Controllable stress facilitates increased effort in social and nonsocial tasks through activation of prefrontal circuitry

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Abstract

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Thesis directed by Dr. Michael Baratta (Assistant Professor)

The relationship between stress and social dominance has been studied and stressors have been shown to produce subordinate status. However, these studies utilize only uncontrollable stressors. This is problematic given that control over a stressor (either perceived or actual) protects against the detrimental effects of the stressor. In rats, animals exposed to a controllable stressor (escapable shock/ES) are protected against the behavioral and neurochemical changes that occur following a physically identical uncontrollable stressor (inescapable shock/IS). This protection produced by control only occurs when the instrumental escape response is learned using prefrontal circuitry. Others have shown that similar prefrontal circuitry is both sufficient and necessary for winning in social competitions. Here I will show that 1) controllable stress facilitates dominance through thalamocortical circuitry, 2) stable dominance relies on corticostriatal circuitry and results in protection against inescapable stress similar to having control, and 3) controllable stress increases instrumental effort in non- social tasks. Together these results indicate that both social dominance and controllable stress rely on a thalamocorticostriatal system used to increase effort in goal oriented instrumental tasks, and that robust activation of this system (i.e. by ES) increases performance in other tasks that rely on instrumental effort.

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Chapter 1:

General Introduction

Part I. Overview

Exposure to stressors, especially highly salient or chronic/sustained stressors, can lead to the development of disorders such as major depressive disorder (MDD) and post-traumatic stress disorder (PTSD) (Agaibi & Wilson, 2016; Paunovic, 2010). Traumatic events are a common human experience, but only a minority of individuals that experience a traumatic event go on to develop a psychiatric disorder (Horn et al., 2016; Shin & Liberzon, 2010). Thus, understanding the circumstances that provide resilience to the outcome of adverse events is of clinical importance. Human studies have revealed that the outcome of a stressor is potently modulated by the individual's self-efficacy, ability to successfully employ coping mechanisms, and ability to exert control over the outcome of the stressor (Chorpita & Barlow, 1998; Paunovic, 2010; Southwick et al., 2005). Among these factors capable of modulating the detrimental outcomes of a stressor, behavioral control is one of the few that can be manipulated in an experimental setting in both humans and animals. As a result, the impact of behavioral control on stress can be studied translationally.

Control over a stressor (specifically the ability to terminate, avoid, or escape said stressor) potently mitigates the outcomes of that stressor compared with a physically-identical uncontrollable stressor (Southwick et al., 2005). The protection offered by control extends to both the behavioral consequences of that stressor as observed in human and rodent studies and the neurochemical response (Amat et al., 1998a; Seligman & Maier, 1967). There are various methods of providing behavioral control to rodents, but the triadic stress model (Maier, 2015a; Seligman & Maier, 1967) was the ideal choice for the studies presented in this thesis. In this model, three rats are placed into identical plexiglass boxes with a wheel mounted on the front wall and their tails extending out the rear to attach shock electrodes. One rat receives 100 intermittent tail shocks, each of which terminates when they turn the wheel on the front wall of their box. Thus, the subject has control over the termination of the shock (escapable shock, ES). The second rat (inescapable shock, IS) is yoked to the ES rat, meaning that their tail is connected to in the same circuit with the ES rat's tail, so they receive identical and synchronous tail shock to that of the ES rat (Figure 1.1). Thus, the only difference between these two animals is that the wheel mounted in the IS animals' enclosure is fixed in place and cannot be turned. The shock for the IS rat terminates only when its yoked ES partner turns the wheel thus terminating the shock for both animals. The third rat does not receive shock and serves as a home cage control (HC). Tail shock is used because the manipulation of control requires a repeatable stressor that can be rapidly initiated and terminated such that ES and IS subjects receive the identical physical stimulus. The stimulus must also be one that allows subjects to readily learn the instrumental escape response. These criteria cannot be met with other stress paradigms, such as restraint or social defeat.

Over fifty years of research have characterized the behavioral changes that occur following inescapable stress (Maier & Seligman, 2016). Following shock, the IS animal displayed decreases in juvenile social interaction (JSE), learning of instrumental responses for appetitive rewards, aggression and sexual behavior (Maier & Watkins, 2010). These animals also displayed increases in fear

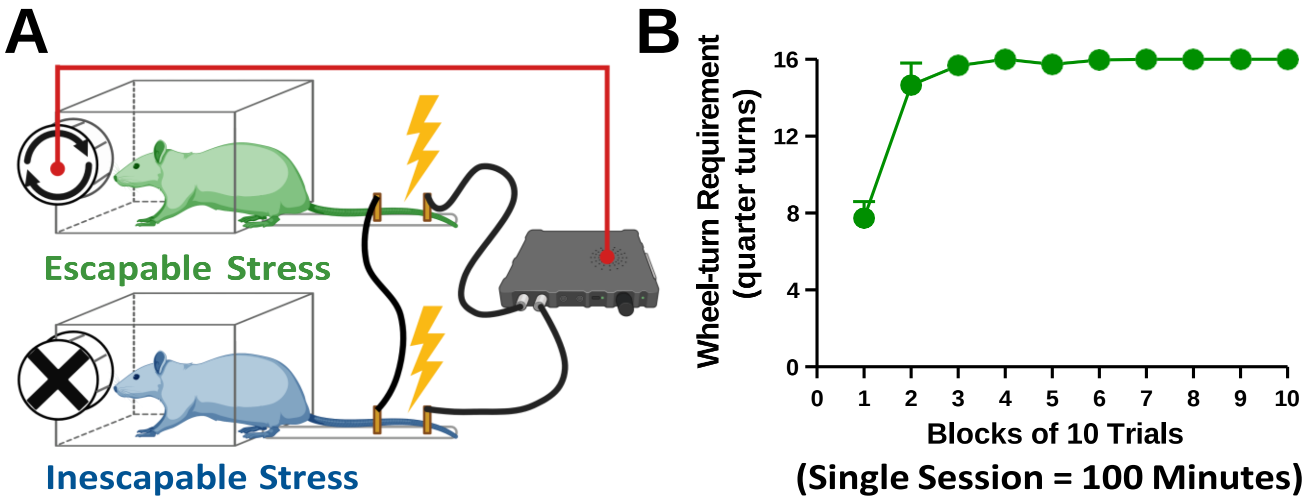

Figure 1.1. Schematic of the wheel turn escape paradigm. A. Representative schematic of escapable stress (ES/green) and inescapable stress (IS/blue) yoked together to receive identical shocks under terminative control by the ES animal. The ES animal must turn the wheel mounted in the front of its cage to turn off shock for both animals. The procedure begins with a one quarter turn (FR1) and is subsequently increased up to the final requirement of four full wheel turns. **B.** Representative wheel turn data for a cohort of 10 male ES subjects.

responses, latency to extinguish fear responses, neophobia, and sensitivity to the rewarding effects of opiates (Baratta et al., 2015; Christianson et al., 2008; Maswood et al., 1998). These behavioral changes mirrored that previously reported by (Overmier & Seligman, 1967) in which fixed-electrode shock created deficits in shuttle box escape. This constellation of behavioral changes was termed "learned helplessness" and was observable following inescapable tail shock starting 24 hours after stress exposure. It is important to also recognize that this phenotype extends across contexts, meaning that IS animals show behavioral changes when tested in contexts that are completely unique from that of the initial stressor.

The advantage of the triadic stress model over models that deliver foot shock through a grid lies in the inability for the animal experiencing tail shock to modulate shock exposure through postural adjustment (e.g. lifting feet and jumping). Remarkably, this ES animal does not display the same behavioral changes despite having been exposed to a physically identical stressor (Seligman & Maier, 1967). To understand why two physically identical stress experiences can have such drastic differences in their behavioral outcomes it is important to investigate the effect of control at the molecular, circuit, and behavioral levels.

Part II. The neural circuitry of controllable and uncontrollable stress.

i. Inescapable Stress

Controllable stress does not result in all the same behavioral changes that are observed following inescapable stress, but that does not mean it is without impact. In fact, many markers of stress, such as plasma adrenocorticotropic hormone (ACTH). Thus, the differences in behavioral sequelae between ES and IS must lie at a higher level of stress-processing given that the circulating levels of ACTH do not vary between control conditions. To explain why these behavioral changes occur following IS, and more importantly why they are absent in ES animals, it is necessary to examine differences in neutral activation between controllable and uncontrollable stressors. One of the largest differences is found in the stress-induced activation of dorsal raphe nucleus (DRN) serotonergic (5-HT) neurons. During IS, but not physically identical ES, DRN extracellular 5-HT increases between 2 and 5 times that of baseline levels (Maswood et al., 1998). When 5-HT signaling is blocked in the DRN during IS (through administration of an agonist for the inhibitory auto receptor 5-HT1_A), the behavioral changes that typically occur following IS are blunted/eliminated (Maier et al., 1995). This indicates that the increased 5-HT release in the DRN is necessary for the IS-induced learned helplessness phenotype. It should be noted that administration of the same 5-HT1_A receptor agonist (8-OH-DPAT) in the absence of stress does not by itself alter behavioral outcomes (i.e. shuttle box escape) further supporting that the behavioral changes are due to stress-dependent 5-HT activity (Maier et al., 1995). In fact, pharmacological activation of the DRN in the absence of stress is sufficient to produce these behavioral changes, further indicating that this system is driving the learned helplessness phenotype (Maier et al., 1995). Together these data indicate the route of IS behavioral changes is the stressed induced increase in DRN 5-HT release, and that if this is blocked it prevents these changes.

DRN 5-HT activity has been implicated in a variety of behaviors from fear to social dominance (Amat et al., 1998b). This drastic increase in DRN 5-HT release during IS subsequently results in an increase in 5-HT release in DRN projection regions such as the striatum, amygdala, nucleus accumbens, and PAG (Amat et al., 1998a; Christianson et al., 2010; Lowry et al., 2008; Maswood et al., 1998; Stamper et al., 2017, 2017). Blockade of 5HT2C in the basolateral amygdala prevented social avoidance following IS (Christianson et al., 2010) and in striatum prevented shuttle box escape deficits (Strong et al., 2011), further implying that DRN 5-HT release in projection areas that serve as the proximal mediator of the behavior is responsible for the effects of inescapable shock(Figure 1.2). It should also be mentioned that the stress-sensitive regions that project to the DRN and drive 5-HT release discussed above, are not sensitive to control (Amat et al., 2001, 2006; McDevitt et al., 2009). Specifically, regions that are activated by stress and have direct projections to the DRN (such as the locus coeruleus, lateral habenula, and bed nucleus of the stria terminalis), respond equally to escapable and inescapable stress. This provides evidence that the difference in neural activation between ES and IS lies in the DRN.

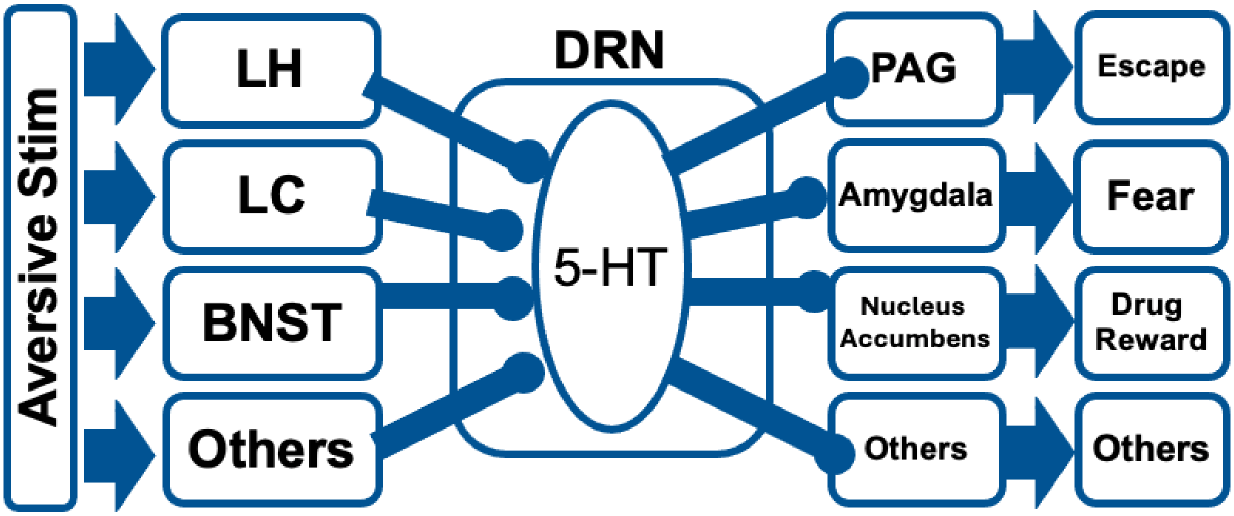

Figure 1.2. Schematic diagram of dorsal raphe nucleus inputs and outputs activated by stress. Inescapable stress (IS) activates a variety of regions known to process aversive stimuli, including the lateral habenula (LH), locus coeruleus (LC), and bed nucleus of the stria terminalis (BNST). All of which project onto dorsal raphe nucleus (DRN) 5-HT neurons, which increases 5-HT release within the DRN and its projection regions. This increase in 5-HT in regions such as the periaqueductal gray (PAG), amygdala, nucleus accumbens, and others, leads to the battery of behavioral changes seen following IS.

ii. Escapable Stress

The above evidence directly indicates that the DRN 5-HT response to IS, and the blunted response to ES, is responsible for the protective effects of control. As mentioned above, IS produces a 200-500% increase in DRN 5-HT release (Maswood et al., 1998). This response is blunted in ES, but not absent. Subsequent studies revealed that during ES, 5-HT levels rise in a similar manner to IS but then quickly drops back to near baseline levels in the first ~40 minutes of the ~100 minute stress exposure (Amat et al., 2001). As mentioned above, the input regions to the DRN responsible for this stress-induced 5-HT release are not sensitive to control, implying that the DRN itself is blunting the stress-induced 5-HT release. However, given that the DRN is a relatively small region that does not receive direct input from motor and somatosensory cortices the 5-HT “clamp” occurring during ES might be the result of a higher-level brain region than the DRN itself (Commons, 2020). The DRN receives information about control through glutamatergic projections from layer V neurons in the prelimbic (PL) region of the medial prefrontal cortex (mPFC) (Gabbott et al., 2005). These projections provide the majority of the cortical input to the DRN and given that the detection of control is an instrumental learning task, this cortical pathway is the perfect candidate to use the presence of control to blunt the effects of stress at the level of the DRN. In fact, this pathway preferentially synapses on gamma-aminobutyric acid (GABA)ergic neurons within the DRN, again indicating that it could be responsible for the 5-HT clamp that occurs during ES (Celada et al., 2001; Hajós et al., 1998; Jankowski & Sesack, 2004).

The PL and the neighboring infralimbic (IL) portion of the medial PFC are heavily implicated in contingency learning (the animal is sensitive to the outcome being contingent on performing an action) of the type necessary to learn the wheel-turn escape response, although the majority of this literature focusses on appetitive tasks (Corbit & Balleine, 2003a; Killcross & Coutureau, 2003; Liljeholm et al., 2011a). To briefly summarize a very large body of literature (which will be discussed in greater depth later), contingency learning relies on a set of regions including the mediodorsal thalamus (MDT), the PL, and the dorsomedial striatum (DMS).

Subsequent work from our laboratory tested the PL-DRN controllability hypothesis by both inhibiting and activating the PL to assess the effect on stress response. Inhibition of the PL in ES animals with the GABA agonist muscimol negated the protective effects of control (Amat et al., 2005), and activation of the PL with picrotoxin in IS animals was sufficient to produce protective effects in IS animals that mirror those observed in ES animals (Amat et al., 2008). Notably, inhibition of the PL had no effect on IS animals nor did it change the ES animals’ ability to learn the controlling response (the latter point will be discussed shortly in the context of instrumental learning). To summarize, the protection by ES lies in the top-down inhibition of the DRN by the PL, which is activated by contingency learning during the wheel-turn escape task in a similar manner to its activation by appetitive instrumental learning (**Figure 1.3**).

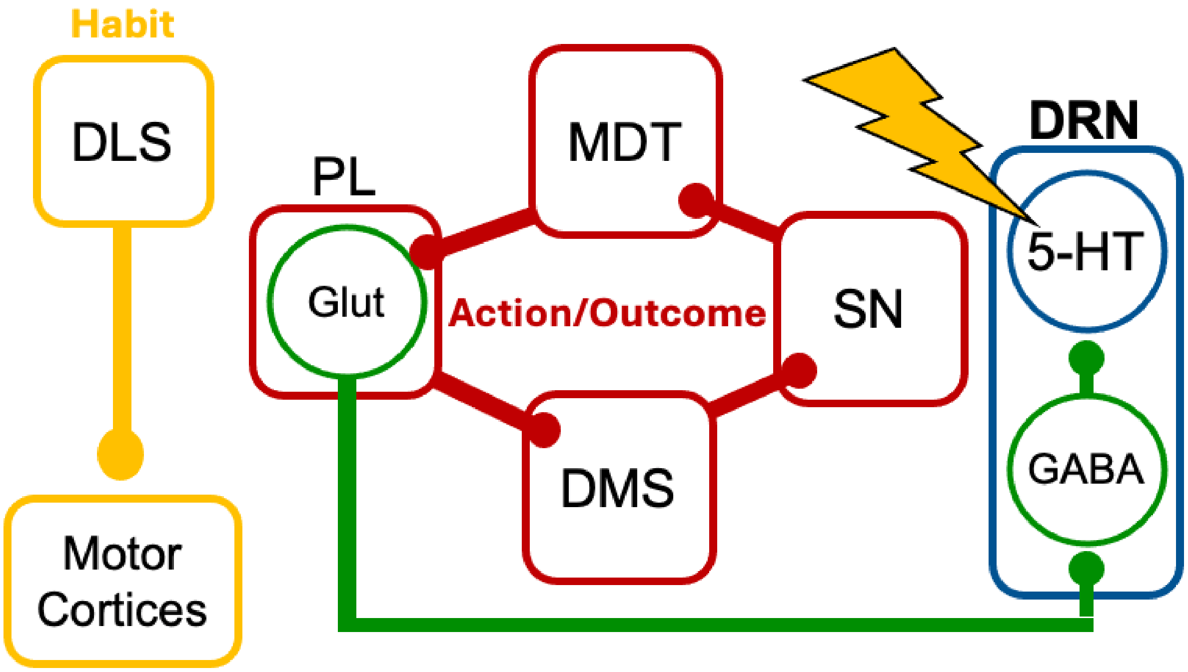

Figure 1.3. Representative Schematic of the circuitry of stress and instrumental learning. Stress potently activates the DRN 5-HT system (blue). This activation can be blunted by the presence of control through activation the Act/Outcome learning circuitry (red) and the glutamatergic PL projections to DRN GABA neurons (green). However, if control is learned with the cortically independent habit system (yellow) there is no inhibition of DRN 5-HT and control is not protective.

