nucleus accumbens
PVT	Paraventricular nucleus of the thalamus
rFSS	Repeated forced swim stress
R-I assay	Resident-intruder assay
SDS	Social defeat stress
TST	Tail suspension test
vlVMH	Ventrolateral division of the ventromedial hypothalamus

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Chapter 1. Introduction

CRF activates the neuroendocrine stress response via the HPA axis

The biological stress response is an intricate and tightly orchestrated adaptation that has presumably been influenced by natural selection to enhance the ability of organisms to cope with novel situations that require action or defense. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis by stimulating the release of the neuropeptide corticotropin-releasing factor (CRF) from parvocellular neurons of the paraventricular nucleus of the hypothalamus (PVH), the primary site of CRF-containing cell bodies (Merchenthaler, 1984; Swanson & Simmons, 1989), into portal circulation for delivery to the anterior pituitary, where CRF triggers the immediate release of adrenocorticotropic hormone (ACTH) (Holsboer & Barden, 1996; Owens & Nemeroff, 1991; Rivier & Vale, 1983; Souza, 1995; Vale et al., 1981). ACTH, in turn, acts on the cortex of the adrenal gland to stimulate the release of the glucocorticoid hormone cortisol into the bloodstream. Increased production of cortisol during stress enhances glucose availability, helps maintain blood pressure, reduces inflammation in response to injury, and suppresses the immune response to enable a rapid response to potentially threatening stimuli (Chrousos, 2009). A negative feedback loop, consisting of cortisol acting at the level of the anterior pituitary and the PVH, prevents prolonged activation of the HPA axis.

CRF and its receptors CRF-R1 and -R2 are expressed in the central nervous system

In addition to and independent from its well-established role in regulating the HPA axis, CRF is released in the brain in response to stress and other arousing environmental

stimuli (Cook, 2004; Holly et al., 2016; Merali et al., 2004; Ohmura et al., 2009; B. Wang et al., 2005), where it can act at its centrally-expressed receptors CRFR1 and CRFR2 (Henckens et al., 2016; Sierra et al., 2015; Steckler & Holsboer, 1999; Van Pett et al., 2000) to direct a vast array of adaptive and maladaptive behavioral responses (Hostetler & Ryabinin, 2013). Three CRF orthologs have been identified: urocortin 1 (Vaughan et al., 1995), urocortin 2 (Reyes et al., 2001), and urocortin 3 (Lewis et al., 2001). Whereas CRF binds to both CRFR1 and CRFR2, but with greater affinity for CRFR1, urocortin 1 binds to CRFR1 and CRFR2 with equal affinity, and urocortin 2 and urocortin 3 are selective ligands for CRFR2.

In extra-hypothalamic brain regions, the function of CRF is much less straightforward, as its release can have opposite physiological and behavioral consequences depending on the brain region or cell type comprising the site of action as well as the state/condition of the animal (e.g., whether it has been exposed to acute, chronic, or no stress).

For example, in rodents, intracerebroventricular (ICV) administration of CRF has a multitude of behavioral effects, including decreased food consumption (Britton et al., 1982; Krahn et al., 1990) and weight gain (Cullen et al., 2001), conditioned place aversion (Cador et al., 1992; Land et al., 2008), reduced exploration of novel stimuli or environments (Berridge & Dunn, 1986; Britton et al., 1982), increased grooming (Britton et al., 1982), increased locomotor activity (Sutton et al., 1982), and increased anxiety-like behavior (Bruchas et al., 2009). ICV drug infusions bypass the blood-brain barrier and allow for central drug administration via the cerebrospinal fluid/ventricular system. Drugs that are injected ICV diffuse into the proximal brain parenchyma with drug penetration

decreasing exponentially with distance from the ventricular surface (Blasberg et al., 1975). Despite the lack of regional specificity conferred by ICV drug administration, the studies summarized above nonetheless provide a foundational understanding of the capacity of central CRF to influence a vast array of behaviors.

Numerous studies have investigated the physiological and behavioral consequences of CRF release and/or CRFR signaling in extra-hypothalamic regions including the bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), dorsal raphe nucleus (DRN), and nucleus accumbens (NAc).

In the BNST, CRFR1 signaling increases glutamatergic transmission onto VTA-projecting BNST neurons (Kash et al., 2008; Silberman et al., 2013) and is anxiogenic (Sahuque et al., 2006). It has been shown that local administration of CRF engages CRFR1 to induce reinstatement of cocaine seeking (Erb & Stewart, 1999). Compared to CRFR1 signaling, the function of CRFR2 in the BNST is comparatively less straightforward as there is evidence to support both anxiogenic and anxiolytic roles for CRFR2 signaling (Henckens et al., 2016).

As in the BNST, CRFR1 signaling in the CeA is implicated in the control of anxiety-like behavior. Knockdown of CRFR1 mRNA in the CeA (via infusion of an antisense oligodeoxynucleotide corresponding to rat CRHR1 mRNA) has been shown to reduce anxiety-like behavior in rats that were subjected to social defeat stress (Liebsch et al., 1995), indicating that CRFR1 signaling in the CeA is anxiogenic. CRFR1 signaling has been shown to mediate both glutamatergic and GABAergic transmission. The reported heterogeneity of the effects of CRFR1 signaling on CeA neuronal activity is thought to

reflect the heterogenous neuronal subpopulations in this region as well as stress-related changes in CRF sensitivity (as reviewed by Yuval Silberman & Winder, 2015).

The CeA contains a population of CRF-expressing neurons that project to the LC where the release of CRF increases tonic LC activity and anxiety-like behavior via CRFR1 signaling (McCall et al., 2015).

The DRN is also innervated by CRF-expressing neurons whose axon terminals primarily contact GABA-containing (rather than 5-HT-containing) dendrites (Waselus et al., 2005) to ultimately inhibit DRN discharge and decrease 5-HT release in the NAc (Lukkes et al., 2008), lateral striatum (Price et al., 1998) and the lateral septum (Price & Lucki, 2001). In the DRN, low concentrations of CRF selectively activate CRFR1 to produce the inhibitory physiological effects described above. However, higher concentrations of CRF engage CRFR2 receptors in the DRN to ultimately enhance neuronal activity (Kirby et al., 2000). The opposing actions of CRFR1 and CRFR2 are hypothesized to facilitate active and passive behavioral coping, respectively (Valentino & Commons, 2005). In response to swim stress, redistribution of the CRFR subtypes occurs: CRFR1, following activation via CRF release due to swim stress exposure, is internalized whereas CRFR2 is trafficked from the cytoplasm to the plasma membrane (Waselus et al., 2009). Indeed, this redistribution, which occurs within twenty-four hours of the swim stress, corresponds to 1) the shift in DRN neuronal responsivity to CRF (from inhibition to excitation), and 2) the shift in coping strategies/mechanisms (from active to passive) that may be used to adaptively respond to a subsequent stressor.

Previous studies demonstrate that, through its actions in the NAc, CRF can increase mesolimbic dopamine release in a CRFR1- and CRFR2-dependent manner

(Lemos et al., 2012, 2019) as well as promote appetitive behaviors in the form of accelerated partner preference in monogamous prairie voles (Lim et al., 2007), enhanced Pavlovian-Instrumental transfer (Peciña et al., 2006), and conditioned place preference for local administration of NAc CRF (vs. NAc vehicle; Lemos et al., 2012). These effects are modulated by a prior stress experience: following exposure to a stressor, CRF loses the ability of to increase mesolimbic dopamine release (Lemos et al., 2012, 2019) and produces an aversive behavioral response as measured with the conditioned place aversion assay (Lemos et al., 2012).

Dysfunction of the CRF system underlies stress-related pathologic sequelae

Importantly, experience-dependent dysregulation of the CRF system has been posited as a major contributor to vulnerability for stress hyperresponsivity as well as a variety of psychiatric disorders including depression, anxiety disorders including posttraumatic stress disorder (PTSD), eating disorders, and substance use disorders. One clinical indicator of a CRF-related pathology is that in the disease state there is elevated CRF in the cerebrospinal fluid (CSF) compared to healthy controls. The first support for a connection between CRF signaling and depression was described in a study conducted by Nemeroff and colleagues in 1984. Here, the investigators found that treatment-naïve individuals with diagnosed depression presented with significantly elevated concentrations of CRF in the CSF compared to patients with other psychiatric conditions and healthy controls (Nemeroff et al., 1984). Clinical studies that investigated the role of CRF signaling in anxiety disorders have found that CSF CRF concentrations are increased in PTSD but not in panic disorder and generalized anxiety disorder (Baker et al., 1999; Bremner et al., 1997; Fossey et al., 1996). Additionally, elevated levels of

CSF CRF have similarly been reported in anorexic patients compared to healthy individuals (Hotta et al., 1986). At present, there have not been any studies that have measured CSF CRF within the context of substance abuse disorder. However, there has been one study that measured CSF CRF in alcohol-dependent patients that were undergoing acute withdrawal and then again following three weeks of abstinence and found that CSF CRF levels were significantly higher during acute withdrawal compared to three weeks after the cessation of alcohol consumption (Adinoff et al., 1996).

Stress-related pathologies and CRF signaling in females

Stress vulnerability has a high degree of sexual dimorphism, with females being generally more stress-sensitive than males to psychiatric pathologic sequelae (Bale & Epperson, 2015; Hodes & Epperson, 2019). This increased vulnerability has been attributed to neuroendocrine effects in women, at least in part, because there are specific windows of vulnerability during periods of extraordinary hormonal flux, such as adolescence, menopause, pregnancy and the post-partum period (Young & Korszun, 2010). Changes in vulnerability are also observed across the estrous cycle, as indicated by the incidence of premenstrual syndrome and premenstrual dysphoric disorder (American Psychiatric Association, 2013). However, the interaction between ovarian steroids and stress is complex. There are reports that rising levels of estrogen during proestrus increases the sensitivity of cognitive function to stress (Shansky et al., 2004; Shors et al., 1998). In contrast, other studies suggest that estrogen plays a protective role against the effects of stress on cognition (Bowman et al., 2002; Wei et al., 2014). Moreover, the sexually dimorphic effects of stress on mental health are not simply quantitative (i.e., males and females show the same pattern of traits but to different

extents) but also qualitative (i.e., a process varies fundamentally by sex or is only present in one sex) (Beltz et al., 2019). For instance, whereas stress is more likely to trigger the onset of depressive disorders in females than males (Kessler et al., 2003), it is more likely to precipitate substance use disorders in males than females (Mchugh et al., 2018).

With regard to CRF function, sex differences are observed in the expression of CRF peptide (Dunčko et al., 2001; Iwasaki-Sekino et al., 2009; Sterrenburg et al., 2012; Viau et al., 2005), and the trafficking and signal-transduction coupling of CRF receptors (Bangasser et al., 2010; Valentino et al., 2013). Collectively, these sexually dimorphic effects indicate that the identification of neural mechanisms underlying stress-induced psychopathological traits in males has limited predictive capacity for females.

Animal models of stress-related pathologies

Animal models of psychiatric diseases have traditionally been evaluated using three criteria: construct validity, face validity, and predictive validity (Willner, 1984). Briefly, construct validity is how well the mechanism used to induce the disease phenotype in animals reflects the currently understood disease etiology in humans, face validity refers to how well a model replicates the disease phenotype in humans, and predictive validity is defined as the measure of how well a model can be used to predict future disease manipulations (e.g., therapeutic outcomes) in humans.

The three principal animal models of stress-related pathologies are 1) chronic mild stress (CMS), 2) social defeat stress (SDS), and 3) physical, non-social stress (also known as learned helplessness). Three varieties of physical, non-social stress are widely used: foot shock shuttle box, restraint stress, and forced swim stress (FSS).

CMS is considered to have very good construct validity, face validity, and predictive validity and it is thus regarded as the animal model of stress-induced depression with the greatest translational potential. This model consists of exposing animals to a variety of mild stressors, such as periods of food or water deprivation, wet bedding, noise, cold temperatures, cage tilt, light-dark cycle reversal, and others over a period of several weeks. Exposure to this stress paradigm causes animals to reduce their sensitivity to natural rewards, reflecting one facet of depression as it manifests in humans. One barrier to widespread utilization that this model faces, however, is a lack of reproducibility both in an intra-lab and an inter-lab sense (Willner, 2017).

SDS has good predictive validity for therapeutic outcomes in men but not women. One shortfall of this model is that only male rodents can be reliably used since females in most rodent species are not inherently aggressive. Investigators have tried, with varying degrees of success, to develop SDS models that are effective in females.

In the foot shock shuttle box model (variant of physical, non-social stress/learned helplessness), animals are subjected to repeated, inescapable, and uncontrollable foot or tail shocks over the course of one or several sessions or days. Eventually, animals subjected to this stressor develop depression-like behavior in the form of learned helplessness: when animals are eventually given the opportunity to avoid the aversive stimulus they do not attempt to do so (Maier & Watkins, 2005). The Porsolt FSS has traditionally been conceptualized as an additional form of physical, non-social stress (Porsolt et al., 1977). As animals progress through the swim stress sessions they transition from using active coping strategies (i.e., swimming and attempting to climb out of the water bucket) to passive coping strategies (i.e., floating/immobility). With increasing

exposure to FSS sessions, the bouts of immobility increase in frequency and duration (Lemos et al., 2012; Mul et al., 2016). This model has good predictive validity, as treatment with antidepressants promotes active rather than passive coping during swim stress (Nestler et al., 2002). However, this model lacks strong face and construct validity. Additionally, the progressive increase in immobility that is observed over time has been posited to instead reflect an adaptive, learned behavioral response and not depression-like behavior (Molendijk & de Kloet, 2015).

Finally, stress vulnerability can extend beyond individuals who directly engage with a conventional stressor. Indeed, stress effects can be broadcast between domestic partners (Bolger et al., 1989). Stress transmission between individuals in a population is a phenomenon that has also been documented and modeled in non-human animals wherein behavioral, endocrine, and/or physiological changes have been engendered in conspecifics of stressed individuals (Bartal et al., 2011; Bruchey et al., 2010; Burkett et al., 2016; Jeon et al., 2010; Langford et al., 2006; Sterley et al., 2018; Zalaquett & Thiessen, 1991). This model has promising construct validity, as it relies on social transmission and can be studied in both sexes.

Chapter 2. Social transmission of a stress-related neuroadaptation

Introduction

Stress is a ubiquitous condition that has serious physical and mental health consequences. Some stressors have clear effects on behavior in some individuals, whereas others induce more nuanced effects that can remain undetected by an observer. The current research describes a process wherein mice that are subjected to a physical stressor exhibit a robust physiological adaptation in the absence of discernable behavioral consequences. Moreover, this stress-related neuroadaptation can subsequently be transmitted to stress-naïve conspecifics, indicating insidious contagion throughout populations. Thus, analogous to the transmission of some deadly viruses, there can be asymptomatic spread of stress-related pathology within communities.

Results

To ascertain whether the effects of CRF on dopamine release in the NAc are sexually dimorphic, we selectively monitored dopamine release evoked by a single biphasic electrical pulse in acute coronal brain slices collected from stress-naïve animals (Fig. 2.1A) using fast-scan cyclic voltammetry at carbon-fiber microelectrodes. Vehicle (0.07% acetic acid in artificial cerebrospinal fluid) or CRF (100 nM or 1 µM) was applied to the slice for 30 minutes after a stable baseline was achieved with four consecutive dopamine recordings where the peak responses were within 10% of each other. In stress-naïve males, it has been shown that CRF increases NAc dopamine release in a concentration-dependent manner with maximal effects at 100 nM (Lemos et al., 2012). We replicated this effect with 100 nM CRF, which significantly increased dopamine

release (Fig. 2.1B) relative to vehicle beginning 12 minutes after drug application and persisting for the duration of the recording (two-way repeated measures analysis of variance (RM ANOVA) with Bonferroni's post-hoc multiple comparisons tests; interaction of Time x Drug, $F_{15, 270} = 7.651$, $P<0.0001$; main effect of time, $F_{15, 270} = 6.374$, $P<0.0001$; main effect of drug, $F_{1, 18} = 11.41$, $P = 0.0034$; Fig. 2.1C). The resultant effect was quantified by averaging the evoked maximum dopamine current in the final 10 minutes of the recording (unpaired t-test; $t(18) = 3.387$, $P = 0.0033$; Fig. 2.1D). The interaction between CRF and dopamine has not been previously examined in females. Therefore, we tested the effect of 100 nM and 1 μM CRF on NAc dopamine release. Similar to males, CRF increased NAc dopamine (Fig. 2.1E) over time relative to vehicle treatment (two-way RM ANOVA with Bonferroni's post-hoc tests; interaction of Time x Drug, $F_{30, 330} = 2.146$, $P<0.001$; main effect of time, $F_{4.551, 100.1} = 2.459$, $P = 0.0431$; main effect of drug, $F_{2, 22} = 12.69$, $P<0.001$; Fig. 2.1F) and this effect was concentration-dependent (one-way ANOVA with Dunnett's post-hoc tests; $F_{2, 23} = 10.36$, $P<0.001$; Fig. 2.1G). In striatal slices from females, the effect of 100 nM CRF appeared to be modest. However, when appropriately accounting for vehicle, comparison of this effect between sexes was not statistically significant (two-way ANOVA with Bonferroni's post-hoc tests; interaction of Sex x Drug, $F_{1, 34} = 2.750$, $P = 0.1064$; Fig. S2.1). These data indicate that the effect of CRF on NAc dopamine release is qualitatively similar across sexes without significant quantitative sex differences.

Co-activation of CRF's cognate receptors CRFR1 and CRFR2 was previously found to be required for CRF-mediated potentiation of NAc dopamine release in stress-naïve males (Lemos et al., 2012). Therefore, we sought to characterize the receptor

subtypes that are responsible for mediating this response in stress-naïve females. We attempted to replicate these historical findings in stress-naïve males by bath-applying the selective CRFR1 antagonist antalarmin or the selective CRFR2 antagonist antisauvagine-30 (ASVG-30) to the striatal slice beginning at least 20 minutes before CRF application. In disagreement with historical data, which demonstrates that application of 1 μM antalarmin or 250 nM ASVG-30 prevented CRF-mediated potentiation of NAc dopamine release in stress-naïve males, application of antalarmin (1 or 2 μM) or ASVG-30 (250 nM, 500 nM, or 1 μM), failed to block the CRF-induced increase in NAc dopamine release (one-way ANOVA with Dunnett's post-hoc tests; antalarmin: $F_{2, 22} = 1.712$, $P = 0.2037$; ASVG-30: $F_{3, 25} = 1.555$, $P = 0.2250$; Fig. 2.2A and B). However, co-application of antalarmin and ASVG-30 to slices collected from stress-naïve males appeared to attenuate CRF-induced dopamine release (one-way ANOVA with Dunnett's post-hoc tests: $F_{2, 17} = 3.123$, $P = 0.0700$; Fig. 2.2C). In stress-naïve females, application of antalarmin (2 μM) and ASVG-30 (500 nM) did not affect dopamine release in response to 1 μM CRF (one-way ANOVA with Dunnett's post-hoc tests: $F_{2, 15} = 1.988$, $P = 0.1715$; Fig. 2.2D). Unfortunately, we were unable to evaluate the effect of co-application of antalarmin and ASVG-30 on CRF-induced potentiation of dopamine release in stress-naïve females. These data thus indicate that the receptor subtypes responsible for mediating CRF-induced potentiation of NAc dopamine release are qualitatively similar across sexes.

