The Effect of Chronic Stress on Valence Learning in Male and Female Mice

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Table of Contents

Abstract

Major depression is one of the most damaging diseases in society boasting the largest share of societal burden of any mental disorder due to its high prevalence and severity of symptoms. Despite years of research, relatively little is known about its underlying symptomatology particularly how it alters valence processing in the brain (degree of pleasurableness or aversiveness of experiences). Questions of valence are tied directly to stress as the major risk factor for depression, as well as sex and gender due to significant differences in stress susceptibility and depression prevalence across sexes and genders. The present thesis was concerned with how valence learning is altered by chronic stress in both male and female mice, in the hopes of further untangling this complicated web of factors.

The first section of this project was focused on developing a tool that would allow for effective investigation into questions about valence learning. Prior to this project, there was no published task that could train freely behaving mice on both rewarding and aversive valence contingencies within the same session. We combined effective protocols for separate Pavlovian reward and fear conditioning tasks into the Pavlovian Valence Discrimination task. This protocol was capable at training mice to discriminate between rewarding, aversive, and neutral cues over the course of seven days.

In the second section of this project, we utilized the PVD task to probe for effects of stress on valence learning and discrimination in both male and female mice. Our results demonstrated that both male mice exposed to chronic social defeat stress and females exposed to sub-chronic variable stress have deficits in reward learning compared to stress-naïve controls. Male stressed animals additionally demonstrated

potentiated freezing responses that did not influence aversive learning. Female mice did not show any alteration in fear behaviour from stress.

This thesis presents a novel valence discrimination task capable of detecting alterations in valence learning due to stress in both male and female mice. Although the scope of the current project was limited to describing these stress differences at the behavioural level, the PVD task opens valence research to new questions that could not previously be answered in single valence tasks. Through further implementation of this task, uncertainties behind how stress influences emotional dysfunction at the root of major depression can finally be resolved.

Resumé

La dépression est une des pathologies les plus dévastatrices de notre société. De toutes les maladies mentales, elle représente le plus gros fardeau pour la société en raison de sa forte prévalence et de la sévérité des symptômes. Malgré des années de recherche, les symptômes sous-jacents, tels que l'effet de la dépression sur la façon dont le cerveau traite la valence (caractère agréable ou désagréable d'une situation), sont peu connus. L'étude de la valence est directement liée au stress, facteur de risque majeur pour la dépression, ainsi qu'au sexe et au genre d'un individu en raison de différences significatives de susceptibilité au stress et de prévalence de la dépression entre les sexes et les genres. Cette thèse s'est intéressée à la façon dont le stress chronique affecte l'apprentissage de la valence chez les souris mâles et femelles, avec pour but de comprendre l'impact de ces facteurs entremêlés.

La première partie de ce projet s'est focalisée sur le développement d'un outil qui permettrait d'évaluer de façon adéquate l'apprentissage de la valence. Au préalable, il n'existait aucune tâche publiée pour entraîner des souris libres de leur mouvement sur des situations de valences opposées au cours de la même session de conditionnement. Nous avons combiné des protocoles efficaces d'apprentissage pavloviens de récompense et d'aversion pour créer la tâche Pavlovienne de Discrimination de Valence (PDV). Ce protocole de 7 jours a permis d'entraîner des souris à faire la différence entre un signal de récompense, un signal de stimulus aversif, et un signal neutre.

Dans la deuxième partie de ce projet, nous avons utilisé la tâche PDV pour étudier les effets du stress chronique sur l'apprentissage et la discrimination de la valence chez des souris mâles et femelles. Nos résultats montrent que les souris mâles exposées à de

la défaite sociale chronique et les souris femelles exposées à du stress variable sous-chronique présentent des déficits d'apprentissage de la récompense en comparaison avec des souris contrôles. Les souris mâles soumises au stress ont également démontré une potentialisation de la réponse d'immobilisation subite qui n'a pas affecté l'apprentissage aversif. Les souris femelles stressées n'ont montré aucune altération dans leur réponse au stimulus aversif.

Cette thèse présente une nouvelle tâche de discrimination de valence capable de détecter des déficits d'apprentissage de la valence dus au stress chronique chez des souris mâles et femelles. Ce projet était limité aux effets comportementaux du stress chronique, mais la tâche PDV ouvre la voie à des questions plus poussées sur la valence qui ne pouvaient pas être étudiées avec les tâches de valence classiques. En utilisant notre nouvelle tâche, il sera enfin possible de déterminer comment le stress affecte le dysfonctionnement émotionnel présent au cœur de la dépression.

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Contribution of Authors

Chapter 1: Introduction

Chase Clark wrote this section in collaboration with Dr. Rose Bagot.

Chapter 2: Literature Review

Chase Clark wrote this section in collaboration with Dr. Rose Bagot.

Chapter 3: Rationale and Hypothesis

Chase Clark wrote this section in collaboration with Dr. Rose Bagot

Chapter 4: Methods

Chase Clark designed experiments and protocols with guidance from Dr. Rose Bagot.

Chapter 5: Results

Stress protocols, behavioural training, and testing were conducted by Chase Clark with assistance from Gabrielle Siemonsmeier and Alejandra Martinez. Behavioural analysis and scoring were conducted by Chase Clark, Dr. Joelle Lopez, Gabrielle Siemonsmeier, and Alejandra Martinez. Dr. Rose Bagot contributed to interpretations of behavioural data.

Chapter 6: Discussion

Chase Clark wrote this section in collaboration with Dr. Rose Bagot.

Chapter 1: Introduction

Recognizing the valence of an event – whether it is positive or negative – and associating it with predictive stimuli is critical for the selection of an appropriate behavioural response and, ultimately, for survival. Deficiencies in learning these associations can lead to suboptimal behaviour and put one in life threatening situations. Mood disorders, such as major depression and anxiety disorders, can lead to such alterations in valence learning (Denny and Hunt, 1992; Rottenberg et al., 2005; Stuhrmann et al., 2013). Chronic stress is a major risk factor for the development of depression; however, it is not yet known exactly how stress impacts valence learning when positive and negative contingencies are learned concurrently. There are substantial sex differences in the stress response of humans and other animals (Bale and Epperson, 2015; Kudielka and Kirschbaum, 2005) and women are twice as likely to develop depression as men, furthering research interest into the interaction between stress and sex (Ford and Erlinger, 2004; Kessler et al., 1997). Until recently, psychiatric and neuroscience research has historically neglected studying females, citing concerns of female sex hormones increasing variability, a claim that has been refuted (Beery and Zucker, 2011; Mogil and Chanda, 2005; Prendergast et al., 2014; Shansky, 2019; Zucker and Beery, 2010). To address these knowledge gaps, this thesis investigated how sex and stress interact to modulate valence learning. To meet this goal, the author developed a novel behavioural paradigm, the Pavlovian Valence Discrimination task, to train mice to simultaneously discriminate between cues predictive of either positive or negative outcomes. By using this task to probe for deficits in valence learning in male

and female mice exposed to chronic stress we gained critical insight into how stress influences an animal's ability to learn about its environment.

Chapter 2: Literature Review

2.1 Depression

Major depression is one of the leading contributors to disability in the world and is currently the most burdensome mental illness due to its high morbidity and severe impact on life (Whiteford et al., 2013). Individuals have a 17% chance of experiencing major depression at some point in their lives (lifetime prevalence) and 4-5% of the population is depressed at any given time (Kessler, 1993; Kessler et al., 1997; Patten et al., 2015). Unlike other diseases with a similarly large impact, our understanding of the etiology of depression and current treatments are limited. Heterogeneity of symptom profiles and therapeutic efficacy across individuals has made defining depression difficult and the basic mechanisms of depression remain unknown. Despite its broad symptomology, deficits in emotional valence processing are a major component of many clinical definitions of depression.

2.1.1 How is valence linked to depression?

Outcomes or experiences can be categorized by where they lie on a spectrum defining their relative “goodness” or “badness”. This characteristic is known as valence and is a major component of emotion (Russell, 1980; Tye, 2018). The valence of an experience can be inherent to it, such as the appetitive nature of food, or the aversive nature of noxious stimuli. Valence judgment of an experience can also be learned based on experience. For example, a neutral stimulus may begin to take on a negative valence if it is consistently paired with an inherently negative noxious stimulus. This phenomenon is known as valence learning and is necessary for driving appropriate and predictive behaviour. The inability to learn about appetitive or aversive outcomes and to

select a correct behaviour can indicate a deficit in valence learning. Such deficits are known to exist in mood disorders such as depression and anxiety. Anhedonia, the loss of interest or enjoyment of things usually found to be pleasurable is one of the cardinal symptoms of depression (American Psychiatric Association, 2013), however, it can also be generalized as reflecting an abnormality in processing positive information. Due to its prevalence, anhedonia – specifically decreased sensitivity to positive stimuli – is often considered the primary manifestation of valence dysfunction in major depression. However, there are two major issues with this characterization. First, many studies investigating anhedonia are unable to discern whether it is driven by an inability to gain pleasure from experiences (i.e. an abnormality in processing positive valence processing) or a lack of motivation independent of valence (Treadway and Zald, 2011). Second, there is uncertainty with how major depression influences emotional sensitivity across the valence spectrum. For example, there is considerable evidence that persons with major depression have blunted responses to positive events – a phenomenon known as positive attenuation, as seen in anhedonia (Rottenberg et al., 2005). However, the literature is less consistent with respect to negative valence. Some studies provide evidence that major depression leads to a potentiation of emotional responses to negative stimuli, while others observe a decrease in emotional responsiveness across the valence spectrum. This has led to the development of the theory of emotional context insensitivity which posits that depression leads to a flattening of affect and reduced reactivity to both positive and negative emotional stimuli (Rottenberg et al., 2005). A wealth of literature has also been published regarding the negative cognitive bias present in most manifestations of depression which further

implicates valence in depression symptomatology (Beck, 1963; Haaga et al., 1991) Considering the importance of valence learning for survival, a further understanding of how major depression compromises it is of utmost importance. Before we delve into the intricacies of valence learning, it is necessary to review evidence and theories of the basic emotional deficits known to arise in individuals with depression: anhedonia and negative bias.