Historically, there are two distinct neural systems that are used for instrumental learning tasks (Liljeholm et al., 2011b). The first of these systems is commonly referred to as the action/outcome system (A/O), and it is so named because learning in this system associates an effortful behavior/action (pulling a lever, pushing a button, turning a wheel, etc.) with the outcome of that behavior (sucrose reward, drug reward, shock termination, etc.). A/O learning is contingency sensitive, meaning that it is sensitive to the probability of the desired outcome given both the presence and absence of an action. For an animal using the A/O system, contingency dependent performance in this task would be sensitive to the relationship between the lever press and the sucrose. Initially, sucrose is presented as a single pellet after a single lever press, in a 1:1 ratio. If this relationship changes (non-contingent probability of an action is increased, termed contingency degradation), for example the animal must press 3 times for a single reward an animal using contingency learning will reduce responding if presented with less effortful option that yields the identical reward. The animal will also reduce responding if the outcome (e.g., sucrose) is devalued, which can be accomplished by satiating them to that reward or swapping the reward for a less desirable one, thus changing the value of the outcome without changing the effort required in the action (Corbit & Balleine, 2003b). A/O contingency learning requires the MDT-PL-DMS circuitry, and if this circuitry is disrupted animals are forced to use the second system, which is insensitive to contingency (Bradfield et al., 2013; Dezfouli & Balleine, 2012; Liljeholm et al., 2011a). It is important to emphasize that performance in this type of learning task is not impossible without this A/O circuitry, it just occurs without regard to contingency. This implies that a second system can be used if the A/O system is offline.

The second system capable of instrumental learning is referred to as the Habit System, primarily due to the nature of the stimulus/response relationship that characterizes habitual behaviors. In contrast to the goal-directed A/O system, the habit system has no sensitivity to contingency (Killcross & Coutureau, 2003). Learned behaviors performed with the Habit system are simply a response to the stimulus associated with the learned action, such as an animal pressing a lever because it is presented instead of performing an action to attain a specific outcome. Using the previous example of lever presentation/lever press/sucrose reward, habit learning associates the presentation of the lever with the immediate action of pressing the lever, and this reaction does not rely on the presence, absence, or value of the reward. This means that this type of responding will not change if the outcome is devalued or contingency is degraded. The Habit system, unlike the A/O system, is PFC-independent and consists of projections from the dorsolateral striatum (DLS) to motor cortices (Balleine and Dezfouli, 2012). From an evolutionary standpoint, the A/O and Habit systems should work together to ensure organisms are able to quickly learn association in a contingency sensitive manner (A/O system) to rule out non-rewarding outcomes, and then transition those tasks to the less cortically dependent Habit system, thus freeing up cognitive resources. In fact, in the appetitive literature animals will transition from the A/O system to Habit system if "overtrained" (300+ trials of lever pressing), as evidenced by the loss of their initial contingency sensitivity in the early trials (Barnes et al., 2005; Jog et al., 1999).

The majority of the instrumental learning literature has been conducted using appetitive paradigms, but as previously mentioned ES is an instrumental learning task.

The stimulus for ES is shock presentation, the response is a wheel turn, and the outcome is shock termination. This implies that the wheel turn escape response can be acquired and controlled by either the A/O system (spinning the wheel to terminate shock), or the habit system (spinning the wheel in response to shock). Similarly to appetitive tasks, ES can be learned without the PL (Amat et al., 2005). However, without the PL activation taken there is no top-down inhibition of the DRN 5-HT release, thus the animal is not protected against the outcomes of the stress experience (Amat et al., 2005). Without the A/O system online, the animals (in theory) had to learn to respond to the stimulus with the PFC-independent habit circuitry, which would not activate the PL-to-DRN projections but would allow them to learn to spin the wheel in response to shock. ES leads to greater activation (relative to IS) in the A/O mediated DMS, but there is no controllability effect on activation of the habit mediated DLS (Amat et al., 2014), meaning that the DMS is activated more by ES than IS, but the DLS seems to be equally activated by both. Later experiments from our group showed a similar effect when the DMS was inhibited, ES animals were still able to learn the controlling response, but they were not protected against the behavioral changes seen following IS (Amat et al., 2014). This experiment also included a group that went through ES with the DLS inhibited to take the habit system offline and there was no effect on either learning the wheel turn response or gaining the protection that comes with control. This implies that the protection from ES lies in the learning of the wheel turn escape response with the prefrontal-dependent A/O system.

One critical piece of information that has yet to be discussed is the fact that only male rats are protected by having control over the shock (Baratta et al., 2018). This sex-dependent effect of ES was discovered far more recently as the field of Neuroscience shifted away from single sex cohorts, and as such the experiments concerning ES discussed up to this point were conducted in male rats only. The mechanism(s) for this difference again lies in the instrumental learning circuits. The presence of control in females does not activate the PL-to-DRN glutamatergic projection and thus does not provide top-down inhibition of DRN 5-HT release (Baratta et al., 2018; Fallon et al., 2020). Control is protective in females with either i) lesions of the DLS or ii) pharmacologic blockade of dopaminergic signaling in the PL via injection of a D1 receptor antagonist (McNulty et al., 2023). The first manipulation results in protection, in theory, due to the necessity for females to learn the wheel-turn escape response with the A/O system if the Habit system is fully offline following the DLS lesion. The second manipulation provides protection in a similar manner, but by disinhibiting the A/O system through modulating prefrontal dopamine (DA) release. During ES, females have a sustained increase in extracellular levels of DA within the PL, which yields a decrease in PL activity (McNulty et al., 2023). This contrasts with the males, in which the presence of control attenuates the PFC stress-induced DA efflux. This elevated DA release in females suggests that the lack of protection is due to DA-mediated silencing of the PL, thus requiring that instrumental learning occur through the Habit circuit. It remains to be seen how lesioning the DLS directly effects this DA efflux in the PL, as DLS lesioned females are protected by ES.

In review, both controllable and uncontrollable stressors activate the DRN through regions including the LH and LC, resulting in increased 5-HT release to downstream regions such as the DMS, PAG, amygdala, and nucleus accumbens. This increase in 5-

HT release then causes a battery of behavioral changes that occur within 24 hours of the stressor onset and can last as long as 3 weeks. However, this does not occur following controllable stressors despite an initial increase in DRN 5-HT release. This is because the regions activated by learning the wheel turn escape response, together comprising the contingency sensitive A/O system, include the PL. This activation of the PL then results in activation of PL-to-DRN glutamatergic neurons which preferentially synapse on DRN GABA neurons, thus inhibiting the DRN 5-HT neurons and ‘clamping down’ on the stress-induced 5-HT release that causes the learned helpless phenotype (Figure 3). This only occurs if the ES task is learned with the A/O system and if the A/O system is taken offline animals are forced to use the non-PL mediated habit system to terminate the shock. This does not interfere with the animal’s ability to learn to spin the wheel, but it also does not activate the PL-to-DRN projection and thus does not interfere with DRN 5-HT release. This is evidenced by the fact that females use the Habit system to learn ES and are not protected by the presence of control.

Part II. Social Dominance.

In the over-fifty years since the development of the triadic stress model by Maier and Seligman, this model has rarely been used to investigate the effect of stressors on social dominance. Many researchers have investigated a similar interaction between stress and dominance, but these studies have exclusively used uncontrollable stressors - that is, adverse events that cannot be escaped, avoided, or diminished by the organisms’ behavioral responses (Cooper et al., 2017; Deuter et al., 2016; Park et al., 2018; Šabanović et al., 2020).

The exiaitence of stable dominance hierarchies is remarkably conserved across social species, and within those species an organism's dominance status has direct effects on physical and psychological wellbeing (Johns et al., 2010; Jupp et al., 2016; Nader et al., 2012; Sapolsky, 2004; Wolffgramm & Heyne, 1991; Zhou et al., 2017). From an evolutionary standpoint, the ability to recognize one's hierarchical position within a social group reduces conflict and injury, thus increasing overall fitness for the individual and the group at large (Schjelderup-Ebbe, 1922). Since the first scientific characterization of a dominance hierarchy in 1922, these social structures have been studied extensively through the lenses of a wide variety of scientific disciplines. Due to the complicated nature of social interactions and variability within species, the term "social dominance" can be used in a number of different ways. Research on human subjects often focuses on socioeconomic status as a metric, and often those with lower socioeconomic standing mirror the effects of a lower hierarchical position in animal models (Sapolsky, 2004; Jupp et al., 2016; Johns et al., 2010). This means that while there is crossover, the term 'social dominance' is not always used to describe the same effects even within a species, much less with translational studies. For the purposes of this work, social dominance refers to the ability for one animal to beat another in a social competition, thus becoming the "winner" and by extension creating a "loser".

Rodent models are ideal to study the neural circuitry of dominance given the intrinsic social nature of most rodents and the relative ease with which regions of interest can be studied during and following social interaction. As a result, there is a large body of research devoted to the neural correlates of dominance and social interactions at large (Amat et al., 2001; Cao et al., 2017; Jupp et al., 2016; Padilla-

Coreano et al., 2022a). For the purposes of this document however, the role of the prefrontal cortex and it's various efferent and afferent projections are of particular interest. Sensitivity to one's position relative to another individual requires recognition of the other individual, interpretation of the social signals of that individual, determination of the appropriate approach for the interaction, monitoring of the other individuals' actions, and adjustment of behavior depending on all of these variables (Wang et al., 2011a; Williamson et al., 2017; Zhou et al., 2017a). This set of complicated processes would require collaboration between multiple brain regions, but the prefrontal cortex would be the focus of this complicated problem solving. As a result there has been a recent rise in the focus of prefrontal circuitry, including pathways in the A/O system, in the context of dominance (Fetcho et al., 2023a; Morrison et al., 2013a; Padilla-Coreano et al., 2022b; Park et al., 2018a; Wang et al., 2011a). These findings largely indicate that the dorsomedial PFC (dmPFC), which includes the PL and the anterior cingulate cortex (ACC), ts sufficient and necessary for winning in a social competition. However, stimulation of solely the ACC is not sufficient to produce dominance, implying that the PL is vital for winning. Others have shown that much like the PL, the MDT projection to the PL is sufficient/necessary for dominance and dominant behavior (Zhou et al., 2017); specifically, increasing plasticity in this pathway increases rank in subordinates and a subsequent decrease in plasticity returns animals to their original subordinate rank.

The MDT-PL pathway is also implicated in instrumental learning through the action/outcome circuit (Bradfield et al., 2013), specifically for goal-directed learning. As previously mentioned, ES is an instrumental learning task that requires activation of the

PL for the experience to be protective (Maier, 2015a). ES also increases plasticity within the PL, in theory increasing the strength of the pathways activated by ES in the PL (Baratta et al., 2019a). The major excitatory input to the PL originates in the MDT (Gabbott et al., 2005; Kelly & Martina, 2018), so it stands to reason that the MDT-PL pathway would be activated by controllable stress, thereby increasing the strength of this pathway and facilitating later dominance.

The tube test is by far the most common paradigm for studying the neural substrates of winning on a circuit level (Cao et al., 2017; Fan et al., 2019; Garcia-Font et al., 2022; Pallé et al., 2019; van den Berg et al., 2015a). There are various forms of this paradigm, but the common thread between them is that two animals meet in the center of a tight tube at which point one must push the other out to proceed. The initial experiments using this task employed a training and food restriction protocol for mice, in which the animals were food restricted and taught that there would be a food reward after reaching the end of the tube (training takes place without an opponent blocking the tube). More recent applications of the tube test, including our own, avoid the use of appetitive reinforcement during training. This approach avoids any confounds created by food deprivation/motivation and is easily attainable in Sprague Dawley rats (for a more in-depth description see Chapter 3: Methods). The other dominance test employed in the present studies is the Warm Spot Test. This test was developed by the Hailan Hu laboratory, and allows for competition between a larger group of animals (here a triad) to attain a single warm spot on an otherwise cold cage floor (Zhou et al., 2017a). The obvious advantage of this model is the ability to test animals in all three stress conditions (ES/IS/HC) against each other in a social competition. Unlike other dominance tests, the cold cage floor is intrinsically motivating and does not require any training for the animals to compete for the single warm spot. Early findings in the mouse indicated that much like the tube test, winning in the warm spot test is linked to PL and MDT-to-PL activation (Zhou et al., 2017a). Combining the above two social dominance paradigms allows us to then determine if any dominance effects are trans-situational and do not rely solely on aversive stimuli (i.e. cold cage floor).

Part III. Introduction Conclusions and Thesis Organization.

The overlap in the circuitry underlying stressor controllability and social dominance is clear. Both paradigms rely on A/O regions, specifically the PL (Amat et al., 2005; Wang et al., 2011a). Increases in plasticity in the PL and it's projections from the MDT actually increases dominance (Zhou et al., 2017a). Controllable stress specifically increases plasticity within the PL (Baratta et al., 2019a) and the process of learning the wheel turn escape response must be learned with A/O circuitry in order for control to be protective (Amat et al., 2014, p. 201; McNulty et al., 2022). It stands to reason that stressor controllability and social dominance would have an effect on each other given this overlap in circuitry. Thus, this thesis is designed to determine if 1) prior experience with behavioral control facilitates social dominance, 2) that facilitation relies on A/O circuitry, and 3) there is an effect of social dominance on stress.

In Chapter 2 I will explore the effect of stressor controllability on social dominance in the warm spot test. My initial findings indicate that escapable stress elicits dominance over inescapably stressed and non-stressed animals one week later. This effect is sex-dependent and like other ES effects is not present in females. I then determined that for this ES facilitated dominance to occur, the wheel turn escape must be learned with the PL online. I then sought to determine if A/O circuitry was necessary for dominance independent of stress, as this would suggest ES facilitated stress occurs because of an increase in A/O circuitry. I found that inhibition of the PL or the DMS in a dominant animal significantly and irreversibly caused that animal to lose in a social competition they had initially won. Given the similarity in circuit activation between winning and ES, I sought to investigate if winning would provide similar protection as does ES to subsequent IS. Prior to this experiment however, I determined that there was an underlying sex difference in the stability of dominance hierarchies with males being the more stable. For this reason, I moved forward with males on this experiment as well. I found that repeated winning blunted the effect of later inescapable shock both at the behavioral level and at the level of 5-HT release in the DRN.

In Chapter 3 I will explore the role of the MDT-to-PL projection in ES facilitated dominance using the tube test. This chapter begins with an experiment designed to separate non-social instrumental effort from the social dominance tasks in order to determine if an increase in effort underlies ES facilitated dominance. I use a novel behavioral paradigm to test the hypothesis that this facilitation does not rely on the social component of dominance, as ES also facilitates an increase in non-social effort as measured by weight pushing. This task requires the animals to push a weighted bottle through the tube instead of a competitor, and ES animals will push significantly more weight following stress exposure. The following experiment will show that ES but not yoked IS, causes an increase in calcium activity in the MDT-to-PL pathway. I will then show that the ES facilitated increase in dominance extends to the tube test, and that this facilitation requires activation of the MDT-to-PL pathway at the time of stress.

Chapter 4 will discuss the implications of this work in the fields of controllable stress and social dominance. I will summarize my findings and discuss possible interpretations as well as potential next steps for the research.

Chapter 2: Prior experience with behavioral control over stress facilitates social dominance

Abstract

Dominance status can drastically alter both physical and mental fitness, and an individual’s relative position within a hierarchy is highly dependent on previous experiences. A variety of considerations suggest that the experience of behavioral control over stressors should produce winning in dominance tests and that winning should blunt the impact of later stressors, as does prior control. To determine the relationship between winning in a social competition and controllable stress, we first examined the impact of controllable stress on performance in a social competition using a version of the warm spot test modified for rats. Prior experience of controllable stress, but not uncontrollable stress, increased winning in the warm spot test through an increase in effortful behavior. This effect was reversed by pharmacological inhibition of the prelimbic (PL) cortex during behavioral control. Controllable stress animals were not dominant, despite learning the wheel turn escape response. Next, we sought to determine if the PL and the DMS were required for repeated winning. After an initial session to establish dominance, we reversibly inhibited the PL or DMS of the dominant animal. This resulted in an irreversible loss of dominance status in both cases. Given this similarity in circuitry necessity for repeated winning and controllable stress, we sought to determine if repeated winning would produce resilience against the typical behavioral changes seen following uncontrollable stress. Rats competed for five sessions to establish dominance status; to establish dominance status, triads of rats were given five sessions of warm spot competition. Stable dominance blunted the later stress-induced increase in dorsal raphe nucleus (DRN) serotonergic (5-HT) activity, as well as

prevented stress-induced social avoidance. In contrast, endocrine and neuroimmune responses to uncontrollable stress were unaffected, indicating a selective impact of prior dominance. Together, these data demonstrate that instrumental control over stress promotes later dominance, but also reveal that winning experiences buffer against the neural and behavioral outcomes of future adversity.

Introduction

Dominance status has important consequences for an individual’s access to resources, health outcomes, and rates of reproductive success and survival. There is considerable interest in the mechanisms by which experiential/behavioral variables influence hierarchy formation, as well as how dominance status impacts an individual’s response to future adversity. The medial prefrontal cortex (mPFC) has been implicated in (i) driving the effortful behavior necessary to attain a higher dominance rank in a social competition and (ii) the ability to generalize this rank to novel social contests (Cooper et al., 2015; Padilla-Coreano et al., 2022a; Zhou et al., 2017b). Winning experiences activate distinct circuits within the prelimbic cortex (PL) and lead to changes in the synaptic strength of layer V pyramidal neurons, the main output layer of the mPFC (Garcia-Font et al., 2022; Wang et al., 2011a; Zhang et al., 2022). Moreover, manipulations that inhibit or activate the PL result in a downward or upward movement in social rank, respectively (Zhou et al., 2017b).

In addition, repeated winning experiences can buffer against *social* stress outcomes (Karamihalev et al., 2020; LeClair et al., 2021). This is noted here because an experience with behavioral control over adverse events also activates PL layer V output (Baratta et al., 2009), and this PL output activation produces enduring protection against both future social (Amat et al., 2010) and non-social stressors (Amat et al., 2006). Controllable (escapable, ES), but not physically identical uncontrollable (yoked inescapable shock, IS) stressors increase the intrinsic excitability of layer V pyramidal neurons in the PL (Varela et al., 2012). Moreover, intra-PL blockade of NMDA receptor activity or inhibition of its downstream effector pathway (ERK/MAPK) prevent ES-induced resistance against the typical neurochemical and behavioral outcomes of later IS that occurs in a novel environment (Christianson et al., 2014). Thus, ES elicits a generalized resistance to adversity experienced in new environments with new task demands.

Given that an initial experience of behavioral control over stress produces long-lasting alterations to the PL, then it might be expected that ES would facilitate later dominance in a social competition that depends upon PL activation. Furthermore, any effect of ES on later dominance should be dependent on PL activation during the initial experience of control. We also hypothesize that these effects are restricted to male rats, as control in females is not protective across a variety of parameters and does not activate the same circuitry (Baratta et al., 2018, 2019a; McNulty et al., 2022). Given dominance procedures such as the tube test involve food reinforcers during training (which can be devalued be stress), we examined the influence of controllability on subsequent performance in the warm spot test (WST). Here we used a modified version of WST for rats, in which a triad competes for sole occupancy of a warm spot on a cold cage floor (Zhou et al., 2017b).

We next characterize the differences in the formation of stable hierarchies between males and females using the warm spot test. We further investigated whether repeated winning in the warm spot competition produces similar resilience phenomena as does behavioral control. Specifically, we examined if the circuits involved in the enduring effects of ES are also engaged in the development of repeated winning. Additionally, we addressed whether stable winning buffers against the behavioral and neurochemical outcomes of IS. Robust activation of serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN) is necessary and sufficient to elicit the behavioral sequelae of IS (for review, see Maier and Watkins, 2005), including the production of the behavioral endpoint measured here, IS-induced social avoidance (Christianson et al., 2008). Thus, we measured extracellular levels of DRN 5-HT during IS and examined subsequent social interaction. The overarching hypothesis is that if behavioral control over *non-social* stressors (shock) and the experience of *social* winning activates the same prefrontal circuitry, they should be fungible.