In striatal slices from males, the effect of CRF on NAc dopamine release was previously found to be sensitive to prior repeated forced swim stress (rFSS) experience (Lemos et al., 2012). In the present experiments, we tested whether this effect generalized to females using a direct stressor in the form of rFSS (Fig. 2.3A). We

observed escalating increases in immobility over rFSS sessions in all animals. This effect was not different between sexes (two-way RM ANOVA with Bonferroni's post-hoc tests; interaction of Swim Session x Sex, $F_{4, 56} = 0.5415$, $P = 0.7059$; Fig. 2.3B). One week after direct rFSS exposure animals were evaluated for changes in CRF-induced modulation of evoked NAc dopamine release. In striatal slices from males, there was a significant interaction between time and drug in the absence of significant main effects of time and drug (two-way RM ANOVA with Bonferroni's post-hoc tests; interaction of Time x Drug, $F_{15, 120} = 3.606$, $P<0.0001$; main effect of time, $F_{4.011, 32.09} = 1.100$, $P = 0.3734$; main effect of drug, $F_{1, 8} = 0.011$, $P = 0.9190$; Fig. 2.3C). This is a qualitative replication of previous findings (Lemos et al., 2012). Similarly, 100 nM and 1 μM CRF did not significantly increase evoked NAc dopamine in direct stress-exposed females over time relative to vehicle (two-way RM ANOVA with Bonferroni's post-hoc tests; interaction of Time x Drug, $F_{30, 180} = 1.169$, $P = 0.2632$; Fig. 2.3D). Therefore, we did not observe sexual dimorphism in this stress-related neurochemical adaptation.

Next, we tested whether the CRF/dopamine interaction was sensitive to stress experienced indirectly (i.e., via social transmission from animals exposed to a direct, physical stressor). To induce indirect stress, mice were housed with cage mates that underwent rFSS (Fig. 2.4A). Again, we observed escalating increases in immobility over rFSS sessions in all animals. This effect was not different between sexes in the directly stressed group that was used to provide indirect stress (two-way RM ANOVA with Bonferroni's post-hoc tests; interaction of Swim Session x Sex, $F_{4, 48} = 0.4469$, $P = 0.7741$; Fig. 2.4B). Moreover, no differences in swim immobility were observed between the direct stress groups (two-way RM ANOVA with Bonferroni's post-hoc tests; interaction of Swim

Session x Sex, $F_{4, 112} = 1.688$, $P = 0.1577$; Fig. 2.4C), indicating that cohousing with rFSS-naïve animals after the first stress session did not impact swim immobility behavior of stress 'donors' on day two. Surprisingly, the CRF-dopamine interaction was also lost in indirectly stressed animals when evaluated one week after their cage mates were exposed to direct stress. This lack of effect was present in both males and females (two-way RM ANOVA with Bonferroni's post-hoc tests; males: interaction of Time x Drug, $F_{15, 120} = 1.428$, $P = 0.1449$; females: interaction of Time x Drug, $F_{30, 270} = 1.150$, $P = 0.2761$; Fig. 2.4D and E). Thus, these data indicate that social transmission of the stress-related physiological adaptation had occurred in both sexes and, like with the direct stress effect, was not sexually dimorphic.

When data from male and female groups were combined to increase power for statistical analysis, these results held up. CRF significantly increased evoked NAc dopamine release in stress-naïve animals over time relative to vehicle (two-way RM ANOVA with Bonferroni's post-hoc tests; interaction of Time x Drug, $F_{15, 540} = 6.686$, $P<0.0001$; main effect of time, $F_{15, 540} = 5.018$, $P<0.0001$; main effect of drug, $F_{1, 36} = 18.47$, $P = 0.0001$; Fig. 2.5A). Although we observed a significant interaction between time and drug in animals tested one week after direct and indirect stress exposure, there were not significant main effects of time or drug (two-way RM ANOVA with Bonferroni's post-hoc tests. Direct stress: interaction of Time x Drug, $F_{15, 270} = 5.525$, $P<0.0001$; main effect of time, $F_{15, 270} = 1.309$, $P = 0.1960$; main effect of drug, $F_{1, 18} = 1.403$, $P = 0.2517$. Indirect stress: interaction of Time x Drug, $F_{15, 315} = 1.740$, $P = 0.0426$; main effect of time, $F_{15, 315} = 1.306$, $P = 0.1966$; main effect of drug, $F_{1, 21} = 0.5198$, $P = 0.4789$; Fig. 2.5B and C). Directly comparing the resultant effects of 100 nM CRF versus vehicle on evoked NAc

dopamine release in the final 10 minutes of the recording between stress pretreatment groups, we found that CRF significantly increased NAc dopamine relative to vehicle in stress-naïve mice, but this effect was attenuated in direct stress- and indirect stress-exposed groups, resulting in a significant group by drug interaction (two-way ANOVA with Bonferroni's post-hoc tests; interaction of Stress Pretreatment x Drug, $F_{2, 75} = 5.690$, $P = 0.0050$; main effect of pretreatment, $F_{2, 75} = 6.736$, $P = 0.0020$; main effect of drug, $F_{1, 75} = 13.48$, $P<0.001$; Fig. 2.5D). We identified the estrous cycle stage in a subset of the female mice used in these experiments but did not observe an effect of cycle stage on CRF's regulation of NAc dopamine release (one-way ANOVA with Dunnett's post-hoc tests; stress-naïve: $F_{2, 23} = 10.36$, $P<0.001$; direct stress: $F_{2, 13} = 1.588$, $P = 0.2145$; indirect stress: $F_{2, 19} = 1.236$, $P = 0.3130$; Fig. S2.2A through C). Thus, as assayed here, the consequences of stress were especially pervasive as not only did stress perturb a veritable neurochemical interaction in the animals that were directly exposed to rFSS, but also in their cage mates.

Given that the neurochemical perturbations were robust in both direct and indirect stress conditions, we sought to compare the behavioral impact of these manipulations. First, we investigated whether stress exposure affects social interaction using the three-chamber social approach assay (Fig. 2.6A) where the relative time that mice interact with a novel mouse versus a novel object was assessed. Since the neurochemical changes in the indirectly stressed animals likely arose through social interactions, we hypothesized that the performance of this group would be particularly affected in the social approach task. However, when tested one week after stress exposure, neither directly nor indirectly stressed mice exhibited sociability deficits when males and females were combined for

analysis (two-way RM ANOVA with Bonferroni's post-hoc tests; interaction of Stress Pretreatment x Zone, $F_{2, 18} = 2.005$, P = 0.1570; Fig. 2.6B). This was also the case when male and female groups were analyzed independently (two-way ANOVA with Holm-Sidak's post-hoc tests; males: interaction of Stress Pretreatment x Zone, $F_{2, 12} = 1.542$, P = 0.2534; females: interaction of Stress Pretreatment x Zone, $F_{2, 18} = 2.534$, P = 0.1072; Fig. S2.3A and B). Notably, we did not observe sexual dimorphism in this behavior as the direct comparison of sociability exhibited by males versus females failed to reach significance (two-way ANOVA with Bonferroni's post-hoc tests; interaction of Stress Pretreatment x Sex, $F_{2, 15} = 0.8526$, P = 0.4460; Fig. S2.3C).

Next, we utilized the tail suspension test (Fig. 2.6C) to evaluate depression-like behavior in the form of immobility. When male and female groups were combined for analysis, stress exposure had no effect on depression-like behavior compared to stress-naïve controls (one-way ANOVA with Dunnett's post-hoc tests; $F_{2, 28} = 0.1883$, P = 0.8294; Fig. 2.6D). Similarly, we did not observe differences in immobility between stressed animals and stress-naïve controls when males and females were evaluated separately (one-way ANOVA with Dunnett's post-hoc tests; males: $F_{2, 14} = 0.2990$, P = 0.7462; females: $F_{2, 11} = 1.921$, P = 0.1925; Fig. S2.4A and B). We did not observe sexual dimorphism in this behavior as the direct comparison of immobility in males versus females failed to reach significance (two-way ANOVA with Bonferroni's post-hoc tests; interaction of Stress Pretreatment x Sex, $F_{2, 25} = 1.804$, P = 0.1854; Fig. S2.4C).

To assess whether mice exhibit anxiety-like behavior following stress exposure we used the elevated plus maze (EPM; Fig. 2.6E), where a decrease in open-arm time is characteristic of an anxiety-like state. We found that stress exposure did not affect

locomotor activity in the EPM when male and female groups were combined (one-way ANOVA with Dunnett's post-hoc tests; $F_{2, 83} = 0.3935$, $P = 0.6760$; Fig. 2.6F), as well as when males and females were evaluated separately (one-way ANOVA with Dunnett's post-hoc tests; males: $F_{2, 40} = 0.8016$, $P = 0.4557$; females: $F_{2, 40} = 0.0733$, $P = 0.9295$; Fig. S2.5A and B), indicating that all groups explored the apparatus. Combining males and females for analysis, our examination of open arm time revealed that indirectly stressed animals spent 24% of total time in the open arms of the EPM, which was significantly less than the directly stressed mice who spent 38% of total time in the open arms (although neither group were different to stress-naïve mice who spent 30% of total time in the open arms), indicating that indirectly stressed animals exhibited significantly more anxiety-like behavior than mice that were subjected to direct stress (one-way ANOVA with Tukey's post-hoc tests; $F_{2, 83} = 3.969$, $P = 0.0226$; Fig. 2.6G). This effect was driven by males in the indirectly stressed group, who spent significantly less time in the open arms of the EPM (20% of total time) compared to stress-naïve and directly stressed males who spent 38% and 41% of total time in the open arms, respectively (one-way ANOVA with Dunnett's post-hoc tests; $F_{2, 40} = 4.477$, $P = 0.0176$; Fig. S2.5C). In contrast to males, direct and indirect stress had no effect on anxiety-like behavior in females as compared to stress-naïve controls (one-way ANOVA with Dunnett's post-hoc tests; $F_{2, 40} = 2.466$, $P = 0.0977$; Fig. S2.5D). Direct comparison of open arm time between sexes revealed that whereas stress-naïve females spent significantly less time in the open arms of the EPM than stress-naïve males, this sex difference in open arm exploration was normalized by stress exposure, resulting in a significant group by sex interaction (two-way ANOVA with Bonferroni's post-hoc tests; interaction of Stress Pretreatment x Sex,

$F_{2, 80} = 3.240$, $P = 0.0443$; main effect of stress pretreatment, $F_{2, 80} = 4.230$, $P = 0.0179$; main effect of sex, $F_{1, 80} = 1.733$, $P = 0.1918$; Fig. S2.5E). A subset of the directly and indirectly stressed mice used in the EPM experiments were subsequently used for voltammetry experiments. Notably, vehicle- and CRF-mediated NAc dopamine release did not significantly differ between EPM-naïve and EPM-exposed directly stressed and indirectly stressed mice (two-way ANOVA with Bonferroni's post-hoc tests; direct stress: interaction of Drug x EPM exposure, $F_{1, 17} = 0.7162$, $P = 0.4092$; indirect stress: interaction of Drug x EPM exposure, $F_{1, 19} = 0.4550$, $P = 0.5081$; Fig. S2.6A and B). Thus, following stress exposure we did not observe behavioral differences between males and females despite preexisting sex differences in anxiety-like behavior in stress-naïve animals. Overall, these results demonstrate the absence of rFSS-induced enduring, overt behavioral manifestations despite the robust physiological changes that can be broadcast to other members of the population.

We designed and carried out an additional behavioral experiment to evaluate if stress-induced dysregulation of CRF-dopamine interactions in the NAc disrupts a learned behavior. This assay allowed us to test the hypothesis that CRF in the NAc functions as a salience signal to promote dopamine release and learning, ultimately leading to an adaptive behavioral response. Thus, it would follow that in the stress-naïve state, when NAc CRF-induced dopamine release is intact, a mouse placed in a mildly aversive context wherein an auditory cue predicts the onset of an escapable foot shock would learn to make an adaptive behavioral response. However, stress exposure and the resultant disruption of NAc CRF-induced dopamine release would impair learning and therefore the ability to respond in an adaptive manner. To carry out this assay, we used a two-

chamber apparatus with an experimenter-controlled door (connecting the two chambers) and electrified grid floor (Fig. 2.7A). Mice could explore the apparatus before being briefly confined to one chamber where they were exposed to a conditioned stimulus (CS; tone)-unconditioned stimulus (US; shock) pairing (Fig. 2.7B). The door leading to the non-shock chamber was opened at the onset of the CS-US pairing, allowing for escape. Four pairings in total occurred during the training session. A probe test was conducted twenty-four hours later wherein mice were again placed in the two-chamber apparatus and following a brief period of exploration they were momentarily confined to the shock chamber. The CS was played and latency to reach the door leading to the non-shock chamber was measured. We found, using male mice, that direct stress exposure did not affect locomotor activity in the apparatus (unpaired t-test; $t(4) = 1.679$, $P = 0.1684$; Fig. 2.7C) or time spent in the apparatus zones (i.e., shock chamber, non-shock chamber, and door; two-way RM ANOVA with Sidak's post-hoc tests; interaction of Zone x Pre-treatment, $F_{2, 8} = 2.249$, $P = 0.1679$; Fig. 2.7D) during the exploration period immediately preceding the probe test. Moreover, we did not observe a significant effect of direct stress on latency to reach the escape door in the probe test (unpaired t-test; $t(11) = 1.808$, $P = 0.0980$; Fig. 2.7E), indicating that this behavior is not sensitive to direct stress. To directly probe the role of endogenous NAc CRF in mediating aversive learning in the shuttlebox assay, we used stress-naïve males with bilateral NAc cannula that received a microinjection of vehicle or the CRF receptor antagonist α-helical CRF (500 ng/200 nL of vehicle/hemisphere) 15 minutes before training and the probe test. We found that CRF receptor antagonism did not probe session did not affect locomotor activity in the apparatus (unpaired t-test; $t(5) = 1.510$, $P = 0.1915$; Fig. 2.7F) or time spent in the

apparatus zones (two-way RM ANOVA with Sidak's post-hoc tests; interaction of Zone x Drug, $F_{2, 10} = 0.2857$, $P = 0.7574$; Fig. 2.7G) during the exploration period immediately preceding the probe test. Finally, we did not observe a significant difference between stress-naïve males that were pretreated with vehicle or α-helical CRF in latency to reach the escape door upon probe test CS presentation (unpaired t-test; $t(5) = 0.4195$, $P = 0.6922$; Fig. 2.7H), indicating that this behavior is not regulated by NAc CRF. Interestingly, the results do not support the initial hypothesis as direct stress exposure and NAc α-helical CRF administration resulted in a modest decrease in latency to reach the escape door upon CS presentation in the probe test relative to the respective controls.

In males, it has previously been shown that the effects of intra-NAc CRF on place conditioning are stress-sensitive: whereas stress-naïve males exhibit a preference for the CRF-paired chamber over the vehicle-paired following conditioning, males that underwent a direct rFSS seven days prior to the start of conditioning exhibit an aversion for the CRF-paired chamber compared to the vehicle-paired chamber following conditioning (Lemos et al., 2012). We attempted to replicate these findings with the goal of evaluating the effect of CRF on this behavior in stress-naïve and stress-exposed females as well as in males that were subjected to indirect stress. In disagreement with historical data, our findings show that stress-naïve males do not exhibit a conditioned-place preference for the CRF-paired chamber nor do males that previously underwent direct stress exhibit a conditioned-place aversion to the CRF-paired chamber (two-way RM ANOVA with Bonferroni's post-hoc tests; stress-naïve: interaction of Drug x Preference test, $F_{1, 22}=2.581$, $P=0.1224$; direct stress: interaction of Drug x Preference test, $F_{1, 28} = 0.1794$, $P = 0.6751$; Fig. 2.8A and B). However, we were able to replicate

historical, unpublished data that was generated in our lab that shows that in stress-naïve males, intra-NAc CRF decreases conditioning session locomotor activity relative to intra-NAc vehicle (unpaired t-test; $t(14) = 2.309$, $P = 0.0367$; Fig. 2.8C). Interestingly, this intra-NAc CRF-induced decrease in conditioning session locomotor activity was absent in direct stress-exposed males (unpaired t-test; $t(14) = 0.1756$, $P = 0.8631$; Fig. 2.8D). Because we were unable to reproduce the conditioned-place preference and aversion findings in stress-naïve and direct stress-exposed males, respectively, we refrained from expanding this study to include indirectly stressed males and stress-naïve, directly stressed, and indirectly stressed females.

Discussion

The principal findings reported here are threefold: 1) The regulation of dopamine by CRF in the NAc of stress-naïve animals is qualitatively similar between sexes, 2) stress exposure can engender a loss of this regulation in the absence of any overt behavioral manifestations in both sexes, and 3) this stress-induced neurochemical change can be socially transmitted to animals not directly exposed to the primary stressor. These findings support the notion that a stress-related physiological adaptation can be transmitted between members of a population, even when the "infectious" individual does not exhibit overt symptoms of the stress experience.

With regard to potential sexual dimorphism, we have been cautious in asserting the absence of qualitative sex differences in the regulation of dopamine by CRF, without excluding the possibility of small quantitative differences. In striatal brain slices from stress-naïve males and females we observed that bath application of CRF significantly increased evoked dopamine release relative to vehicle. Although 100 nM CRF resulted

in a more robust increase in NAc dopamine release in males than females, this comparison did not reach statistical significance. The vehicle-controlled analysis was sufficiently powered ($1 - \beta = 0.80$, $\alpha = 0.05$) to observe effect sizes greater than $f = 0.47$. Similar outcomes between sexes were also observed in how stress altered CRF regulation of dopamine, and its social transmission. This absence of robust sex differences is in stark contrast to CRF function on some other neural processes where both quantitative and qualitative differences across sexes have been demonstrated (D. A. Bangasser et al., 2010; Rita J. Valentino et al., 2013).

One week after rFSS exposure, we did not observe changes in any of the behavioral metrics tested, specifically, social interaction, depression-like behavior, and anxiety-like behavior. While this testing does not comprehensively rule out every potential behavioral consequence of the stressor exposure, it does demonstrate that the classic behavioral phenotype of pathological stress was not present in these individuals. These data corroborate a previous report, which found that male C57BL/6 mice did not exhibit anhedonia (sucrose preference), depression-like behavior (tail suspension test), or anxiety-like behavior (open field test) when assessed about a month after rFSS exposure (Mul et al., 2016). The one test in this previous study where enduring behavioral effects were observed is the forced swim test. This finding is similar to work from our laboratory (Lemos et al., 2012). However, it is important to note that shorter latencies to immobility when introduced to the FSS apparatus with repeated exposure can be a learned adaptive phenomenon confounding the ability to infer depression-like behavior (Molendijk & de Kloet, 2019; Mul, 2018). With that in mind, it appears that the long-term behavioral consequences of the stressor exposure were relatively benign. Despite this apparent

absence of behavioral manifestation, we replicated our previous finding that the regulation of dopamine release by CRF in the NAc is ablated following rFSS (Lemos et al., 2012). An even more surprising outcome was that this neurochemical adaptation was socially transmitted from the stressor-exposed animals. Thus, while an enduring behavioral phenotype indicative of pathological stress was not present in individuals following the aversive rFSS experience (i.e., they would be considered largely asymptomatic), they robustly transmitted physiological adaptations engendered by the experience.

In humans, stress contagion is a recognized and ubiquitous phenomenon that pervades all domains of social organization: It can occur on a micro-level (between individuals or within a family unit), meso-level (within a city or statewide community), and macro-level (within a national population or globally) (Boroson, 2011). The current work demonstrates that, in a controlled experimental system, stress-related phenotypes can penetrate the population through social transmission in a manner that is undetected through behavioral observations. This process underscores the pervasive nature of stress on our society.

Materials and Methods

Animals. Male and female C57BL/6 mice aged >7 weeks old were maintained under a 12-h light–dark cycle with access to standard food and water *ad libitum*. All procedures on animal subjects were approved by the University of Washington Institutional Animal Care and Use Committee. Stress-naïve or directly stressed mice were singly or group housed (up to five same-sex mice per cage). Direct stress (donors) and indirectly stressed mice were housed in groups of four with two animals belonging to each group.