2.1.2 Anhedonia

Anhedonia, is a cardinal symptom of depression characterized in the DSM-5 as a markedly diminished *interest* or *pleasure* in activities for the duration of the day over most days (American Psychiatric Association, 2013). Anhedonia, along with depressed mood are two symptoms that must be present for a diagnosis of major depression to be made. However, anhedonia itself is not a unitary symptom, but instead is a highly heterogeneous collection of symptoms. Its breadth has lead to difficulties in treatment, and its presence is a predictor of poor treatment response (Spijker et al., 2001). Importantly, the clinical definition of anhedonia does not differentiate between a decreased motivation to participate in pleasurable events and a reduction in experienced pleasure (Treadway and Zald, 2011). This has led to difficulties in developing both a general understanding of anhedonia and effective ways to treat it. Clinical assessment of anhedonia is often made through self-reports, like other depressive symptoms. However, it should be noted that most of these self-reporting tools focus exclusively on deficits in experienced pleasure, while questions probing for decreased motivation are absent (briefly reviwed by Treadway & Zald, 2011).

Research has been conducted in patient populations to determine physiological and behavioural features that may explain anhedonia. A well reported finding is that reactivity to positive emotional stimuli is decreased in depressed individuals (Gruber et al., 2011). Such effects have been confirmed by a meta-analysis that included data from 19 studies. Their analysis used a multidimensional definition of reactivity, including self-reports, behavioural changes, and physiological responses. It revealed that depressed individuals have decreased emotional reactivity to positive stimuli across their broad definitions of reactivity (Bylsma et al., 2008).

Impaired reactivity to positive stimuli may be responsible for deficits in reinforcement learning that are observed in depressed patients. Several studies have found that depressed individuals are less able to bias their responding towards rewarded stimuli and are less capable of learning reward contingencies, particularly those requiring discrimination between several cues. In a response bias task, depressed and control subjects were made to distinguish between three cues, where selected would lead to either a reward, punishment, or nothing (Henriques et al., 1994). Although the two groups did not differ on their response bias towards the neural and negative cues, depressed subjects showed decreased bias towards the positive cue indicating an deficits in approach related behaviour (Henriques et al., 1994). In a probabilistic reward learning task, depressed patients demonstrated decreased bias towards the more rewarding stimuli than controls, indicating that the reward association was not made (Pizzagalli et al., 2008). Further imaging studies from the same group noted that this reward deficit may be modulated by decreased reactivity in the nucleus accumbens and caudate, regions highly implicated in reward processing (Pizzagalli et

al., 2009). Associative reward learning is a key mechanism for guiding appropriate behaviour and is a prominent valence deficit present in anhedonia symptoms of depression.

2.1.3 Negative bias in depression

Separate to motivational and affect-based symptoms of depression, depressed individuals demonstrate altered cognitive processing which can take the form of negative biases. Beck's analysis of depressed patients led to his cognitive theory of depression which postulates that major depression alters internal conceptions of self and the environment towards negative conclusions (Beck, 1963, 1987). Negative bias alters the interpretation of different experiences through a negative lens (Beck, 1963, 1987). Negative conceptions at the level of the self, world, and future interact with one another forming a "cognitive triad" which generalizes a poor outlook on life that becomes all encompassing (Beck, 1987). Extensive studies on self-reported negative cognition provide a consensus that depressed patients experience heightened negative thinking (Haaga et al., 1991). These negative conceptions do not just exist at the internal level but alter external interactions with the world. For example, a study where depressed patients and control individuals interacted with opposite-sex strangers revealed that the depressed group found the interaction less favourable. Upon recall depressed patients evaluated the experience as even more negative than their initial evaluation, indicating that negative bias further distorts perceived experiences through time (Gotlib, 1983).

Cognitive manifestations of negative bias have often been studied in the context of facial expression discrimination. In one study, depressed patients performed poorly in

such a task, judging happy faces as being neutral and neutral faces as being sad (Gur et al., 1992). The degree of negative bias tended to correlate with severity of depressive symptoms (Gur et al., 1992). Like in controls, right amygdala reactivity tracks negative bias in facial recognition, with this bias predictive of increased depression severity and length (Dannlowski et al., 2007). A systematic review of facial expression discrimination fMRI studies found abnormalities in key nodes of the facial processing network where depressed patients showed hyperactivation to negative and hypoactivation to positive face stimuli (Stuhrmann et al., 2011). Behavioral manifestations of these cognitive and fMRI precipitate during fear extinction learning. Depressed patients show increased skin conductance during fear extinction which is thought to be tied to impaired-top down emotional regulation leading to amygdala hyperreactivity (Dibbets et al., 2015).

From these studies, it is clear that the basic processing of valence information is compromised in depressed patients leading to a wide array of behavioural deficits. What these studies do not tell us, however, is how these deficits are produced. The etiology of depression is a complicated question with many currently unsatisfactory answers, however extensive research has been conducted to investigate the role of chronic stress as a causative factor.

2.1.4 Stress as a risk factor for depression

Since the early 20th century it was observed that depressive episodes appear to be preceded by highly stressful life events, and that these events appear to cause “permanent internal changes” (Kraepelin, 1921). Further research throughout the century has determined this to be true and we now know that life stress is the single largest risk factor for the development of depression. A meta-analysis of several case-

control studies tracking life stress found that depressed patients are more likely to have experienced at least one adverse life event (Mazure, 1998). Studies have suggested that stressful life events may have a causal effect on the onset of depressive episodes (Kendler et al., 1999, 2000). Interestingly, the strength of this effect is decreased as an individual experiences more depressive episodes implying that stress plays a major role in the early development of depression (Kendler et al., 2000). Indeed, further studies support this “kindling hypothesis” where stress has its largest effect on the development of the first episode of major depression than on subsequent episodes, and that this sensitization may be encoded at the level of gene expression (Post, 1992). The largest risk factor of subsequent episodes is not stress but number of previous depressive episodes (Kendler et al., 2000).

Life stress, and in particular childhood trauma, sensitizes the stress response and produces neuroendocrine and hippocampal alterations that parallel features of major depression (Heim et al., 2008). Sex differences in stress response may also contribute to the higher prevalence of depression in women (Bale and Epperson, 2015). Animal research has found that chronic stress has long term effects on neuroplasticity and functioning that may play a leading role in mechanism of depression (Pittenger and Duman, 2008). To further understand the causative role stress plays in the context of depression, and how its myriad of downstream molecular, genetic, and circuit effects lead to its development, the use of animal models in preclinical research becomes critical (Krishnan and Nestler, 2008).

2.2 Stress models of depression in animals

Animal models for depression have proved useful in furthering our understanding of the basic mechanisms of depression at both the behavioural and neural circuit level, especially as technology to study these has improved (Muir et al., 2019). Useful animal models for depression do not claim to replicate the disease state, however they are essential tools to study specific aspects of depression in a mechanistic manner not possible in humans. While it is not possible to replicate the full symptomatology of depression in non-human animals, we can model specific aspects of the disease, such as deficits in valence learning. One of the most common approaches to model depression-relevant behavioural deficits in rodents is through exposure to chronic stress.

2.2.1 Using chronic social defeat stress to model depression in male animals

Many stress models have been developed over the years to investigate depression in rodents (Duman and Monteggia, 2006; Krishnan and Nestler, 2008; Muir et al., 2019). One of the most common models used today is chronic social defeat stress (CSDS). In this model, C57 male mice are physically defeated every day for 10 days by a larger aggressive mouse and housed on the opposite side of a perforated divider between sessions (Berton et al., 2006; Golden et al., 2011). This high-intensity stressor leads to long lasting behavioural, physiological, and molecular changes (Berton, 2006; Krishnan et al., 2007; Tsankova et al., 2006). Mirroring the human condition, chronic, but not acute treatment with anti-depressants led to a recovery of deficits in social interaction and dysregulated BDNF expression in CSDS-susceptible animals (Berton, 2006; Tsankova et al., 2006).

2.2.2 Modeling stress in female mice

CSDS, along with other models, was originally developed in male mice; however, major sex differences are known to exist between stress responsiveness of male and female mice (Bale and Epperson, 2015) and similarly, women are twice as likely as men to develop depression (Kessler, 1993). Despite this, female mice (and women) have long been neglected as research subjects (Shansky, 2019). The majority of studies have used exclusively males, following a longstanding false belief that the estrus cycle of female mice leads to inherently more variable data (Beery and Zucker, 2011; Prendergast et al., 2014). A major barrier to using females in CSDS experiments is that aggressive male mice fail to reliably attack female C57s. Recently a modified version of CSDS relying on “emotional stress” has been developed where the experimental mouse is not physically defeated but instead witnesses the defeat of another C57 (Sial et al., 2016; Warren et al., 2013). This witness defeat stress (WDS) has been adapted for use in females C57 mice and has been found to reliably induce a stress phenotype in a subset of animals, similar to CSDS in males (Iñiguez et al., 2018).

Sub-chronic variable stress (SCVS) was developed to further research into the effect of stress on female mice. Similar to other stress protocols in males, SCVS produces consistent behavioural and physiological changes in female mice (Hodes et al., 2015; LaPlant et al., 2009). SCVS consists of 3 stressors cycled over 6 days; importantly this length of stress induces a susceptible phenotype in female, but not male mice (Hodes et al., 2015). Protocols like CSDS and SCVS, although all different in the specific type of the stress, provide an excellent set of tools to investigate stress-related phenotypes in both sexes.

2.3 Stress induced valence deficits

We have already discussed how depression alters basic emotional processing. As we are interested in investigating parallel effects in mice, it is necessary to discuss how stress alters basic valence learning in animal models. Through this review we will gain insight into how stress alters positive and negative valence learning in rodents. In the discussion below – and the thesis generally – we will primarily refer to positive valence in the context of appetitive (food) rewards and negative valence in the context of punishments (foot shocks).

2.3.1 Positive valence and reward learning

Chronic stress has been demonstrated to have negative effects on various aspects of reward-related behaviours. Reward behaviours can be broadly broken down into two main groups, consummatory and appetitive behaviours (Berridge and Robinson, 1998; Panksepp and Ikemoto, 1996). In the context of valence, consummatory behaviours refer to the eating or drinking of rewards and the associated inherent hedonic responses, while appetitive behaviours are anticipatory, occurring when a reward is expected or before it is consumed (Von Frijtag et al., 2002).