Materials and Methods

Subjects. Adult male (275-300 g) and female (225-250 g) Sprague–Dawley rats (Envigo) were pair-housed on a 12-h light–dark cycle (lights on at 0700 h) with *ad libitum* access to standard laboratory chow and water. Only subjects implanted with guide cannula were individually housed to avoid potential damage from cage mates. Rats were allowed to acclimate to colony conditions for at least one week before experimentation. All behavioral procedures were carried out between 0900 and 1400 h. All experiments were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Colorado Boulder Institutional Animal Care and Use Committee.

Wheel-turn ES/yoked IS procedure. For manipulation of stressor controllability, subjects were run in a triadic design as previously described (Baratta et al., 2007). One subject of each triad received ES, a second received yoked IS, and a third remained undisturbed in the colony room (home cage, HC). Each ES and IS rat were placed in a 14 × 11 × 17 cm (length × width × height) Plexiglas box with a wheel mounted in the front. The tail was secured to a Plexiglas rod extending from the back of the box and affixed with two copper electrodes and electrode paste. The single stress session consisted of 100 tail shock trials administered by a current-regulated shocker (Coulbourn Instruments). Tail shocks (1.0 mA) were presented on a 60-s variable interval schedule. Initially, the shock could be terminated by a quarter turn of the wheel by the ES rat. When trials were completed in less than 5 s, the response requirement was increased by one-quarter turn of the wheel, up to a maximum of four full turns of the wheel. The requirement was reduced if the trial was not completed in less than 5 s. If the trial was not completed in 30 s, the shock was automatically terminated, and the requirement was reset to a one-quarter turn of the wheel. For yoked IS rats, the onset and offset of each tail shock were identical to those of its ES partner. A computer equipped with Graphic State 4 (Coulbourn Instruments) controlled the experimental events and recorded the wheel turn requirement and escape latency for each trial.

Inescapable shock procedure. For behavioral experiments that only involved inescapable stress, rats were placed in a Plexiglas restraint tube (17.5 cm in length × 6.0 cm in diameter) with a Plexiglas rod protruding from the rear to which the rat's tail was taped and affixed with two copper electrodes. Rats received a single session of 100 inescapable tail shocks (5 s duration, 1.0 mA each) with a variable inter-trial interval ranging from 30–90 s (average of 60 s).

Warm spot. The warm spot competition was adapted from the protocol previously described in mice (Zhou et al., 2017b). An empty housing cage (48.3 cm in length × 26.7 cm in width × 20.3 cm in height) was placed on ice to cool the cage floor (0°C). In one corner of the cage, an inverted circular lid (diameter: 8.9 cm; peripheral lip height:

1.6 cm) containing a thin toe warmer covered with paper nesting material served as the ‘warm spot’ (~35°C). The warm spot was sufficiently large to accommodate only a single adult rat and was affixed to the cage floor to prevent displacement during the competition. Prior to experimentation, rats were individually habituated to the above warm spot-cold cage environment for 20 min.

For all experiments, the warm spot competition involved a triad of non-cage-mates. Triads were first introduced into an empty housing cage with a cold floor that did not contain a warm spot. After 10 min, the triad was transferred to the warm spot-cold cage environment in which subjects competed over a 20 min period for access to the warm spot. All sessions were videotaped to minimize experimenter presence in the procedure room. Subjects were marked with different colors for unique identification within the triad and to ensure videos were scored blinded to treatment. Competition behavioral measures included the number of 1) pushes initiated (not in response to being pushed by another triad member); 2) resistance bouts (either withstanding or pushing back after being pushed); and 3) retreats (withdrawing from warm spot in response to the actions of another triad member). Occupancy time of the spot for each triad subject was calculated and expressed as a percentage of the total time the warm spot was occupied during the 20 min session.

Juvenile social exploration (JSE). As described in Christianson et al. (2010), animals were moved from their colony room to a novel procedure room (150 lux at the position of the animal) and placed in a standard Plexiglas housing cage with bedding and a wire lid. Following a 1 h habituation period, a juvenile stimulus rat (28-35 days old male Sprague-Dawley) was introduced to the cage for 3 min and exploratory behaviors (sniffing, pinning, chasing, and allogrooming) initiated by the adult were scored by an observer blind to treatment.

Stereotactic surgery. All surgeries were performed under inhaled isoflurane anesthesia (5% induction, 2% maintenance in 2.5 L/min O_2; Piramal Critical Care). For drug microinfusion studies, cannula (26 gauge; P1 Technologies) were implanted bilaterally in either the PL (A/P: +2.6; M/L: +/-0.5; D/V: -1.8 mm from the pial surface) or DMS (A/P: -0.2; M/L: +/-2.1; D/V: -3.0 mm from the pial surface) and secured to the skull with stainless steel screws and acrylic cement. Internal guide cannula were inserted to keep the cannula patent and were held in place with a fitted dust cap (P1 Technologies). Subjects were given 10-14 days to recover before experimentation. For *in vivo* microdialysis, general surgical procedures for guide cannula implantation into the midline DRN (A/P: -7.8, M/L: 0.0, D/V: -4.9 mm from the pial surface) were similar to above. However, a screw cap from a 15-mL conical centrifuge tube (with the central portion removed) was affixed to the skull in an inverted orientation so that it encircled the guide cannula. This was done to protect the microdialysis guide cannula during tail shock. Subjects were allowed 7-10 days to recover. All subjects were given postoperative subcutaneous injections of an extended-release nonsteroidal anti-inflammatory (meloxicam, 4.0 mg/kg; Vetmedica) and an antibiotic (CombiPen-48, 0.25 mL/kg; Bimeda). At the end of the experiment, brains were collected, sliced at 30 µm, and stained with cresyl violet for verification of cannula placement. Subjects were only included in the data analysis if tissue damage from the cannula tip fell within the target structure.

Drug microinfusion. For pharmacology studies, subjects received microinjections 30-45 min prior to stress treatment or warm spot competition. Microinjections were made in a quiet room near the testing area. Subjects were gently restrained and microinjectors (33 gauge; P1 Technologies), attached to a 25-µl Hamilton syringe with PE 50 tubing, that extended 1 mm beyond cannula tips were inserted. Syringes were mounted in a Kopf microinjection unit (Model 5000) and delivered either the $GABA_A$ agonist muscimol (50 ng/0.5 µl/side; Sigma-Aldrich) to the PL, the NMDA antagonist AP5 (3 µg/0.5 µl/side; Tocris Bioscience) to the DMS, or equal volume sterile saline to either brain region. Injectors were left in place for 80 s following injection to allow for diffusion. All drugs were dissolved in 0.9% sterile saline and doses were chosen based on our prior work in male Sprague-Dawley rat (Amat et al., 2005, 2014).

In vivo microdialysis. A CMA 12 microdialysis probe (0.5 mm diameter, 1 mm length, 20 kDa cut-off) was inserted through the cannula guide to the midline of the DRN the afternoon before sample collection. A portion of a 15-mL conical tube was screwed onto the skull-mounted screw cap, through which the dialysis tubing, protected within a metal spring, was entered, and attached to the probe. Each subject was placed individually in a Plexiglas bowl and infused with artificial cerebrospinal fluid (pH 7.2, 145.0 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl, 1.0 mM $MgCl_2$) at a rate of 0.2 µL min^{-1}. The following day the flow rate was increased to 0.8 µL min^{-1}. After a 90-min stabilization period, four baseline samples were collected. Subjects were then transferred to a Plexiglas open-top box where they received 100 trials of inescapable tail shocks (5 s duration, 1.0 mA, 60 s average inter-trial interval) or no stress. Following stress treatment, subjects were moved back to the bowls, where three additional samples were collected. During the baseline, stress, and post-stress phases, dialysates were collected at 20 min intervals and stored in an -80°C freezer until analysis. Microdialysis data are expressed as a percentage of baseline, defined as the mean of four consecutive samples collected prior to the stress phase.

5-HT analysis. 5-HT concentration was measured in dialysates using high-performance liquid chromatography (HPLC) with electrochemical detection. The system consisted of an online Shimadzu DGU-2045 degasser, an ESA 584 pump, a Dionex UltiMate 3000 electrochemical detector with a 6041 RS ultra amperometric cell and autosampler, and an ESA 5020 guard cell. The analytical column was an Acclaim RSLC PolarAdvantage II (2.1 × 100 mm; Thermo Fisher Scientific), maintained at 38°C, and the mobile phase was the ESA buffer MD-TM. The analytical cell potentials were kept at +220 mV, and the guard cell at +250 mV. External standards (Sigma-Aldrich) dissolved in artificial cerebrospinal fluid were run each day to quantify 5-HT.

Tissue dissection. Rats were given a lethal dose of sodium pentobarbital. Cardiac blood was collected prior to performing transcardial perfusion with ice-cold saline (0.9%) for 3 min to remove peripheral immune leukocytes from central nervous system vasculature. Brain was rapidly extracted, placed on ice, and the hypothalamus dissected. Tissue samples were flash frozen in liquid nitrogen and stored at -80°C.

Enzyme-linked immunosorbent assay (ELISA). Cardiac blood was centrifuged (10 min, 14,000 × g, 4°C) and serum stored at -80°C. Serum corticosterone (CORT) was measured using a competitive immunoassay (Enzo Life Sciences) as described in the manufacturer’s protocol. Serum CORT levels were expressed as μg/dL.

Quantitative real-time PCR. Total RNA was isolated from hypothalamus using TRI Reagent (MilliporeSigma) and a standard method of phenol:chloroform extraction (Chomczynski & Sacchi, 1987). Total RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). cDNA synthesis was performed using the SuperScript II Reverse Transcriptase kit (Thermo Fisher Scientific). A detailed description of the PCR amplification protocol has been published previously (Frank et al., 2006). cDNA sequences were obtained from Genbank at the National Center for Biotechnology Information. Primer sequences were designed using the Operon Oligo Analysis Tool and tested for sequence specificity using the Basic Local Alignment Search Tool at NCBI (Altschul et al., 1997). Primers were obtained from Thermo Fisher Scientific, and specificity was verified by melt curve analyses. All primers were designed to span exon/exon boundaries and thus exclude amplification of genomic DNA. Primer sequences: interleukin-1β (*Il1b*), F: CCTTGTGCAAGTGTCTGAAG, R: GGGCTTGGAAGCAATCCTTA; interleukin-6 (*Il6*), F: AGAAAAGAGTTGTGCAATGGCA, R: GGCAAATTTCCTGGTTATATCC; C-X3-C motif chemokine receptor 1 (*cx3cr1*), F: AGCTGCTCAGGACCTCACCAT, R: CCGAACGTGAAGACAAGGGAG; β-actin (*Actb)*, F: TTCCTTCCTGGGTATGGAAT, R: GAGGAGCAATGATCTTGATC. PCR amplification of cDNA was performed using the Quantitect SYBR Green PCR Kit (Qiagen). Formation of PCR products was monitored in real time using the CFX96 Touch Real-Time PCR Detection System (BioRad). Relative gene expression was determined using *Actb* as the housekeeping gene and the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001).

Statistical analysis. Data analyses were performed using Prism software (GraphPad, RRID:SCR_002798). The effect of treatment was analyzed with unpaired *t* test, one-way, two-way, or repeated measures ANOVA. Main effects and interactions were considered significant if $p < 0.05$. When appropriate, *post-hoc* analyses were performed with Tukey's multiple comparison test or unpaired *t* test. For nonparametric data (latency to spot, rank), Mann-Whitney U or Kruskal-Wallis (for comparison of more than two groups) followed by Dunn's multiple comparison test were used. In all cases, data are expressed as the mean ± SEM.

Results

Effects of stressor controllability on later social dominance

To evaluate the impact of stressor controllability on subsequent dominance behavior, male rats were individually habituated to the warm spot apparatus 24 h prior to receiving ES, yoked-IS, or HC treatment (Fig. 2.1*A*). One-week later ES/IS/HC triads were exposed to the warm spot competition. One-way ANOVA showed a significant effect of prior stress condition ($F_{2,30} = 5.79$, $p = 0.007$, $n = 11$ per group), with ES subjects spending significantly more time on the warm spot than did both IS and HC subjects (p's < 0.05; Fig. 2.1*B*). The impact of the stressor on later dominance was selective to controllable stress; the percentage of warm spot occupation did not differ between IS and HC subjects ($p > 0.999$). Importantly, once the competition was initiated, the latency time to first access the warm spot did not differ between groups, indicating that all groups exhibited similar levels of recall for the warm spot location ($p = 0.120$, Kruskal-Wallis; data not shown). Across triads, ES subjects consistently ranked higher than IS

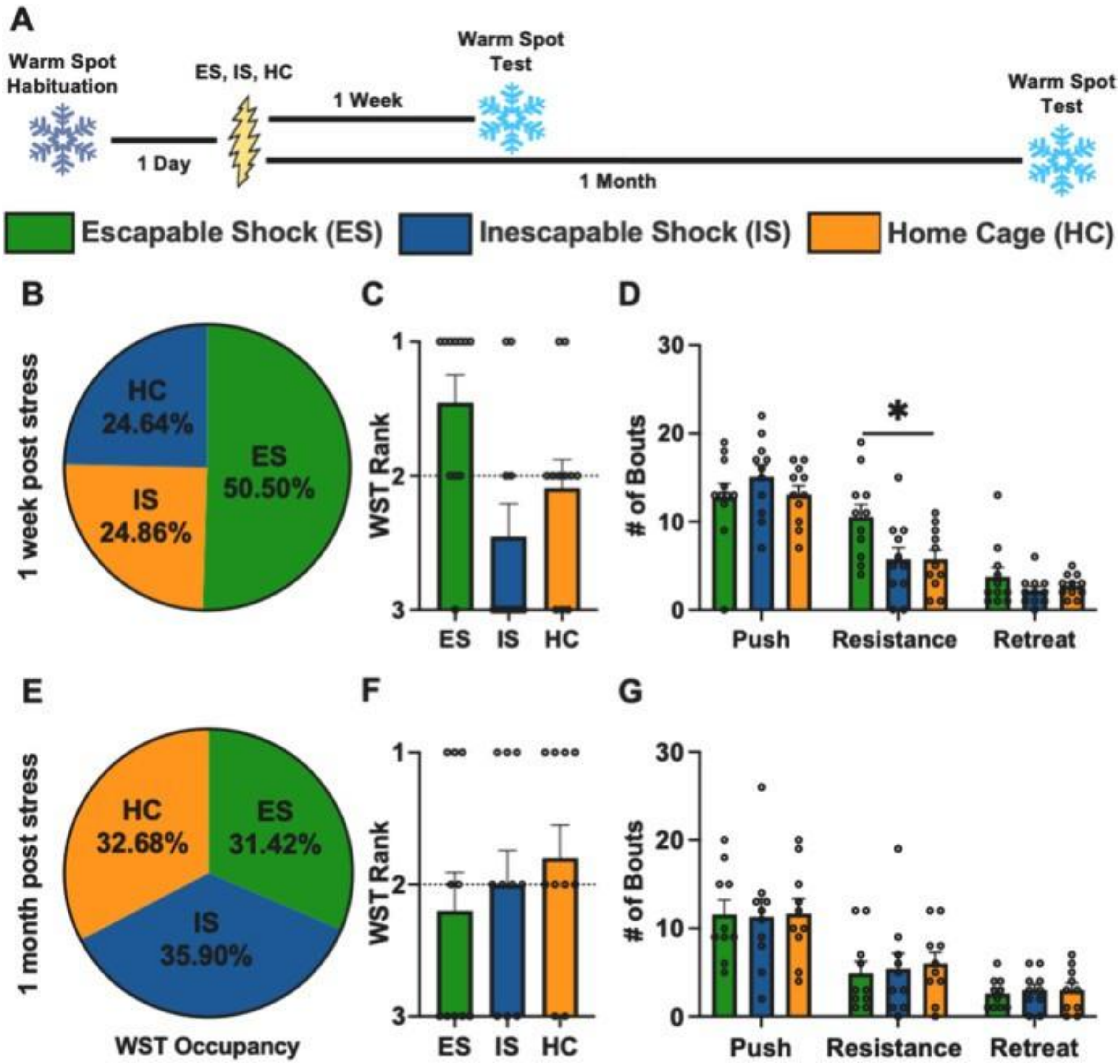

Figure 2.1. Behavioral control increases dominance in the warm spot competition 1 week later. ***A***, Experimental timeline. ***B***, Percent occupancy in the warm spot test (WST) 1 week after stress treatment. Each triad consisted of one member from escapable stress (ES), yoked-inescapable stress (IS), and no stress (homecage, HC) groups. ***C***, Average rank positions across warm spot triads. The dotted line indicates expected ranking if there was no impact of prior stress treatment. ***D***, Number of pushes initiated, resistance bouts (pushbacks, withstanding a push) and retreats. ***E***, Percent occupancy in the WST 1 month after stress treatment. ***F***, Average rank positions across warm spot triads. ***G***, Number of individual behaviors. Values represent the mean ± SEM. $^*p < 0.05$, Dunn's (rank) and Tukey's *post hoc* tests.

subjects ($p = 0.014$, Dunn's; Fig. 2.1*C*) and exhibited a distinct behavioral profile during the 20 min WST competition. Prior ES increased the number of resistance behaviors (pushbacks, maintaining spot occupation when pushed by another member of the triad) compared to IS and HC (p's = 0.033; Fig. 2.1*D*). In contrast, there were no significant group differences in the mean number of pushes initiated ($F_{2,30} = 0.883$, $p = 0.424$) and passive responses (retreats from the warm spot; $F_{2,30} = 1.179$, $p = 0.322$; Fig. 2.1*D*). The ES-induced facilitation of dominance was both time-limited and sex-specific. In a separate male cohort, no significant group differences emerged when the warm spot competition occurred 30 days post-stress treatment ($n = 10$ per group; Fig. 2.1*E-G*). Warm spot occupation was similar between ES, yoked-IS, and HC groups ($F_{2,27} = 0.085$, $p = 0.919$). An impact of behavioral control is often reported to be absent in female rats (Fallon et al., 2020), and here we tested whether ES in females would produce dominance in the warm spot competition one week later as it does in males (Fig. 2.2*A*). Males and females typically perform the wheel-turn escape response with equal proficiency, and this was true here as well. Repeated-measures ANOVA indicated no differences in ES performance between females and males (male data from Fig. 2.1 *B*) in the wheel-turn response requirement throughout the entire 100-trial session (sex: $F_{1,22} = 0.008$, $p = 0.931$; Fig. 2.2*B*). Despite a similar level of wheel-turn acquisition and motivation to escape across trials, prior ES in females had no impact on overall warm spot occupancy ($F_{2,39} = 0.875$, $p = 0.425$, $n = 14$ per group; Fig. 2.2*C*), rank ($p = 0.390$, Kruskal-Wallis; Fig. 2.2*D*), or individual behavioral measures (p's > 0.05; Fig. 2.2 *E*).