Fast-scan cyclic voltammetry. Mice were quickly decapitated, and the head placed in pre-oxygenated ice-cold sucrose-based artificial cerebrospinal fluid (aCSF) solution (in mM: 248.3 sucrose, 3 KCl, 2 $Mg_2SO_4.7H_2O$, 1.3 NaH_2PO_4 monobasic, 10 D-glucose anhydrous, 26 $NaHCO_3$, 0.1 $CaCl_2$ dihydrate). The brain was rapidly removed and blocked to isolate the anterior forebrain. This block of tissue was secured by the caudal surface to the specimen disc in the vibratome (Leica VT1000 S Vibratome) buffer tray using Loctite Super Glue Gel Control. Coronal slices (250 µm) containing the NAc (+0.62-1.78 mm rostral to bregma) were prepared in oxygenated ice-cold sucrose-aCSF. Prepared slices were then transferred to a holding chamber in a water bath maintained at 32-35°C for a minimum of 50 min. The holding chamber contained oxygenated (non-sucrose) aCSF (in mM: 124.1 NaCl, 3 KCl, 2 $Mg_2SO_4.7H_2O$, 1.3 NaH_2PO_4 monobasic, 10 D-glucose anhydrous, 26 $NaHCO_3$, 2.5 $CaCl_2$ dihydrate). After 50 min, the holding chamber was removed from the water bath and left at room temperature for an additional ten min before slices were used for the experiment. Slices were placed in a recording chamber and continuously perfused (1.5-2.0 mL/min) with oxygenated aCSF maintained at 31-33°C. Fused-silica-insulated carbon-fiber microelectrodes (Clark et al., 2010), fabricated in-house, were inserted in the NAc core, just ventral or ventrolateral to the anterior commissure. The potential at a carbon fiber microelectrode was held at -0.4V versus Ag/AgCl, ramped to 1.3 V and back to -0.4 V (400 V/s) every 100 ms. A single biphasic electrical pulse (2 ms per phase, 120-200 µA) was applied to the slice to evoke dopamine release every two minutes. Data were collected using TarHeel CV (University of North Carolina, Chapel Hill, NC).

Repeated forced swim stress. Mice were subjected to a two-day, modified Porsolt forced swim stress (Porsolt et al., 1977) as described previously (McLaughlin et al., 2003). On day one, animals were placed in a vessel of 30-32°C water for 15 minutes. Twenty-four hours later (day two), animals underwent four six-minute swims separated by six-minute recovery sessions in their home cage. Animals were then returned to their home cage for seven days before voltammetry or behavioral testing was conducted. Overhead video footage of all swim trials was recorded using a Canon ZR90 camcorder and time spent immobile in the first five minutes of each swim session was analyzed using EthoVision (version 3.0; Noldus Information Technology) with the default immobility threshold of 20%.

Behavioral tests. Mice were handled daily on the three days preceding behavioral testing. At least one hour before the start of testing, mice were moved to the procedure space, located close to their housing, and allowed to acclimate. Mice were returned to their home cage once all cage mates had been tested. The three-chamber social approach, tail suspension test, and elevated plus maze behavioral tests are described in detail below.

Three-Chamber Social Approach. Social preference was assayed using the three-chamber social approach assay as described previously (Stein et al., 2019). Twenty-four hours before the beginning of the experiment, sex- and age-matched target (novel) mice were habituated to being enclosed in an inverted wire pencil cup (10.16 cm width x 10.16 cm depth x 10.8 cm height, Galaxy Pencil & Utility Cup; Spectrum Diversified Designs, LLC) for one hour in an open field box. A custom-built white opaque acrylic three-chambered apparatus (62.23 cm length x 31.75 cm width x 31.12 cm height with two

transparent internal partitions measuring 15.24 cm high x 29.21 cm wide, 5.08 cm x 5.08 cm square openings in the center to allow for travel in between the three chambers; TAP Plastics, Seattle, WA) was utilized for these studies. Following a 10-minute habituation period, in which the experimental mouse was placed into the center chamber and allowed to freely explore the apparatus, the mouse was briefly removed and placed in a holding cage. Two pencil cups were inverted and placed in the far corners of the apparatus. One cup remained empty and a target mouse was placed in the other inverted cup. Weighted cylindrical bottles were placed atop the inverted cups to prevent the experimental mouse from climbing on top of said cups. The experimental mouse was subsequently reintroduced into the center chamber and was free to explore the apparatus for an additional 10 minutes. Target mice were not used for consecutive trials and each target mouse was used for no more than four trials in one day. The side of the chamber that contained the target mouse was counterbalanced between trials and the pencil cups and the apparatus were cleaned with 35% ethanol and paper towels in between each trial. The experimental mouse's movement was recorded with a HD ceiling mounted camera (Panasonic WV-CP504). Time spent in each chamber and time spent in a proximal circle extending 9 cm beyond each of the wire enclosures was recorded and analyzed using EthoVision XT (version 14.0; Noldus Information Technology). Heatmaps of activity were generated using Ethovsion XT (version 11.0; Noldus Information Technology).

Tail suspension test (TST). The TST was employed to evaluate depression-like behavior and was conducted as previously described (Can et al., 2012). Mice were suspended by their tails from one half of a conditioned-place preference (CPP) chamber constructed from clear acrylic (20 cm length x 20 cm depth x 20 cm height) and placed

on its side, which was nested within a white acrylic open field (OF) box (40.6 cm length x 40.6 cm width x 30 cm height) placed on its side. The CPP-OF box setup was used to improve video contrast and facilitate mouse movement tracking. The CPP-OF apparatus was positioned at the edge of the lab bench (3 ft height) in the procedure room, such that when suspended by their tails, mice dangled over the side of the lab bench. Prior to the experiment, a hollow cylindrical tube used to ensheathe the tail to impede tail climbing behavior once suspended and a piece of tape used to attach the tail to the CPP box were prepared for each experimental mouse. Specifically, a Falcon 3 mL Transfer Pipet (Corning Inc.) that had the bulb and tip cut off was trimmed down to a 4-cm long hollow cylindrical tube and a 13-cm long piece of VWR General-Purpose Laboratory Labeling Tape (12.7 mm width; VWR International, LLC) was marked with a permanent marker at 5.5 cm, 10 cm, and 11 cm relative to one end. To carry out the experiment, a mouse's tail was passed through the hollow tube and the section of prepared tape between 10-11 cm was wrapped around the end of a mouse's tail leaving 2-3 mm of tail exposed at the end. Experimental mice were transported from their home cage in the palm of the experimenter's hand and the tape position marked at 5.5 cm was attached to the top of the CPP box in the center, such that mice could not contact the sides of the CPP box. Mice were then suspended from their tails for the 6-minute trial. The CPP-OF apparatus was cleaned with 70% ethanol and paper towels in between trials. Mouse movement was recorded with a Canon ZR90 camcorder affixed to a tripod to capture side-on immobility. Immobility was analyzed using EthoVision (version 3.0; Noldus Information Technology) with an immobility threshold of 10%.

Elevated plus maze (EPM). EPM testing was conducted as previously described (Bruchas et al., 2009) with noted modifications. The EPM (38 cm arm length x 7.62 cm width x 75 cm height; Med Associates, Inc.) was constructed out of black acrylic, and the open and closed arms were outfitted with strips of laminated white paper (5.5 cm width) in order to enhance video contrast and mouse tracking. On the day of the experiment, the EPM position and lighting were adjusted to minimize shadows in the closed arms and such that light detected at the ends of the open arms measured 15 lux. To conduct the experiment, the experimental mouse was transported from its home cage to the EPM in the palm of the experimenter's hand. The mouse was gently placed in the center of the EPM facing a corner formed by an open and closed arm and allowed to freely explore the apparatus for six minutes. In between trials the apparatus was cleaned with 70% ethanol and paper towels. Movements were video recorded using a Canon ZR90 camcorder and analyzed using EthoVision (version 3.0; Noldus Information Technology) and heatmaps of activity were generated using Ethovsion XT (version 11.0; Noldus Information Technology). We used open arm time expressed as a percentage of total time as the primary measure of anxiety-like behavior. A subset of the directly and indirectly stressed mice used in the EPM experiments were subsequently used for voltammetry experiments.

NAc core cannulations. Bilateral guide cannulae (C235G-2.0/SPC with 4.5 mm length; Plastics1/Invivo1) were implanted above the NAc core using the following stereotaxic coordinates: AP +1.35, DV -3.75, ML ±1.00. Bilateral dummy internal cannulae were inserted into guides to prevent clogging (C235DC/SPC fit to 4.5 mm C235G-2.0/SPC without projection; Plastics1/Invivo1) and were kept in place by a nylon dust cap (303DC/1; Plastics1/Invivo1). Internal cannulae (C235I/SPC fit to 4.5 mm C235G-

2.0/SPC with 0.5 mm projection; Plastics1/Invivo1) were used to microinject drug into the NAc core. Animals were handled in a progressive manner on the five days prior to the start of the experiment, as described previously (Lemos et al., 2016).

Shuttle box assay. A shock box with two chambers connected by a guillotine door was used (35 cm wide × 18 cm deep × 28 cm high; Coulbourn Instruments, Allentown, PA). Training occurred on the first day of the experiment. Briefly, a mouse was placed in the shuttle box and could freely explore both chambers for two minutes. Following this initial period, the mouse was briefly confined to the chamber with the electrified grid floor. (Unfortunately, we were not able to counterbalance the chamber that training occurred in; only one chamber was outfitted with a functional electrified grid floor.) The door connecting to the other chamber was opened at the onset of the conditioned stimulus (CS)-unconditioned stimulus (US) pairing, which consisted of a five-second tone that co-terminated with a one-second 0.3 mA foot shock. A 90-second inter-pairing interval ensued, followed by three additional pairings for a total of four pairings followed by an inter-pairing interval. Twenty-four hours later, the mouse was placed in shuttle box and could once again explore for two minutes. Next, the mouse was confined to the chamber with the electrified floor grid and a probe test was conducted. The guillotine door was opened while the CS was played. Movements were video recorded. An experimenter later reviewed the footage and used a stopwatch to quantify the latency to reach the door leading to the non-shock chamber upon CS presentation. In mice that had cannula implanted above the NAc core, drug was microinjected 15 minutes before the start of the training session and probe test.

Conditioned place preference and conditioned place aversion. Experiments were conducted as described previously (Lemos et al., 2012).

Statistical analysis. Statistical analyses were performed, and graphs were generated using Prism 8 (GraphPad Software Inc.). Details of statistical tests employed can be found in text. Post-hoc power calculations were conducted using G*Power 3.1.9.4 (Heinrich-Heine-Universität Düsseldorf).

Chemicals and drugs. Sucrose and NaH_2PO_4 monobasic were obtained from Sigma-Aldrich. KCl, $Mg_2SO_4.7H_2O$, Dextrose (D-glucose) anhydrous, $CaCl_2$ dihydrate, NaCl, $NaHCO_3$, and glacial acetic acid were obtained from Fisher Scientific. CRF (human, rat; catalog no.: 1151), antisauvagine-30 (catalog no.: 2071), antalarmin hydrochloride (catalog no.: 2778), and α-helical CRF 9-41 (catalog no.: 1184) were obtained from Tocris Bioscience. Concentrated stock solutions of CRF (100 µM or 1 mM) and its vehicle (7% acetic acid in molecular biology grade water) were prepared and stored at -20°C for up to one week. Concentrated stocks were thawed on ice prior to being pipetted directly into oxygenated aCSF. Final concentrations of CRF and its vehicle were 100 nM (from 100 µM) or 1 µM (from 1 mM) and 0.07% by volume, respectively.

Figures 2.1-2.8

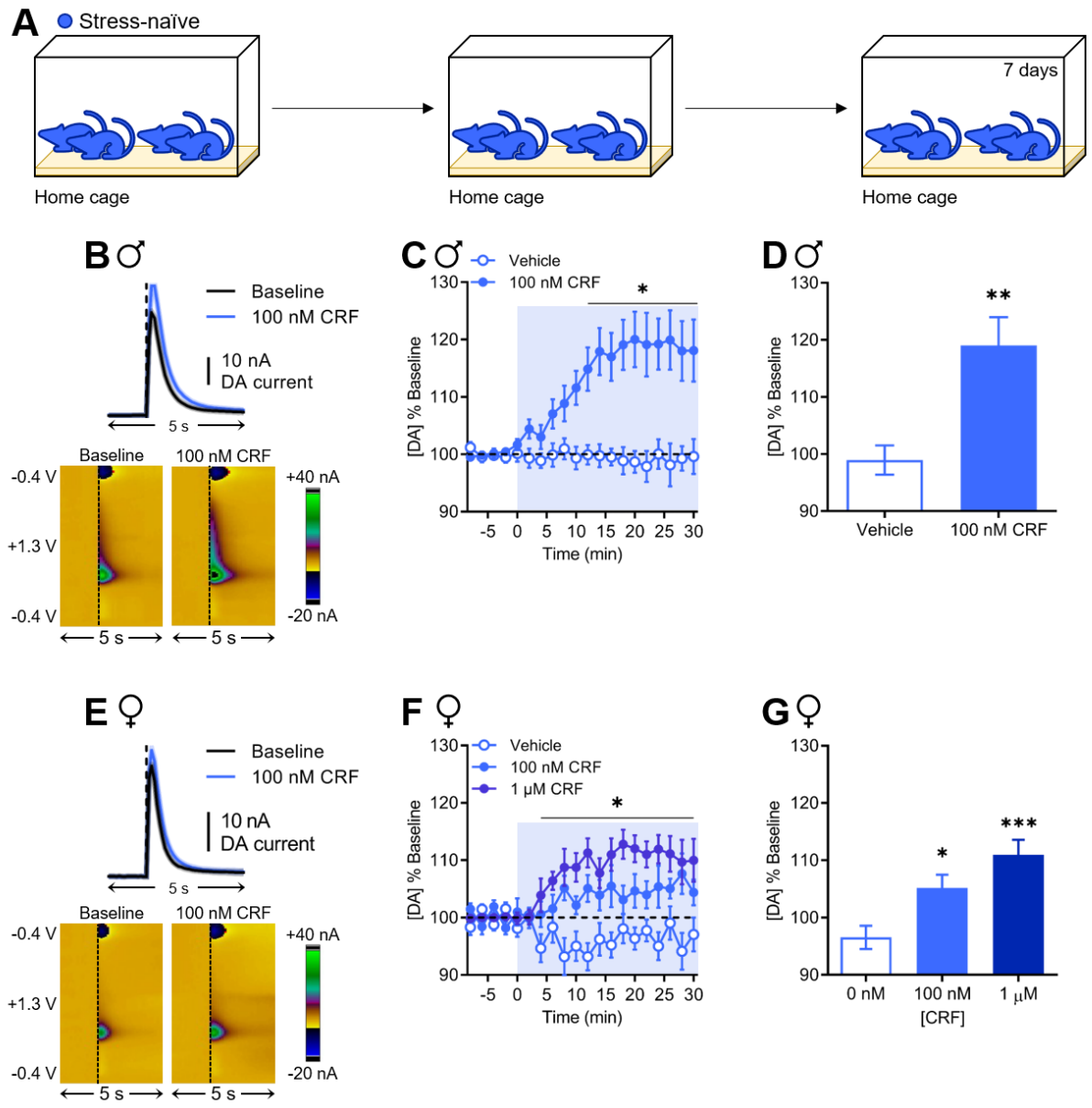


Figure 2.1. CRF increases dopamine release in the nucleus accumbens (NAc) in stress-naïve males and females. (A) In the stress-naïve experimental group, mice remained in their home cage for the duration of the experiment. **(B)** Representative dopamine release evoked by electrical stimulation (dashed line) before and after application of 100 nM CRF to a NAc brain slice collected from a stress-naïve male (mean ± SEM for 5 consecutive stimulations, top) and corresponding two-dimensional plots depicting changes in faradaic current (pseudocolor) with time as the abscissa and applied potential as the ordinate (bottom). **(C)** Baseline-normalized mean peak amplitude of electrically evoked dopamine release over time in response to vehicle or 100 nM CRF

application to NAc slices from stress-naïve males (n = 9-11). **(D)** Baseline-normalized mean peak amplitude of dopamine release 20-30 min after vehicle or 100 nM CRF application to NAc slices from stress-naïve males (n = 9-11). **(E)** Representative dopamine release evoked by electrical stimulation (dashed line) before and after application of 100 nM CRF to a NAc brain slice from a stress-naïve female (mean ± SEM for 5 consecutive stimulations, top) and corresponding color plots (bottom). **(F)** Baseline-normalized mean peak amplitude of electrically evoked dopamine release over time in response to vehicle, 100 nM CRF, or or 1 μM CRF application to NAc slices from stress-naïve females (n = 8-10). **(G)** Baseline-normalized mean peak amplitude of dopamine release 20-30 min after vehicle, 100 nM CRF, or 1 μM CRF application to NAc slices from stress-naïve females (n = 8-10). Error bars, SEM. *P<0.05. **P<0.01, ***P<0.001.

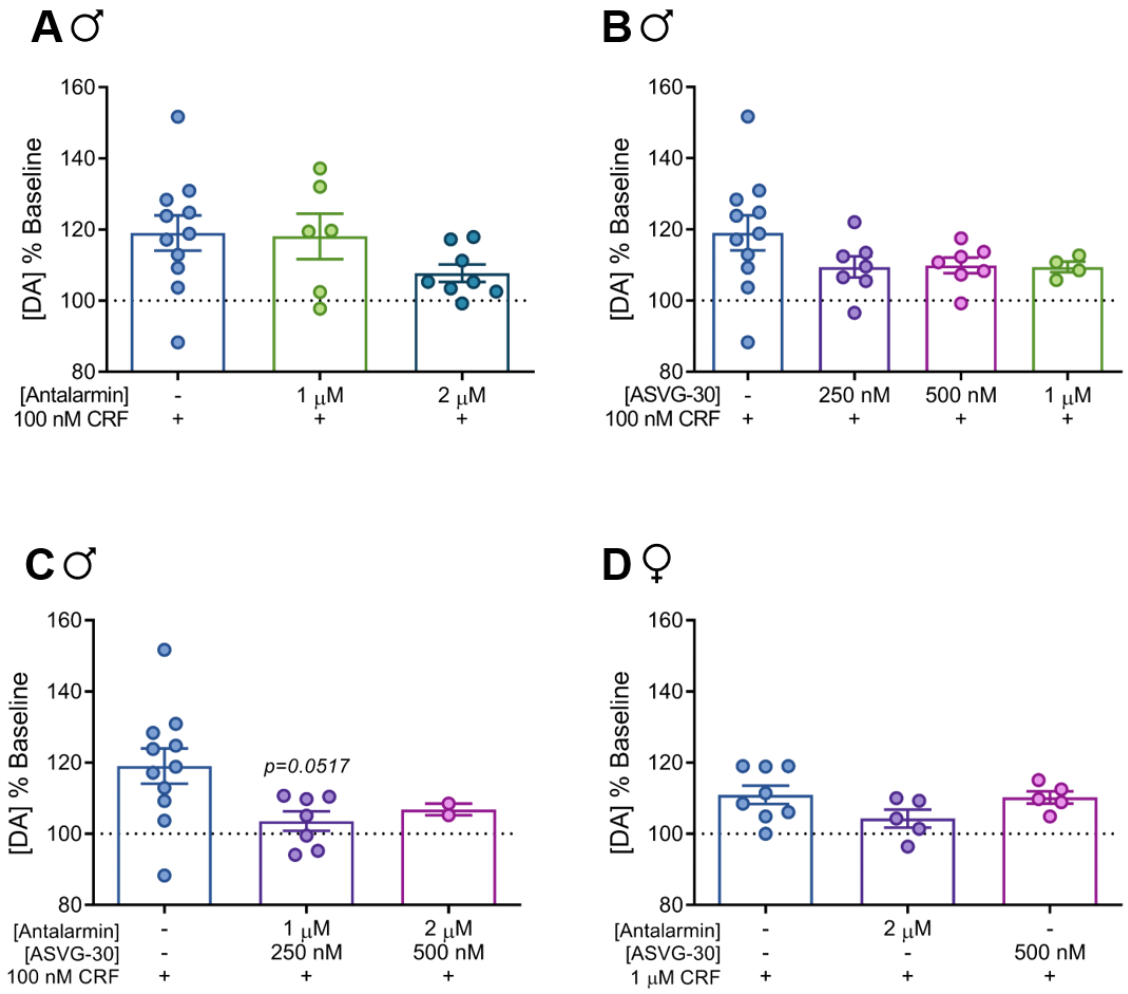


Figure 2.2. Characterization of the roles of CRFR1 and CRFR2 in mediating CRF-induced potentiation of NAc dopamine release in males and females. (A) Baseline-normalized mean peak amplitude of dopamine release 20-30 min after 100 nM CRF application to NAc slices from stress-naïve males that had been pretreated with the CRFR1 antagonist antalarmin (n = 6-11). **(B)** Baseline-normalized mean peak amplitude of dopamine release 20-30 min after 100 nM CRF application to NAc slices from stress-naïve males that had been pretreated with the CRFR2 antagonist ASVG-30 (n = 4-11). **(C)** Baseline-normalized mean peak amplitude of dopamine release 20-30 min after 100 nM CRF application to NAc slices from stress-naïve males that had been pretreated with the CRFR1 antagonist antalarmin and the CRFR2 antagonist ASVG-30 (n = 2-11). **(D)** Baseline-normalized mean peak amplitude of dopamine release 20-30 min after 1 μM CRF application to NAc slices from stress-naïve females that had been pretreated with the CRFR1 antagonist antalarmin or the CRFR2 antagonist ASVG-30 (n = 5-8). Error bars, SEM.