Chronic stress has an impact on consummatory behaviours, for example SCVS and CSDS reliably lead to decreased sucrose preference in female and male mice, respectively (Hodes et al., 2015; Krishnan et al., 2007). Decreased sucrose preference is a common measure used to model anhedonic behaviour in rodents. In the context of depression, differences in consumption between patients and controls are generally not as consistent as differences observed in reinforcement tasks, signifying the importance of reward learning and motivation to the disease (Treadway and Zald, 2011). For this

reason, we will restrict our investigation to reward learning tasks. We will broadly split our discussion of the effect of stress on appetitive behaviours into those utilizing instrumental or Pavlovian reward learning.

Instrumental reward learning comprises a broad number of tasks where an animal performs a specific action to receive a reward. Many studies have investigated the effect of stress on reward learning and motivation though the lens of instrumental behaviour. For example, a study in rats found that both control animals and those exposed to chronic unpredictable stress increased lever pressing for food rewards even as the effort necessary to receive rewards increased (Dias-Ferreira et al., 2009). However, after the reward was devalued, stressed animals did not update their behaviour: responding on both valued and devalued levers remained at a similar level. This transition from motivated to habitual behaviour suggests that stressed animals become insensitive to alterations in the value of the outcomes of their actions (Dias-Ferreira et al., 2009). These results were corroborated by another study, where chronic corticosterone (CORT) administration in drinking water induced habitual behaviour in rats and mice after reward devaluation in an instrumental task (Gourley et al., 2012). The study also found that mice were less motivated for rewards. Chronic antidepressant treatment (amitriptyline) rescued habitual behaviour phenotype in rats (Gourley et al., 2012). A later study by the same group demonstrated that chronic CORT administration led to an overall decrease in performance on an instrumental-reward task and decreased motivation in mice and rats (Olausson et al., 2013). Similarly, chronic amitriptyline administration rescued these deficits in both species (Olausson et al., 2013). These studies provide clear examples of how stress inhibits appropriate reward

learning in instrumental tasks through the replacement of goal-directed behaviour with habitual behaviour or by decreasing reward motivation.

A different study investigating instrumental reward seeking found similar motivational deficits through the extraction of parameters after a mathematical principles of reinforcement (MPR) analysis (Kleen et al., 2006). Animals were initially trained on a lever-pressing task where the number of lever presses necessary to receive a reward varied across blocks. After training, animals went through daily rounds of chronic restraint and instrumental training for 21 days. After several days, chronically stressed animals developed a motivational deficit during the instrumental task, illustrated by a decrease in the motivational parameter, while the locomotion parameter was unaffected indicating that the stress induced deficit to motivation was not related to locomotor ability (Kleen et al., 2006).

As we see above, chronic stress can interfere with goal directed behaviour and motivation to work for rewards. However, these are two very different deficits and their inconsistent presence in the above studies should be noted. Although these results are important, they do not model the clear deficits in cue-reward associations seen in humans (Henriques et al., 1994; Pizzagalli et al., 2008), such results would be best modeled in animals in Pavlovian and Pavlovian-to-Instrumental Transfer (PIT) tasks.

Overall, the literature provides little evidence that stress has a major negative impact on simple associative behavioural paradigms. The relationship between stress and associative reward learning appears to be more nuanced, with deficits appearing only during “difficult” tasks that involve discrimination or multiple associations. This is consistent with our above discussion of anhedonia in humans where depressed patients

are less able to learn reward discrimination tasks. For example, a recent study found that chronically restrained rats were able to learn a simple reward-cue Pavlovian association just as well as stress-naïve rats (Xu et al., 2017). However, it should be noted that although both groups were able to learn the task, stressed rats showed a decrease in task engagement (fewer conditioned responses overall) implying that there may be underlying deficits in motivation or arousal (Xu et al., 2017).

Pavlovian-to-Instrumental Transfer (PIT) refers to the phenomenon where a cue previously predictive of an outcome (e.g. reward) invigorates instrumental responding for either the same outcome or another outcome of the same valence in an animal (Holmes et al., 2010). Therefore, PIT is an excellent tool for investigating how reward conditioned cues can influence goal-directed behaviours (Holmes et al., 2010). A study in rats found that although chronic unpredictable stress had no effect on learning Pavlovian cue-reward or instrumental contingencies in isolation, these animals did demonstrate a deficit in PIT indicating that stress may have interfered with the ability for cue-reward related information to invigorate goal-directed reward seeking in a different modality (Morgado et al., 2012).

Another study in rats approached this question in a purely Pavlovian manner. In the study several groups of rats were stressed through a foot-shock paradigm where one group could escape the shocks (controllable stress), another could predict the shocks but not escape them (predictable-uncontrollable), and the last could neither predict nor escape them (unpredictable-uncontrollable, DeCola & Rosellini, 1990). After the stress, all animals were trained on a simple Pavlovian cue-reward contingency task. After training, all animals showed similar conditioned responding to the cue, suggesting

no learning deficit (DeCola and Rosellini, 1990). Animals were then trained on a conditioned inhibition task where they were exposed to the previous cue (reinforced) and a compound cue (previous cue + white noise, unreinforced). Rats in the unpredictable-uncontrollable group did not significantly reduce their conditioned responding to the compound cue as compared to all other stress and control groups suggesting that this type of stress impaired the ability of the rats to inhibit reward seeking behaviour under changing conditions (DeCola and Rosellini, 1990). Such evidence suggests that stress leads to deficits in complicated associative tasks where an animal must utilize the associative information it has learned and apply it to a new context (e.g. conditioned inhibition or PIT).

An apparent exception to this observation is a study where rats that were housed alone and defeated show a deficit in a Pavlovian conditioning task (Von Frijtag et al., 2000, 2002). In this experiment, rats were trained to associate a compound visual/auditory cue with the availability of a sucrose solution. Stressed rats demonstrated reduced anticipatory behaviour similar to the group where the cue and reward were not paired (Von Frijtag et al., 2000, 2002). This stress-induced deficit was rescued after chronic imipramine administration (Von Frijtag et al., 2002). The methodology of this experiment differed from the above examples in several important ways that may explain these results. First, unlike the above experiments, the US was not a discrete event, but rather a period of 5-minutes where sucrose solution was made available; additionally, there was a 2.5- to 10-minute interval between CS presentation and US availability. Most importantly, the behavioural measure for learning was less specific, recording general behavioural changes over the interval period (e.g. rearing,

sniffing, sitting, freezing, grooming, etc., Von Frijtag et al., 2000, 2002). This general increase in behavioural activity is not as specific as those observed in the above studies (such as anticipatory food-port entries, instrumental responding) and may therefore involve a separate phenomenon than what was described above.

The above literature demonstrates that stress negatively influences positive valence learning, however the specific deficits were variable and inconsistently present. In some but not all studies of instrumental behaviour stressed animals demonstrated a predisposition for habitual behaviour or decreased motivation for rewards. Literature surrounding Pavlovian discrimination tasks were very limited and should be expanded on. However, the valence learning deficits observed are likely related to symptomology observed in depression, specifically anhedonia (Treadway and Zald, 2011; Willner et al., 1992). Although positive valence deficits are those most closely associated with depression anecdotally, deficits on negative valence learning are also implicated in depression. Therefore, it is crucial to consider the implications stress has on negative valence learning in rodents as well.

2.3.2 Negative valence and fear learning

Literature investigating the effect of stress on negative valence learning is broad and contains many inconsistent findings. Most of these studies utilize a form of classical fear conditioning analogous to appetitive Pavlovian conditioning described above. In classical fear conditioning, foot-shocks are repeatedly paired with a conditioned stimulus (CS), such as a discrete cue (auditory or visual; cued fear conditioning) or a context (contextual fear conditioning). Most studies use freezing as an index for fear

behaviour and probe learning at three different points in time: during fear conditioning, the day following conditioning (retrieval), and during fear extinction.

Several studies have found that chronic restraint stress increases freezing to a stimulus that has been paired with a foot shock compared to stress-naïve control animals. Repeated restraint stress (21 days) before fear conditioning facilitates fear learning in rats to both the cue and context compared to stress-naïve rats (Conrad et al., 1999). Similarly, another study found that 21 days of restraint stress led to increased freezing after contextual fear conditioning compared to stress-naïve rats (Sandi et al., 2001). This implies that stressed animals either learn about the shock contingency more quickly, or that stressed animals are more likely to freeze generally when presented with a threat. A separate study found a marginal increase in freezing after cued fear conditioning in rats exposed to 21 days of restraint stress as well as elevated levels of freezing during extinction trials (Hoffman et al., 2014). This suggests that stressed animals are less able to form fear extinction memories. Another study found that 21 days of restraint did not lead to a stress induced fear conditioning facilitation, which the authors attribute to the inclusion of pre-conditioning habituation trials (Baran et al., 2009). This study was primarily interested in the effect of stress on fear extinction in both male and female rats. Similar to Hoffman et al. they found that males showed a deficit in extinction memory formation. However, stressed females instead showed a facilitation of extinction compared to stress-naïve females (Baran et al., 2009).

Rats exposed to a less intense stress (seven days of restraint) do not show a facilitation of fear learning, but do demonstrate a deficit in extinction memory formation (Miracle et al., 2006). Another study investigating fear learning after a seven day

restraint stress in males and females also found no facilitation of fear learning (Blume et al., 2019). However, male animals demonstrated an increase in freezing during the first three extinction trials (initial fear expression) but showed similar acquisition of the extinction memory during later trials (Blume et al., 2019). Stressed females demonstrated the opposite: similar initial freezing expression, but enhanced freezing during extinction implying a deficit in extinction memory formation (Blume et al., 2019). Another study exposed rats to seven days of chronic variable stress before a simple contextual fear conditioning paradigm. Stressed rats demonstrated minor increases in freezing directly after conditioning and during late extinction sessions (McGuire et al., 2010). Importantly, stressed rats demonstrated increased fear recall after a reminder shock seven days after conditioning compared to stress-naïve animals, implying a facilitation of the fear memory and insufficient extinction memory formation (McGuire et al., 2010).

Interestingly, one study investigating the effect of social instability stress on fear conditioning in adult and adolescent rats found conflicting results (Morrissey et al., 2011). In this study they found that stress had no effect on either fear conditioning or extinction memory formation in either age group, however, stressed adolescent rats showed decreased fear retrieval the day after conditioning suggesting a deficit in memory consolidation (Morrissey et al., 2011). Other studies found that early life stress increased fear to safety and uncertain cues after fear conditioning in females but not males, with the effect persisting in extinction trials (Walker et al., 2018).