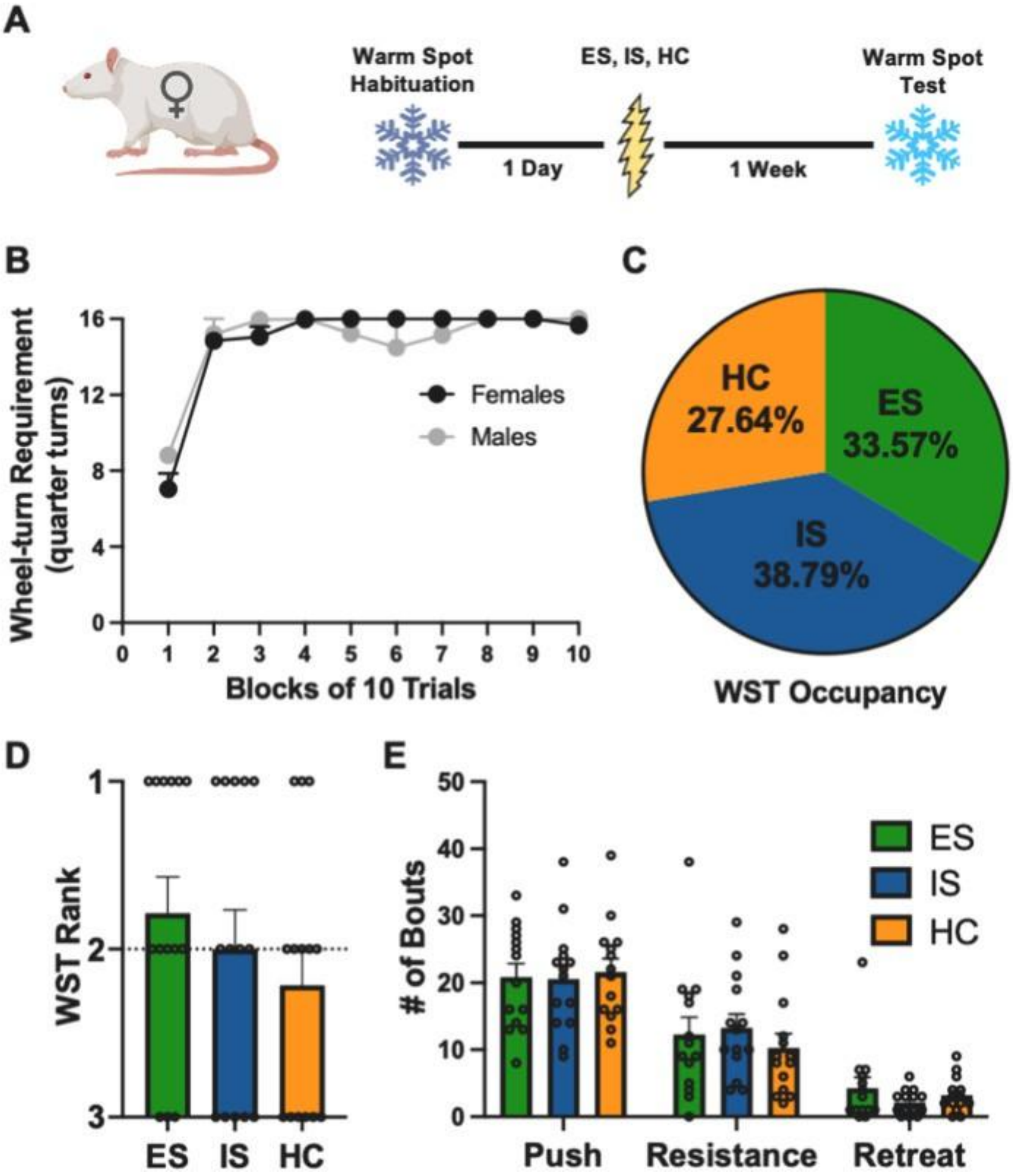

Figure 2.2. Behavioral control in female rats does not produce dominance in the warm spot competition. ***A***, Experimental timeline. ***B***, Comparison of female wheel-turn escape behavior with that of males in Figure 4. Number of quarter turns of the wheel attained as the escape requirement for each trial. ***C***, Percent occupancy of the warm spot test for escapable shock (ES), yoked-inescapable shock (IS), and no stress (homecage, HC) groups 1 week after stress treatment. ***D***, Average rank positions across warm spot triads. The dotted line indicates expected ranking if there was no impact of prior stress.

Prelimbic cortex involvement in the dominance-producing effects of behavioral control

A variety of data supports the idea that the enduring effects of behavioral control requires PL activity during the control experience (Baratta et al., 2009; Christianson et al., 2014). Thus, we addressed whether pharmacological inactivation of the PL during ES would prevent its selective impact on later warm spot behavior (Fig. 2.3*A*). ES subjects received intra-PL bilateral microinfusions of either muscimol (ES-M; 50 ng/0.5 μl/side, $GABA_A$ agonist) or saline vehicle (ES-V) prior to stress (cannula placements shown in Fig. 2.3*B*). IS and HC triad members received only saline vehicle. Importantly, efficiency in wheel-turn escape behavior was identical between ES-V and ES-M groups (drug: $F_{1,17} = 0.332$, $p = 0.572$; Fig. 2.3*C*). Once again, ES-V produced dominance 1 week later in the 20-min WST compared to IS-V and HC-V groups ($F_{2,30} = 8.261$, $p = 0.001$, $n = 11$ per group; Fig. 2.3*D*). ES-V spent a significantly greater amount of time on the warm spot compared to HC-V ($p = 0.035$) and IS-V ($p = 0.001$). In contrast, intra-PL muscimol given before ES completely eliminated the dominance-producing effect of ES, that is, ES subjects no longer showed increased spot occupation times (Fig. 2.3*E*). These conclusions were confirmed by ANOVA ($F_{2,21} = 12.52$, $p < 0.001$, $n = 8$ per group). HC-V now had significantly greater occupancy time than ES-M and IS-V groups ($p = 0.005$ and $p < 0.001$, respectively), whereas ES-M and IS-V did not differ ($p = 0.456$). Intra-PL muscimol also reduced the effects of ES on later rank ($p = 0.029$, Mann-Whitney U test; Fig. 2.3*F*) and the number of resistance bouts ($p = 0.036$, unpaired t test; Fig. 2.3*G*).

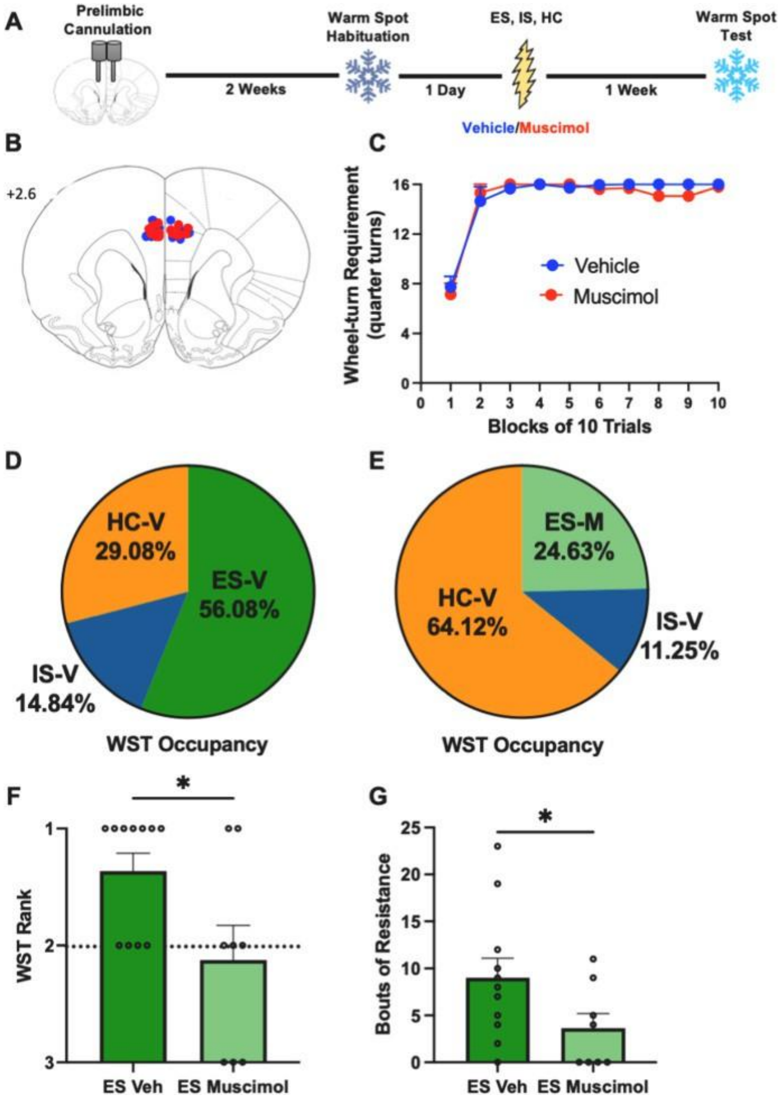

Figure 2.3. Prefrontal involvement in the dominance-producing effects of behavioral control. ***A***, Experimental timeline. ***B***, Cannula placement in the prelimbic region of the medial prefrontal cortex for vehicle- (blue dots) and muscimol-treated (red dots) escapable stress (ES) subjects. Number indicates distance (mm) anterior to bregma. ***C***, Comparison of wheel-turn escape behavior between intra-PL vehicle- and muscimol-treated ES subjects. Number of quarter turns of the wheel attained as the escape requirement for each trial. ***D***, Percent occupancy of the warm spot test for triads in which the ES subject received vehicle or ***E***, muscimol. ***F***, Average rank position of ES vehicle and muscimol groups. The dotted line indicates expected ranking if there was no impact of prior stress treatment. ***G***, Number of resistance bouts. Values represent the mean ± SEM. $^*p < 0.05$, Mann-Whitney U (rank) and unpaired *t* test (resistance).

Sex Differences in the formation of dominance hierarchies.

Given the absence of control facilitated dominance, and prior work indicating sex differences in the formation and maintenance of dominance hierarchies, we wished to characterize the formation of dominance hierarchies in male and female Sprague Dawley rats before investigating the neural mechanisms of winning. Male and female rats were placed into triads and following individual habituation, competed for seven sessions in the warm spot task (Fig. 2.4A). Each competition was recorded for later behavioral analysis. Stables hierarchies, defined as groups with stable rankings at least 5 of the seven days, were achieved in 7 out of 8 male triads (Fig 2.4F) and 5 out of 8 female triads (Fig. 2.4M). One way ANOVA revealed no significant effect of S1 body weight in males ($F_{2,18} = 1.799$, $p = 0.194$, $n = 6\text{-}8$ per group, Fig 2.4C), or females ($F_{2,21} = 0.0993$, $p = 0.906$, $n = 8$ per group, Fig 2.4J). One way ANOVA revealed a significant effect of S7 body weight on rank in males ($F_{2,18} = 4.396$, $p = 0.028$, $n = 6\text{-}8$ per group, Fig 2.4D), with 2nd rank animals weighing more than 1st rank (p=0.035), but no significant effect of S7 body weight on rank in females ($F_{2,21} = 0.994$, $p = 0.906$, $n = 8$ per group, Fig 2.4J). In males, a stable first rank appeared around S3, at which point second rank dropped down to near third rank occupancy (Fig 2.4G). A repeated measures ANOVA comparing the mean area under the curve for occupancy showed significant effects of the interaction between rank and occupancy ($F_{2,18}= 23.07$, $p<0.0001$; Fig. 2.4H). First ranked animals occupied the spot significantly more than second ($p<0.001$) or third ($p<0.0001$) but there was no significant difference between second and third ranked animals ($p=0.3622$). In females, a stable first rank appeared around S3, at which point second rank dropped down to near third rank occupancy (Fig

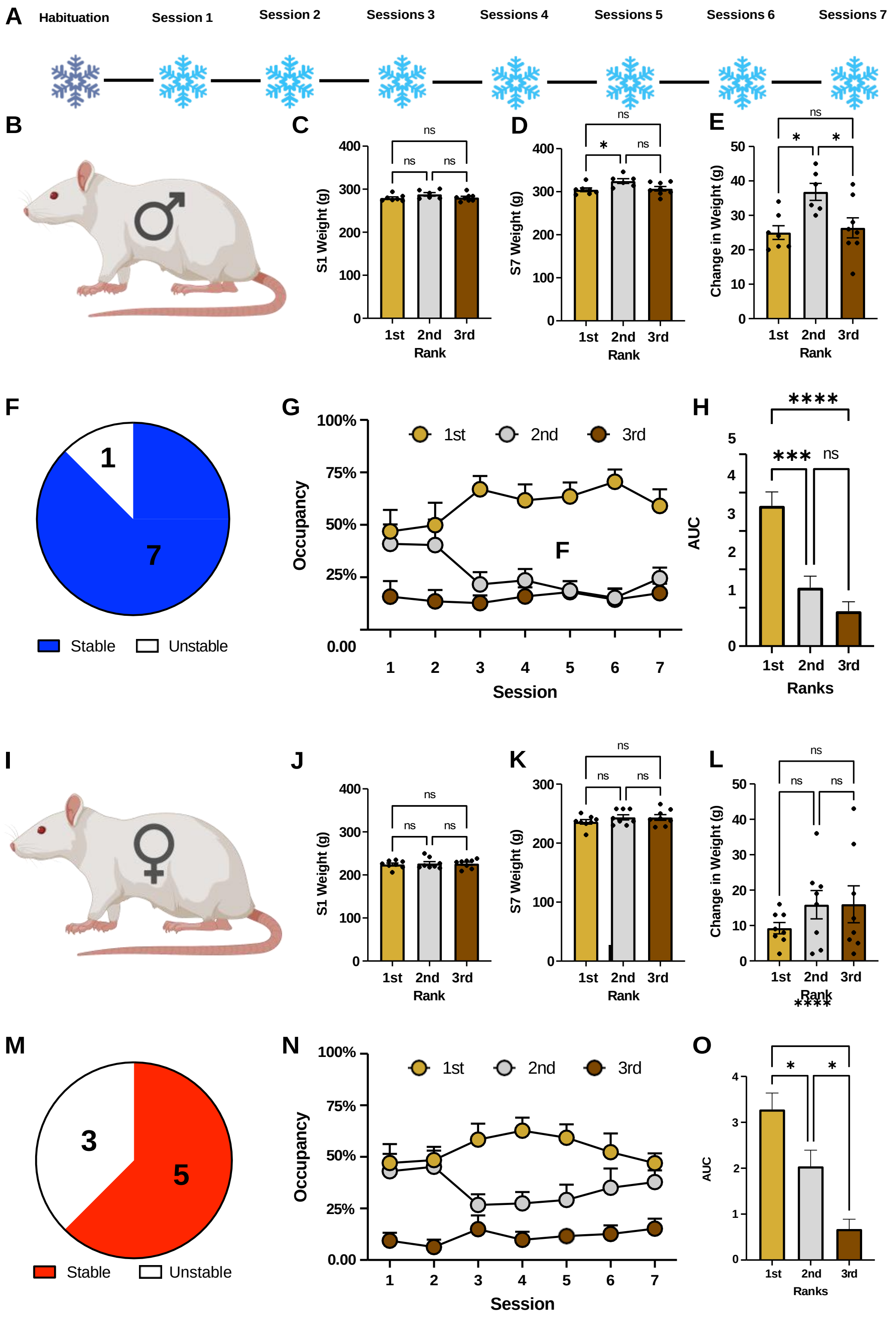

Figure 2.4. **Hierarchy stability in males *B-H* and females *I-O*. *A.*** Experimental Timeline. ***C/J.*** Session 1 weights (grams) for future ranks 1-3. ***D/K.*** Session 7 body weights by rank (grams). ***E/L.*** Change in body weight from session 1 to session 7 by final rank. ***F/M.*** Number of stable triads out of 8 total. ***G/N.*** Percent occupancy of the

warm spot by rank per session. ***H/O.*** Area under the curve analysis of occupancy by rank per session as shown in *H/O.* Values represent the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

2.4N). However, second rank slowly began to raise back towards first rank occupancy approaching S7. A repeated measures ANOVA comparing the mean area under the curve for occupancy showed significant effects of the interaction between rank and occupancy ($F_{2,21}$= 17.12, $p<0.0001$; Fig. 2.4O). First ranked animals occupied the spot significantly more than second ($p=0.0286$) or third ($p<0.0001$), and second occupied significantly more than third ranked animals ($p=0.0156$).

Stable dominance in the warm spot requires the corticostriatal system

Similar to an experience with behavioral control, prior history of winning in a social competition can also increase the probability of winning in future contests ('winner effect'; Landau, 1951; Dugatkin, 1997). We next addressed whether corticostriatal structures are involved in the development of sustained winning in the warm spot test since both the PL and DMS are necessary for the protective effects of behavioral control (Amat et al., 2014; Christianson et al., 2014). Given the results of our previous experiment that females form less stable dominance hierarchies (Fig 2.4F/M) and have less significant difference between first and second ranked animals (Fig 2.4H/O), we opted to use male cohorts to study stable winning. First, rats were implanted with bilateral cannula in the PL (Fig. 2.5*B*) 2 weeks prior to a series of 20-min warm spot triad competitions. There was a total of 5 competitions, each separated by 48 h. Winners in the initial warm spot session (subjects that had the highest occupancy time in each triad) received either intra-PL muscimol or vehicle 30 min prior to the second session. All other members of the triad received saline vehicle. In session 1 winners, vehicle treatment had no impact on subsequent performance in session 2. PL inactivation, however, produced a marked decrease in the percentage of spot occupancy. Repeated-measures ANOVA indicated a significant main effect of drug condition ($F_{1,13} = 7.993$, $p = 0.014$, $n = 7\text{-}8$ per group), but no significant interaction between drug and session ($F_{4,52} = 1.819$, $p = 0.139$; Fig. 2.5*D*). Surprisingly, muscimol treatment not only reduced session 2 dominance ($p = 0.005$; Fig. 2.5*E*), but also in subsequent sessions when subjects were drug-free ($p = 0.017$; Fig. 2.5*F*).

Similar to the above, a second cohort was implanted with bilateral cannula targeting the DMS rather than the PL (Fig. 2.5*C*). Half of the winners from the first warm spot session received intra-DMS microinjections of AP5 prior to session 2, whereas the remaining half received saline vehicle. Repeated-measures ANOVA showed significant effects of drug condition ($F_{1,13} = 10.26$, $p = 0.007$, $n = 7\text{-}8$ per group) and interaction between drug and session ($F_{4,52} = 3.137$, $p = 0.022$; Fig. 2.5*G*). Once again, vehicle-treated session 1 winners continued to show high levels of spot occupancy across all sessions. Intra-DMS AP5 decreased occupation time during session 2 ($p = 0.009$; Fig. 2.5*H*), and this loss of dominance was evident throughout the remaining sessions ($p = 0.005$; Fig. 2.5*I*).