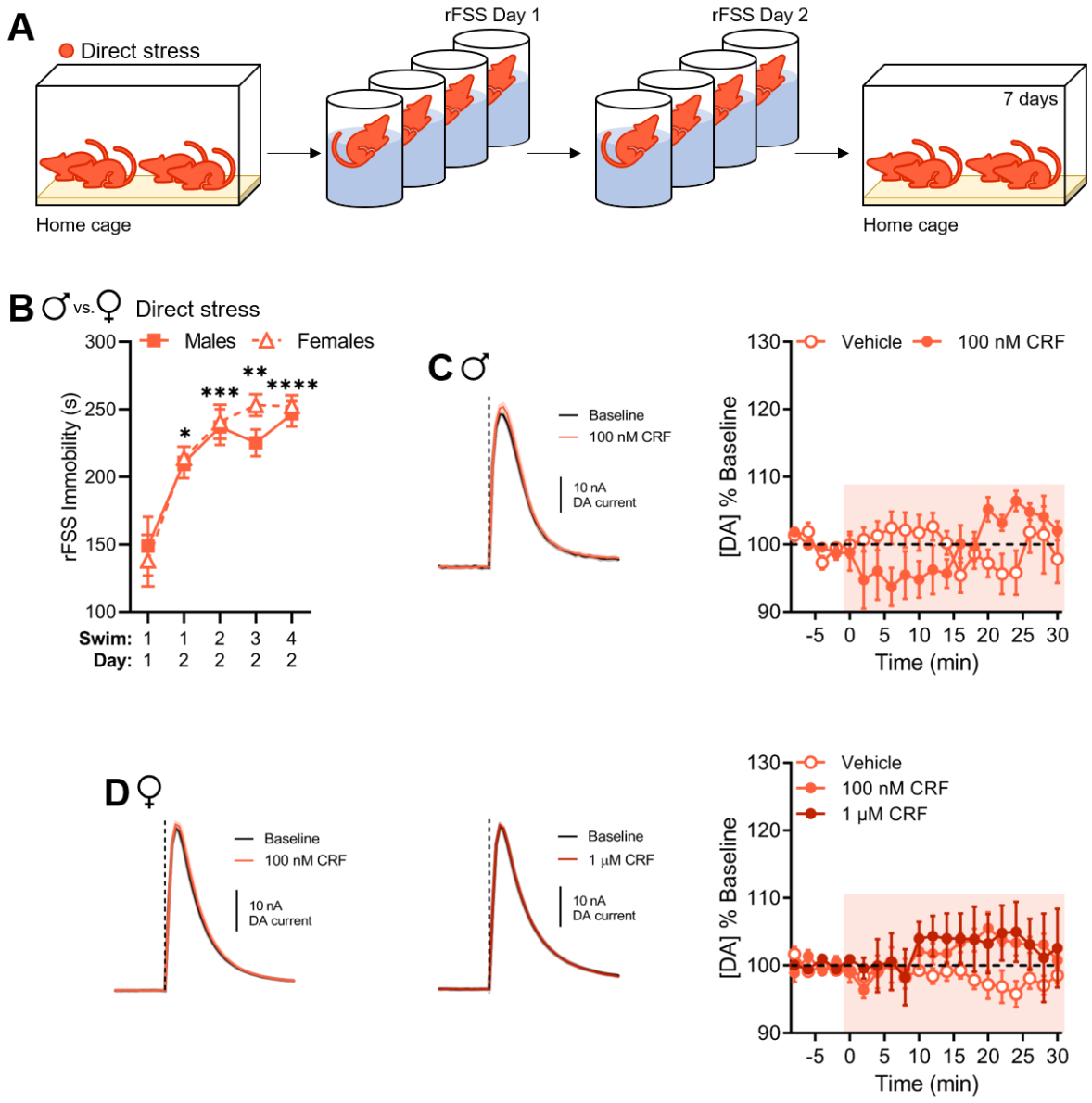


Figure 2.3. Direct stress disrupts CRF-mediated dopamine release in the NAc one week after repeated forced swim stress (rFSS) exposure in males and females. (A) In the direct stress experimental group, mice were exposed to rFSS and tested seven days later. **(B)** Cumulative time spent immobile for the first five min of Swim 1 on rFSS Day 1 and Swims 1-4 on rFSS Day 2 in males and females belonging to the direct stress group (n = 6-10). **(C)** Representative dopamine release evoked by electrical stimulation (dashed line) before and after application of 100 nM CRF to a NAc brain slice (mean ± SEM for 5 consecutive stimulations, left) and baseline-normalized mean peak amplitude of electrically evoked dopamine release over time in response to vehicle or 100 nM CRF application to NAc slices (n = 5, right) from directly stressed males one week after stress exposure. **(D)** Representative dopamine release evoked by electrical stimulation before and after application of 100 nM CRF (left) or 1 µM CRF (middle) to a

NAc slice (mean ± SEM for 5 consecutive stimulations) and baseline-normalized mean peak amplitude of electrically evoked dopamine release over time in response to vehicle, 100 nM CRF, or 1 μM CRF application to NAc slices (n = 5, right) from directly stressed females one week after stress exposure. Error bars, SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

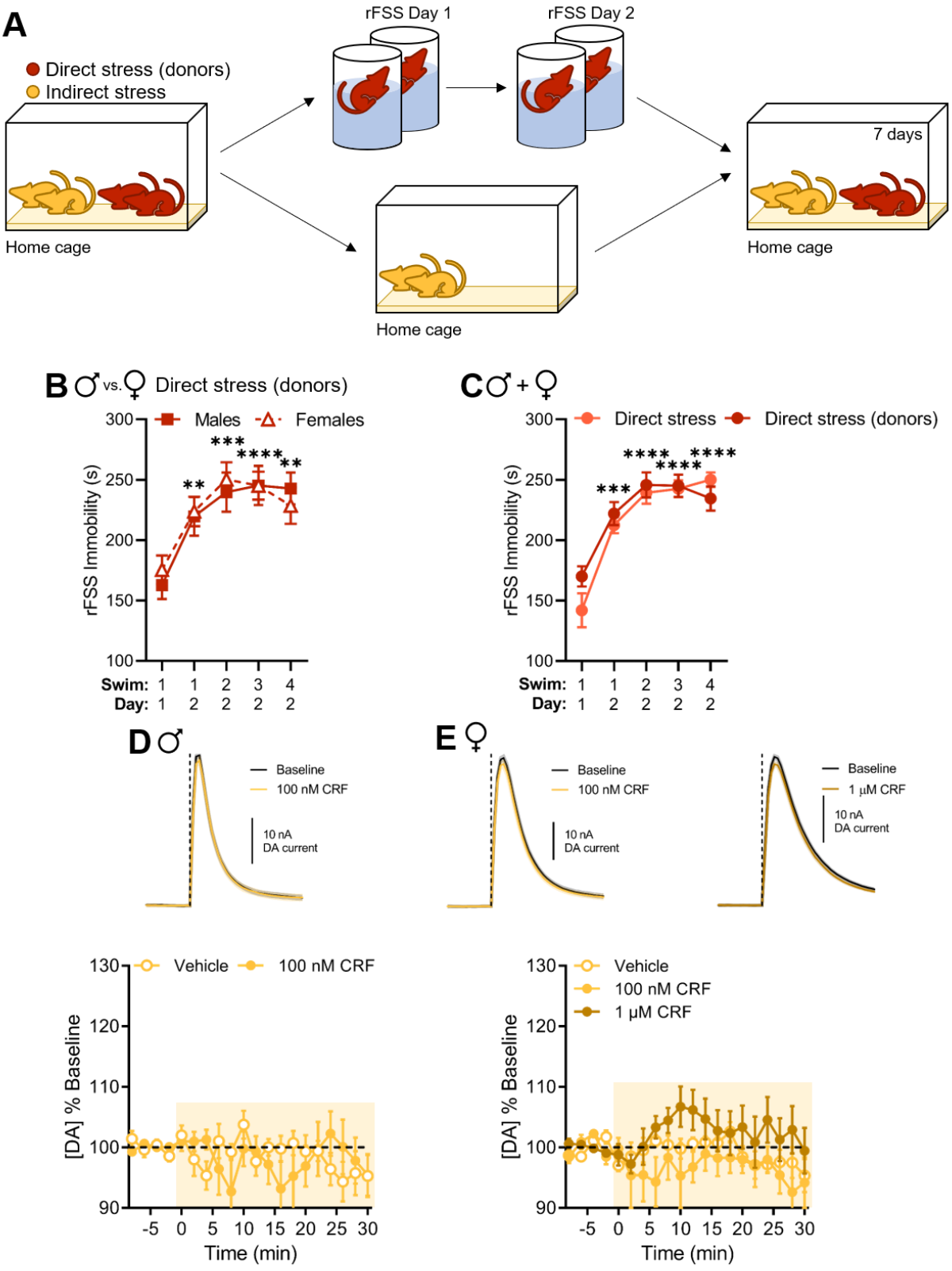

A
Direct stress (donors)
Indirect stress
rFSS Day 1
rFSS Day 2
7 days
Home cage
Home cage
Home cage
B ♂ vs. ♀ Direct stress (donors)
Males
Females
rFSS Immobility (s)
Swim: 1 1 2 3 4
Day: 1 2 2 2 2
C ♂ + ♀
Direct stress
Direct stress (donors)
D ♂
Baseline
100 nM CRF
10 nA
DA current
E ♀
Baseline
100 nM CRF
1 µM CRF
Vehicle
100 nM CRF
1 µM CRF
[DA] % Baseline
Time (min)

Figure 2.4. Indirect stress disrupts CRF-mediated dopamine release in the NAc in males and females one week after the onset of stress exposure. (A) Mice were housed in groups of four and two mice were subjected to direct stress. The two mice that did not undergo direct stress, termed the indirect stress group, remained in the home cage for the duration of the experiment. (B-C) Cumulative time spent immobile for the first five min of Swim 1 on rFSS Day 1 and Swims 1-4 on rFSS Day 2 in **(B)** males and females that were housed with indirectly stressed animals (n = 6-8) **(C)** mice (male and female groups combined) belonging to each of the direct stress groups (n = 14-16). **(D)** Representative dopamine release evoked by electrical stimulation (dashed line) before and after application of 100 nM CRF to a NAc brain slice (mean ± SEM for 5 consecutive stimulations, top) and baseline-normalized mean peak amplitude of electrically evoked dopamine release onuver time in response to vehicle or 100 nM CRF application to NAc slices (n = 4-6, bottom) from an indirectly stressed males one week after cage mates were directly stressed. **(E)** Representative dopamine release evoked by electrical stimulation before and after application of 100 nM CRF (top, left) or 1 μM CRF (top, right) to a NAc slice (mean ± SEM for 5 consecutive stimulations) and baseline-normalized mean peak amplitude of electrically evoked dopamine release over time in response to vehicle, 100 nM CRF, or 1 μM CRF application to NAc slices (n = 5-8, bottom) from indirectly stressed females one week after cage mates were directly stressed. Error bars, SEM. **P<0.01, ***P<0.001, ****P<0.0001.

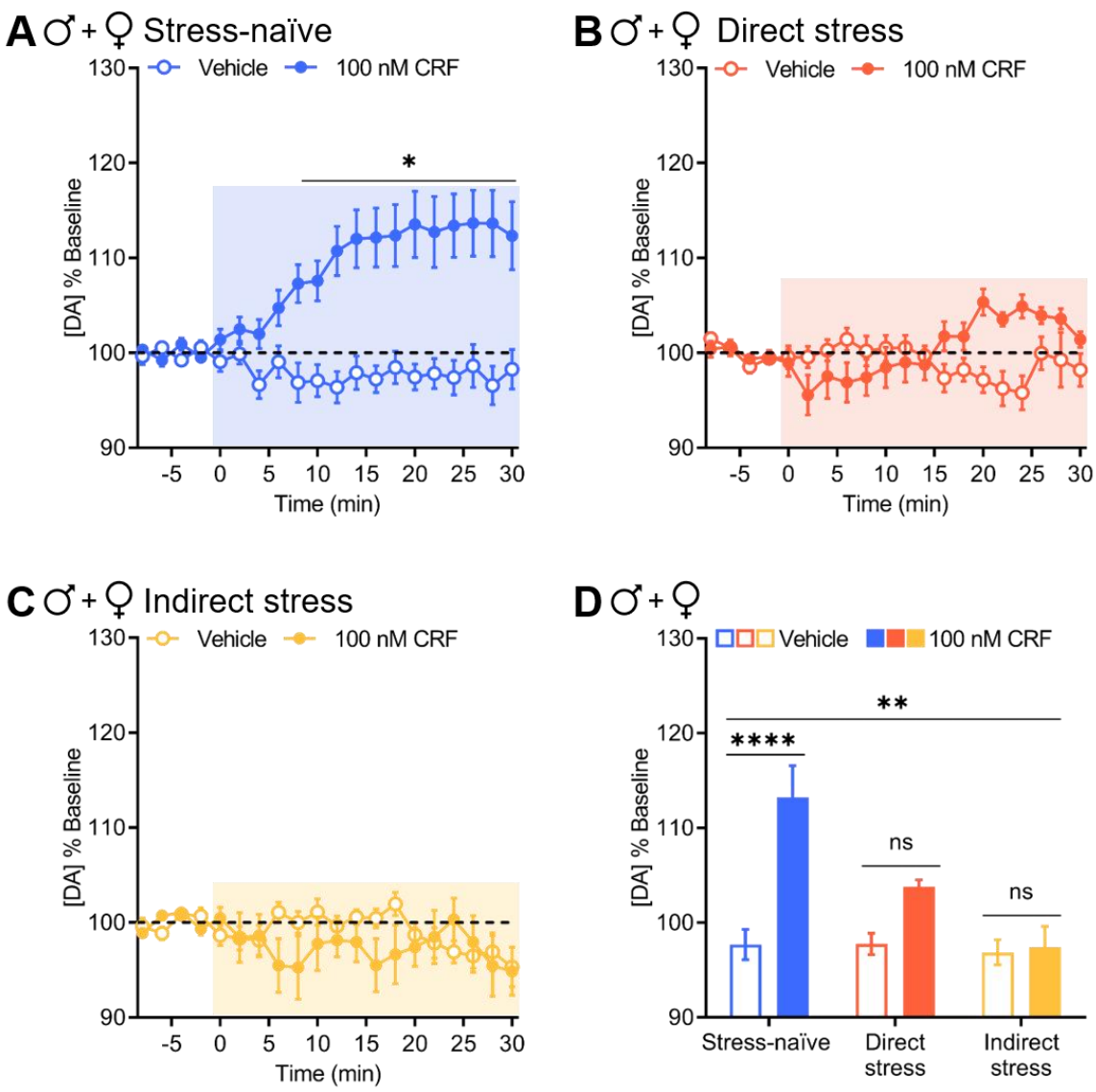


Figure 2.5. Direct and indirect stress disrupt CRF-mediated dopamine release in the NAc one week after stress exposure. Baseline-normalized mean peak amplitude of electrically evoked dopamine release over time in response to vehicle or 100 nM CRF application to NAc slices collected from **(A)** stress-naïve males and females (n = 15-17), **(B)** directly stressed males and females one week after rFSS exposure (n = 10), and **(C)** indirectly stressed males and females one week after cage mates were subjected to direct stress (n = 11-12). **(D)** Baseline-normalized mean peak amplitude of dopamine release 20-30 min after vehicle or 100 nM CRF application to NAc slices from stress-naïve, directly stressed, or indirectly stressed males and females (n = 10-17). Error bars, SEM. NS P>0.05, *P<0.05, **P<0.01, ****P<0.0001.