These results paint a complicated and inconsistent picture of the effect of stress on fear conditioning, especially between the sexes. Although the trend through most

experiments is of increased freezing behaviour due to stress, it is necessary that further controlled experiments be conducted to confirm this finding in both males and females during the same task. It is also critical that future experiments explore discrimination between fear and neutral cues, which was generally ignored in the above literature.

From this investigation into the literature of stress induced valence deficits we see that stress plays an important role in altering emotional responses to stimuli. Despite this, the above review found many inconsistencies in behavioural results. The current thesis provides us the opportunity to reveal how the literature stands up to a controlled accounting of the different factors of at play (stress, sex, and valence).

2.3.3 Inconsistencies and ethological validity

The above literature demonstrates the variety of effects chronic stress may have on the learning about both positive and negative stimuli. However, there is little consistency across stress types, animal models, inclusion of both sexes, and valence learning protocols used. This has led to a lack of consistent evidence across the literature. Importantly, in many of the Pavlovian conditioning paradigms (positive and negative valence) an unpaired cue was not included. This makes it difficult to establish whether reported dysfunctional valence learning is specific to the paired cue or would be generalized to unpaired cues. This is especially important in the context of the fear conditioning experiments where increased freezing to the conditioned cue is often reported. Without an unpaired cue, it is not possible to determine if increased freezing is specific to the paired cue or if fear behaviour is generalized.

A major issue with the above research is that positive and negative contingencies were not investigated concurrently, only in isolation. This is problematic for several

reasons. First, in the real world, animals rarely learn about positive and negative contingencies in isolation. Potential deficits caused by stress may be more likely to be present when animals are in a situation where learning about the environment is challenging. Experimental protocols where animals must learn about both appetitive and aversive stimuli are more ethologically and clinically valid (Belzung and Lemoine, 2011). Second, as mentioned by Treadway and Zald, experiments where animals must discriminate between different cues are a particularly useful tool when investigating deficits in valence associations related to symptoms of depression (Treadway and Zald, 2011). This was made obvious in our discussion of deficits in appetitive Pavlovian conditioning (see above). By probing behavioural discrimination between positive and negative cues (as well as neutral ones) it is possible to discern whether behavioural responses (freezing and approach) are specific to their associated cue, or if they generalize. Such distinction is important as it can provide more insight into what cognitive properties are being compromised by stress. Currently, no such bivalent Pavlovian conditioning paradigm exists in mice. Therefore, in the current work, we strived to develop an appetitive and aversive Pavlovian conditioning paradigm that is simple to implement to probe for stress induced deficits in valence learning.

2.4 Utility of simultaneous valence conditioning to probe neural correlates of valence processing

Including the above ethological justification, there are several important reasons why a behavioural paradigm that concurrently trains animals on appetitive and aversive cue-outcome contingencies would be desirable. Although positive and negative valence appear to be opposing processes current research suggests that several key limbic

regions that are involved in processing differentially valenced stimuli. For example, the ventral tegmental area (VTA) was traditionally thought to exclusively encode reward learning, however, recent studies have found distinct subpopulations of cells that encode appetitive and aversive behaviours (Lammel et al., 2012). Similarly, projection neurons in the basolateral amygdala (BLA) are predisposed to respond to appetitive or aversive outcomes or their associated cues depending on their projection target (Beyeler et al., 2016, 2018). The nucleus accumbens (NAc) plays a major role in integrating contextual, emotional and internal information to select appropriate output behaviours (Goto and Grace, 2008). A large body of research has accumulated demonstrating that the NAc is sensitive to appetitive and aversive stimuli and can drive both approach and avoidance behaviours (Al-Hasani et al., 2015; Lemos et al., 2012; Reynolds and Berridge, 2002; Roitman et al., 2005; Soares-Cunha et al., 2016).

In order to further study how neurons in these regions encode cue-outcome associations of opposite valence and how stress influences these associations, it is necessary to have a robust behavioural paradigm where animals are exposed to both contingencies within the same session. Such a behavioural paradigm would be indispensable for tracking the development of valence encoding as associations are learned. These future goals played a major role in the decision to develop a bivalent Pavlovian conditioning paradigm.

Chapter 3: Rationale and Hypothesis

Limited research has probed how stress influences valence learning and discrimination which is thought to be a key factor in depression symptomatology. Even fewer studies have investigated how these effects are influenced by sex. Most studies have only been able to investigate positive and negative valence separately. Such designs – although simpler to implement – have limited ethological validity. Currently, there is no behavioural task for valence discrimination in mice that is simple to implement. Therefore, our aims for this project were as follows:

1) **Develop a Pavlovian task to train mice to discriminate between appetitive and aversive contingencies.**
2) **Probe for deficits in valence learning and discrimination in male mice after chronic social defeat stress**
3) **Probe for deficits in valence learning and discrimination in female mice after sub-chronic variable stress**

We hypothesize that under non-stressed conditions, animals will learn both appetitive and aversive contingencies and be able to discriminate between them. We also hypothesize that animals exposed to stress will demonstrate a deficit in positive valence learning and discrimination and a potentiated response to the aversive cue. As female mice are generally more susceptible to stress, we hypothesize that the effects on learning and discrimination will be increased in females.

Chapter 4: Methods

4.1 Animals

Adult (8 week) male and female C57 BL/6 mice from Jackson Laboratory (Bar Harbour, Maine) were used in all experiments. Animals were not disturbed for at least one week after their arrival in the facility. Animals were group housed for the duration of experiments (four mice per cage) unless the stress manipulation required single housing (see Chronic Social Defeat Stress section below). Food and water were provided *ad libitum* for the duration of the experiment, except when food was restricted during appetitive conditioning, where mice were provided 0.5-1 pellets of food daily after training to keep body weight at 85-90% of pre-experiment baseline. All animals were kept on a 12hr:12hr light:dark cycle, lights on at 7 am. All procedures were conducted in accordance with McGill University's Animal Care Committee and the Canadian Council on Animal Care.

4.2 Stress protocols

4.2.1 CD1 aggression screening

Retired male CD1 breeders from Charles River (Quebec, Canada) were used as aggressors for the defeat stress. Prior to the start of the stress protocol, all CD1 retired breeders were screened for aggressiveness. Non-experimental C57s were introduced to the home cage of a CD1 for three minutes or after 5 attacks (whichever came first); latency to the first attack and total number of attacks were recorded for each CD1 over three days. Animals were selected as aggressors if on day 3 their attack latency was < 30 seconds and they made at least five attacks. Aggressive CD1s were also used in the social interaction task (see below).

4.2.2 Chronic social defeat stress

The chronic social defeat stress protocol was implemented as previously reported (Berton, 2006; Golden et al., 2011). Defeat cages (46x24 cm) were separated lengthwise by a perforated plexiglass divider. 24 hours prior to stress onset CD1s were placed on one side of the divider and allowed to habituate. On each day of defeat, a novel C57 male was placed on the CD1 side of the cage. Defeat sessions lasted 8 minutes or until the C57 was attacked 25 times at which point the C57 male was removed and placed on the other side of the divider for 24 hours. On the following days, C57 males were rotated such that they were defeated by a new CD1 each day. This process was repeated until each C57 male was defeated 10 times.

An attack was defined as an interaction during which the CD1 bit, pinned down, or otherwise immobilized the C57 male. Attacks were terminated if they continued for longer than five seconds. If mice spent extended time hanging from the grid roof, they were gently pushed back to the cage floor. After day 10, C57 males were single housed in clean mouse cages and allowed 24 hours of rest before the first behavioural test.

Control animals were housed in the same room as defeat animals in sex-matched pairs, singly housed on opposite sides of a divided 46x24 cm cage. They were only disturbed once a day to be weighed. After the last day of defeat, they were single housed in clean mouse cages for 24 hours before the first behavioural test.

4.2.3 Sub-chronic variable stress

Sub-chronic variable stress consists of unpredictable shocks, tail suspension, and a restraint stress, with a rotation of one different stressor each day for one hour, for

six days. The SCVS protocol was implemented as previously reported (Hodes et al., 2015; LaPlant et al., 2009).

During the shock stressor, mice were separated by sex and placed in a shock chamber (where necessary, sex-matched non-experimental animals were added such that at least 12 mice were shocked together) and were exposed to 100 pseudorandom 1 second 0.45mA shocks. For the tail suspension, mice were hung upside-down by their tails, attached by a piece of lab tape to a wire rack. During the restraint stress, mice were placed headfirst into a 50mL falcon tube with holes drilled in the tip of the tube and the lid for respiration and the tail. All mice were group housed for the duration of the stress.

SCVS controls were group housed in the same colony room as their stress counterparts. For the duration of the stress the mice were not disturbed except for routine cage change.

4.3 Stress behaviour tests

All stress behavioural tests were recorded and analyzed by Ethovision XT (Noldus). All mice were habituated outside of the experimental room one hour before testing. Males and females were tested separately. The social interaction and open field test were conducted four hours apart, while the forced swim test was conducted on the following day. All tests were conducted during the light cycle.

4.3.1 Social interaction test

The social interaction (SI) arena consisted of a 45 x 45 cm open field with a mesh enclosure against one wall. The SI test comprises a no-target trial with an empty

enclosure followed by a target trial (2.5 minutes each) wherein an aggressive CD1 is present in the mesh enclosure. Time spent in the corners furthest from the mesh enclosure and the area surrounding the enclosure (interaction zone), and proximal social interaction (nose-to-grid events (NTG) were recorded. NTG events were defined as periods when the C57 placed its nose against the mesh enclosure. NTG events were scored offline by a blinded experimenter.

In the SCVS experiment, the target mouse was an age and sex matched C57 whereas in defeat experiments an aggressive CD1 was used. SCVS mice and controls were single housed 24 hours before the test and were re-grouped at the end of the day.

The social interaction nose-to-grid ratio was calculated as follows:

$$NTG\ Ratio\ = \frac{cumulative\ duration\ of\ NTG\ events_{(target)}}{cumulative\ duration\ of\ NTG\ events_{(no-target)}}$$

The SI test was conducted in red light with background white noise provided by a small speaker.