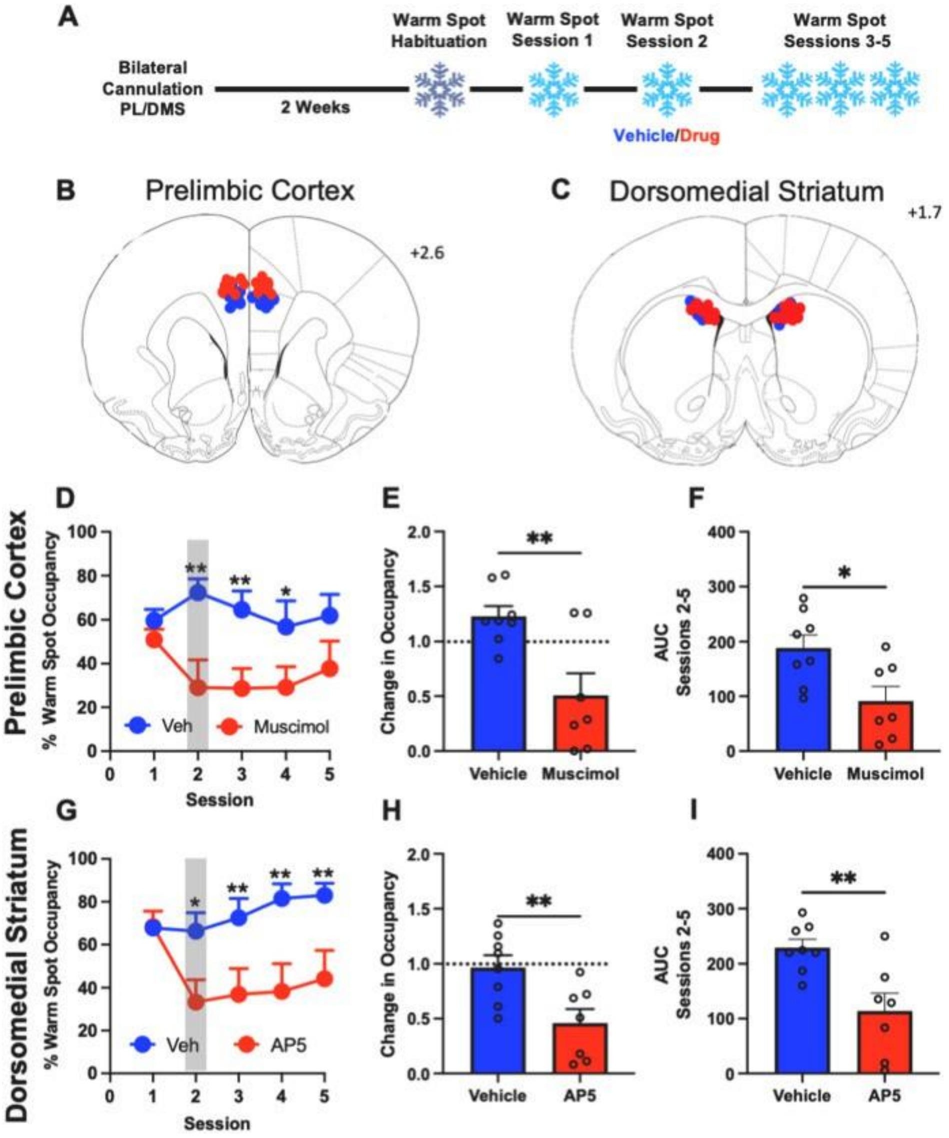

Figure 2.5. The prelimbic cortex and the dorsomedial striatum are required for the development of stable dominance. ***A***, Experimental timeline. ***B***, Cannula placements for session 1 winners that received vehicle (blue dots) or muscimol (red dots) in the prelimbic cortex (PL) cortex or ***C***, vehicle (blue dots) or AP5 (red dots) in the dorsomedial striatum (DMS). Numbers indicate distance (mm) anterior to bregma. ***D***, Percent occupancy of the warm spot test for initial winners that received intra-PL vehicle or muscimol prior to session 2. Gray bar indicates drug-treatment session. ***E***, Change in spot occupancy (session2/session1) for initial session winners. ***F***, Histogram depicting the mean area under the curve (AUC) for warm spot occupancy, sessions 2-5 only. ***G***, Percent occupancy of the warm spot test for initial winners that received intra-DMS vehicle or muscimol prior to session 2. Gray bar indicates drug-treatment session. ***H***, Change in spot occupancy (session2/session1) for initial session winners. ***I***, Histogram depicting AUC for warm spot occupancy, sessions 2-5. Values represent the mean ± SEM. *$p < 0.05$, **$p < 0.01$, unpaired *t* test.

Impact of repeated winning on the behavioral and neurochemical sequelae of inescapable stress

Given the involvement of the PL and DMS in the development of stable dominance, and the prior work showing the necessity of these structures for the occurrence of the protective effects of behavioral control, we next sought to determine if the experience of repeated winning buffers against the behavioral and neurochemical outcomes of future IS, as does prior ES (Fig. 2.6*A,B*). Triads received 5 daily warm spot competitions prior to receiving a single session of IS (100 trials, 1.0 mA and 5 s duration each) or no stress (HC). JSE was assessed 24 h later. As is typical, exposure to IS reduced JSE in rats that did not undergo the warm spot procedure (IS-No WST; Fig. 2.6*C*). Prior history of repeated winning (top rank position for at least 4 out of the 5 sessions) completely blocked the social avoidance produced by IS. Importantly, the impact was specific to winning. Repeated losing (lowest rank position for at least 4 out of the 5 sessions) led to reduced social interaction, independent of stress treatment (data not shown). To control for the reduced amount of time spent on the cold floor by winners, we also included a group in which subjects were matched to stable winners such that they received an equivalent amount of time exposed to both the warm spot and cold cage floor but did not have to compete for gaining spot access (“Exposure”). Matching exposure to the warm spot-cold cage environment to that of winners did not buffer against IS-induced social avoidance. These conclusions were confirmed by ANOVA showing a main effect of stress condition ($F_{1,47} = 10.35$, $p = 0.002$, $n = 8$-10 per group) and a significant interaction between stress and warm spot experience ($F_{2,47} = 4.351$, $p = 0.02$; Fig. 2.6*C*).

Exposure to IS produces a persistent increase in DRN 5-HT efflux indicative of DRN 5-HT activation, and activation of DRN 5-HT neurons is both necessary and sufficient for the behavioral sequelae of IS, such as social avoidance (Baratta et al., 2023; Christianson et al., 2008; Maswood et al., 1998). To examine whether a history of winning also buffers against IS-induction of DRN 5-HT efflux, subjects were first implanted with a microdialysis probe targeted to the mid-caudal region of the DRN (Fig. 2.6*E*). IS led to a large increase in extracellular DRN 5-HT that remained elevated throughout the entire stress treatment (S1-S5; group: $F_{3,23} = 5.358$, $p = 0.006$, $n = 5\text{-}9$ per group; Fig. 2.6*F*), however this only occurred in repeated warm spot losers (IS-Losers) and subjects that did not undergo warm spot competition (IS-No WST). In contrast, IS led to an initial increase in 5-HT that quickly returned to baseline in subjects with a history of winning (IS-Winners). Comparison of the mean area under the curve ($F_{3,23} = 5.205$, $p = 0.007$; Fig. 2.6*G*) further showed that IS only elevated 5-HT levels in IS-Losers and IS-No WST groups (p's < 0.05), whereas IS-Winners did not differ from no stress subjects (HC-No WST; $p = 0.987$).

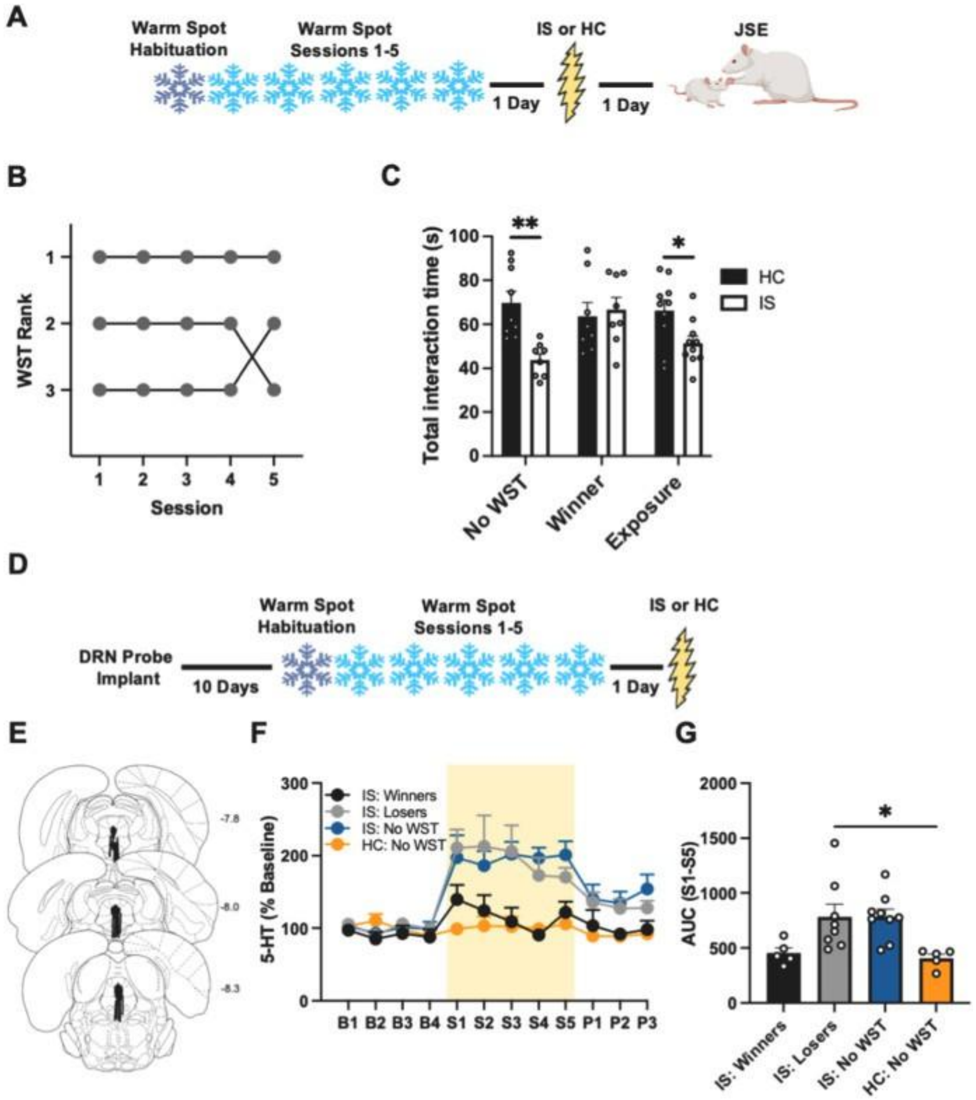

Figure 2.6. Stable dominance buffers against the behavioral and neurochemical outcomes of inescapable shock. ***A***, Experimental timeline of behavioral experiment. Warm spot competition “naïve” subjects (No WST), repeated winners, repeated losers, and controls that received equal exposure to the warm spot environment but not competition were given inescapable stress (IS) or no stress (homecage, HC). ***B,*** Representative rankings across 5 sessions for triads with a repeated winner (left) and a repeated loser (right) across five sessions. ***C***, A 3-min juvenile social exploration (JSE) session was conducted 24 h after stress treatment and data is expressed as the total exploration time. ***D***, Experimental timeline of *in vivo* microdialysis experiment. ***E***, Black bars represent the placement of microdialysis probes in the dorsal raphe nucleus (DRN). Numbers indicate distance (mm) posterior to bregma. ***F***, Extracellular serotonin (5-HT) as a percentage of baseline in the mid-caudal region of the DRN during IS or HC. Samples were collected every 20 min during the baseline (B1-B4), stress (S1-S5; depicted by yellow shaded area), and post-stress (P1-P3) phases. ***G***, Histogram depicting the mean area under the curve (AUC) for extracellular 5-HT during IS (S1–S5). Values represent the mean ± SEM. *$p < 0.05$, **$p < 0.01$, Tukey’s.

Effect of stable dominance on the glucocorticoid and neuroimmune responses to inescapable stress

In addition to DRN 5-HT activity, exposure to IS also induces an array of endocrine (Fleshner et al., 1995; Maier et al., 1986) and central immune responses (Frank et al., 2019; O'Connor et al., 2003). Here we addressed whether the stress-buffering effects of stable dominance (again, top rank position for at least 4 out of the 5 sessions) extends to IS-induced endocrine and neuroimmune changes that are known not to be buffered by behavioral control. Immediately after the IS procedure (or no stress), cardiac blood was collected to obtain a measure of circulating CORT, along with tissue dissection of the hypothalamus to examine cytokine (IL-1β, IL-6) and chemokine (CX3CR1) mRNA expression. As is typical, IS led to a pronounced increase of serum CORT ($F_{1,27} = 541.8$, $p < 0.001$, $n = 7\text{-}9$ per group), but there was no main effect of prior dominance status ($F_{1,27} = 1.685$, $p = 0.205$) nor an interaction between status and stress conditions ($F_{1,27} = 2.207$, $p = 0.149$; Fig. 2.7*B*). The elevation of CORT elicited by IS was similar in both stable winners and losers. IS also increased IL-1β ($F_{1,26} = 36.02$, $p < 0.001$, $n = 7\text{-}8$ per group), but not IL-6 ($F_{1,26} = 4.178$, $p = 0.051$), mRNA levels in the hypothalamus (Fig. 2.7*C*). Once again, the stress-induced increase in hypothalamic IL-1β mRNA was independent of prior winning history ($F_{1,26} = 0.029$, $p = 0.867$) nor was there an interaction between status and stress ($F_{1,26} = 0.166$, $p = 0.687$). We also measured the gene expression level of the chemokine receptor CX3CR1. Expressed on the surface of microglia, the primary innate immune cell of the brain, CX3CR1 is thought to constitutively maintain microglia in a quiescent state through interactions with its ligand expressed on neurons (Cardona et al., 2006). IS-induced neuroinflammation may be mediated, in part, by IS-induced downregulation of CX3CR1. IS significantly downregulated CX3CR1 gene expression ($F_{1,26} = 59.78$, $p < 0.001$; Fig. 2.7*C*) and this reduction did not differ between winners and losers.

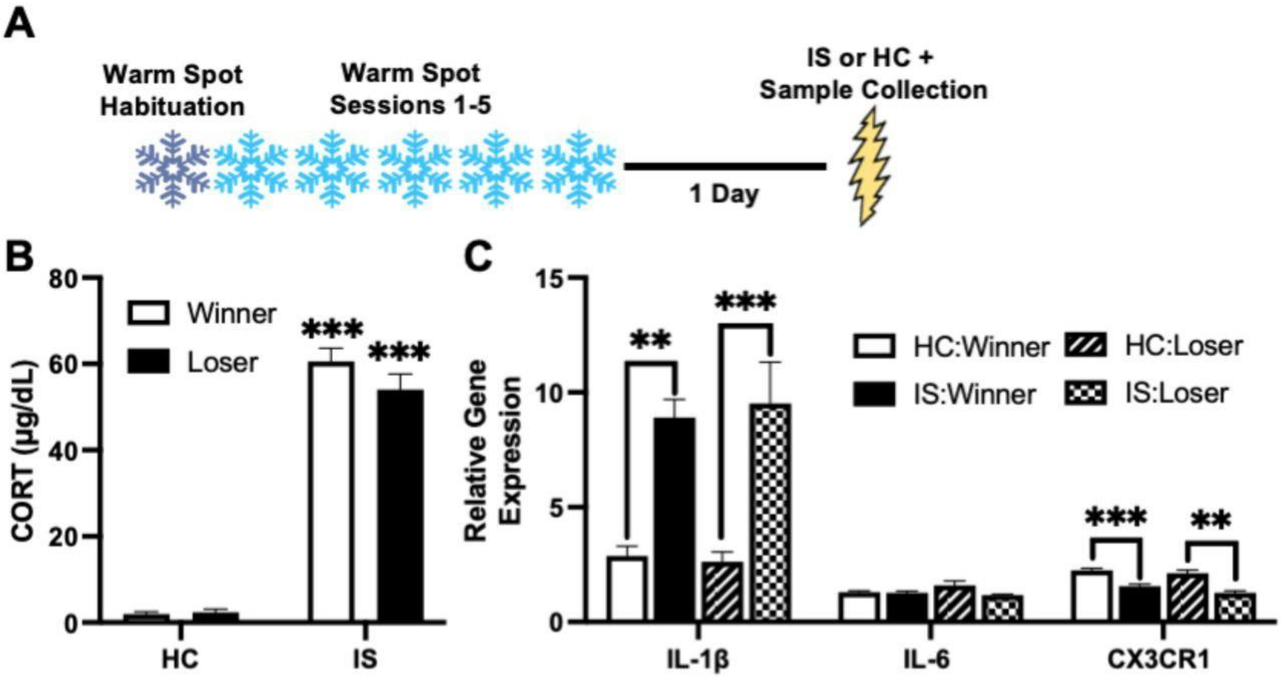

Figure 2.7. Dominance status does not impact endocrine and neuroinflammatory sequelae of inescapable shock. ***A***, Experimental timeline. Repeated winners and losers in the warm spot test (WST) were given inescapable stress (IS) or no stress (homecage, HC). ***B***, Measurement of serum corticosterone (µg/dL, CORT) collected immediately after stress treatment. ***C***, Expression of neuroinflammatory-related genes in hypothalamus dissected immediately after stress treatment. Relative gene expression of (left to right) interleukin-1β (*Il1b*), interleukin-6 (*Il6*), and C-X3-C motif chemokine receptor 1 (*cx3cr1*) mRNA. Values represent the mean ± SEM. **$p < 0.01$, ***$p < 0.001$, Tukey's.

Discussion

The current set of experiments led to two primary findings. First, behavioral control in males increases the number of effortful behavioral epochs during future competitive interactions and facilitates their winning, an outcome that requires PL activation during the control experience. Second, the development of stable dominance involves corticostriatal structures (both PL and DMS), and once established, buffers against the typical neurochemical and behavioral sequelae of IS. We also observed that IS-induced endocrine and neuroimmune outcomes were unaffected by dominance rank, suggesting boundary conditions to the stress buffering effects of stable dominance.

Exposure to adverse events can shape future dominance hierarchies (Park et al., 2018a; Šabanović et al., 2020a). Our focus on the controllability of the stressor is motivated by the fact that a single experience of behavioral control leads to long-lasting resistance against future adversity in a prefrontal-dependent manner (Maier, 2015b). Similarly, prefrontal circuits have been implicated in a number of features related to dominance behavior, such as competitive success and its transitivity (Wang et al., 2011a; Zhou et al., 2017b), effort expenditure (Porter & Hillman, 2021; Zhou et al., 2017b), detection of the relative social rank of self and others (Garcia-Font et al., 2022; Padilla-Coreano et al., 2022a), and working memory function that may contribute to these processes (D’Esposito et al., 2000; Miller, 2013). If behavioral control and winning strengthen and are regulated by the same circuits, then experiencing control should facilitate later winning. Indeed, we found that in males ES, but not physically identical IS, enhances dominance one week later, with PL activation being required during ES.

In contrast to males, prior ES in females had no impact on resistance behavior and spot occupancy one week later. Rather, all female groups (ES, IS, HC) displayed similar levels of dominance. This was not due to differences in learning the controlling response. Females readily acquired and maintained the instrumental controlling response to the same extent as males (Fig. 5*B*). However, prior work supports the idea that the operation of control in females (i) does not lead to the same stress-buffering effects as in males and (ii) the learning of the controlling response is accomplished with a different instrumental learning system thanin males, namely a dorsolateral striatum (DLS) ‘habit’ system rather than a corticostriatal ‘action-outcome’ system (Fallon et al., 2020; McNulty et al., 2022). If the DLS is inactivated, shifting instrumental responding to the DMS, control now leads to protection in females. Although speculative, future work should investigate whether ES in females would facilitate later dominance if corticostriatal structures (PL and DMS) were activated during the ES experience.

As mentioned, there is similarity in the mechanisms that mediate winning in social encounters and the protective effects of behavioral control over aversive events. This led us to hypothesize that corticostriatal structures (PL and DMS) would be required for the development of stable dominance. We opted to use males to investigate this hypothesis given that we discovered males to have a more stable first rank and greater overall stability. We show that for winners of the initial warm spot competition, subsequent inactivation of the PL or blockade of the NMDA-dependent glutamatergic signaling in the DMS, the downstream striatal target of the PL, led to a lowering of dominance rank. Reduced status extended to subsequent drug-free competitions, thus interfering with the stability of dominance observed in vehicle-treated winners.