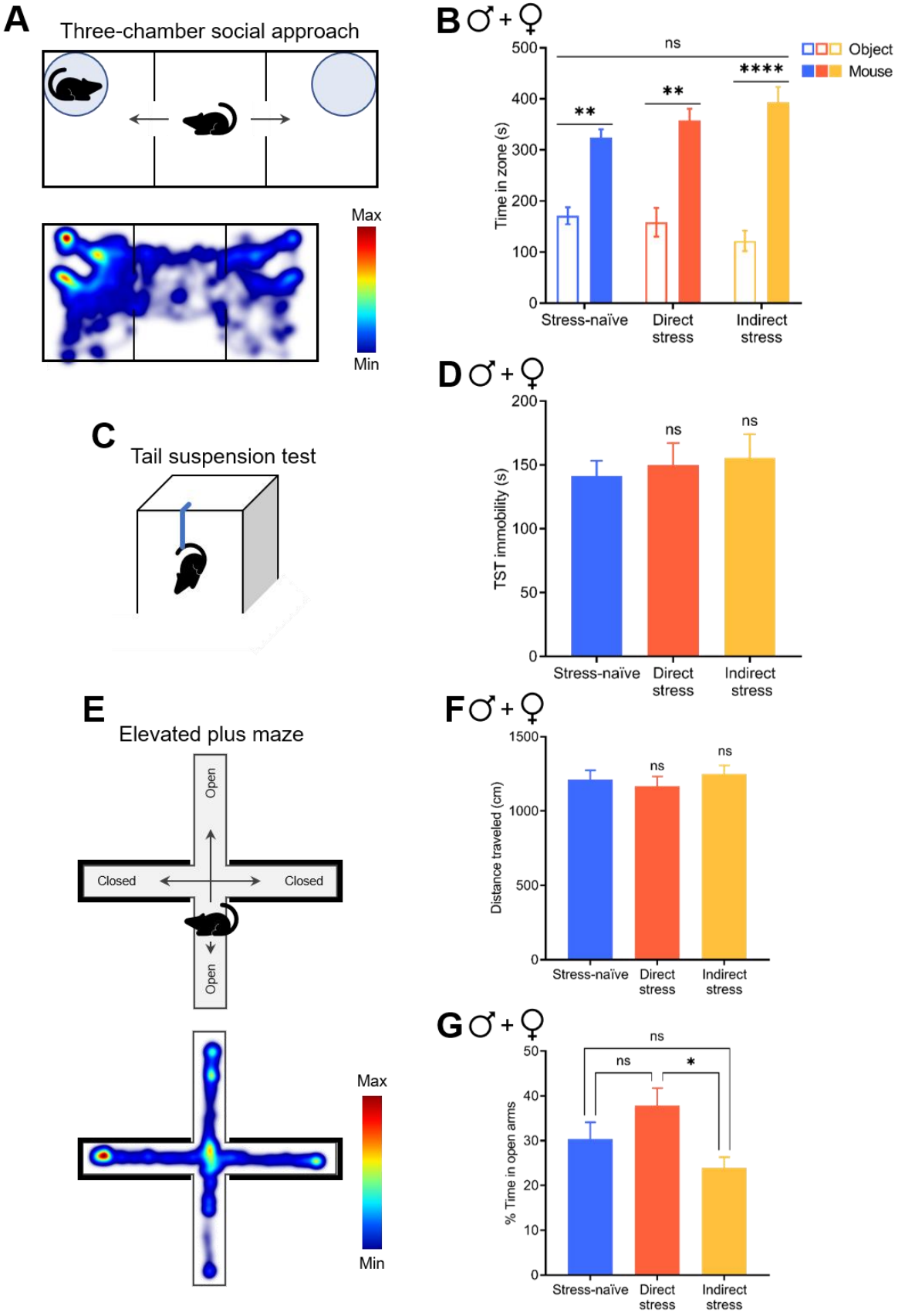

A
Three-chamber social approach
Max
Min
B ♂+♀
ns
Object
Mouse
****
**
**
Time in zone (s)
500
400
300
200
100
0
Stress-naïve
Direct stress
Indirect stress
C
Tail suspension test
D ♂+♀
TST immobility (s)
200
150
100
50
0
ns
ns
Stress-naïve
Direct stress
Indirect stress
E
Elevated plus maze
Open
Closed
Closed
Open
Max
Min
F ♂+♀
Distance traveled (cm)
1500
1000
500
0
ns
ns
Stress-naïve
Direct stress
Indirect stress
G ♂+♀
% Time in open arms
50
40
30
20
10
0
ns
ns
*
Stress-naïve
Direct stress
Indirect stress

Figure 2.6. Direct and indirect stress do not affect social interaction, depression-like behavior, or anxiety-like behavior one week after stress exposure. (A) Cartoon depicting the three-chamber social approach assay (top) and a representative heatmap of activity during this test (bottom). **(B)** Time spent in the novel object and novel mouse zones in the three-chamber social approach assay in stress-naïve, directly stressed, and indirectly stressed males and females (n = 6-8). **(C)** Cartoon depicting the tail suspension test. **(D)** Time spent immobile for the six-min duration the tail suspension test in stress-naïve, directly stressed, and indirectly stressed males and females (n = 10-11). **(E)** Cartoon depicting the elevated plus maze (EPM, top) and a representative heatmap of activity in the EPM (bottom). **(F)** Distance traveled in the elevated plus maze (EPM) in stress-naïve, directly stressed, and indirectly stressed males and females (n = 25-32). **(G)** Percent of total time spent exploring the open arms of the EPM in stress-naïve, directly stressed, and indirectly stressed males and females (n = 25-32). Error bars, SEM. NS P>0.05, *P<0.05, **P<0.01, ****P<0.0001.

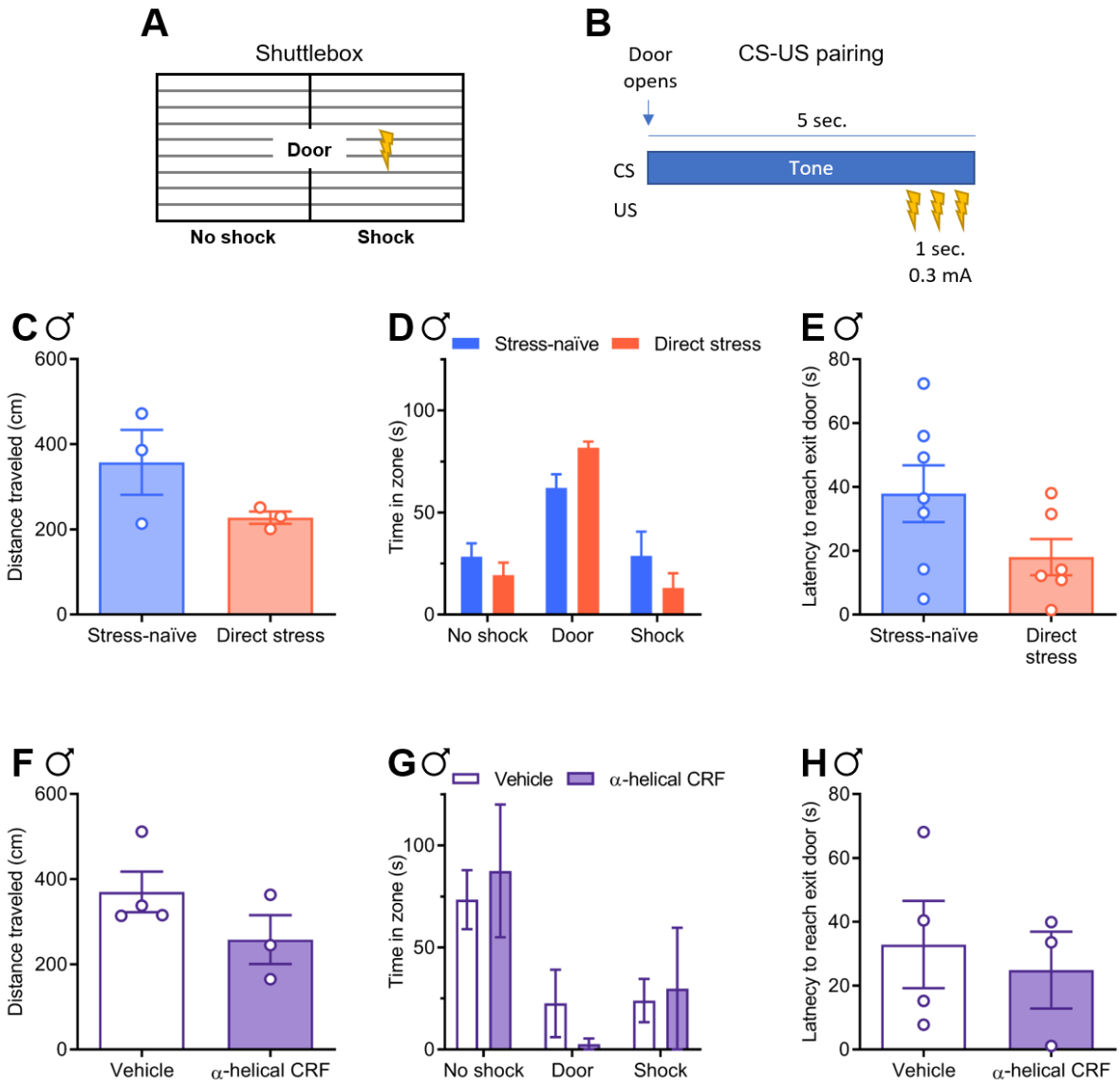


Figure 2.7. Characterizing the effect of direct stress and the role of NAc CRF in aversive learning. (A) Cartoon depicting the two-chamber shuttlebox apparatus. **(B)** Cartoon depicting the CS-US pairing used in the training session. **(C)** Distance traveled in the shuttlebox apparatus in stress-naïve and directly stressed males in the exploration period immediately preceding the probe test (n = 3). **(D)** Time spent in the zones of the shuttlebox apparatus in stress-naïve and directly stressed males in the exploration period immediately preceding the probe test (n = 3). **(E)** Latency of stress-naïve and directly stressed males to reach the escape door upon CS presentation in the probe test (n = 6-7). **(F)** Distance traveled in the shuttlebox apparatus in stress-naïve males that received NAc microinjections of vehicle or α-helical CRF in the exploration period immediately preceding the probe test (n = 3-4). **(G)** Time spent in the zones of the shuttlebox apparatus in stress-naïve males that received NAc microinjections of vehicle or α-helical CRF in the

exploration period immediately preceding the probe test (n = 3-4). **(H)** Latency of stress-naïve males that received NAc microinjections of vehicle or α-helical CRF to reach the escape door upon CS presentation in the probe test (n = 3-4).

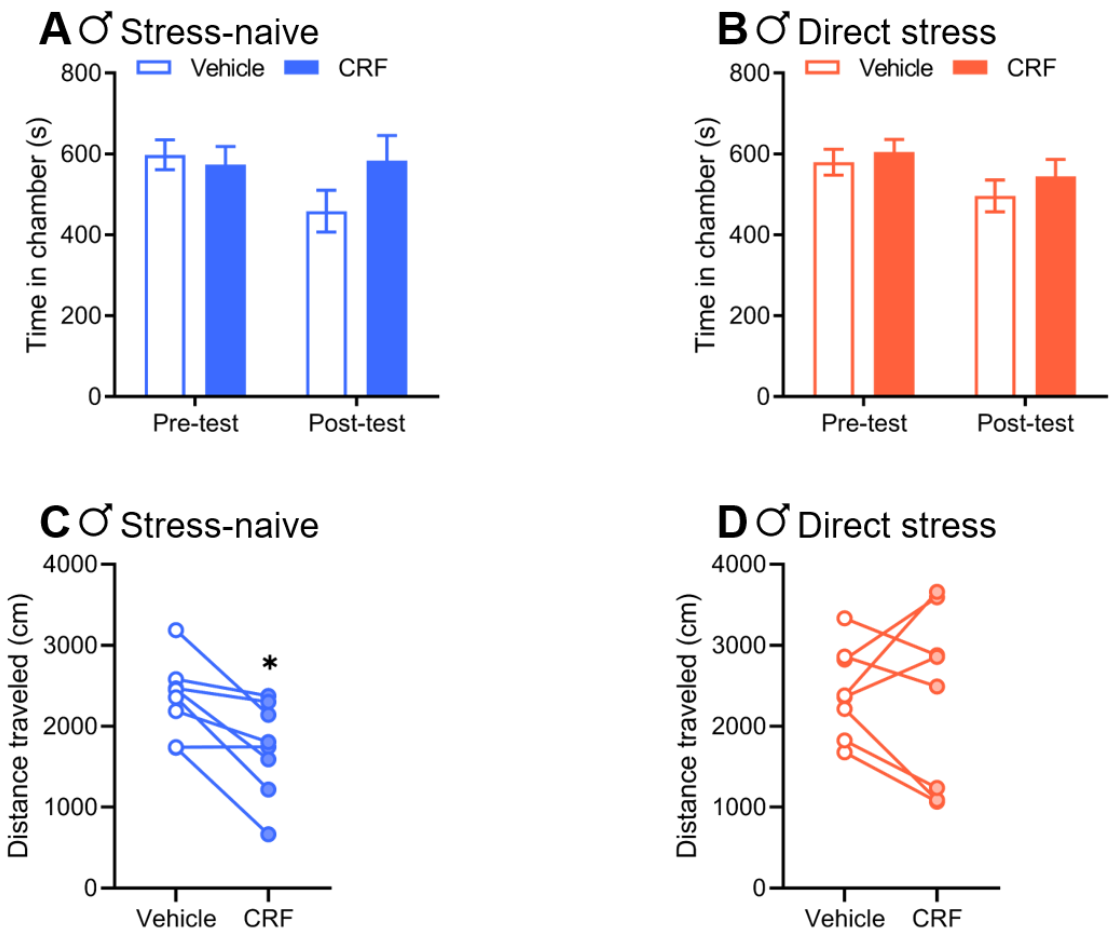
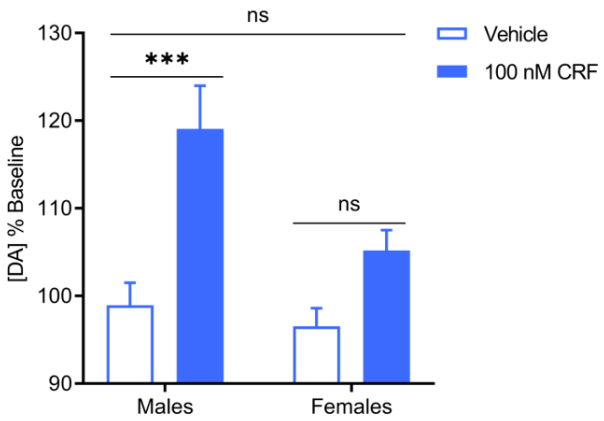
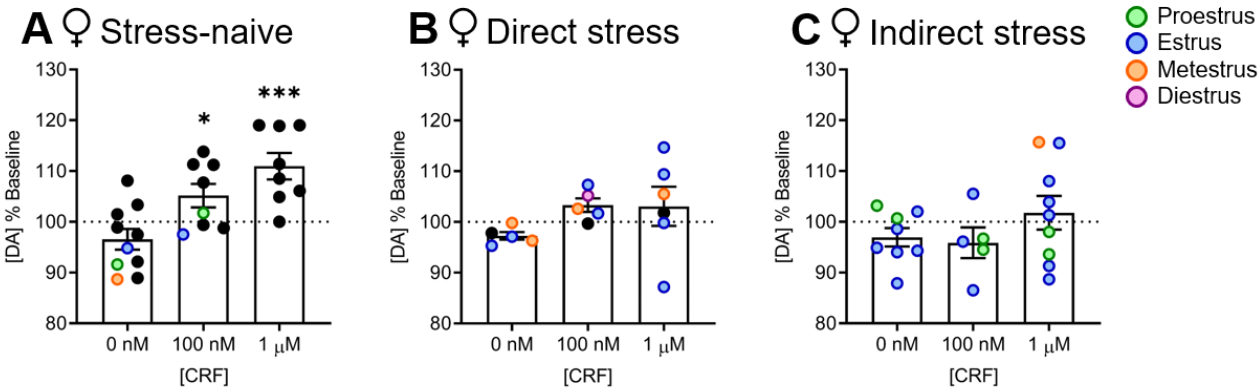


Figure 2.8. Intra-NAc CRF decreases locomotor activity in stress-naïve but not direct stress-exposed males. Mean difference in time spent in the CRF-paired chamber compared to the vehicle-paired chamber before and after conditioning in **(A)** stress-naïve males (n = 12) and **(B)** males that underwent direct stress seven days before the start of conditioning (n = 15). Distance traveled in the vehicle and CRF conditioning sessions averaged across days one and two of conditioning in **(C)** stress-naïve males (n = 8) and **(D)** males that underwent direct stress seven days before the start of conditioning (n = 8). Error bars, SEM, *P<0.05.

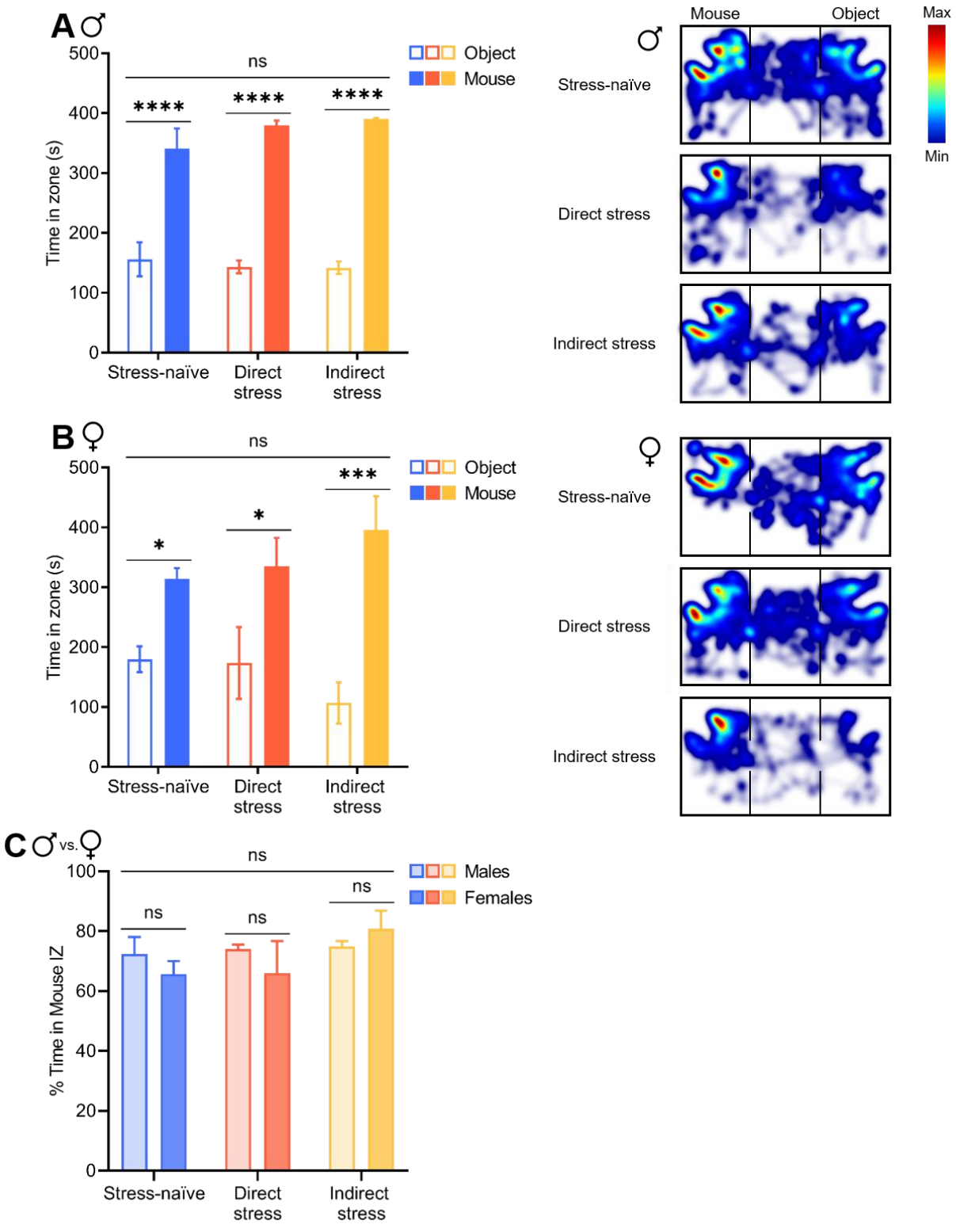
Supplemental Figures S2.1-2.6



Supplemental Figure 2.1. Effects on 100 nM CRF on NAc dopamine transmission is not significantly different between males and females. Baseline-normalized mean peak amplitude of dopamine release 20-30 minutes after Vehicle or 100 nM CRF application to NAc slices collected from stress-naïve males and females (n = 8-11). Error bars, SEM. NS P>0.05, ***P<0.001.