4.3.2 Open field test

Mice were placed in a 45 x 45 cm open field and allowed to freely explore for 5 minutes. Time spent in the centre, middle, and periphery (defined by three concentric squares of decreasing area) of the arena and mean velocity were recorded. The open field test (OFT) was conducted in red light with background white noise.

4.3.3 Forced swim test

Mice were placed in a beaker filled with 3L of 25°C water for five minutes. Time spent immobile as well as latency to immobility were recorded by Ethovision. Water was

changed between male and female mice. The FST was conducted in bright light with background white noise.

4.4 Pavlovian conditioning

All conditioning experiments were conducted in standard Med Associates mouse chambers controlled by Med-PC V software using custom code developed by the author (Appendix A). Sessions were recorded by cameras attached to the celling of each sound attenuating chamber. Before each training session, animals were transferred from the colony room to a habituation area outside of the conditioning room for one hour to allow them to acclimatize. In all below conditioning paradigms, animals experienced a pre-exposure session the day before training commenced. In these sessions, mice were exposed to four presentations of each CS in the protocol without reinforcement to reduce unconditioned orienting responses that could interfere with conditioning. Data from pre-exposure sessions were not included in our analyses.

All CS presentations were delivered in a pseudorandom order. At the start of each trial a cue was selected from an array at random without replacement. Experiment parameters did not allow the same cue to be presented more than twice in a row. Variable ITI lengths and US delivery delays were selected at the start of every trial in a similar pseudorandom manner. CS intensity was 75dB, background noise in the conditioning room was between 40-50dB.

In the appetitive and PVD conditioning protocols, animals received a small number of chocolate pellets in their home cage the day before training once the pre-exposure session was complete in order to reduce reward novelty.

4.4.1 Appetitive conditioning

Mice were trained to associate one auditory cue (CS^{R+}) with the delivery of a food reward (US^{R}, chocolate pellet) to the food-port while another distinct auditory cue (CS^{-}) was unpaired (neutral). The cues were a 9kHz tone (CS^{R+}) or a 10Hz clicker (CS^{-}). Trials consisted of a two-minute habituation period, followed by 15 second cue presentations separated by a 180 second variable inter-trial interval (vITI). Animals were trained over 14 days, with daily sessions consisting of 12 CSR^{+} and 4 CS^{-} presentations presented in a pseudorandom order with the US delivered pseudorandomly within the last five seconds of the CS^{R+}. Latency to enter the food-port after CS onset and the number of head entries made into the food-port during the first 10 seconds of the CS were recorded by Med-PC for further analysis. Percent time spent in the food port was recorded but not reported as it was not as reliable a measure as latency to head entry.

4.4.2 Aversive conditioning

Mice were trained to associate one auditory cue (CS^{S+}) with a shock delivered via the grid floor (US^{S}, 0.5mA, 0.5 seconds). A distinct auditory cue was unpaired (CS^{-}). The cues were white noise (CS^{S+}) or a 2Hz clicker (CS^{-}). Trial structure was identical to that of appetitive conditioning (see above). Animals were trained for four days with four daily presentations of each CS per session. Shocks were delivered pseudorandomly within the last five seconds of the CS^{S+}. Video recordings were used to score freezing behaviour offline. Experimenters, blind to CS identity, recorded the time animals spent freezing during the 10 seconds prior to CS onset and the first 10 seconds of each CS.

4.4.3 Pavlovian valence discrimination task

The Pavlovian Valence Discrimination task (PVD) trained animals to discriminate between cues paired with a food reward (chocolate pellet), shock (0.5mA for 0.5 seconds), or nothing. CS^{R+} and CS^{S+} tone identities were counterbalanced across animals (9kHz tone or 5Hz clicker). The CS^{-} was always white noise. Trial structure was identical to appetitive and aversive conditioning. Mice were trained on the task for seven days, each day consisting of 12 CS^{R+}, four CS^{S+}, and four CS^{-} presentations presented pseudorandomly identical to the individual protocols outlined above.

4.5 Data analysis

All data were analysed and graphed by Prism8 (GraphPad). Behavioural data was extracted, organized, and cleaned with custom code built with Python (Appendix A).

4.5.1 Discrimination scores

Discrimination scores are an index to determine the specificity of conditioned responding to different cue types. A value of 1 or -1 indicates that an animal responds exclusively to one cue and not the other, a value of 0 indicates equivalent responding between cues.

The discrimination score is calculated as follows, where x is the behavioural read-out (latency or percent freezing) and $CS_{1/2}$ are the cues of interest:

$$Discrimination\ Score = \frac{x_{CS_1} - x_{CS_2}}{x_{CS_1} + x_{CS_2}}$$

4.5.2 Statistics

All comparisons were made between control and stress groups within sexes or between male and female control mice. Unpaired t-tests were used to compare stress behaviour results between control and stress groups. A two-way repeated measures ANOVA was used to compare appetitive and aversive Pavlovian acquisition and discrimination time course results between control and stress groups or CS type. Sidak's post hoc tests were used when ANOVA results indicated group differences or interaction effects. All alpha values were set to $\alpha = 0.05$.

Chapter 5: Results

5.1 Stress-naïve animals can learn appetitive and aversive Pavlovian contingencies separately

5.1.1 Appetitive Pavlovian conditioning

Male (n = 3) and female mice (n=5) were trained on a two-tone appetitive conditioning task where one tone was paired with a chocolate pellet reward (CS^{R+}) and the other was unpaired (CS^{-}) (Fig. 2a). As animals learned the association, their latency to enter the food port was significantly reduced during the CS^{R+} compared to the CS^{-}, as shown by a two-way RM ANOVA Time x CS-type interaction $F_{(13,91)} = 10.55$, $p < 0.0001$ (Fig. 2b). Post-hoc Sidak's tests found significantly reduced latency during the CS^{R+} on all days after day 7 compared to the CS^{-} ($p < 0.05$). Further post-hoc Sidak's tests reveal that compared to day 1, latency to enter during the CS^{R+} was significantly reduced on all days following day 8 ($p < 0.05$). Time effects on latency within CS^{-} trials were not significant. Anticipatory head entries to the CS^{R+} were significantly increased compared to the CS^{-} as revealed by a two-way RM ANOVA Time x CS-type interaction $F_{(13,91)} = 8.903$, $p < 0.0001$ (Fig. 2c). Post-hoc Sidak's tests found significantly increased head entry rate during the CS^{R+} on all days after day 8 compared to the CS^{-} ($p < 0.05$). A time effect on head entry rate was detected within the CS^{R+} but not the CS^{-}. Post-hoc Sidak's tests found head entry rates during the CS^{R+} were significantly elevated on all days following day 8 compared to day 1 ($p < 0.05$); these were not significant for CS^{-} trials. Head entry rates were also significantly increased during the CS^{R+} compared to the 10 second period immediately preceding the cue onset (Pre-CS), as shown by a two-way RM ANOVA Time x CS-period interaction $F_{(13,91)} = 9.074$, $p < 0.0001$ (Fig. 2d).

Post-hoc Sidak's tests found significantly increased head entry rate during the CS^{R+} on all days after day 8 compared to the pre-CS period ($p < 0.01$). There was no significant time effect on head entry rate during the Pre-CS period, post-hoc Sidak's test were all non-significant ($p > 0.99$). Discrimination scores were calculated to compare latency and head entry rates during the CS^{R+} and the CS^{-} over time. Discrimination scores for both head entry rate and latency increased over time indicating that conditioned responding became specific to the CS^{R+} and not the CS^{-} (Fig. 2e, f).

5.1.2 Aversive Pavlovian conditioning

A separate cohort of stress-naïve male (n = 5) and female mice (n = 3) were trained on the aversive conditioning task with one tone paired with a mild foot-shock (CS^{S+}) and the other tone unpaired (CS^{-}) (Fig. 3a). After several days, animals learned the contingency, and showed anticipatory freezing behaviour towards the shock-cue, but not the unpaired cue, as demonstrated by a two-way RM ANOVA Time x CS-type interaction $F_{(3,21)} = 3.826$, $p = 0.0248$ (Fig. 3b). On days 2 through 4, the mice showed significantly increased freezing during the CS^{S+} but not the CS^{-} ($p < 0.001$, Sidak's multiple comparisons test). Interestingly, there was no significant time effect; within group post-hoc Sidak's tests did not detect significant differences in freezing to the CS^{S+} across days. However, discrimination between the cues did increase over time (Fig. 3c). A two-way RM ANOVA comparing freezing during the CS^{S+} and the pre-CS period revealed a trending interaction effect: $F_{(3,21)} = 2.850$, $p = 0.0619$. Post-hoc Sidak's test (from the trending interaction) revealed a significant difference in freezing between CS^{S+} and CS^{-} trials on day 4: $p = 0.0021$, (trending significance on days 2 and 3 ($p = 0.0677$ and 0.0742, respectively, Fig. 3d).

5.2 Stress-naïve animals can learn the Pavlovian valence discrimination task

Male (n = 3) and female mice (n = 5) were trained on the Pavlovian valence discrimination (PVD) task over seven days. Three distinct tone cues were paired with either delivery of a reward (CS^{R+}), a foot-shock (CS^{S+}), or nothing (CS^{-}) (Fig. 4a). Latency to head entry was decreased significantly during the CS^{R+} as animals were trained: two-way RM ANOVA Time x CS-type interaction $F_{(12,84)} = 2.039$, $p = 0.0303$ (Fig. 4b). Post-hoc Sidak's multiple comparisons tests found latency significantly reduced during the CS^{R+} compared to the CS^{S+} from day 4 onward ($p < 0.01$; significance was trending on day 6, $p = 0.0578$) but did not reach significance when compared to CS^{-} (trending on day 7 $p = 0.0973$, Fig. 4b). Discrimination scores comparing head entry latency between the CS^{R+} and both the CS^{S+} and CS^{-} increased across days (Fig. 3c).

Head entry rates were significantly increased during the CS^{R+}: two-way RM ANOVA Time x CS-type interaction $F_{(12,84)} = 2.801$, $p = 0.0029$ (Fig. 4d). Post-hoc Sidak's tests indicated that head entry rates during the CS^{R+} were significantly elevated compared to CS^{s+} on days 6 ($p = 0.0046$) and 7 ($p < 0.0001$), and significantly elevated compared to CS^{-} on day 7 ($p < 0.0001$, Fig. 4d). Discrimination scores comparing head entry latency between the CS^{R+} and both the CS^{S+} and CS^{-} increased across days (Fig. 3c).