Dominance interactions

are learning experiences as well as social interactions, and perhaps disruption of corticostriatal function interferes with the subject’s ability to use information from their previous success – such as the contingency between instrumental effort and outcome (e.g., occupying the warm spot) – to guide their performance in subsequent competition. The role of the corticostriatal system in aversively, rather than appetitively, motivated tasks is not well studied. However, the same manipulations used here that interfered with repeated winning also eliminate the resilience-producing effects of behavioral control over stress (Amat et al., 2005, 2014).

The data from above suggest that repeated winning in the warm spot test might mimic the proactive effects of behavioral control. It is known that reduced social investigation produced by IS is mediated by potent activation of 5-HT neurons in the DRN (Amat et al., 1998a; Christianson et al., 2008, 2010), and this activation is inhibited by control. Indeed, we found that a history of winning prior to IS exposure blocked the increased levels of extracellular 5-HT in the DRN produced by IS and prevented social avoidance. These buffering effects were specific to winning, as repeated losing produced a very different neurochemical and behavioral pattern. Losing, independent of IS exposure, led to reduced juvenile social exploration, a finding that replicates previous work in mice (Šabanović et al., 2020a). Losing also failed to modify the DRN 5-HT response to IS, with elevated 5-HT levels persisting throughout the entire shock session. It should be noted that other acute stressors, such as social defeat, activate DRN 5-HT cells (Amat et al., 2010; Cooper et al., 2009; Paul et al., 2011) and produce similar behavioral outcomes as does IS, such as social avoidance and shuttle box escape failure (Amat et al., 2010). This defeat-induced DRN 5-HT activation is selectively suppressed in animals with dominant status (Cooper et al., 2017). Taken together, these findings highlight several properties of winning as a resilience factor. Competitive success buffers against adversity that takes place in a novel context (generalization) and provides resistance to the effects of both nonsocial and social adverse events.

The impact of winning on the stressor response was selective. Neuroendocrine and central immune responses are also elicited by adverse events, although changes to these measures are typically not sensitive to the controllability of the stressor (Frank et al., 2007; Helmreich et al., 1999; Maier et al., 1986). Different energetic requirements are associated with social status and competition outcomes, and the levels of glucocorticoids and innate immune function can play a correlative or even causal role in the formation of hierarchies (Audet et al., 2010; Avitsur et al., 2007; Knight & Mehta, 2017; Wingfield et al., 1990). We assessed whether dominance status would block IS-induced changes to circulating corticosterone and hypothalamic cytokine (IL-1β, IL-6) and chemokine (CX3CR1) mRNA expression levels. All measures (except IL-6) were impacted by IS, but the magnitude of change did not differ between winners and losers. The foregoing suggests that dominance status does not make the IS experience more or less "aversive" or "stressful"; rather it constrains the circuit (serotonergic) response to IS, thereby preventing the behavioral outcome (social avoidance). There are any number of experience-dependent intrinsic and extrinsic mechanisms that would alter how the dorsal raphe responds to IS. One possibility is that repeated winning experiences might strengthen PL top-down regulation of the DRN during IS (Grizzell et al., 2020).

In summary, our findings demonstrate that winning in social encounters and control over non-social stressors are fungible. The operation of behavioral control over adverse events, a key aspect of coping, facilitates later competitive success. Repeated winning in social encounters produces resilience against the non-social stressors such as uncontrollable shock. Given that control and stable dominance are implicated in positive health outcomes, the involvement of corticostriatal structures for both may represent a circuit-level endophenotype in the production of resilience.

Chapter 3: The role of thalamocortical circuitry in control facilitated competitive success

Abstract

Instrumental control over stress (escapable stress, ES) prevents both the neurochemical and behavioral changes that follow a physically identical uncontrollable stressor (inescapable stress, IS) if acquired by the goal directed circuitry.. We have previously shown that in males ES facilitates winning relative to IS and home cage (HC) rats in a social dominance task one- week post stress. This control-facilitated dominance is dependent on activation of the prelimbic (PL) region of the medial prefrontal cortex during ES. Given that this facilitated dominance is dependent on the same circuitry that guides goal-directed learning, it may be that controllable stress increases the effort an animal exerts towards a goal (thus increasing dominance) regardless of whether the behavior occurs in a social or non-social context. To test this idea we developed a non-social tube test, in which a single animal pushes a sled through the tube in place of an opponent. After each successful trial the weight in the sled is increased until failure (breakpoint) occurs. We found that ES increases the breakpoint weight one-week post stress. There was no significant change in breakpoint for IS and HC treated rats. Others have shown that the synaptic strength of the PL input from the mediodorsal thalamus (MDT) directly correlates with winning in dominance tasks and instrumental effort in the face of challenge. Thus, we next investigated whether the MDT-to-PL pathway is necessary during ES for later control facilitated dominance using an intersectional chemogenetic strategy. All animals received CNO 30 minutes prior to stress (ES/IS/HC) and were tested for dominance one week later against non-stressed home cage opponents. In mCherry controls, ES facilitated winning against HC rats in the social tube test. ES hM4Di animals were subordinate to HC one week post stress.

IS rats were equally dominant to HC rats regardless of viral group (mCherry/hM4Di).

Introduction

Dominance hierarchies exist in a large number of species from insects to humans, and an organism's overall fitness is directly affected by its status in that hierarchy (Fan et al., 2019; Fetcho et al., 2023b; Larrieu & Sandi, 2018; Pallé et al., 2019; Šabanović et al., 2020a; Zhou et al., 2017a). Recently, there has been growing interest in the experiential factors that dictate an individual's position within those hierarchies. Whereas initially, dominance research focused on intrinsic factors such as strength and size, the field has now shifted to studying the mechanism through which an individual's experience can either increase or decrease their dominance. One such mechanism is stress. Historically, the effect of stress on dominance has been studied through the lens of uncontrollable stressors, such as chronic social defeat stress or chronic variable stress models (Boersma et al., 2017; Deuter et al., 2016; Iñiguez et al., 2018; Karamihalev et al., n.d.; Morrison et al., 2013b; Park et al., 2018b; Šabanović et al., 2020b; Sapolsky, 2004). However, we have recently shown that experiencing a single non-social controllable stressor can facilitate dominance over animals that experienced an uncontrollable stressor and animals that were not stressed at all (Coleman et al., 2024).

Controllable stressors (in this case escapable tailshock) potently activate the medial pre frontal cortex (mPFC), a region in which activity is also correlated with increased dominance (Baratta et al., 2009; Wang et al., 2011a). In fact, escapable stress (ES) results in an increase in plasticity within layer V of the mPFC, especially the prelimbic region (PL) (Varela et al., 2012). This is of note here because it has been shown that increasing or decreasing the strength of the mediodorsal thalamic

(MDT) projection to the PL, a projection responsible for the majority of the excitatory input to the PL and strongly represented in layer V (Gabbott et al., 2005; Kelly & Martina, 2018), can directly lead to an increase or decrease in winning in the mouse tube test (Zhou et al., 2017a). Specifically, using optogenetically induced LTP/LTD, the Hu lab made tube test losers into winners (LTP in the MDT-to-PL path), then quickly reversed and made the now winners losers once again (LTD in the MDT-to-PL path). This findings, together with their related finding that increasing/decreasing AMPA receptor signaling in the vmPFC increased/decreased (respectively) that animals rank (Wang et al., 2011a), implies that increasing the strength of PL inputs from the MDT will directly increase dominance. We have yet to determine the effect of ES on the MDT-to-PL pathway, but given the increased PL plasticity following ES (Baratta et al., 2019a) and the necessity for activation of act/outcome regions during ES for ES to be protective (Amat et al., 2014; McNulty et al., 2022) it is highly likely the MDT-to-PL pathway is robustly activated by ES. This activation could provide a mechanism for increased dominance through higher plasticity and activation of the PL via glutamatergic MDT projections.

The MDT-to-PL pathway has been implicated in a variety of behavioral paradigms, including working memory, instrumental learning, social dominance, and effort (Bradfield et al., 2013; Chakraborty et al., 2019; Corbit et al., 2003; Mitchell et al., 2007; Wolff & Halassa, 2024; Zhou et al., 2017c). This system is comprised of multiple projections from the various portions of the MDT into the vmPFC, although the lateral portion (MDTl) is of specific interest here given its involvement in instrumental effort and social dominance (Collins et al., 2018; Gabbott et al., 2005; Hoover & Vertes, 2007). The neurons in the MDTl preferentially target the anterior cingulate cortex (ACC) and

PL, but the extent of their collateralization is such that a single neuron in the rat MDTl can innervate as much as 1mm in diameter of the vmPFC. This is mentioned here to point out that while our interest is on the PL specifically, it is highly likely that any effect of MDT-to-PL manipulation is also due to a change in MDT-to-ACC activity. Activity in this vmPFC circuitry is correlated with an increase in persistent effort in the face of challenge, and these PFC glutamateric cells receive the majority of their input from the MDT (Elston & Bilkey, 2017; Holec et al., 2014). This work was done in nonsocial tasks, in which animal were required to expend more or less effort for a higher or lower reward. Our previous work indicated that the ES facilitation of dominance coincided with an increase in instrumental effort in the form of actively resisting being pushed off the spot in the warm spot task (Coleman et al., 2024). It is unclear if the increase in effort is a result of increased dominance, or vice versa. As such we decided to develop a novel effort tube test in which animals must push a weighted object out of the tube in the place of an opponent. Previous research would indicate that any increase in MDT-to-PL activity resulting from ES shoiuld lead to an increase in both social and non-social effort.

The present studies aimed to verify that the social tube test could be used to form stable dominance hierarchies in rats, assess if the control facilitated increase in effort extends to non-social tasks, and if the thalamocortical system is required during controllable stress for facilitated dominance. Thus, we first use the tube test to form stable triadic hierarchies and assess the effect of body weight on dominance in the tube test. Additionally, we determine if controllable stress has any effect on non-social effort using a novel effort tube test. Finally, we investigate the necessity of the MDT-to-PL pathway for ES facilitated dominance.

Materials and Methods

Subjects.

Adult male (275-300 g) Sprague–Dawley rats (Envigo) were pair-housed on a 12-h light–dark cycle (lights on at 0700 h) with ad libitum access to standard laboratory chow and water. Rats were allowed to acclimate to colony conditions for at least one week before experimentation. All behavioral procedures were carried out between 0900 and 1400 h. All experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Colorado Boulder Institutional Animal Care and Use Committee.

Wheel-turn ES/yoked IS procedure.

Subjects were run in the same triadic design as previously used to model controllable stress (Coleman et al., 2024). Each triad consisted of one subject that received ES, one that received IS, and a third that non-stressed animal that remained in the colony room (HC). For ES and IS, rats were placed in 14 × 11 × 17 cm (length × width × height) Plexiglas boxes, each with a wheel mounted in the front. A plexiglass rod extended out the rear of the box to affix the tail with copper electrodes and electrode paste or shock delivery. The stress session itself consisted of 100 individual tail shock trials administered by a current regulated shock controller (Coulbourn Instruments). Each shock (1.0mA) was administered randomly at either 30, 60, or 90 second intervals. The wheel turn requirement to terminate shock starts at a single quarter-turn of the wheel by the ES rat. If the animal successfully terminates within 5 seconds of shock initiation this requirement increases to two quarter turns, then a full turn, until gradually reaching four full turns. The requirement is reduced to the previous stage if that animal does not terminate the shock within 5 seconds and is reset fully to a single quarter turn if the animals fails to terminate by the time the shock automatically stops at 30 seconds. For yoked IS rats, the onset and offset of each tail shock were identical to those of its ES partner. A computer equipped with Graphic State 4 (Coulbourn Instruments) controlled the experimental events and recorded the wheel turn requirement and escape latency for each trial.

Effort Tube Test.

The same tube test enclosure used for social competition is used for a progressive weight paradigm. Animals undergo 30 minutes of habituation to the tube test enclosure with the center door up. Then, on the same day, the center door is added and a hollow white plastic cylinder weighing 45 grams is place at the at the very end of the tube on the opposite side of the door from the animal. The door is removed once the animal reaches the center of the tube and the animal is free to investigate the cylinder, which is placed in such a way that a light touch from the animal will cause it to fall out of the tube. The door is then replaced, and the cylinder is moved to the other side of the tube, placed slightly closer to the center door so that the animal has to push it farther to move it out of the tube. This is continued until the cylinder is placed in the center of the tube flush against the door, requiring the animals to push it the same distance they would have to push an opponent in the social tube test. At this point following every successful tria.l a 45g led weight is placed into the cylinder until the animal is pushing roughly their body weight, at which point they are considered trained. The following day the empty bottle is placed in the center of the tube and again weight is added every time the animal pushes the weight from the tube. Weight is continually added until the animal fails to push the cylinder fully out of the tube within 5 minutes, this trial is then deemed their initial “breakpoint” or BP1. Animals are then divided up into high and low pushers based on their BP1 and randomly assigned to either ES, IS, or HC. BP2 is measured in the same way one week after stress. Heavier final weight values are equated with higher effort expenditure. Body weights are taken before training, BP1, and BP2.

Social Tube test.

The tube test enclosure consists of two identical cages connected by a clear plexiglass tube (length:48cm; diameter:6.5cm). The tube has a door placed in the center. Rats receive a 30-min habituation to the apparatus with initial placement in one cage, exploration of the tube, and eventual exit into the cage on the opposite side. Importantly, pretraining food deprivation/reward is not used. For the round robin style tube test experiment in Figure 2.1, animals are then placed into non-cage mate triads. Each animal in the triad competed against the other two for five trials each day (10 trials total per day). The order in which animals compete was randomized and animals were weighed on the first and last day. For the chemogenetic experiments, the day following habituation animals are randomly assigned to ES/IS/HC. One week later, subjects are paired with an unfamiliar non-stressed home cage competitor. Each rat competes in 5 trials per day for 5 days, against a new HC opponent each day. For each trial one competitor is placed in each of the two cages. The trial is initiated when each subject enters the tube and reaches the center door, which is then removed to start the competition. The subject that retreats or is forced out of the tube is designated as the “loser”, and the subject remaining in tube “winner”. Behavioral epochs (pushes-initiated, pushbacks and resistance bouts, retreats) were identified with frame-by-frame video analysis (EthoVision). Behaviors were not scored for the HC hM4Di group due to a camera error which corrupted the videos from this cohort.

Stereotactic surgery and CNO injection

All surgeries were performed under inhaled isoflurane anesthesia (5% induction, 2% maintenance in 2.5 L/min O2; Piramal Critical Care). Animals were injected with AAV2-eSYN-eGFP-T2A-iCRE-WPRE (Vector Biolabs) bilaterally into the PL (1.0 μl/side; A/P: +2.6; M/L: ± 0.5; D/V: -1.8mm) and with either AAV.hSyn.DIO.hM4D.mCherry or AAV.hSyn.DIO.mCherry bilaterally into the MDT (1.0 μl/side; A/P: -2.5; M/L: ± 0.9; D/V: -4.6mm). All subjects were given postoperative subcutaneous injections of an extended-release nonsteroidal anti-inflammatory (meloxicam, 4.0 mg/kg; Vetmedica) and an antibiotic (CombiPen-48, 0.25 mL/kg; Bimeda). Animals were be pair-housed within their stress group (ES/IS/HC). Once recovered from surgery, animals were habituated to the tube test apparatus as described in as described above. Animals were then be placed in one of three stress conditions (ES/IS/HC) and 30 minutes prior to stress animals received an injection of clozapine-N-oxide (CNO, 3.0 mg/kg, i.p.; NIMH Chemical Synthesis Program). hM4Di-induced inhibition of the PL-MDT pathway sufficiently decreases activity of MDT glutamatergic projections to the PL during ES (Figure 3.3) and that this inhibition has no effect on acquisition of the wheel turn escape response. At the end of the experiment, brains were collected, sliced at 30 μm, and stained with cresyl violet for verification of cannula placement. Subjects were only included in the data analysis if the viruses were successfully targeting the MDT-PL glutamatergic projection.

Tissue dissection.

Rats were deeply anesthetized via isoflurane prior to performing transcardial perfusion. Brain was rapidly extracted and flash frozen in isopentane at 38 degrees Celsius for 80 seconds. Brains were then sectioned at 30μm for verification of viral expression. *Fiber Photometry*

Animals received intra-PL (1.0 μL/side; A/P: +2.6; M/L: ±0.5; D/V: -3.1 mm from skull surface) bilateral microinjections of AAV2.retro-hSyn-jGCaMP8m (Addgene).The total injection volume (1.0 μL) and flow rate (0.1 μL/min) were controlled with a microinjection pump, and the 31-gauge needle was left in place for an additional 10 min to allow diffusion. Following 2 weeks of viral expression, a 5.1mm fiber optic cannula(400μM fiber core, 0.66 NA; Doric) was implanted above the unilateral MDT (A/P: -2.5; M/L: ±1.2; D/V: -4.9 mm). This coordinate is designed to place the tip of the fiber on the dorsal edge of MDTl cells. Cannula were secured to the skull with stainless steel screws and acrylic cement. They were not implanted during the initial viral surgery to ensure that cement headcaps were sufficiently robust during tail shock and was slowly lowered at 0.5 mm intervals (waiting 2 min between each step) to minimize tissue damage in the recording area.

Following two weeks of surgical recovery, animals were attached to a fiber-optic patch cable. The cable was protected within a metal spring fixed between a plastic collar on the animal and a Counter-Balanced Lever Arm (Instechlabs) to further protect the patch cable. Each subject was allowed 30 minutes to habituate to the recording apparatus in a cage with bedding. Rats were then placed in wheel-turn boxes for tailshock.

Photometry Analysis

GCaMP8m expressing tissue was excited at two wavelengths (474nm calcium-dependent signal and 405nm isosbestic) through a fiber patch cord. Recorded fluorescence was optically separated into 474nm and 405nm signals and detected with a photoreceiver at a sampling rate of 1kHz. Analysis of the signal was performed using custom written MATLAB scripts. Fluorescence signals were low-pass filtered and downsampled to 30Hz. The 405nm control signal was fit to the 474nm calcium signal using a least-squares polynomial regression to generate a predicted 405nm signal scaled to the 474nm signal as in prior reports[42,44]. Individual traces were created by normalizing (z-score) changes in fluorescence to a baseline (5 s before shock) and event-locked to either the onset or offset of shock for each animal averaged over the full 100, first 10, or last 10 trials. Mean traces were created from the average of each group’s individual traces. AUC of traces was calculated between shock onset and 2.5s after shock onset. Peak dF was determined from the mean traces.

Results

The tube test can be used to form stable dominance ranks in rats.

We initially sought to verify that with our procedures the tube test could be used in rats to form stable dominance hierarchies without food deprivation/motivation. In order to do this animals were habituated to the tube individually, then placed in non-cage mate triads in which they competed for 5 sessions against each of the two other triad members (10 total trials per animal per day) for five days (Fig 3.1A). One way ANOVA revealed no significant effect of session 1 bodyweight (grams) on final rankings ($F_{2,45} = 1.551$, $p = 0.223$, $n = 16$ per group, Fig 3.1B). However, there was a significant interaction between S5 body weight and final rank ($F_{2,21} = 4.787$, $p = 0.0194$, $n = 8$ per group, Fig 3.1C), with 2nd ranked animals weighing significantly more than first ranked animals ($p = 0.0174$, Tukey's, Figure 3.1C). There was a clear difference in number of wins between 1st, 2nd, and 3rd ranked animals (Figure 3.1D).