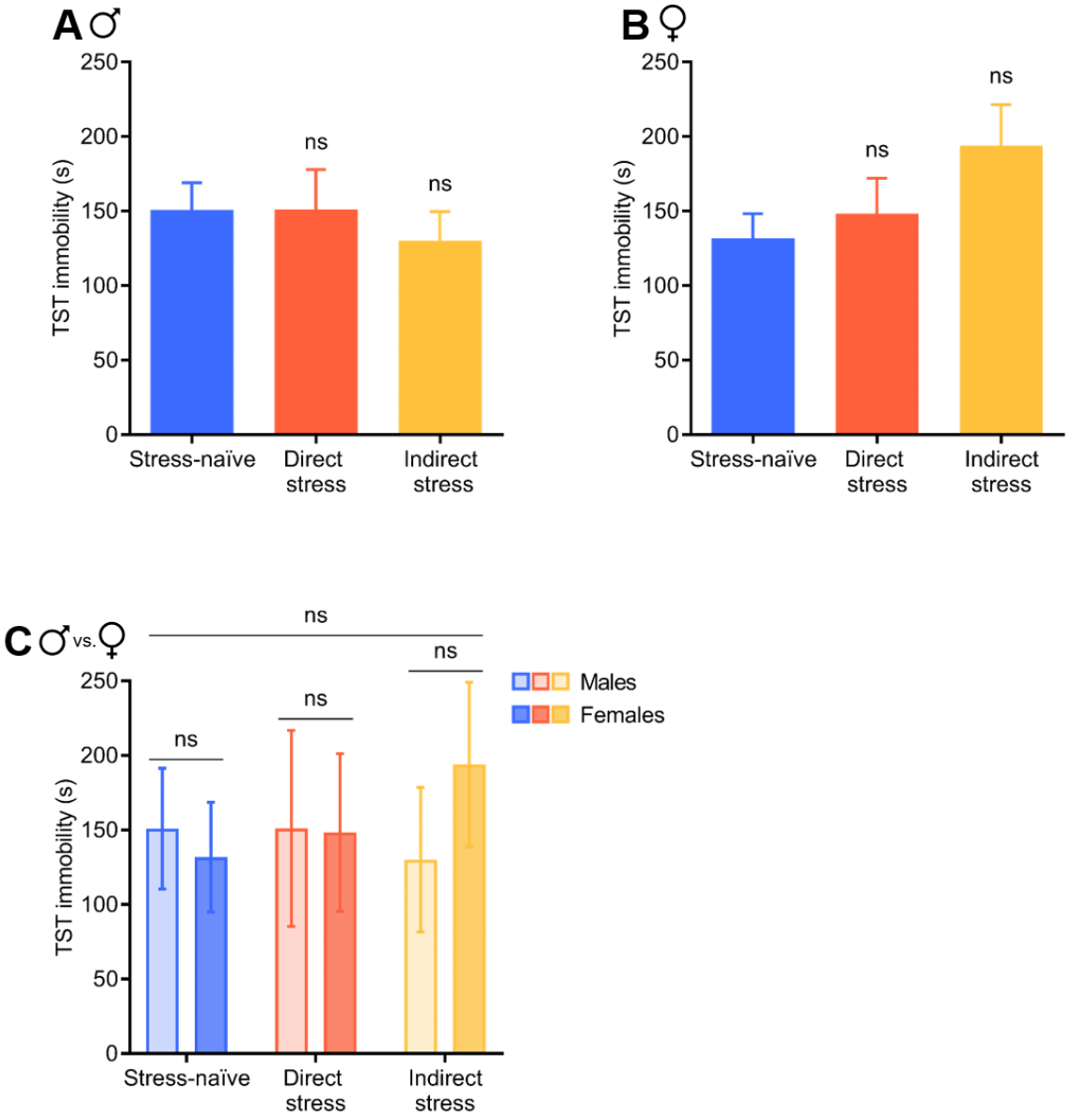


Supplemental Figure 2.2. Estrous cycle stage does not affect CRF's regulation of NAc dopamine release. (A) Baseline-normalized mean peak amplitude of dopamine release 20-30 min after vehicle, 100 nM CRF, or 1 μM CRF application to NAc slices from stress-naïve females with estrous cycle stage indicated (n = 8-10). **(B)** baseline-normalized mean peak amplitude of electrically evoked dopamine release over time in response to vehicle, 100 nM CRF, or 1 μM CRF application to NAc slices from directly stressed females one week after stress exposure with estrous cycle stage indicated (n = 5-6). **(C)** Baseline-normalized mean peak amplitude of electrically evoked dopamine release over time in response to vehicle, 100 nM CRF, or 1 μM CRF application to NAc slices from indirectly stressed females one week after cage mates were directly stressed with estrous cycle stage indicated (n = 5-8). Error bars, SEM. *P<0.05. ***P<0.001.

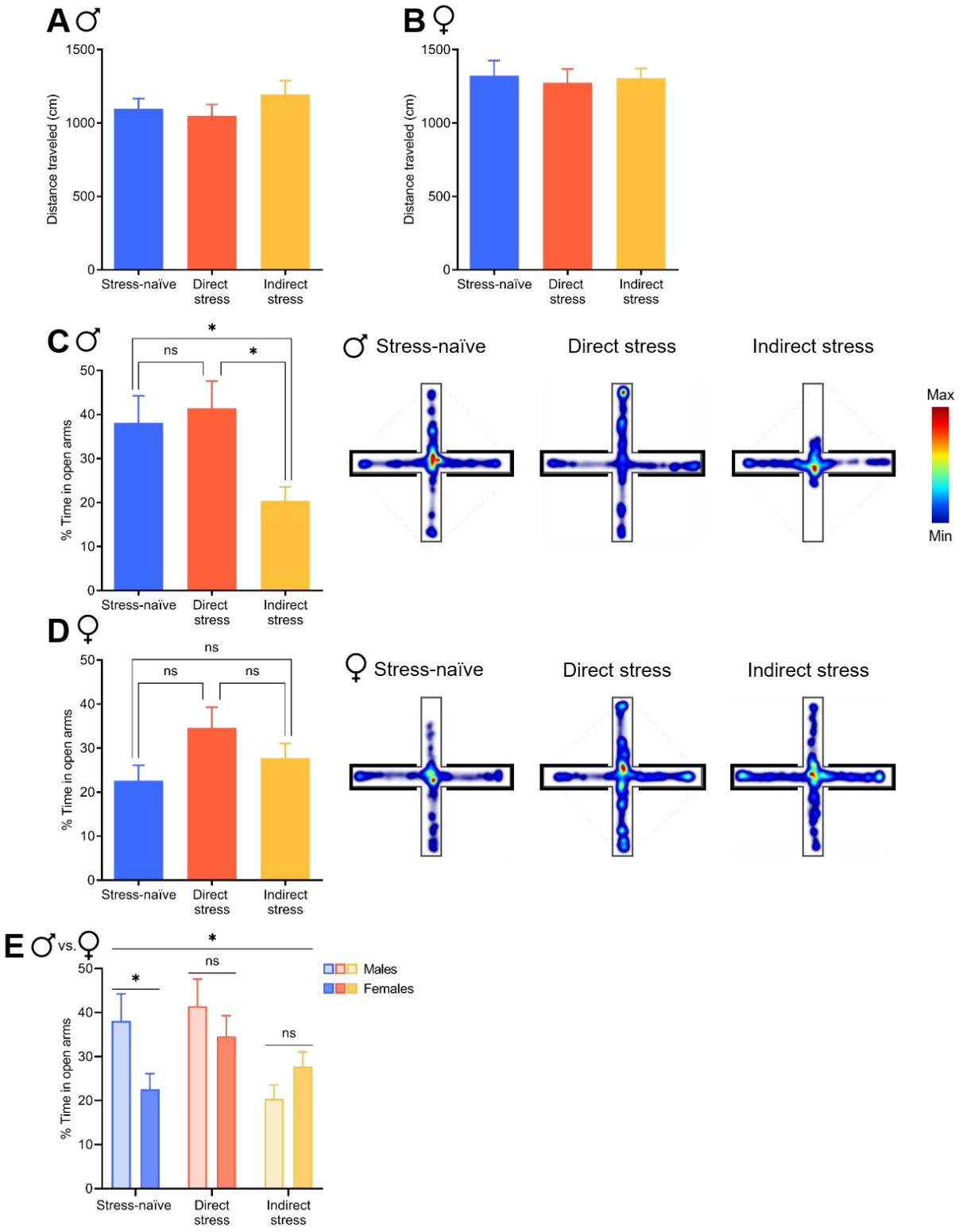


Supplemental Figure 2.3. Direct and indirect stress do not affect social interaction one week after stress exposure. (A) Amount of time spent in the novel object and novel

mouse zones in the three-chamber social approach assay in stress-naïve, indirectly stressed, and directly stressed males (n = 3, left) and representative heatmaps of activity in this assay (right). **(B)** Amount of time spent in the novel object and novel mouse zones in the three-chamber social approach assay in stress-naïve, indirectly stressed, and directly stressed females (n = 3-5, left) and representative heatmaps of activity in this assay (right). **(C)** Comparison of the percentage of total interaction zone exploration time (defined as time in novel object interaction zone + time in novel mouse interaction zone) that was spent in the novel mouse interaction zone in stress-naïve, indirectly stressed, and directly stressed males and females (n = 3-5). Error bars, SEM. NS $P>0.05$, *$P<0.05$, ***$P<0.001$, ****$P<0.0001$.

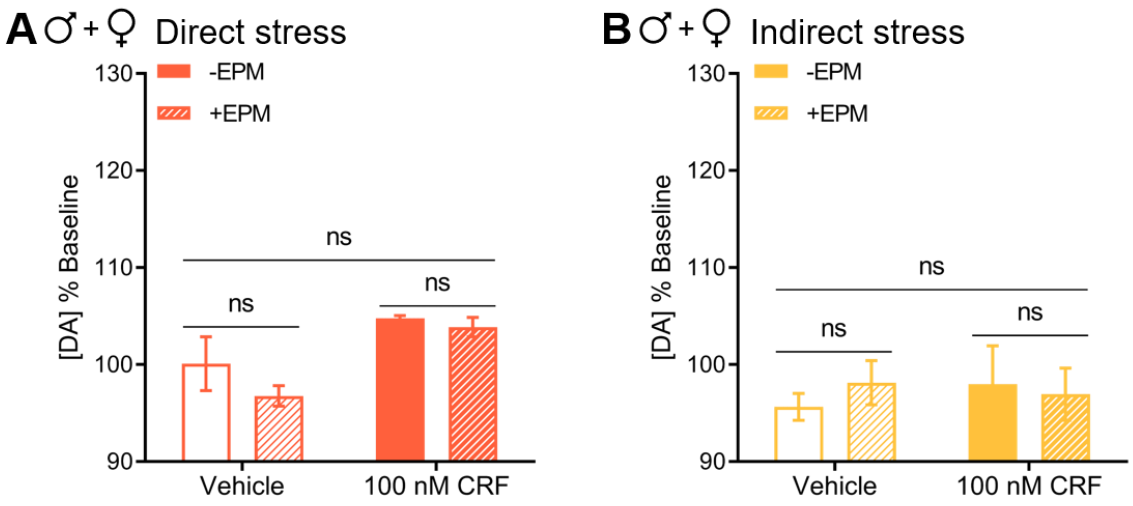


Supplemental Figure 2.4. Direct and indirect stress do not affect depression-like behavior one week after stress exposure. (A) Cumulative time spent immobile for the duration the tail suspension test in stress-naïve, directly stressed, and indirectly stressed males (n = 5-6). **(B)** Cumulative time spent immobile for the duration the tail suspension in stress-naïve, directly stressed, and indirectly stressed females (n = 4-5). **(C)** Cumulative time spent immobile for the duration the tail suspension test in stress-naïve, directly stressed, and indirectly stressed males and females (n = 4-6). Error bars, SEM. NS P>0.05.



Supplemental Figure 2.5. Direct and indirect stress differentially affect anxiety-like behavior in males and females one week after stress exposure. (A) Distance traveled in the elevated plus maze in stress-naïve, directly stressed, and indirectly stressed males

(n = 12-16). **(B)** Distance traveled in the elevated plus maze in stress-naïve, directly stressed, and indirectly stressed females (n = 13-16). **(C)** Percent of time spent exploring the open arms of the elevated plus maze in stress-naïve, directly stressed, and indirectly stressed males (n = 12-16, left) and representative heatmaps of activity in the EPM (right). **(D)** Percent of time spent exploring the open arms of the elevated plus maze in stress-naïve, directly stressed, and indirectly stressed females (n = 13-16, left) and representative heatmaps of activity in the EPM (right). Error bars, SEM. *P<0.05. **(E)** Comparison of percentage of total time spent exploring the open arms of the elevated plus maze in stress-naïve, directly stressed, and indirectly stressed males vs. females (n = 12-16). Error bars, SEM. NS P>0.05, *P<0.05.



Supplemental Figure 2.6. Exposure to the EPM does not affect CRF-mediated NAc dopamine release in mice that have undergone direct or indirect stress. (A) Baseline-normalized mean peak amplitude of dopamine release 20-30 min after vehicle or 100 nM CRF application to NAc slices from directly stressed males and females (n = 3-8). **(B)** Baseline-normalized mean peak amplitude of dopamine release 20-30 min after vehicle or 100 nM CRF application to NAc slices from indirectly stressed males and females (n = 5-6). Error bars, SEM. NS P>0.05.

Chapter 3. Modeling social defeat stress in female C57BL/6 mice

Introduction

Despite higher lifetime prevalence of depression and anxiety in women compared to men, and existing gender differences in etiology and response to treatment for neuropsychiatric disorders, a majority of basic research studies aimed at elucidating the neurobiological mechanisms underlying depression have been conducted using male animals. Exposure to repeated social defeat stress (SDS) produces a validated animal model of depression-like behaviors with ethological significance and face validity (Krishnan & Nestler, 2011). Traditionally, this model exploits a strong innate territorial aggression in male rodents towards other males. Using this model, male C57BL/6 mice repeatedly subjected to bouts of SDS by a larger, dominant male mouse (aggressor) demonstrate dynorphin-mediated social defeat behaviors (McLaughlin et al., 2006). While other species, such as the California mouse (*Peromyscus californicus*), exhibit female intrasexual territorial aggression (Trainor et al., 2011), we confirm that female C57BL/6 mice (*Mus musuclus*) do not spontaneously exhibit robust aggression towards unfamiliar males or females. Because lesioning the lateral septum (LS) is known to engender "septal rage" in males (Slotnick et al., 1973), we tested whether LS inhibition using a DREADD-mediated approach induces social aggression by bilaterally targeting AAV-hSyn-hM4Di-mCitrine to the LS (LS^{hM4Di}) of C57BL/6 male and female mice. After allowing two weeks for hM4Di expression to occur, singly housed LS^{hM4Di} males were screened for aggressive behavior with the resident-intruder (R-I) paradigm, in which an unfamiliar male mouse (intruder) was placed in the home cage of the LS^{hM4Di} male (resident). On test day, two R-I assays were conducted: one in the morning (t = 0) in which the LS^{hM4Di} resident male

was saline-pretreated, and a second assay at t = 4 hours in which the same LS^{hM4Di} resident male was CNO-pretreated (3 mg/kg) to induce LS inactivation.

Here, we found that relative to saline-pretreatment, CNO-pretreatment significantly enhanced intrasexual aggression exhibited by LS^{hM4Di} males towards intruders, effectively inducing social defeat of the intruder male. In contrast to the robust aggression exhibited by CNO-pretreated LS^{hM4Di} males, CNO-pretreatment in LS^{hM4Di} female residents did not promote intrasexual aggression towards intruders. However, LS^{hM4Di} males exhibited aggression (not sexual behavior) resembling that of male intrasexual aggression towards female intruders if the female intruder had previously been swabbed with urine collected from a male mouse. Socially defeated female intruders exhibited social defeat behaviors mirroring those of socially defeated male intruders. Overall, through this work, we demonstrate

Results

We tested whether DREADD-mediated inhibition of the lateral septum (LS) induces aggression by bilaterally targeting AAV-hSyn-hM4Di-mCitrine to the LS (LS^{hM4Di}) of C57BL/6 male and female mice (Fig. 3.1). After allowing two weeks for hM4Di expression to occur (Fig. 3.2A), singly housed LS^{hM4Di} males were screened for aggressive behavior using the resident-intruder (R-I) paradigm, in which an unfamiliar male mouse (intruder) was placed in the home cage of the LS^{hM4Di} male (resident). On test day, two R-I assays were conducted: one in the morning (t = 0) in which the LS^{hM4Di} resident male was saline-pretreated, and a second assay at t = 4 hours in which the same LS^{hM4Di} resident male was CNO-pretreated (3 mg/kg) to induce LS inactivation. Relative to saline-pretreatment, CNO-pretreatment significantly enhanced LS^{hM4Di} resident

aggression (exemplified by increased frequency and duration of attacks) towards male intruders (paired t-test; $t(9) = 4.325$, $P = 0.0019$; Fig. 3.2B), effectively inducing social defeat of the intruder male, which was characterized by the presence of submissive postures, immobility, escape attempts, and defensive upright stances. CNO-induced male intrasexual aggression was reversible and repeatable (data not shown).

In contrast to the robust aggression exhibited by CNO-pretreated LS^{hM4Di} males, CNO-pretreated LS^{hM4Di} female residents exhibited significantly less aggression (in the form of mounting and chasing) towards intrasexual intruders compared to when they were pretreated with saline 4 hours earlier (paired t-test; $t(11) = 3.779$, $P = 0.0031$; Fig. 3.2C). The initial level of aggression observed at saline t = 0 could be recovered: Mice that were saline-pretreated and retested at t = 24 hours exhibited levels of aggression that were not significantly different than that displayed at t = 0 (one-way RM ANOVA with Dunnett's post-hoc tests: $F_{1.252,7.511} = 8.397$, $P = 0.0180$; Fig. 3.2D). Finally, to test if the CNO-mediated decrease in aggression was in fact due to CNO-induced inactivation of the LS, we next conducted an experiment wherein female LS^{hM4Di} mice were re-treated with saline at t = 4 hours. Here, we found that saline re-treatment at t = 0 decreased aggression relative to the saline t = 0 pretreatment (paired t-test; $t(5) = 2.450$, $P = 0.0579$; Fig. 3.2E), indicating that the decreased aggression observed in response to CNO pretreatment at t = 4 hours was likely due to retesting rather than LS inactivation. Indeed, analyzing the data collected from all groups without matching revealed that pretreatment with CNO or saline at t = 4 hours but not with saline at t = 24 hours significantly decreased aggression relative to saline pretreatment at t = 0 (one-way ANOVA with Dunnett's post-hoc tests: $F_{3,33} = 6.733$, $P = 0.0011$; Fig. 3.2F).

We also evaluated the effect of DREADD-mediated activation (hM3Dq) of the LS and its downstream site implicated in mediating aggression in males, the ventrolateral division of the ventromedial nucleus of the hypothalamus (vlVMH; Wong et al., 2016), on female intrasexual aggression. Female LS^{hM3Dq} mice displayed less aggression when pretreated with CNO at t = 4 hours and saline at t = 4 and 24 hours relative to when they were pretreated with saline at t = 0 (saline t = 0 vs. CNO t = 4 and saline t = 24, one-way RM ANOVA with Dunnett's post-hoc tests: $F_{1.939,11.64}$=6.419, P=0.0137; saline t = 0 vs. 4 hours, paired t-test t(1) = 2.091, P = 0.2840; Fig. 3.3A and B). Analyzing the data collected from all groups without matching revealed that pretreatment with CNO at t = 4 hours or saline at t = 24 hours but not with saline at t = 4 hours significantly decreased aggression relative to saline pretreatment at t = 0 (one-way ANOVA with Dunnett's post-hoc tests: $F_{3,19}$ = 5.293, P = 0.0080; Fig. 3.3C). Notably, to complete this data set, the number of subjects in the LS^{hM3Dq} saline t = 4 hours group needs to be increased. At a sample size of n = 2 mice, analyses conducted with this data are currently underpowered. Female $vlVMH^{hM3Dq}$ mice exhibited decreased aggression towards intruders when they were pretreated with CNO at t = 4 hours, but by t = 24 hours, following treatment with saline, they recovered their initial levels of aggression (one-way RM ANOVA with Dunnett's post-hoc tests: $F_{1.019,2.037}$=6, P=0.0154; Fig. 3.3D). The t = 4 hours saline group is an important control that the $vlVMH^{hM3Dq}$ dataset currently lacks.

We were unable to induce reliable intrasexual aggression in female LS^{hM4Di}, LS^{hM3Dq}, and $vlVMH^{hM3Dq}$ mice under myriad circumstances, including the utilization of both virgin and lactating female intruders, as well as resident LS^{hM4Di} mice that were postpartum (maternal aggression model), singly-, or group-housed. Interestingly, LS^{hM4Di}

males exhibited aggression (not sexual behavior) resembling that of male intrasexual aggression towards female intruders if the female intruder had previously been swabbed with urine collected from a male mouse. Socially defeated female intruders exhibited social defeat behaviors mirroring those of socially defeated male intruders (data not shown). Please see Table 3.1 for a summary of the experiments that were conducted with the goal of developing a model for female social defeat stress.