A two-way RM ANOVA Time x CS-type interaction revealed percent freezing was increased during the CS^{S+} ($F_{(12,84)} = 8.690$, $p = 0.0009$; Fig. 4e). Post-hoc Sidak's tests found that compared to the CS^{R+}, freezing was increased during the CS^{S+} on all days except for day 2 ($p < 0.01$). In comparison to the CS^{-}, freezing was significantly

increased during the CS^{S+} on all days ($p \leq 0.01$, Fig. 4e). Further post-hoc Sidak's tests revealed a time effect on freezing within the CS^{S+} where freezing was significantly elevated on all days compared to day 1 ($p < 0.001$). Discrimination scores comparing freezing between the CS^{S+} and CS^{R+} or CS^{-} show a strong positive trend (Fig. 4f).

5.3 The effect of CSDS on the PVD task in males

5.3.1 CSDS produces a strong behavioural susceptible effect in males

Male (n = 7) mice were exposed to 10 days of CSDS. After the stress, they completed three behavioural tests to probe for a susceptible phenotype. Compared to stress-naïve control males (n = 7), stressed males spent less time in the centre or middle of an open field (OFT), preferring to stay near the walls (unpaired t-test $t_{(13)} = 3.259$, $p = 0.0062$; Fig. 5a). Stressed animals also moved at a lower velocity on average (unpaired t-test $t_{(13)} = 4.864$, $p = 0.0003$; Fig. 4b).

Stressed males did not demonstrate any differences in time spent immobile during the forced swim test (FST) compared to stress-naïve controls (Fig. 5c). In the social interaction (SI) test, stressed males showed a strong deficit in social interaction behaviour. Indeed, control animals spent more time proximally interacting with the mesh enclosure when a target mouse was present than when absent, compared to defeated mice (unpaired t-test $t_{(13)} = 2.389$, $p = 0.0327$; Fig. 5d).

5.3.2 CSDS alters both appetitive and aversive learning in males

Stressed and stress-naïve male mice were trained on the PVD task for seven days. Male defeated mice demonstrated increased latency to enter the food port during the CS^{R+} compared to control males, as shown by a two-way RM ANOVA Stress effect $F_{(1,12)} = 7.724$, $p = 0.0167$ (Fig. 6a). A two-way RM ANOVA Stress effect revealed both

CS^{R+} vs CS^{S+} and CS^{R+} vs CS^{-} discrimination were reduced in stressed mice ($F_{(1,12)}$ = 8.076, p = 0.0148 and $F_{(1,12)}$ = 5.308, p = 0.0399, respectively; Fig. 6b, c). Post-hoc tests were not significant.

Male stressed and control mice also demonstrated marked differences in their freezing response. A two-way RM ANOVA revealed a significant stress effect indicating that stressed mice demonstrated increased freezing during the CS^{S+} compared to their stress-naïve counterparts, $F_{(1,12)}$ = 8.924, p = 0.0133 (Fig. 6d). Despite this group difference in general freezing, there were no significant differences in discrimination scores (CS^{S+} vs CS^{R+} or CS^{S+} vs CS^{-}) between stressed and control mice. Instead, discrimination increased significantly in both groups across time. Discrimination between the CS^{S+} vs CS^{R+} increased over time, as shown by a two-way RM ANOVA time effect $F_{(6,72)}$ = 24.56, p < 0.0001. Post-hoc Sidak's tests indicated that discrimination scores on days 3 through 7 were significantly elevated compared to days 1 and 2 (p < 0.0001, Fig. 6e). Similarly, a two-way RM ANOVA revealed an effect of time on CS^{S+} vs CS^{-} discrimination scores, $F_{(6,72)}$ = 7.706, p = 0.0004. Here again post-hoc Sidak's tests revealed that discrimination scores were significantly increased on days 4 through 4 compared to day 1 (p < 0.05, Fig. 6f).

5.4 The effect of SCVS on the PVD task in females

5.4.1 SCVS did not produce behavioural stress effects in females

After six days of SCVS, animal behaviour was tested with the same battery of tests as above. In the OFT no differences were observed in either centre occupancy or velocity between female stress (n = 8) and control (n = 8) mice (Fig. 7a, b). Additionally, no differences in immobility during the FST or nose-to-grid behaviour in the SI test were

observed between stress and stress-naïve females (Fig. 6c, d). These results are unusual, and the lack of any stress effects implies that the SCVS may have been weaker than expected.

5.4.2 SCVS produces an appetitive but not aversive learning deficit in females

Female SCVS and control animals were trained on the PVD task for seven days. In females, appetitive acquisition was decreased in stressed females compared to controls, as revealed by a significant two-way RM ANOVA Stress x Time interaction, $F_{(6,84)} = 2.944$, $p = 0.0117$ (Fig. 8a). Post-hoc Sidak's tests revealed a near significant difference in latency during CS^{R+} on day 6 ($p = 0.0652$). Discrimination between CS types was also significantly influenced by stress. A two-way RM ANOVA revealed a significant effect of stress on CS^{R+} vs CS^{S+} discrimination, $F_{(1,14)} = 6.671$, $p = 0.0217$ (Fig. 8b, centre) was observed, indicating latency discrimination between the CS^{R+} and CS^{S+} was decreased in stressed animals. Discrimination between the CS^{R+} and CS^{-} was also significantly reduced in stressed animals as revealed by a significant two-way ANOVA Stress x Time interaction effect, $F_{(6,84)} = 2.506$, $p = 0.0280$ (Fig. 8c). As in the CSDS experiment, post-hoc tests were non-significant.

Unlike in the male CSDS experiment, SCVS did not appear to influence general freezing responses to the CS^{S+}. A two-way RM ANOVA revealed no significant effect of stress or any interaction effects. However, a strong effect of time was detected, indicating that both groups effectively learned the aversive contingency, $F_{(6,84)} = 55.56$, $p < 0.0001$ (Fig. 8d). Post-hoc Sidak's tests revealed that freezing behaviour on days 3 through 7 was significantly elevated compared to day 1 ($p < 0.001$). Interestingly, a two-way RM ANOVA revealed a significant interaction effect on CS^{S+} vs CS^{R+} freezing

discrimination, $F_{(6,84)} = 2.300$, $p = 0.0418$ (Fig. 8e). Post-hoc Sidak's tests revealed that on day 2, freezing discrimination between the CS^{S+} and CS^{R+} was significantly reduced in control animals ($p = 0.0004$). However, such effects were not detected in the CS^{S+} vs CS^{-} discrimination scores (Fig. 8f). Both groups were able to learn to discriminate these CS types over time as revealed by a two-way RM ANOVA (main effect of time, $F_{(6,84)} = 17.14$, $p < 0.0001$).

Chapter 6: General Discussion

The purpose of this thesis was to further current investigations into the process of valence learning in mice and to infer how it is influenced by stress. As mentioned above, there are valence studies in both humans and rodents, yet in most circumstances each valence is investigated in isolation. Although simplifying experiments, this does not correspond to reality where several conflicting emotional stimuli can be experienced in a similar timeframe. This conflicting emotional information is a key regulator of appropriate behaviour in a given context (Baumeister et al., 2007). For example, if a child smells a freshly baked pie on a windowsill they might be tempted to steal it, however if the baker catches them, they will be punished. If the child has experienced this before there will be a conflict between two oppositely valenced emotions, a positive one promoting approach, and a negative one promoting caution. This contrasting emotional information plays a role in determining which choice the child makes. As mentioned in the introduction, stress can have a major impact on emotional processing and therefore on valence learning. This thesis set out to start a new chapter in this large field, focusing on how positive and negative contingencies are learned together and how stress can alter this process.

Stress has a marked effect on emotionality and therefore can greatly alter how emotional information conditions behaviour. It is believed that this plays a major role in the development of depressive-like behaviours. The present study set out with two primary goals. The first was to establish a behavioural paradigm that would allow for the investigation of valence learning (both appetitive and aversive) within a single task and

the second was to determine how stress alters the ability of male and female mice to learn these opposing valence associations at the same time.

To achieve the second goal, it was imperative to find a behavioural paradigm that met several conditions. These were as follows: the task must be possible for mice to learn, training on both valence types must occur within the same sessions, the task must allow for full freedom of movement (i.e. not head-fixed), must be simple enough that animals can learn it within a relatively short period of time, and it must rely on Pavlovian learning. Some criteria (e.g. no head-fixed behaviour, Pavlovian conditioning) were included to ensure the same task could be used for future experiments outside the scope of this thesis. Although there are several valence training paradigms in the literature, our investigation found none that met all the above conditions. Paradigms that demonstrated robust learning in mice generally required head-fixing or utilized instrumental responses, and those that allowed for free movement were conducted in rats (Sangha et al., 2014). For these reasons we decided to develop our own paradigm.

6.1 Interpretation of results

6.1.1 Animals effectively learned both valence contingencies separately

Before testing our combined Pavlovian valence task, we initially confirmed that mice could learn either valence contingency in isolation. Most reward Pavlovian protocols are implemented over long training periods often with many days of training and dozens of trials per day. On the contrary, most aversive conditioning protocols are very short, with training usually completed within a day. Considering that we were interested primarily with how these different valences are learned together, we needed to decrease the difference in these timeframes. To do this we developed a shorter

appetitive conditioning paradigm that utilized a longer variable ITI. This increased ITI served two purposes: to increase the time for consolidation between trials and to increase the value of the reward by increasing the length between rewards (Gallistel and Gibbon, 2000). Our thought was that by increasing the value and attention to the cue, mice may be able to learn the task more quickly. Early pilot experiments comparing short versus long ITI found that appetitive conditioning was facilitated by a longer ITI (data not shown).

The results of our appetitive only experiment demonstrate that our line of thinking was correct. Latency to enter the food port during the CS^{R+} was significantly reduced compared to the CS^{-} after day 7 (Fig. 2b). From day 8 on we saw that mice entered the food port before the 5 second reward period, indicating that animals began to anticipate the reward delivery (Fig. 2b). This was complimented by their head entry rate during the 10 seconds prior to the reward period. Head entry rates during this period were significantly increased during the CS^{R+} compared to the CS^{-} from day 8 on (Fig. 2c). With increased conditioned responding to the reward cue we confirmed that the mice successfully learned to associate the reward with the CS^{R+}. We also compared head entry rates during the CS^{R+} and the 10 seconds prior to it to ensure that conditioned responding was limited to the cue period. Similarly, from day 8 onwards conditioned responding was significantly increased during the reward cue but not in the last 10 seconds of the vITI (Fig. 2d). With these data we were able to conclude that the mice were able to learn the association in a relatively short period of time.