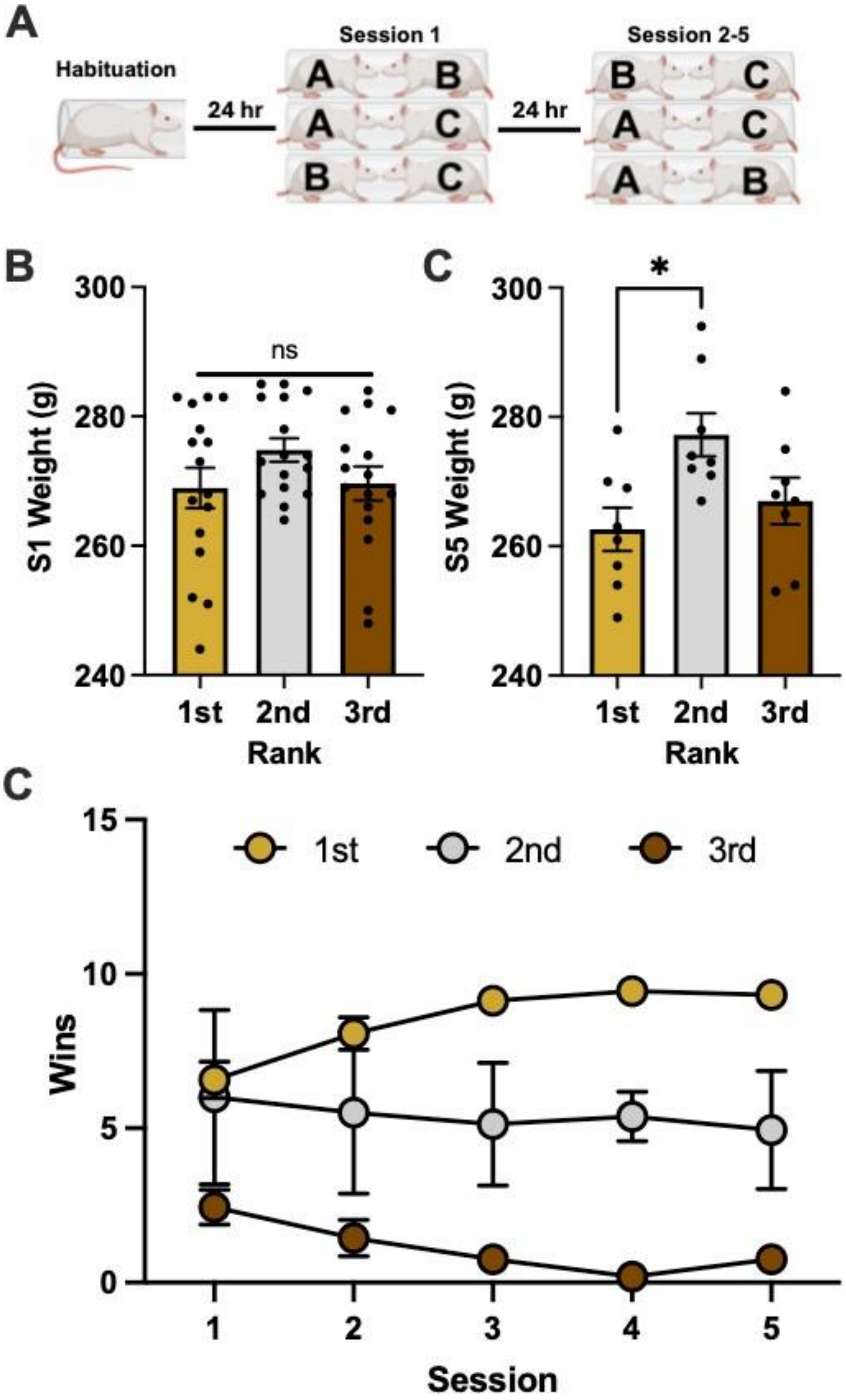

Figure 3.1. The tube test can be used to create stable dominance hierarchies in rats. ***A.*** Experimental timeline. ***B.*** Session 1 weights (grams) for future ranks 1-3. ***C.*** Session 5 body weights by rank (grams). ***C.*** Number of wins per session for first, second, and third ranked animals.

Controllable stress improves performance in a non-social effort task.

To assess the effect of uncontrollable versus controllable stressors on non-social effort, male rats were individually habituated to the test enclosure and trained to push a weighted bottle. Animals initially were trained to push up to their body weight, then the following day the first breakpoint (BP) was determined as the weight at which the animal failed to push the bottle from the tube. The following day animals were stressed using the triadic design, and a post stress BP was recorded one week later (Fig. 3.2A). Each stress group received high and low pushers as measured by their prestress BP. There was no difference in Pre-stress BP between groups ($F_{2,33}$= 1.603, p=0.2167; Fig. 3.2B). There was also no significant difference in body weight between groups prior to the post-stress BP ($F_{2,33}$=1.101, p=0.3445; Fig. 3.2G) nor was there a correlation between post-stress BP (regardless of group) and BP weight (R^2=0.03521, p=0.2526; Fig. 3.3D). Repeated measures ANOVA revealed a significant interaction between stress and time ($F_{2,34}$= 3.452, p=0.04; Fig.

3.2F), ES animals pushed significantly more post-stress (p<0.05, Šídák). There was no significant change in pre- vs. post-stress for IS (p=0.92) or HC (p>0.99). ES animals also had a less significant correlation (R^2=0.017, p=0.021; Fig. 3.2G) between push time (seconds) and weight (grams), compared with IS (R^2=0.165, p<0.001; Fig.3.2H) and HC (R^2=0.064, p<0.001; Fig. 3.2I).

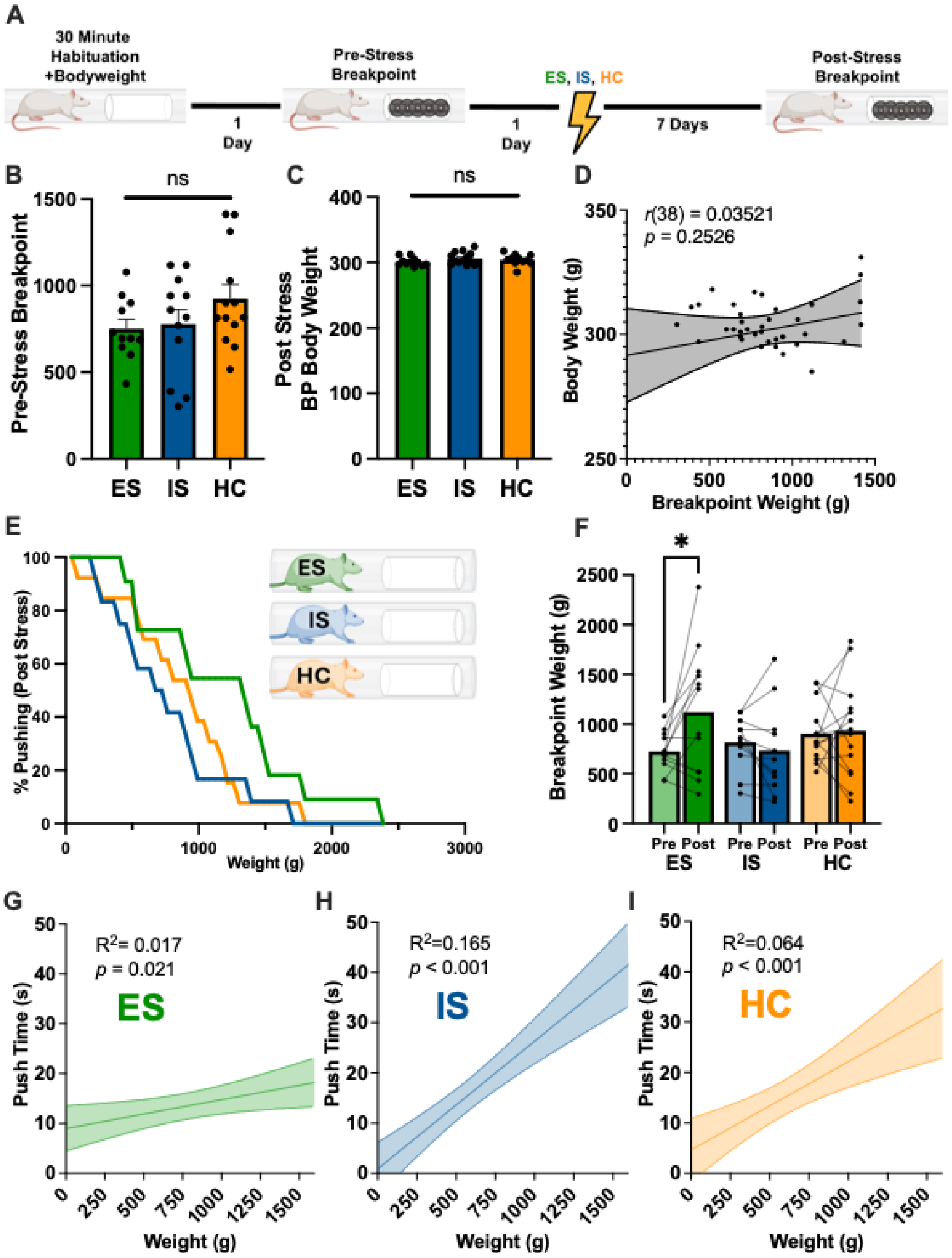

Figure 3.2. Controllable stress increases performance in the effort tube test. *A.* Experimental timeline. *B.* Pre-stress breakpoint weights for escapable shock (ES/green), inescapable shock (IS/blue) and homecage (HC/orange) groups. ***B.*** Body weights (grams) for ES/IS/HC animals immediately prior to post-stress breakpoints. ***D.*** Pearson's correlation for body weights and post stress breakpoints of all animals. ***E.*** Percentage of animals still pushing by group (ES/IS/HC) as weight increases. ***B.*** Change in breakpoint for all groups. ***G-I.*** Pearson's Correlation of total pushing time (time in seconds actively pushing the bottle) and weight (grams) for ES (***A***), IS (***B***), and HC (***C***).

Role of the mediodorsal thalamus in control facilitated dominance.

Given the role of the PL in ES facilitated dominance, and the prior work showing a role for the MDT-to-PL projection in dominance (Zhou et al., 2017), we sought to evaluate the role of the MDT-to-PL pathway during controllable stress. We ran a small preliminary study in which we injected a retrograde hSyn.GCaMP into the PL and placed recording fibers in the MDT to allow for calcium recording during ES. We found that during controllable stress, shock increases the calcium signal in the MDT-to-PL pathway, whereas during IS, shock decreased the calcium signal. This difference was clear for the first 10 trials but seemed to be absent in the last 10 trials. Given this, we next investigated the necessity of activity in the MDT-to-PL pathway for ES to facilitate dominance in the tube test. First, we verified that we could significantly reduce cellular activity in this pathway during ES (Fig.3.4A/B). CNO significantly reduced the number of cfos positive cells in the in the MDT-PL pathway compared to vehicle treated animals (p=0.0015).

To evaluate the role of the MDT-PL pathway in ES induced dominance, male rats were individually habituated to the tube test apparatus and 24 h later received an i.p. injection of CNO 30 minutes prior to ES, yoked-IS, or HC treatment (Fig.3.5A). One week later each experimental animal competed in five trials against a novel HC mCherry animal opponent in the tube test. This was repeated for five days against a different opponent each day. A win ratio was generated for each subject based on the number of wins out of the 25 total trials. Each group was then compared to the theoretical mean of 0.5 wins using a one-sample t-test with a Wilcoxon correction for multiple comparisons. ES mCherry animals were significantly more likely to be winners (t_7=4.472, p=0.0029,

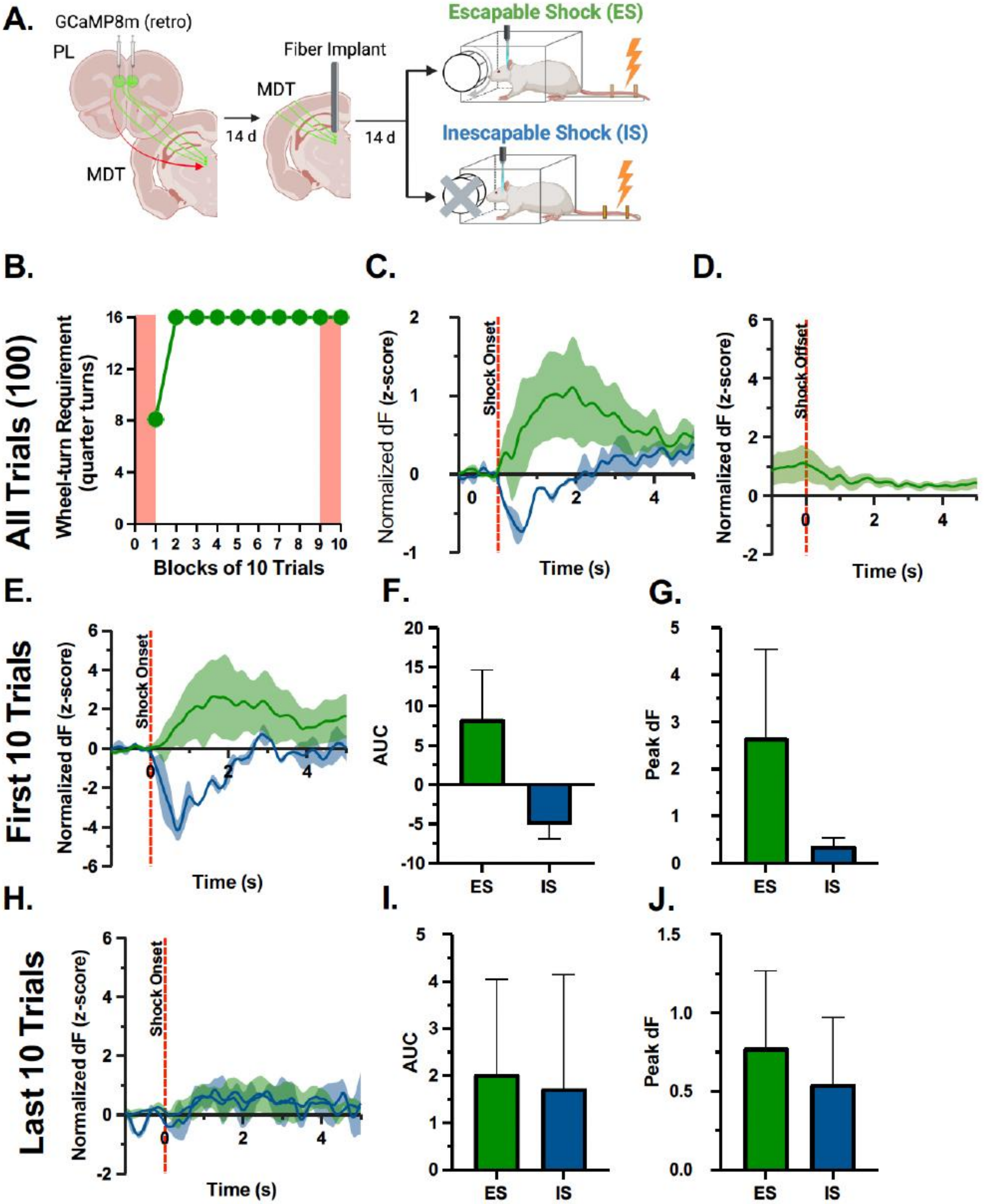
A.
GCaMP8m (retro)
PL
MDT
14 d
Fiber Implant
MDT
14 d
Escapable Shock (ES)
Inescapable Shock (IS)
All Trials (100)
B.
Wheel-turn Requirement (quarter turns)
16
12
8
4
0
0 1 2 3 4 5 6 7 8 9 10
Blocks of 10 Trials
C.
Normalized dF (z-score)
2
1
0
-1
Shock Onset
0
2
4
Time (s)
D.
Normalized dF (z-score)
6
4
2
0
-2
Shock Offset
0
2
4
Time (s)
First 10 Trials
E.
Normalized dF (z-score)
6
4
2
0
-2
-4
-6
Shock Onset
0
2
4
Time (s)
F.
AUC
20
15
10
5
0
-5
-10
ES
IS
G.
Peak dF
5
4
3
2
1
0
ES
IS
Last 10 Trials
H.
Normalized dF (z-score)
6
4
2
0
-2
Shock Onset
0
2
4
Time (s)
I.
AUC
5
4
3
2
1
0
ES
IS
J.
Peak dF
1.5
1.0
0.5
0.0
ES
IS

Fig 3.3. GCaMP recordings from the MDT-PL pathway during stress. ***A.*** Timeline for viral injections, fiber implants, and stress. ***B-C.*** Data from all 100 shock trials. ***B.*** Wheel turn acquisition. ***C.*** Normalized dF (z-score) for ES (n=6) and IS animals (n=2) at shock onset. ***D.*** Normalized dF (z-score) for ES (n=6) and IS animals (n=2) at shock offset. Values represent the mean ± SEM. $^{**}p < 0.01$. ***E-G.*** Data represent average of the first 10 trials. ***E.*** Normalized dF (z-score) for ES (n=6) and IS animals (n=2) ***F.*** Area under the curve of E. ***G.*** Peak dF from E. ***H-J.*** Data represent average of the last 10 trials. ***H.*** Normalized dF (z-score) for ES (n=6) and IS animals (n=2). ***F.*** Area under the curve of H. ***G.*** Peak dF from H.

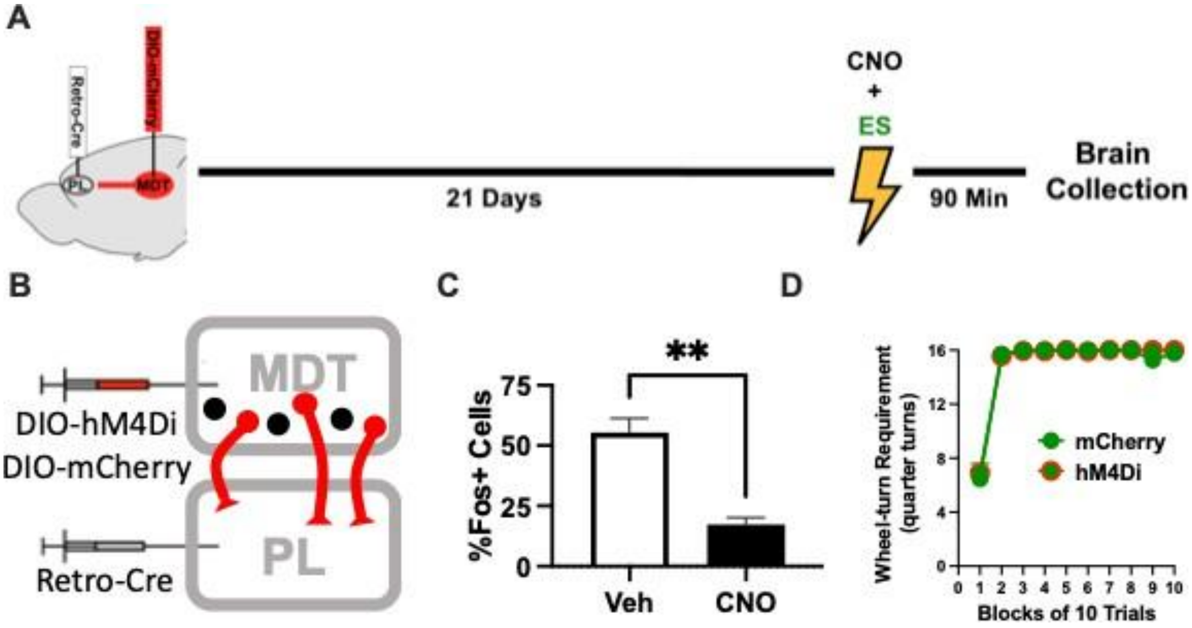

Fig 3.4. DREADD Verification. ***A.*** Timeline for viral injections and stress. ***B.*** Representative schematic for viral injections. ***C.*** Percentage of mCherry cells that are positive for cfos. ***D.*** Wheel-turn data for animals expressing either mCherry or hM4Di. Values represent the mean ± SEM. $^{**}p < 0.01$.