In contrast to LS^{hM4Di} and LS^{hM3Dq} females following CNO pretreatment as well as relative to the within-subject saline pretreatment at t = 0, $vlVMH^{hM3Dq}$ females pretreated with CNO at t = 4 hours exhibited behavior that appeared to reflect a state of agitation (i.e., mice displayed a preponderance of digging behavior and locomotor activity in the R-I assay; data not shown). Therefore, we next subjected saline- and CNO-pretreated $vlVMH^{hM3Dq}$ females to the elevated plus maze (EPM) to ascertain if vlVMH activation was anxiogenic. We found that $vlVMH^{hM3Dq}$ females that were pretreated with CNO spent less time exploring the open arms of the EPM than females that were pretreated with saline (unpaired t-test; $t(5)=1.480$, $P=0.1989$; Fig. 3.4A). At a sample size of 2-5 subjects per group, this comparison is underpowered and is thus not significant. Next, given the role of the VMH in regulating food intake (Panksepp, 1971), we investigated whether CNO-induced activation of the vlVMH affected light cycle feeding behavior. We found that CNO-induced activation of the vlVMH induced hyperphagia relative to when mice were pretreated with saline (two-way RM ANOVA with Tukey's post-hoc tests; interaction of Time x Drug, $F_{9, 24}=7.216$, $P<0.0001$; main effect of time, $F_{3,24}=38.26$, $P<0.0001$; main effect of drug, $F_{3, 8}=8.476$, $P=0.0073$; Fig. 3.4B). Furthermore, this effect appeared to be mediated in part by the kappa opioid receptor (KOR), as pretreatment with the long-acting KOR antagonist

norBNI (10 mg/kg) one week prior to the experiment partially abrogated the CNO-induced hyperphagia.

Discussion

The goal of the experiments described above was to develop a social defeat stress model that was effective in female C57BL/6 mice. We first conducted proof-of-concept experiments in males, which demonstrated that hM4Di-mediated inhibition of the lateral septum promoted robust intrasexual aggression. Next, we attempted to adapt this approach to females with numerous efforts detailed in Table 3.1. Although we were unable to successfully induce female intrasexual aggression, we ultimately developed an inconsistent model that sometimes resulted in what appeared to be female social defeat behavior. This model consisted of swabbing the back and anogenital region of female intruders with urine collected from a male mouse. Using LS^{hM4Di} as well as non-injected sexually experienced males, we observed that a subset of these residents exhibited aggression (not mating behavior) towards urine-swabbed female intruders.

These experiments were not continued beyond what is presented in this dissertation for two reasons: 1) the UNC Vector Core stopped producing the hM4Di virus and our efforts to have the plasmid (purchased from Addgene) packaged independently were unsuccessful and 2) in its most evolved form, our model (i.e., swabbing female intruders with male urine) lacked substantial construct and face validity.

Materials and Methods

Animals. Male and female C57BL/6 mice aged >7 weeks old were maintained under a 12-h light–dark cycle with access to standard food and water *ad libitum*. All procedures

on animal subjects were approved by the University of Washington Institutional Animal Care and Use Committee. Mice were singly or group housed (up to five same-sex mice per cage).

Stereotaxic viral injections. Lateral septum (LS) coordinates: AP +1.00, DV -3.10, ML ±0.35. LS injection volume: 200 nL/hemisphere. Ventrolateral VMH (vlVMH) coordinates: AP -1.45, DV -5.75, ML ±0.60. vlVMH injection volume: 25 nL/hemisphere. AAV2-hSyn-hM4Di-mCitrine and AAV8-hSyn-hM3Dq-mCherry were purchased from the UNC Vector Core. Animals were given two weeks between surgery and testing to allow sufficient time for viral infection.

Resident-Intruder assay. In the morning, resident, DREADD-injected mice were injected with saline 30 minutes prior to the start of the R-I assay. The R-I interaction occurred in the resident mouse's home cage and consisted of introducing a novel intruder mouse into said resident's cage. The 10-minute long R-I interaction was video recorded for later behavioral scoring and analysis. At least four hours after the saline injection, residents were injected with 3 mg/kg CNO 30 minutes prior to the start of the afternoon R-I session, as described above.

Drugs. Clozapine N-oxide (CNO; catalog #: C0832-5MG) was obtained from Sigma-Aldrich. Drug solutions were prepared on the day of the experiment at 3 mg/kg in saline.

Figures 3.1-3.4

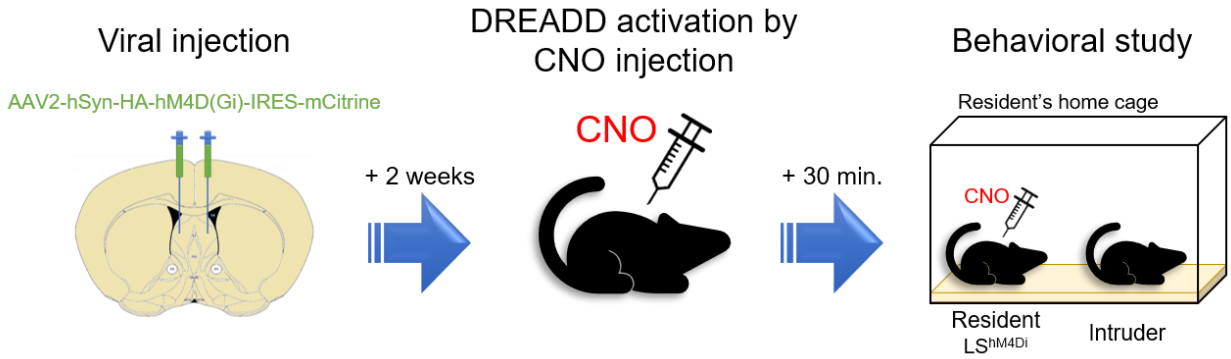
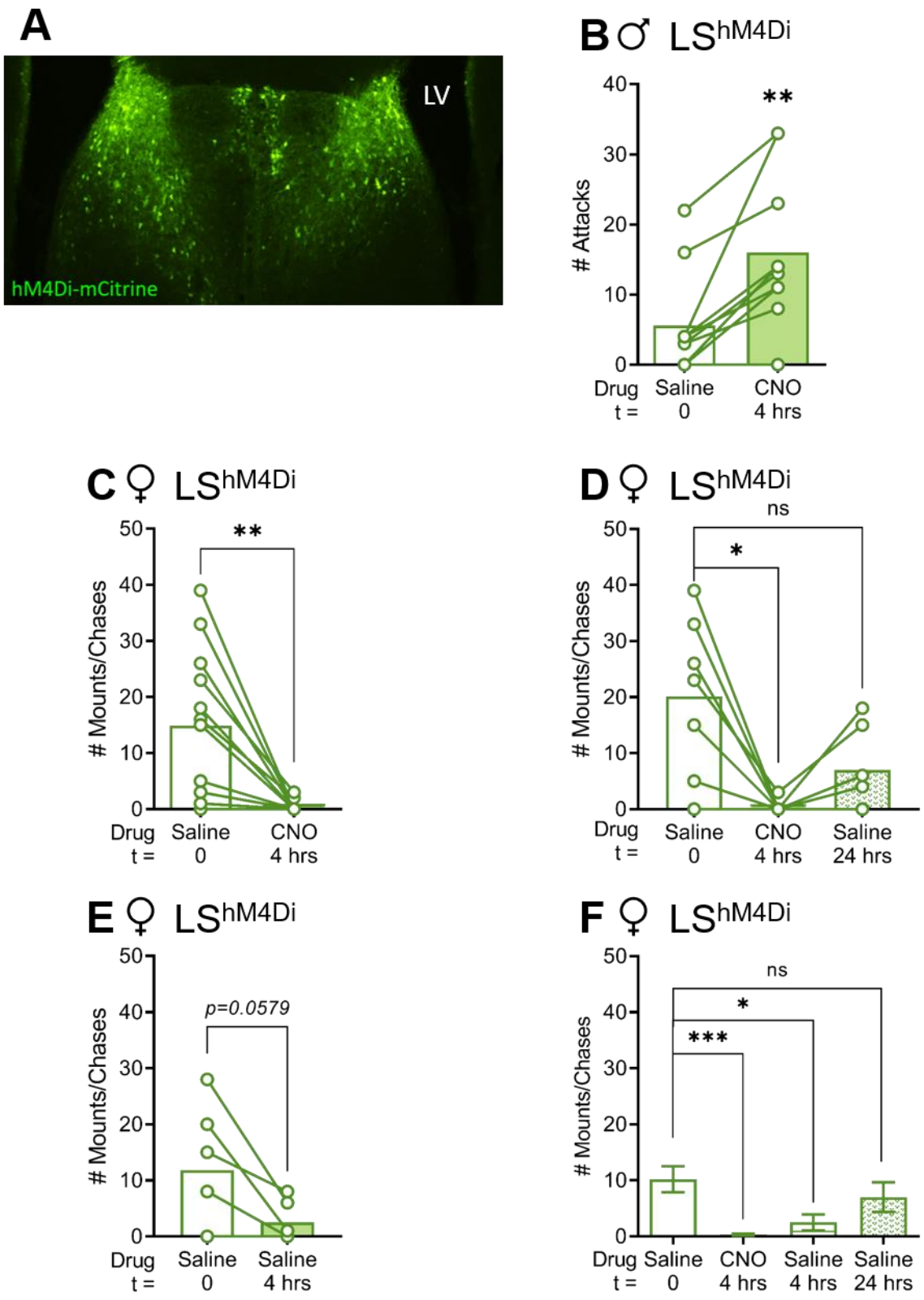


Figure 3.1. Illustration of experimental design. We tested whether LS inhibition induces social aggression using a DREADD-mediated approach: AAV-hSyn-hM4Di-mCitrine was bilaterally targeted to the LS (LS^{hM4Di}) of C57BL/6 male and female mice. After allowing two weeks for hM4Di expression to occur, singly housed LS^{hM4Di} mice were screened for intrasexual aggressive behavior with the resident-intruder (R-I) paradigm, in which an unfamiliar mouse (intruder) of the same sex was placed in the home cage of the LS^{hM4Di} mouse (resident). On test day, two R-I assays were conducted: one in the morning (t = 0) in which the LS^{hM4Di} resident was saline-pretreated (not depicted above), and a second assay at t = 4 hours in which the same LS^{hM4Di} resident was CNO-pretreated (3 mg/kg) to induce LS inactivation.


A
LV
hM4Di-mCitrine
B ♂ LS^hM4Di
**
Attacks
Drug Saline CNO
t = 0 4 hrs
C ♀ LS^hM4Di
**
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Drug Saline CNO
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D ♀ LS^hM4Di
ns
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Mounts/Chases
Drug Saline CNO Saline
t = 0 4 hrs 24 hrs
E ♀ LS^hM4Di
p=0.0579
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Drug Saline Saline
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F ♀ LS^hM4Di
ns
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Mounts/Chases
Drug Saline CNO Saline Saline
t = 0 4 hrs 4 hrs 24 hrs

Figure 3.2. Chemogenetic inhibition of the LS in male mice promotes intrasexual aggression. (A) A coronal brain section illustrating hM4Di expression in the LS of a male C57BL/6 mouse. **(B)** Mean number of attacks made by resident LS^{hM4Di} males towards same-sex intruders following a saline (t = 0) or CNO (t = 4 hours) IP injection (n = 10). **(C)** Mean number of mounts and chases exhibited by resident LS^{hM4Di} females towards same-sex intruders following a saline (t = 0) or CNO (t = 4 hours) IP injection (n = 12). **(D)** Mean number of mounts and chases exhibited by resident LS^{hM4Di} females towards same-sex intruders following a saline (t = 0 or 24 hours) or CNO (t = 4 hours) IP injection (n = 7). **(E)** Mean number of mounts and chases exhibited by resident LS^{hM4Di} females towards same-sex intruders following a saline injection at t = 0 and 4 hours (n = 6). **(F)** Mean number of mounts and chases exhibited by resident LS^{hM4Di} females towards same-sex intruders following a saline (t = 0, 4, or 24 hours) or CNO (t = 4 hours) IP injection (n = 6-12). Error bars, SEM. NS $P>0.05$. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

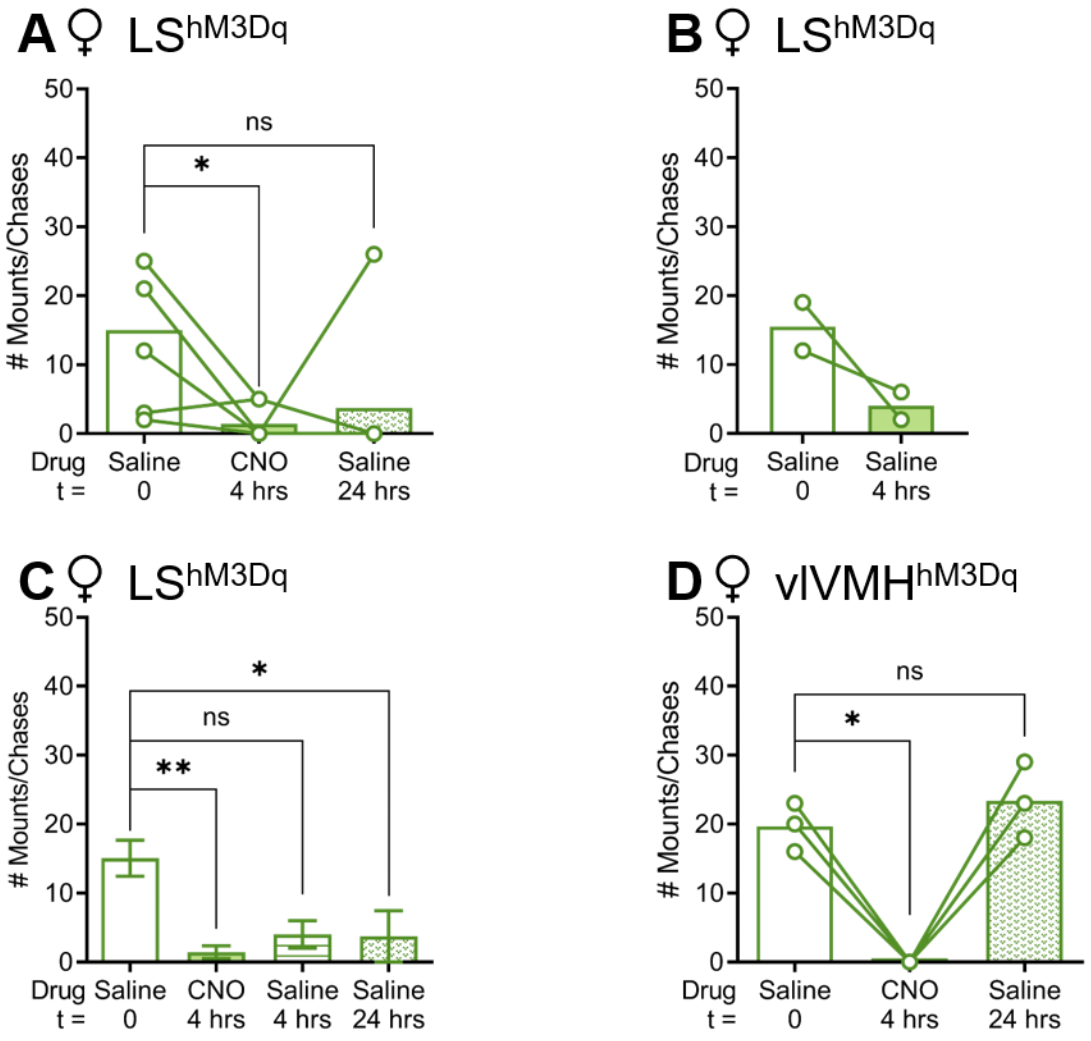

Figure 3.3. Chemogenetic excitation of the LS and vlVMH in female mice has ambiguous effects on female aggression. (A) Mean number of mounts and chases exhibited by resident LS^{hM3Dq} females towards same-sex intruders following a saline (t = 0 or 24 hours) or CNO (t = 4 hours) IP injection (n = 7). **(B)** Mean number of mounts and chases exhibited by resident LS^{hM3Dq} females towards same-sex intruders following a saline injection at t = 0 and 4 hours (n = 2). **(C)** Mean number of mounts and chases exhibited by resident LS^{hM3Dq} females towards same-sex intruders following a saline (t = 0, 4, or 24 hours) or CNO (t = 4 hours) IP injection (n = 2-7). **(D)** Mean number of mounts and chases exhibited by resident vlVMH^{hM3Dq} females towards same-sex intruders following a saline (t = 0 or 24 hours) or CNO (t = 4 hours) IP injection (n = 3). Error bars, SEM. NS P>0.05. *P<0.05, **P<0.01.

Table 3.1 Experiments conducted with the goal of modeling social defeat stress in females.

Resident (R) sex	DREADD	Brain region targeted	Add'l. R manipulations	Intruder (I) sex	I pretreat-ment	R-I observations
Male	hM4Di	LS	N/A	Male	N/A	CNO vs. saline: ↑ Aggression
				Female	N/A	Saline & CNO: Male residents attempted to mate with females
					Swabbed with male mouse urine	Saline & CNO: Aggression
	N/A	N/A	Sexually experienced	Female	N/A	Male Rs tried to mate with females
					Swabbed with male mouse urine	Aggression
Female	hM4Di	LS	N/A	Female	N/A	↓ Aggression*
			Group housed, one R	Female	N/A	No effect
			Group housed, multiple Rs			
			Post-partum, pups removed from cage for R-I			
			Post-partum, pups in cage for R-I			
	hM3Dq	LS	N/A	Female	N/A	↓ Aggression*
			Group housed, multiple Rs	Female	N/A	No effect
	hM3Dq	vlVMH	N/A	Female	N/A	↓ Aggression**
					Lactating (post-partum)	No effect
			Post-partum, pups removed from cage for R-I	Female	N/A	No effect

*Aggression decreased in a drug-independent manner with repeated R-I sessions. **Aggression decreased with CNO at t=4. However, R-I at saline t = 4 hasn't been conducted.

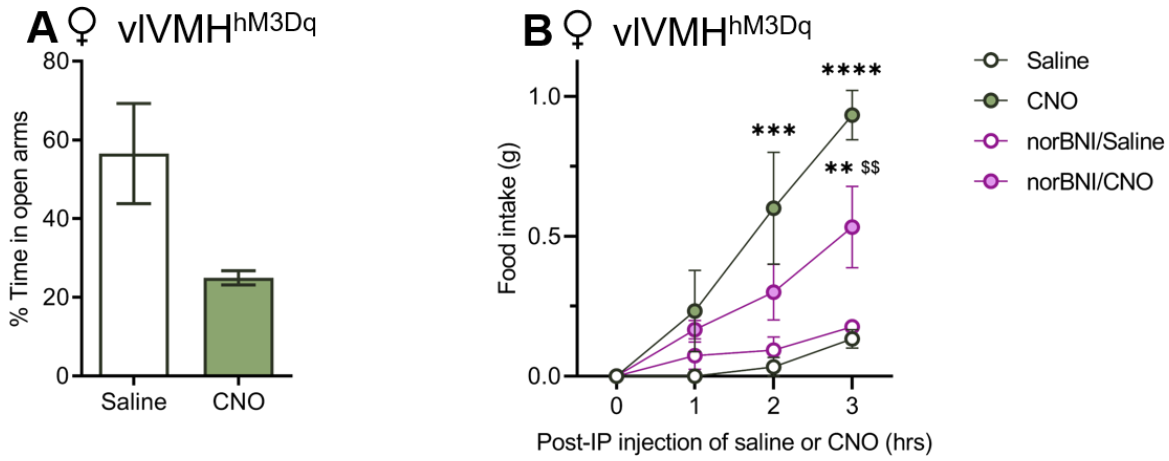

Figure 3.4. Chemogenetic activation of the vlVMH in females is anxiogenic and increases light cycle food intake. (A) Percent of total time spent exploring the open arms of the elevated plus maze in LS^{hM3Dq} females that were treated with saline or CNO (n = 2-5). **(B)** Amount of food consumed during the light cycle in LS^{hM3Dq} females that were treated with saline or CNO (n = 3). Two additional groups were pretreated with norBNI pretreated one week before the experiment (n = 3). Error bars, SEM. NS P>0.05. **P<0.01 vs. saline, $^{\$\$}$P<0.01 vs. CNO, ***P<0.001 vs. saline, ****P<0.0001 vs. saline.