Following this we trained animals on a similar paradigm for fear conditioning to determine the efficacy of the protocol. By day 2 of training animals demonstrated a

significant increase in freezing behaviour during the shock cue compared to the neutral cue (Fig. 3b). This elevated freezing was sustained for the remaining days. Interestingly differences in freezing between the shock cue and the 10 second period prior became significant only on day 4 (Fig. 3c). Pre-CS freezing appeared to remain relatively elevated. One potential reason for this could be that the CS^- acts as a safety cue as the animal learns that it is not associated with the shock. This could have the effect of lowering freezing slightly below baseline in the vITI (Takemoto and Song, 2019). Despite this caveat the animals appeared to effectively discriminate between the aversive and neutral cues. With these data we decided to combine the two protocols together as outlined above into the Pavlovian valence discrimination (PVD) task.

6.1.2 Cue discrimination remained high in the presence of two opposing valence contingencies during the PVD task

We set two goals to determine the efficacy of the PVD task. One was that after training animals would show invigorated conditioned responding (head entry or freezing) to the paired cue, and second, that the conditioned responding would not generalize to the opposite or neutral cues. Overall latency to enter the food port was decreased during the reward cue, however it did not quite reach significance when compared to the neutral cue indicating that animals were entering the food port relatively consistently during the CS^- (Fig. 4b). However, it appeared that the animals could discriminate between the appropriate and inappropriate cues. Discrimination scores comparing latency during the CS^{R+} to the other cues increased throughout training indicating that conditioned responding was becoming specific (Fig. 4c). Head entry data further demonstrates that the animals were able to efficiently learn the contingency and

discriminate the cues. The head entry rate during the CS^{R+} was significantly higher compared to the shock and neutral cues on day 7 (Fig. 4d). Although the discrimination between CS^{R+} and CS^{-} was not clear from the latency data, the head entry data clearly shows that conditioned responding was specifically invigorated during the reward CS^{R+}.

Throughout training freezing was significantly increased during the CS^{S+} compared to the CS^{R+} and CS^{-} indicating that the fear contingency was learned rapidly and retained (Fig. 4e). This was also illustrated by the continued increase in freezing discrimination after day 2 (Fig. 4f). Importantly, there was no evidence that freezing was generalized to the other cues after the contingency was learned. The increased generalization of freezing on day 2 appears just to be a feature of early conditioning. Due to the intensity of fear conditioning, our initial concern was that the shock stimuli would impede the animals' ability to learn the reward association and cause generalized freezing, however the data demonstrated that the presence of shocks did not impede appetitive learning.

It should be noted that we reduced the length of the protocol from 14 days in the original appetitive task to seven for two reasons: first to avoid effects of overtraining on either valence contingency, and second to keep the training period as short as possible. For these reasons training length in all PVD experiments was kept at seven days. The above data demonstrates that stress-naïve mice were able to learn a complicated valence discrimination task in a relatively short period of time. We are not aware of any other Pavlovian valence discrimination protocols published for use in freely moving mice.

6.1.3 Chronic social defeat stress impaired reward learning and elevates conditioned freezing in males

As predicted, the chronic social defeat protocol generated a significant stress phenotype in male mice after 10 days. This phenotype was characterized by decreased locomotion and time spent in open areas of the chamber during the OFT as well as decreased proximal interaction with the target mouse during the SI test (Fig. 5a, b, d). The OFT results are indicative of an anxiogenic phenotype, while the SI results indicate a deficit in social interaction behaviour, both of which are common stress related phenotypes. Interestingly we saw no effect on the FST which is often used to probe "depressive" like behaviours such as passive coping (Fig. 5c). This is not surprising as FST data is known to be unreliable and highly sensitive to minor changes in protocols between studies (Bogdanova et al., 2013). Demonstrably, the CSDS exposed mice expressed a strong stress phenotype when compared to the stress-naïve controls.

Comparisons between stress and stress-naïve male mice on the PVD task revealed significant differences in the learning and discrimination of valence cues. Control animals rapidly learned to anticipate the reward indicated by their low latency to enter the food port during the CS^{R+} (Fig. 6a). Stressed animals did not appear to readily learn the task. When compared to control animals, their latency to enter the food port was significantly higher (Fig. 6a). At the end of training, stressed animals on average entered the food port at the onset of the reward period suggesting that they had still not learned to anticipate reward delivery. This significant group effect implies that mice exposed to CSDS were less able to learn the reward contingency. Our cue discrimination analysis found that unsurprisingly stressed animals that did not learn the

contingency and so were not able to discriminate between the CS^{R+} and the other cues as well as the control animals (Fig. 6b, c). Latency to enter the food port was high during all CS types for stressed animals, indicative of limited or no contingency acquisition. These data corroborate a general trend in the literature where stress appears to cause a decrease in the capability of animals to learn positive contingencies (discussed further below).

A separate effect was observed in the freezing data. Unlike the decreased conditioned responding to the reward cue, stressed male mice demonstrated a potentiated freezing response. Compared to control animals, mice exposed to CSDS showed a general elevation in their freezing response to the CS^{S+} (Fig. 6d). However, there were no differences in the freezing cue discrimination analysis which suggested that control and stressed animals both learned to discriminate the cues at a similar rate (Fig. 6 e, f). These results show that, although the stressed animals freeze relatively more to the CS^{S+} than control animals, this is not indicative of increased fear learning. Since the discrimination scores are the same between groups, the stressed animals must be freezing to all cues at a proportionately higher rate than the control animals, indicating generally increased freezing and not enhanced fear learning. This conclusion is concordant with the anxiogenic phenotype found in these animals during the OFT. Past studies have also found a relationship between decreased locomotor activity and increased fear induced freezing connected to stress induced disturbances in dopamine function (Azzinnari et al., 2014). From these data we can conclude that CSDS has a strong effect on valence learning, particularly that it disrupts Pavlovian reward conditioning but potentiates freezing behaviour in male mice. Importantly, these results

are in conflict with the theory of emotional context insensitivity which would predict that fear responses would be lowered or unchanged compared to controls (Rottenberg et al., 2005).

6.1.4 Sub-chronic variable stress impaired reward learning but not fear learning in females

Unlike in the literature and previous experiments in our lab, SCVS did not produce a stress phenotype in our battery of general behavioural tests (Fig. 7). This is unusual as a 6-day SCVS has reliably produced stress effects in these tests (Muir et al., 2020). Despite this, the PVD task revealed a significant deficit in reward learning and discrimination very similar to that found in males (Fig. 8a, b, c). However, unlike in the male experiment, SCVS exposed females did not show any differences in conditioned fear acquisition compared to controls (Fig. 8d). Although there was a significant interaction effect within the CS^{S+} vs CS^{R+} discrimination scores, this appeared to be a result of the relatively low discrimination in controls on day 2 alone (Fig. 8e). For the remainder of the data no significant differences were noted. From this we can gather that SCVS did not influence the ability of females to learn the aversive contingency in the PVD task, but it did impair their ability to form reward associations.

6.2 Complications with determining differential stress effects across sexes

Studying stress can be a complicated matter due to general variability, however, comparing the stress response between the sexes is particularly challenging. Differences in susceptibility to the same stress protocol can have vastly different effects on either sex (Bale and Epperson, 2015; Hodes et al., 2015; LaPlant et al., 2009). Attempts to achieve the same “level” of stress through altered protocol length in both

sexes are generally not feasible. The situation is further complicated by prominent individual differences in the stress response of animals (Beery and Kaufer, 2015; de Boer et al., 2017; Ellis et al., 2006). Therefore, direct comparisons between the sexes are generally not possible. This limitation was understood at the outset of this study, hence we opted to use protocols that have been well established in either sex. Despite this limiting our ability to compare males and females directly, we saw a similar pattern emerge in the above data in both sexes. Chronic stress impeded the ability of male and female mice to learn the reward contingency in line with our hypothesis. Aversive conditioning and discrimination were not negatively affected by stress – rather stress potentiated the fear response in males. It is important to note this deficit in reward learning was present in females even though they did not initially present a stress phenotype in our behavioural battery. This is contrary to our hypothesis where we expected the stress phenotype to be enhanced in females. These female results also imply that reward conditioning may be a more sensitive test for stress behaviour. This claim is not unfounded as stress is known to directly impede upstream appetitive processes such as reward valuation and motivation (Dias-Ferreira et al., 2009; Kleen et al., 2006; Krishnan et al., 2007; Olausson et al., 2013).

Our fear conditioning results were consistent with some parts of the literature, finding that freezing levels were potentiated in stressed males (Conrad et al., 1999; Sandi et al., 2001). These studies, however, could not distinguish whether stress facilitated fear learning or if it raised freezing levels in general. Our discrimination data provided evidence that although stressed animals froze more to the CS^{S+} compared to control animals, this was not due to an increased learning rate. Rather, stress animals

froze proportionally more to all CS types compared to the controls suggesting that freezing in stressed animals is generally increased. Other studies mentioned above found that less intense stress (seven days of restraint) did not lead to fear potentiation (Blume et al., 2019; Miracle et al., 2006). This was in line with our female data, where a less intense stressor (SCVS) did not lead to fear potentiation. However, to support such a claim it would be necessary to compare females exposed to SCVS and a more stressful paradigm (21-day SCVS). In studies where an effect of stress on fear acquisition was not found, generally a wide variety of differences in extinction learning were present (Baran et al., 2009; Blume et al., 2019; Hoffman et al., 2014; Miracle et al., 2006). Extinction learning was not in the purview of the present study; however, it should be investigated in the future before ruled out.

To further elucidate stress and sex effects on PVD learning, future studies should utilize a variety of stress intensities and protocols. Recent studies have found differences in behaviour and neural activation in key emotional circuits in both males and females exposed to SCVS (Muir et al., 2020). Males and females exposed to variable lengths of SCVS could provide a clearer picture. Additionally, shifting from CSDS to witness defeat stress in both sexes could provide more direct sex comparisons.