Fig.3.5B) and ES hM4Di animals were significantly more likely to be losers (t_{15}=2.347, p=0.0330). There was no difference from chance for IS mCherry (t_9=0.5576, p=0.5907), IS hM4Di, (t_8=1.141, p=0.2868), or HC hM4Di (t_{10}=1.389, p=0.1951). ES mCherry animals also displayed a higher number of pushes than ES hM4Di or either IS group, peaking at session three then returning to normal by session five (Fig.3.5F). A repeated measures ANOVA comparing the mean area under the curve for pushes showed significant effects of the interaction between stress and virus ($F_{1,29}$= 6.940, p=0.0120; Fig.3.5G). ES mCherry animals had more pushes than either ES hM4Di (p=0.0122) or IS mCherry (p=0.0199) but not IS hM4Di (p=0.0812)(Fig.3.5G). There was also a significant effect of the interaction between stress and virus on number of retreats ($F_{1,29}$= 6.940, p=0.012; Fig.3.5G), but no significant difference found between individual groups (Fig.3.5E/I). We also found a significant effect of the interaction between stress and virus on number of resistances ($F_{1,39}$= 4.968, p=0.0316), but again no significant difference found between individual groups (Fig.3.5J/K).

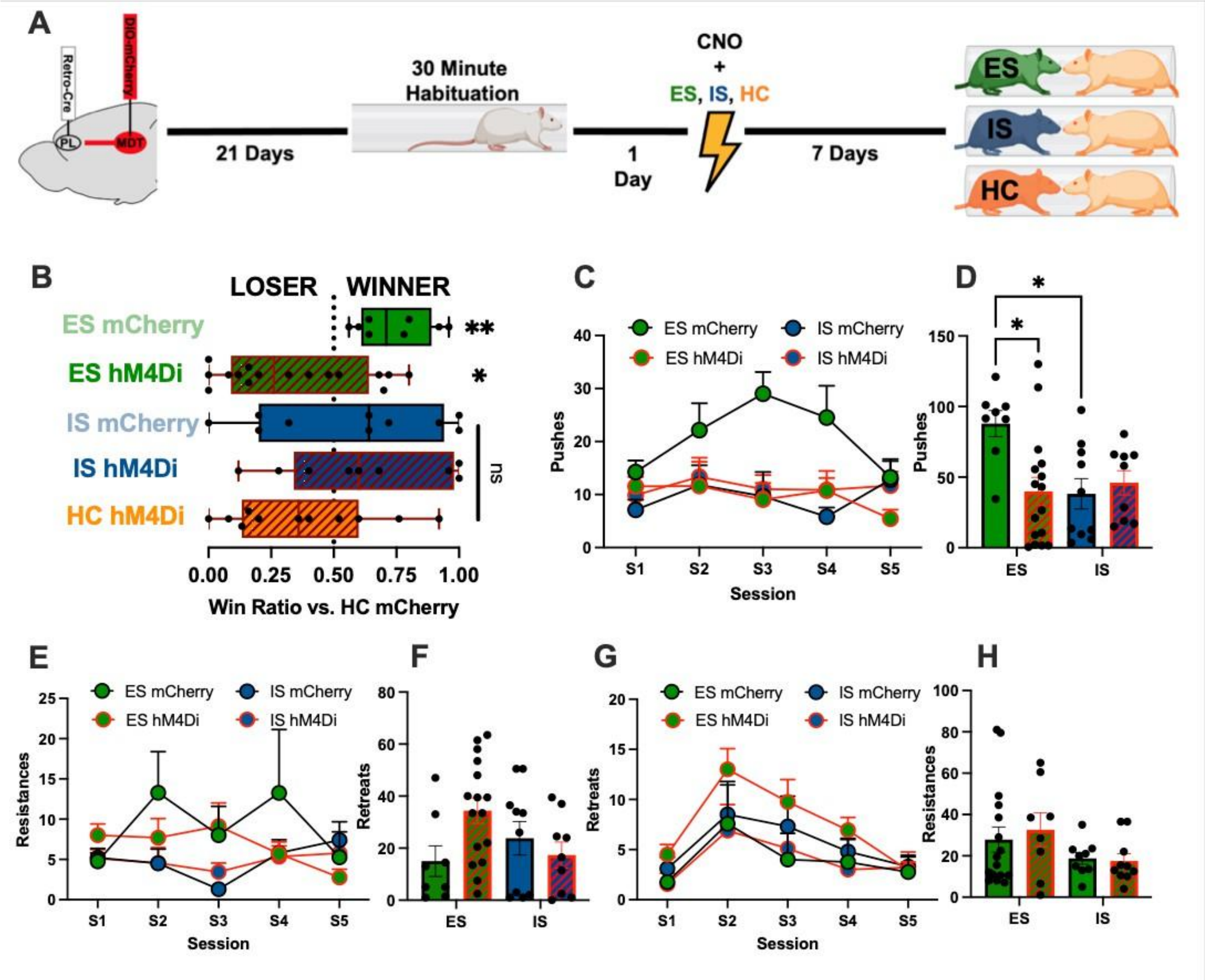

Fig 3.5. MDT-to-PL pathway is necessary for ES facilitated dominance. ***A.*** Timeline for viral injections and stress. ***B.*** Win ratio against HC mCherry opponents across all 5 days for each group. ***C.*** Pushes against HC mCherry across sessions for all groups. ***D.*** Area under the curve analysis of C. ***E.*** Resistances against HC mCherry across sessions for all groups. ***F.*** Area under the curve analysis of E. ***F.*** Retreats across sessions for all groups. ***D.*** Area under the curve analysis of F. Values represent the mean ± SEM. **p* < 0.01, ***p* < 0.001.

Discussion

There were three major findings. First, controllable stress increases performance in a non-social effort tube test. Second, controllable stress facilitates an increase in effortful behavior which leads to dominance in the tube test competition, but only when the controlling response is learned with the MDT-to-PL pathway online. Third, behavioral control facilitates a decrease in dominance if the MDT-PL is offline at the time of stress. Together with our previous findings, these experiments support the idea that controllable stress and social dominance interact through a common thalamocortical circuit used for instrumental effort.

Initially, the effort tube test was developed as a method of 1) training all animals prior to social tube test competitions to push an object similar in size and weight to their own body out of the tube, and 2) proving that any dominance effects were not due to underlying individual differences in body weight or strength. Thus, we developed a novel, non-social, version of the commonly used dominance tube test to assess an animal's ability to push within the tube prior to stress. This task was motivated by the work of the Hailan Hu lab, who trained mice to push an object out of the tube, increasing later social pushing behavior without altering ranks (Fan et al., 2019). However, in our task we train the animals to push the unweighted object, then add weight up to their body weight on the training day. Rats learn this task very quickly without the need for any extrinsic reward, and there is a large amount of variability in the 'breakpoint' weight. Given these findings, we opted to investigate if the amount the animals pushed was affected by prior stressors differing in controllability in the same way as was dominance. We determined that ES significantly increases an animal's performance in this task one week later, and that IS did not differ from HC treatment. These results suggest that our previous finding that ES facilitates dominance in the warm spot test (Coleman et al., 2023) may be due to an underlying increase in effort following ES which manifests as dominance.

We also determined that the ES facilitation of dominance observed in the warm spot test is also present in the tube test, and that it relies on activation of the MDT-to-PL pathway at the time of stress. This data represents one of the first instances in which goal directed Action/Outcome circuitry has been studied in an aversive context as well the first instance in which a non-social version of a dominance task is used to show a potential underlying effect of effort for social dominance. Others have shown that the anterior cingulate (ACC) region of the dorsal medial prefrontal cortex (dmPFC) is involved in effortful tasks, and that increased ACC activity corresponds with an increase in effort in the face of opposition (Holec et al., 2014; Elston and Bilkey, 2017). The ACC, much like the PL, receives input directly from the lateral portion of the MDT (MDTl) (Kuramoto et al., 2017; Wolff and Halassa 2024). In fact, a single neuron projecting from the MDTl can innervate ~1mm in diameter of cortical space in the dmPFC. This MDTl-to-dmPFC projection, of which we targeted a portion here, has long been associated with instrumental learning and executive functions (Corbit et al., 2003; Mitchell et al., 2007; Bradfield et al., 2013; Charkraborty et al., 2019), and as we have already mentioned increasing or decreasing the strength of the MDTl-to-dmPFC pathway directly increases or decreases dominance rank (Zhou et al., 2017). These findings together show that the MDTl, which is the major excitatory input to the dmPFC, is directly involved in stressor controllability, instrumental learning, social dominance, and persistent effort. Obviously, these are not mutually exclusive, as control over the stressor requires instrumental learning, and the majority of social dominance models rely on some form of effort. Nevertheless, it appears that this projection underlies the ES facilitation of dominance because ES robustly activates it, thus likely increasing plasticity in this pathway, which in turn would facilitate performance of subsequent tasks that relies on this pathway. Others have noted that the dmPFC does not underly dominance per se, but instead in responsible for persistence in effortful behavior, evidenced by increase firing of layer V PL neurons while pushing another animal (Wang et al., 2011a).

Further evidence for this suggestion comes from our third finding from this paper, one that is mirrored by our earlier work (Coleman et al., 2023). ES animals who were forced to learn the wheel turn escape task without the MDT-to-PL online were significantly more likely to lose, much like ES animals who learned ES without the PL online.

Historically, it is thought that there are two system through which instrumental actions can be learned. The first is the cortically dependent action/outcome system, and the second the dorsolateral striatum (DLS)-mediated habit system (Corbit and Balleine, 2003; Dezfouli and Balleine, 2012; Liljeholm et al., 2011; Corbit et al., 2003). Our previous work indicates that male rats use the act/outcome circuitry to learn the escape response and females use the habit circuitry (McNulty et al., 2023; Fallon et al., 2020; Baratta et al., 2019). This leads to a lack of protection in females following ES and is likely the reason for the lack of ES- facilitated dominance in females. However, females can be made to use the act/outcome system if the DLS is lesioned, at which point they are protected. The losing effect we have seen with the males who learn ES without the PL, or the MDT-to-PL pathway may operate similarly. It is possible that taking the act/outcome system offline forces males to use the habit system, and the resulting robust activation increases plasticity in the DLS thus increasing the likelihood that these animals will use the habit system for subsequent behaviors such as dominance.

In summary, these experiments show that ES facilitates and increase in effortful behavior in both non-social and social tasks. This increase in effort leads to an increase in dominance status, but this effect is dependent on activation of the MDT-to-PL pathway at the time of stress. Silencing the MDT-to-PL pathway prevents this effect, making an animal more likely to lose in social competitions. This means for a stressor to facilitate and increase dominance effort it must be controllable, and that control must be learned by the thalamocortical instrumental learning system.

Chapter 4:

Discussion

Summary of Findings.

It is well documented that experiencing an adverse event can drastically influence the development of future social hierarchies (Park et al., Sabanovic et al., 2020). However, the majority of this work used solely inescapable stressors and found that these stressors decrease later dominance. With the above work I have shown that stressors can yield an increase in dominance if 1) the stressor is controllable and 2) the controlling response is learned with the thalamocortical pathway. I have also shown that 3) this increase in dominance is due to an underlying increase in effort in both social and non-social tasks. This suggests that the circuitry underlying effortful behavior may be a common link between stress and social dominance. As further evidence of this, I also show here that 4) repeated winning grants protection against the behavioral and neurochemical effects of an uncontrollable stressor. Together, these results provide evidence that an initial experience in which an animal learns to use goal-directed effortful actions to attain a goal (winning, pushing a weight, terminating shock) provides that animal with an advantage in performing subsequent tasks requiring effortful action.

This work was initially motivated by a rising in interest in the role of the dmPFC, and specifically the PL, in dominance. Decades of research on controllable stress have shown a high level of involvement of the PL and the DMS in controllable stress effects, suggesting that the MDT-to-PL-to-DMS thalamocorticostriatal circuit commonly associated with appetitive instrumental learning may also be mediating the beneficial effects of control (Amat et al., 2014; Christianson et al., 2014; McNulty et al., 2022). This activation of the PL during controllable stress yields an increase in plasticity with layer V pyramidal neurons in the vmPFC (Baratta et al., 2019b; Maier, 2015b), the same layer in which an increase in pyramidal neuron firing correlates with dominant effortful behaviors (Wang et al., 2011b). Increasing the strength of glutamatergic inputs to the PL from the MDT can also make a loser a winner, an effect that is reversible if this pathway is then weakened again (Zhou et al., 2017b). This research led to our hypothesis that the activation of the thalamocorticostriatal system during ES would increase subsequent dominance. This hypothesis was strengthened by the fact that ES facilitation of dominance is trans-situational (tube test or warm spot test), only present in males (as females do not use the PL during ES), and is absent in animals who were forced to learn the wheel turn response without the PL or MDT-to-PL pathway online.

Given the results of the initial ES facilitation experiments, I next sought to further characterize the circuitry of social dominance. If winning in a competition was acting in a similar manner to controllable stress, then there should be similar patterns of circuit activity and behavioral outcomes between the two. Independent of stress, both the DMS and the PL were necessary for an animal to maintain dominance. Similarly, inhibiting either of these regions prevents the protective effects of ES. This suggested that repeated winning may mirror other effects of controllable stress, such as immunization against the effects on an uncontrollable stressor. During ES, robust activation of the PL yields activation of a PL-to-DRN pathway which in turn inhibits the DRN 5-HT response to stress. Much like the other PL activation, it appears that activation of the PL-to-DRN pathway during stress increases plasticity of this pathway allowing it to become active during subsequent uncontrollable stress to provide protection (Amat et al., 2008; Baratta et al., 2019b; Maier, 2015b). Given the necessity for the PL and DMS to maintain dominance, as well as ES facilitation of dominance, I next sought to determine the effect of repeated winning on stress induced neurochemical and behavioral changes. I found that animals that had repeatedly won in the warm spot test were protected against the stress induced decrease in juvenile social interaction as well as the stress induced DRN 5-HT release, similar to the response an ES animal has to subsequent uncontrollable stressors. This data further indicated that social dominance and controllable stress may be two very different distinct behavioral paradigms that are controlled through the same circuitry. Specifically, the thalamocorticostriatal system that is responsible for goal directed instrumental effort.

In order to determine if this hypothesis was true, it was then necessary to test the effect of controllable stress on a non-social task. The finding that ES facilitated winning in the warm spot test shows that controllable stress increases winning in an aversively motivated dominance task. Then the finding that ES facilitated winning in the tube test, a task in which the only apparent motivation is the tube itself and the social interaction that occurs within, indicated that this facilitation did not rely on the common aversive motivation for both ES and the WST. However, if it is the same thalamocortical system that is associated with effortful responding (Elston & Bilkey, 2017; Holec et al., 2014), instrumental learning (Chakraborty et al., 2019; Corbit et al., 2003; Mitchell et al., 2007; Wolff & Halassa, 2024), and controllable stress; then ES should improve performance in behavioral paradigms measuring everything from instrumental learning to effort. To test this I developed the Effort Tube Test, in which the animal was given the option to push a bottle of increasing weight back and forth through the tube for no apparent reward. The fact that ES also increased performance in this task indicates that the social component of tasks like the WST and tube test is irrelevant in the context of these experiments.

Increased dominance is simply a result of increased effort, which in turn is due to an increase in the thalamocortical circuitry activation following stress. Of course, further experiments are necessary to strengthen this hypothesis.

4.2. Future Directions.

The ideas I have just described, which link together this body of research, suggest many new exciting experiments. Among the many directions these potential experiments could take, the common themes are 1) the circuitry of winning, 2) the circuitry of controllable stress, and 3) the interaction between tasks commonly activating/relying on thalamocortical circuitry.

Thus far, it is clear that the MDT, PL, and DMS are involved in winning in social dominance tasks. What remains unclear is how these circuits are employed to increase winning, especially between males and females. There is some indication that males and females use different mechanisms to form hierarchies, males using previous experiences and females using intrinsic factors (van den Berg et al., 2015b), which could explain why I found less stability in female hierarchies than males. Of particular interest here is the fact that the only way I have discovered to successfully create a ‘loser’ is by forcing a male to learn ES without the thalamocortical system. This results in animals that lose to HC in both the WST and the tube test. I believe this to be because the males are then forced to learn with the DLS mediated habit system, which then undergoes similar changes as the thalamocortical system (increased plasticity and activation in subsequent tasks). This would mean that these males are then biased to use the habit system during competition, which decreases their instrumental effort and leads to losing. I believe this also explains why the IS hM4Di groups did not differ in dominance, as there is no instrumental learning taking place during IS that would activate the thalamocortical/habit systems. Previous work shows that females learn the ES task without the prelimbic cortex online (McNulty et al., 2022), but I did not find any differences in dominance between ES, IS, and HC females. If the above theory is correct, females may be using completely separate circuitry for dominance, explaining their less stable hierarchies. Additional experiments are necessary to fully determine the circuitry of winning and the sex differences in stable hierarchy formation.

The above work also presents new questions about the circuitry of controllable stress. To my knowledge, this is the first time the MDT-to-PL pathway has been implicated in an aversively motivated task such as ES. Given the rising increase in research in this area, specifically as it pertains to instrumental effort and learning, this data provides the basis for investigating the thalamocortical system in an entirely new context. For example, my findings that ES facilitation of dominance is dependent of the MDT-to-PL suggest that the activation of this pathway by aversively motivated effortful learning increase plasticity (either at the level of the PL or the MDT) which then increase subsequent performance in other effortful tasks. What has yet to be determined is if the aversive nature of the ES task is necessary. I have shown that ES-facilitated dominance extends beyond an aversive task, and the appetitive literature indicated that many non-aversive tasks can robustly activate thalamocortical circuitry, meaning it is entirely possible that ES facilitates performance in other appetitive tasks or that appetitive instrumental learning increases social dominance.

It also must be determined how, if at all, ES can facilitate dominance in females. Females receive the same protection from controllable stress as males when the controlling is learned with the PL disinhibited (using a D1 antagonist), or with the DLS lesioned. Either of which should, in theory, force females to use the thalamocortical circuitry during ES. However, if females are not using this same circuitry to form dominance hierarchies, perhaps instead relying on intrinsic factors, and increase in thalamocortical circuitry would be irrelevant. Again, the fact that females learning ES with the habit system are not at a disadvantage during subsequent dominance testing implies this may be the case. Further work is necessary to determine if biasing females to use thalamocortical circuitry during ES facilitates later dominance.

4.3. Concluding remarks.

Together these data indicate that effortful instrumental learning increases strength of thalamocortical circuitry, thus increasing performance in subsequent tasks requiring instrumental effort. Future work should investigate the extent of effortful behavior influenced by controllable stress and dominance, as well as the circuit determinants of dominance in females.

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