Chapter 4. Conclusion

The primary findings reported in this dissertation are fourfold: 1) The regulation of dopamine by CRF in the NAc of stress-naïve animals is qualitatively similar between sexes with CRF being more potent in males than females, 2) stress exposure can engender a loss of this regulation in the absence of any overt behavioral manifestations in both sexes, 3) this stress-induced neurochemical change can be socially transmitted to animals not directly exposed to the primary stressor and 4) modeling social defeat stress in female C57BL/6 mice is largely unfeasible.

Sexual dimorphism in the CRF system has been reported in other brain regions including the LC, BNST, DRN, hippocampus, and cortex with females exhibiting greater sensitivity to CRF than males (Bangasser & Wiersielis, 2018; Valentino et al., 2013). The finding that CRF is more potent at increasing NAc dopamine release in stress-naïve males than females begs the question of what biological sex differences underlie this observation. Several potential explanations for our findings might include NAc sexual dimorphism in 1) CRFR expression, with males having higher receptor expression levels than females, 2) CRFR trafficking, with males having higher CRFR expression in the plasma membrane than the cytosol relative to females, and/or 3) CRFR coupling and signaling, with CRFR1 in males being more highly coupled to Gs than females, leading to enhanced activation of the cAMP-PKA pathway in males relative to females. Notably, enhanced CRFR1-mediated cAMP-PKA signaling in the LC in females has been linked to increased CRF sensitivity compared to males (Curtis et al., 2006).

To evaluate whether the increase in stress-naïve male sensitivity to CRF as it functions to potentiate NAc dopamine release is due to the third hypothesis (enhanced

Gs coupling leading to increased cAMP-PKA signaling), the same CRF slice voltammetry experiments that I performed (described in Chapter 2 of this dissertation) could be repeated in males and females in the presence of an inert cAMP analog (e.g., Rp-cAMPs), which would block cAMP-dependent signaling. Separate experiments could evaluate the effect of a PKA inhibitor (e.g., PKI 14–22) on CRF-mediated potentiation of NAc dopamine release in stress-naïve males and females. If the hypothesis is correct and males have increased Gs coupling and/or signaling via cAMP-PKA relative to females, I would expect that the cAMP analog and the PKA inhibitor would more profoundly block CRF-induced potentiation of NAc dopamine release in stress-naïve males compared to females.

Another unanswered question that the work presented in this dissertation prompts is what biological function CRF serves in the NAc core. Through my work and my reading of the extant literature, my sense is that CRF in the NAc core serves a highly nuanced function related to cue—and potentially incentive—salience. The experiments that I conducted aimed to ascertain whether stress exposure altered 'innate' behaviors (i.e., social interaction, depression-like behavior, and anxiety-like behavior) on a post-stress time scale that corresponds to when CRF-dopamine interactions in the NAc are not intact. However, apart from indirectly stressed males, which exhibited increased anxiety-like behavior, direct and indirect stress did not discernably affect these behaviors.

To gain insight into what stimuli or contexts promote CRF release in the NAc core, which in turn might function to inform the design of future behavioral experiments that aim to further characterize the role of CRF in the NAc, one approach could be to inject a retrograde GCaMP virus in the NAc core of a CRF-Cre mouse and to implant optic fibers above the NAc. Then, the mouse could be exposed to a variety of behavioral tasks and

calcium activity of CRF NAc afferents could be imaged. Once one or several contexts that lead to increased NAc CRF afferent activity have been identified, depending on whether or not the behavioral assay can be repeated, it would be interesting to do a within-subject study so that recordings could be obtained pre- and post-stress. However, if this is not tenable, a study that includes separate stress-naïve and a stress-exposed groups would suffice.

An alternative approach to using a retrograde GCaMP virus for the experiment described above would be to inject an anterograde GCaMP virus into CRF^{+} neuronal populations with identified NAc afferents, including the paraventricular nucleus of the thalamus, BNST, medial prefrontal cortex, and the basolateral amygdala (Itoga et al., 2019).

Finally, the increased anxiety-like behavior observed in indirectly stressed males but not directly stressed males or females, nor indirectly stressed females, was intriguing and future experiments aimed at better characterizing the mode of indirect stress transmission would likely inform our understanding of the etiology of this sex- and stressor-specific phenotype. Videotaping and analyzing home cage interactions between directly stressed and indirectly stressed mice before and after the directly stressed mice undergo rFSS could be illuminating in this regard. An additional factor to consider, and why I would advocate for analyzing social interactions between cage mates before and after stress exposure, is that the pre-existence of social hierarchies in male rodents has been linked to stress vulnerability (Larrieu et al., 2017). Thus, in both sexes, it would be interesting to examine whether direct and indirect stress exposure alters the social hierarchy within a cage using the social-confrontation tube test (Wang et al., 2011) and

how potential changes in social standing affect anxiety-like behavior in the EPM as well as NAc CRF-dopamine interactions as measured using slice FSCV.

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Jennifer S. Steger

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Education

University of Washington, Seattle, WA 2015-2020
Ph.D., Pharmacology

Brown University, Providence, RI 2009-2013
Sc.B. with Honors in Neuroscience

Research Experience

Neurobiology of Stress 2015-Present
Role: Graduate Student
Advisors:

- **Charles Chavkin, Ph.D.**, Allan & Phyllis Chair of Pain Research and Professor of Pharmacology, University of Washington
- **Paul E. M. Phillips, Ph.D.**, Professor of Pharmacology, Professor of Psychiatry and Behavioral Sciences, University of Washington

- Developed a novel stress model with excellent ethological and face validity and phenotyped mice subjected to aforementioned stress using a) fast-scan cyclic voltammetry to measure sub-second changes in evoked dopamine release from acute nucleus accumbens brain slices and b) a suite of behavioral assays including the tail suspension test, elevated plus maze, and social interaction.

Neural Circuitry of Appetite Regulation 2013-2015
Role: Research Assistant
Advisor: **Bradford B. Lowell M.D., Ph.D.**, Professor of Medicine, Harvard Medical School, Beth Israel Deaconess Medical Center

- Contributed to the design and execution of experiments for several projects utilizing genetic engineering techniques in mice, in conjunction with optogenetics, pharmacogenetics (DREADDs), and rabies mapping, to elucidate discrete neural circuits controlling appetite and feeding behavior.
- Published results describe a novel anorectic brain circuit comprised of MC4R-expressing neurons in the Paraventricular Hypothalamus (PVH) that project to the Lateral Parabrachial Nucleus (LPBN) to promote satiety (please see citation in **Publications** section).
- Skills acquired in this position include:
    - Stereotaxic surgery: intracranial cre-dependent AAV injection, fiber-optic implantation for optogenetics studies.
    - Behavioral phenotyping: optogenetics and DREADDs feeding studies, real-time place preference.
    - Histology: transcardial perfusion and brain extraction, tissue preparation and cryosectioning, immunohistochemistry, mounting/slide preparation, fluorescence and confocal microscopy.
    - Mouse husbandry: breeding, weaning, genotyping (tissue collection, DNA extraction, PCR, gel electrophoresis).

Neuroendocrinology of Obesity 2011-2013
Role: Undergraduate Honors Research Student
Advisor: **Eduardo A. Nillni M.S., Ph.D.**, Brown University, Department of Medicine and Molecular Biology, Cell Biology & Biochemistry

- Designed and performed *in vivo* and *in vitro* experiments to investigate neuroendocrine and cellular mechanisms underlying obesity pathology using the following techniques:
  - *in vivo*: intra-cerebro-ventricular (ICV) cannulation surgeries in Sprague-Dawley rats, ICV hormone infusions, microdissection of arcuate nucleus and paraventricular nucleus, sample collection including brown adipose tissue, liver, skeletal muscle, serum collection.
  - *in vitro*: cDNA and siRNA transfections in AtT-20 anterior pituitary cells, N43 neuronal cells, and human embryonic kidney 293 (HEK 293) cells.
  - Assays: Bradford assay for protein quantification, western blot for protein expression.
- Observed that hypothalamic inhibition of the nutrient-sensing enzyme Sirt1 results in increased energy expenditure in obese rats; identified that this response is mediated by the melanocortin pathway and the HPT axis (please see citation in **Publications** section).

Genetic Susceptibility of Tanoak to Sudden Oak Death Summer 2010
Role: Summer Research Student
Advisor: Richard S. Dodd, Ph.D., University of California, Berkeley, Department of Environmental Science, Policy & Management

- Designed and conducted experiments investigating the genetic susceptibility of Tanoak to the pathogen responsible for Sudden Oak Death.
- Extracted and purified nuclear and chloroplast DNA from leaf samples collected from six Tanoak populations in Northern California.

Genetic Causes of Panamerican Ground Sloth Extinction Summer 2010
Role: Summer Research Student
Advisor: Anthony Barnosky, M.S., Ph.D., University of California, Berkeley, Department of Integrative Biology

- Developed a study to determine if mass-extinction of the Panamerican Ground Sloth at the Tanque Loma site in Santa Elena, Ecuador during the late Pleistocene epoch was due to genetic predisposition to a hyperdisease.
- Sorted through soil samples from the site to collect fossilized bone fragments for DNA analysis.

The Juneau Icefield Research Program (JIRP) Summer 2008
Role: Summer Research Student
Advisor: Maynard M. Miller, Ph.D., University of Alaska Southeast, Juneau Icefield, AK

- Developed and conducted an independent field research project to explore and characterize the existence of distinct microclimates on the Taku Glacier.
- Took advanced University field courses in glaciology, geology, meteorology, hydrology, geophysics, atmospheric sciences, geobotany, lichenometry, surveying, and mapping.

Business & Entrepreneurship Experience

Nanodropper, Inc., Seattle, WA 2018-Present
Role: Co-founder and Chief Scientific Officer
The Nanodropper is an affordable, universal eyedropper adaptor that decreases the volume of oversized eyedrops to reduce cost, waste, and side effects, ultimately increasing access to expensive prescription eye medications.

Company traction:

+ Product launch scheduled for June 2020
+ Raised $560k in dilutive and non-dilutive funding as of June 2020
+ Utility patent (No. 16255152) granted by the USPTO, international patent (PCT/US19/14717) pending
+ Listed with the FDA as a class I 510(k)-exempt medical device

Awards:

+ Air Force (AFWERX) SBIR Phase II grantee, contract-pending
+ VentureWell E-Team Stage I grantee — May 2020
+ Finalist at Social Venture Partners Fast Pitch, Seattle, WA — April 2020
+ Semi-finalist at Arizona State University Innovation Open, Phoenix, AZ — Jan. 2020
+ Air Force (AFWERX) SBIR Phase I grantee — Dec. 2019
+ 1st Place: Entrepreneurs' Organization Global Student Entrepreneurship Awards, Seattle, WA — Nov. 2019
+ 2nd Place: Collegiate Inventors Competition, Graduate Division, Alexandria, VA — Oct. 2019
+ Accepted into Buerk Center for Entrepreneurship Jones Foster Accelerator, Seattle, WA — July 2019
+ 1st Place: Life Science Innovation Northwest Fast Pitch Competition, Seattle, WA — April 2019
+ 2nd Place: Innovation Prize at Unite for Sight Global Health and Innovation Conference, New Haven, CT — April 2019
+ 1st Place: Hollomon Health Innovation Challenge, Seattle, WA — Mar. 2019
+ Top 10 Finalist at Baylor New Venture Competition, Waco, TX — Feb. 2019
+ 1st Place: SEBA Science & Technology Showcase, Seattle, WA — Jan. 2019
+ Prototype funding: UW Buerk Center for Entrepreneurship, Seattle, WA — Jan. 2019
+ Judge's Choice Award: UW Business Case 60' Pitch Competition, Seattle, WA — Jan. 2019
+ Audience Favorite Award: Winter Walleye Tank, Rochester, MN — Dec. 2018
+ 1st Place: Walleye Student Showcase, Rochester, MN — Nov. 2018
+ 2nd Place: Future Founders U.Pitch Competition, Chicago, IL — Nov. 2018
+ Best Healthcare Innovation Award: UW Business Plan Competition, Seattle, WA — May 2018
+ 1st Place: Johns Hopkins Student Healthcare Design Competition, Baltimore, MD — April 2018
+ Honorable Mention: BMEidea — April 2018
+ 4th Place & JARL ("Judges Also Really Liked") Award: Hollomon Health Innovation Challenge, Seattle, WA — Mar. 2018
+ Prototype funding: UW Buerk Center for Entrepreneurship, Seattle, WA — Jan. 2018

Publications

1. **J.S. Steger**, B.B. Land, J.C. Lemos, C. Chavkin, P.E.M. Phillips (2020). Insidious transmission of a stress-related neuroadaptation. In review at Frontiers in Behavioral Neuroscience. Manuscript ID #: 564054.
2. J. Berrios, C. Li, J.C. Madara, A.S. Garfield, **J.S. Steger,** M.J. Krashes, and B.B. Lowell (2020). Food cue inhibition of AgRP neurons by a lateral hypothalamic circuit 1 guides learning. Under revision for publication in Nature. Manuscript ID #: 2019-11-17100.
3. M.M. Li*, J.C. Madara*, **J.S. Steger**, M.J. Krashes, N. Balthasar, J.N. Campbell, J.N. Resch, A.S. Garfield, and B.B. Lowell (2019). The paraventricular hypothalamus regulates satiety and prevents obesity via two genetically distinct circuits. Neuron 102(3):653-667.e6. *Equal Contribution

4. A.S. Garfield*, B.P. Shah*, C.R. Burgess*, M.M. Li*, C. Li, **J.S. Steger**, J.C. Madara, J.N. Campbell, D. Kroeger, T.E. Scammell, B.A. Tannous, M.G. Myers, M.L. Andermann, M.J. Krashes, and B.B. Lowell (2016). Dynamic GABAergic afferent modulation of AgRP neurons. Nature Neuroscience 19(12):1628-1635. *Equal Contribution
5. A.M. Toorie, N.E. Cyr, **J.S. Steger**, R. Beckman, G. Farrah, and E.A. Nillni (2016). Hypothalamic paraventricular Sirt1 regulates the CRH axis by altering the post-translational processing of proCRH in male rats. Journal of Biological Chemistry 291(11):5844-59.
6. A.S. Garfield*, C. Li*, J.C. Madara*, B.P. Shah, E. Webber, **J.S. Steger**, J.N. Campbell, O. Gavrilova, C.E. Lee, D.P. Olson, J.K. Elmquist, B.A. Tannous, M.J. Krashes, and B.B. Lowell (2015). A neural basis for melanocortin-4 receptor-regulated appetite. Nature Neuroscience 18(6):863–871. *Equal Contribution
7. N.E. Cyr, **J.S. Steger**, A.M. Toorie, J.Z. Yang, R. Stuart, and E.A. Nillni (2014). Central Sirt1 Regulates Body Weight and Energy Expenditure Along With the POMC-Derived Peptide α-MSH and the Processing Enzyme CPE Production in Diet-Induced Obese Male Rats. Endocrinology 155(7):2423-35.
8. N.E. Cyr, A.M. Toorie, **J.S. Steger**, M. Sochat, M. Perello, S. Hyner, R. Stuart, and E.A. Nillni (2013). Mechanisms by which the orexigen Neuropeptide Y (NPY) regulates anorexigenic α-melanocyte-stimulating hormone (α-MSH) and thyrotropin-releasing hormone (TRH). American Journal of Physiology-Endocrinology and Metabolism 304(6):E640-50.

Presentations

- **J.S. Steger** (2017). Social Contagion of Stress Disrupts CRF Regulation of Dopamine Release. Oral presentation, UW Pharmacology Department Annual Retreat. Bainbridge Island, WA, 2017.

- **J.S. Steger**, S.D. Johnson, Charles Chavkin (2017). Chemogenetic Inhibition of the Lateral Septum in C57BL/6 Male Mice Induces Social Aggression and Subsequent Social Defeat Behaviors in Both Male and Female Intruders. Poster presentation delivered at the 4th Conference on the Therapeutics Potential of Kappa Opioids. Philadelphia, PA, 2017.

- **J.S. Steger**, N.E. Cyr, R. Stuart, and E.A. Nillni. (2013). Inhibition of Hypothalamic Sirt1 in Diet-Induced Obese Rats Significantly Increases Energy Expenditure Through FoxO1 Acetylation and Akt Signaling. Poster presentation delivered at:
  - ENDO 2013: The Endocrine Society's 95th Annual Meeting & Expo. San Francisco, CA. June, 2013.
  - Brown University 2013 Neuroscience Undergraduate Honors Poster Presentations. Providence, RI. April, 2013.

- **J.S. Steger**, N.E. Cyr, R. Stuart, and E.A. Nillni. (2012). Neuronal Mechanisms Underlying the Interaction Between Leptin and Sirt1 in the Hypothalamus. Poster presentation delivered at:
  - Rhode Island Hospital's 20th Annual Hospital Research Celebration. Providence, RI. October, 2012.
  - Brown University's Summer Research Symposium. Providence, RI. August, 2012.
  - 18th Annual Department of Medicine Research Forum. The Warren Alpert Medical School of Brown University. Providence, RI. June, 2012.

Awards

+ **Husky 100, University of Washington** 2019
  - Awarded to 100 UW students who connect what happens inside and outside of the classroom and apply what they learn to make a difference on campus, in their communities and for the future. https://www.washington.edu/husky100/#name=jennifer-steger

+ **Top Scholar Award, University of Washington** 2015
  - Awarded to one incoming graduate student in the Pharmacology Department in recognition of excellent academic record.

+ **Summer Research Fellowship, The Endocrine Society** Summer 2013
  - Awarded to promising undergraduate students, medical students, and first year graduate school students to pursue careers in Endocrinology. Student award recipients participate in research projects under the guidance of a Society member for 10 to 12 weeks during the summer and are provided with funding to attend The Endocrine Society's annual meeting.
  - Advisor: Eduardo A. Nillni
  - Project: Inhibition of Hypothalamic Sirt1 in Diet-Induced Obese Rats Significantly Increases Energy Expenditure Through FoxO1 Acetylation and Akt Signaling

+ **Honors in Neuroscience, Brown University** May 2013
  - Thesis: Inhibition of Hypothalamic Sirt1 in Diet-Induced Obese Rats Significantly Increases Energy Expenditure Through FoxO1 Acetylation and Akt Signaling
  - Advisor: Eduardo A. Nillni

+ **Undergraduate Teaching and Research Award (UTRA), Brown University** Summer 2012
  - Awarded to Brown University undergraduate students to conduct a summer research project under the guidance of a Brown University faculty member.
  - Advisor: Eduardo A. Nillni
  - Project: An Investigation of the Neuronal Mechanisms Underlying the Interaction Between Leptin and Sirt1 in the Hypothalamus

Leadership and Volunteer Experience

♦ SARJE (Scientists Advocating for Representation, Justice, and Equity) Officer, UW 2018-19
♦ UW Pharmacology Department Student/Post-Doc Seminar Coordinator 2017-18
♦ UW Pharmacology Student Association, Student Representative 2016-17
♦ Neurobiology Community Outreach at the University of Washington 2015-20
♦ CASPAR, Inc. Emergency Service Center volunteer, Cambridge, MA 2013
♦ Women in Science and Engineering (WiSE), Brown University 2009-13
  Peer mentor 2011-13
♦ Matched Advising Program for Sophomores (MAPS), Brown University 2010-13
♦ Sciences, Technology, Engineering, and Math (STEM), Brown University 2009-13
  Student Coordinator 2012-13
♦ A Day on College Hill (ADOCH) volunteer, Brown University 2009-13