6.3 The challenges in determining the "cause" of valence deficits

As mentioned above, the goal of this project was to create a new model for studying valence learning in mice and to determine whether stress alters their ability to learn these valence contingencies. In this regard, the project was certainly successful, however our ability to determine the underlying causes of these stress effects was

limited. This was particularly the case with respect to the appetitive contingency. The general stress phenotype we detected could have been precipitated by deficits in any number of reward processes. Stress induced alteration in consumption, anticipation, motivation for rewards, and more general deficits in cognition and reward learning have been characterized in the literature (Der-Avakian and Markou, 2012). Although these deficits could manifest themselves in the same way behaviourally, the underlying causes are varied and rely on different neural circuits (Der-Avakian and Markou, 2012). A great deal of investigation has been made into determining which processes are the causative factor in stress related reward deficits. The literature is varied on this subject, with some finding primary effects on consumption (Hodes et al., 2015; Krishnan et al., 2007), however, the present data did not find any evidence for differences in reward consumption. Other studies found deficits in motivation (Kleen et al., 2006; Olausson et al., 2013) or reward-discrimination tasks (DeCola and Rosellini, 1990). Others found that effects only manifested themselves in the development of habitual behaviours, indicative of a cognitive deficit (Dias-Ferreira et al., 2009). Interestingly, some studies have found that stress has opposite effects on reward behaviour depending on its intensity, with low intensity or intermittent stress facilitating reward consumption and motivation (Miczek et al., 2011; Riga et al., 2015). Due to the broad effects stress has on emotional and motivational circuit systems, the inconsistency in reward and aversive conditioning literature is not at all surprising. Due to the strong interconnectedness of stress response systems (HPA axis, corticosteroid system) with reward relevant neurotransmitter systems (dopamine, serotonin), limbic, and cognitive neural circuits, as

well as variability in stress sensitivity, variable results should be expected (Cabib and Puglisi-Allegra, 1996; López et al., 1999).

Further studies should be conducted to determine whether our appetitive effects are primarily due to differences in motivation, reward sensitivity, or decreased cognitive ability. Potential methods to investigate such questions could include progressive ratio, reward devaluation, sucrose preference, and a Go No-Go tasks.

6.4 Further limitations

The primary limitation of this study was the use of different stress paradigms for males and females. These paradigms were chosen due to their consistent record of generating stress phenotypes in either sex. As previously stated, using different paradigms between the sexes reduces our ability to investigate sex differences. Future studies should endeavour to use the same protocol (of varying lengths) in both males and females to achieve more comparable results.

Although not a pressing issue for the current study, it is not favourable for the two conditioned responses we record to be based on opposite modalities. Using behavioural excitation and inhibition as the main output measures for appetitive and fear conditioned responding respectively may provide challenges in data interpretation in future studies. This is especially the case if future experiments focus on the nucleus accumbens (NAc), which is directly linked to movement generation/inhibition. A remedy to this issue would be securing resources that will allow further development of the PVD task into an approach/avoidance task by allowing a region of safety for the mouse to escape to during the aversive cue. Thus, the same measure, latency (to the food port or safety),

can be used to compare behavioural invigoration of either valence. This will hopefully reduce movement as a confounding factor in future studies.

6.5 Future directions in valence learning research

The development of the PVD task opens the door to research into a wide variety of questions that could not previously be investigated. The ability to study learning of both aversive and appetitive valence contingencies at the same time will allow for far more detailed investigation into how valence learning occurs. New research has found that many limbic regions process both positive and negative valence information with particular focus on the basal amygdala (BLA) and NAc (Al-Hasani et al., 2015; Beyeler et al., 2016, 2018; Qi et al., 2016; Reynolds and Berridge, 2002; Roitman et al., 2005). This research demonstrates that within these regions there are discrete or distributed cell populations that process both positively and negatively valenced stimuli (Tye, 2018). Further understanding of how these regions dynamically process this information during a learning task such as the PVD task would provide crucial insight into how valence is processed in the brain. Data from the basal amygdala has already provided a great deal of insight. Through *in vivo* electrophysiology during a head-fixed valence discrimination task, researchers were able to determine that although BLA cells respond to both aversive and appetitive cues, the projection target of the cell is predictive of its cue specificity (Beyeler et al., 2016). Cells that project to the NAc are more likely to respond to the appetitive cue, while those projecting to the central amygdala (CeA) generally responded more to the negative cue (Beyeler et al., 2016). These populations were relatively overlapping, but cells selective for the aversive cue were more likely to be located dorsally in the BLA (Beyeler et al., 2018).

Such studies have not been conducted in the NAc, which is posed as a prime candidate for further investigations. This region is of particular interest not only due to its processing of positive and negative experiences (Al-Hasani et al., 2015; Qi et al., 2016; Reynolds and Berridge, 2002; Roitman et al., 2005), but also due to its implication in stress susceptibility (Bagot et al., 2015; Francis and Lobo, 2017; Francis et al., 2015, 2017; Hodes et al., 2015; Muir et al., 2020). Studies have found that stress appears to alter how the NAc processes valence, such as through altering the size of differential valence responsive fields (Reynolds and Berridge, 2008) and by completely inverting the behaviour generated by stress responsive factors in the NAc from appetitive to aversive (Lemos et al., 2012; Wanat et al., 2013). Through minimally invasive imaging techniques such as microendoscopy (Resendez et al., 2016), we could determine how valence learning occurs within the NAc and how this process is altered or disrupted by chronic stress. A detailed analysis of NAc neural population dynamics during PVD learning in both stressed and stress-naïve animals will provide valuable data that will further our understanding of the links between stress and valence processing. Ultimately such research will provide further insight into how depression alters our emotional states and behaviour.

6.6 Conclusion

This project set out to develop a novel behavioural paradigm that allows one to investigate bivalent Pavlovian learning in freely moving mice and to determine how stress alters this process. We were able to demonstrate that through the PVD task mice were able to effectively learn both valence contingencies and discriminate between opposite and neutral cues. Such a test that can be used in freely moving mice has to

our knowledge not been developed before. Due to the inextricable link between stress and emotional behaviour, we wished to test whether stress alters an animal' ability to learn valence contingencies concurrently. As stress has variable effects on the sexes, we conducted this experiment in male and female mice, using stress models known to induce susceptibility in either sex. Our results indicated that stress causes a deficit in appetitive learning in both sexes, however, CSDS in males has the added effect of increasing general fear behaviour. Although these deficits are not new to the literature, this is the first time they have been observed in a valence discrimination task in both sexes. These results demonstrate the similarities in valence processing deficits experienced by both sexes due to stress susceptibility and provides a springboard for further study into these questions. With the PVD task, further questions such as how valence and associated contingencies are learned and processed, how this processing goes awry during stress, and how these differ in male and female mice can now be more easily investigated. With this project we have achieved the first step in a much longer investigation that will be able to shed light on some of the most uncertain questions in the field.

Literature Cited

Al-Hasani, R., McCall, J.G., Shin, G., Gomez, A.M., Schmitz, G.P., Bernardi, J.M., Pyo, C.O., Park, S.I., Marcinkiewcz, C.M., Crowley, N.A., et al. (2015). Distinct Subpopulations of Nucleus Accumbens Dynorphin Neurons Drive Aversion and Reward. Neuron *87*, 1063–1077.

American Psychiatric Association (2013). Diagnostic and statistical manual of mental disorders (Arlington: American Psychiatric Publishing).

Azzinnari, D., Sigrist, H., Staehli, S., Palme, R., Hildebrandt, T., Leparc, G., Hengerer, B., Seifritz, E., and Pryce, C.R. (2014). Mouse social stress induces increased fear conditioning, helplessness and fatigue to physical challenge together with markers of altered immune and dopamine function. Neuropharmacology *85*, 328–341.

Bagot, R.C., Parise, E.M., Pena, C.J., Zhang, H.X., Maze, I., Chaudhury, D., Persaud, B., Cachope, R., Bolanos-Guzman, C.A., Cheer, J.F., et al. (2015). Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. Nat Commun *6*, 7062.

Bale, T.L., and Epperson, C.N. (2015). Sex differences and stress across the lifespan. Nat. Neurosci. *18*, 1413.

Baran, S.E., Armstrong, C.E., Niren, D.C., Hanna, J.J., and Conrad, C.D. (2009). Chronic stress and sex differences on the recall of fear conditioning and extinction. Neurobiol. Learn. Mem. *91*, 323–332.

Baumeister, R.F., Vohs, K.D., DeWall, C.N., and Zhang, L. (2007). How Emotion

Shapes Behavior: Feedback, Anticipation, and Reflection, Rather Than Direct Causation. Personal. Soc. Psychol. Rev. *11*, 167–203.

Beck, A.T. (1963). Thinking and Depression: I. Idiosyncratic Content and Cognitive Distortions. Arch. Gen. Psychiatry *9*, 324–333.

Beck, A.T. (1987). Cognitive models of depression. J. Cogn. Psychother. *1*, 5–37.

Beery, A.K., and Kaufer, D. (2015). Stress, social behavior, and resilience: Insights from rodents. Neurobiol. Stress *1*, 116–127.

Beery, A.K., and Zucker, I. (2011). Sex bias in neuroscience and biomedical research. Neurosci. Biobehav. Rev. *35*, 565–572.

Belzung, C., and Lemoine, M. (2011). Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. Biol. Mood Anxiety Disord. *1*, 1–14.

Berridge, K.C., and Robinson, T.E. (1998). What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? Brain Res. Rev. *28*, 309–369.

Berton, O. (2006). Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress. Science *311*, 864–868.

Beyeler, A., Namburi, P., Glober, G.F., Simonnet, C., Calhoon, G.G., Conyers, G.F., Luck, R., Wildes, C.P., and Tye, K.M. (2016). Divergent Routing of Positive and Negative Information from the Amygdala during Memory Retrieval. Neuron *90*, 348–361.

Beyeler, A., Chang, C.J., Silvestre, M., Leveque, C., Namburi, P., Wildes, C.P., and

Tye, K.M. (2018). Organization of Valence-Encoding and Projection-Defined Neurons in the Basolateral Amygdala. Cell Rep *22*, 905–918.

Blume, S.R., Padival, M., Urban, J.H., and Rosenkranz, J.A. (2019). Disruptive effects of repeated stress on basolateral amygdala neurons and fear behavior across the estrous cycle in rats. Sci. Rep. *9*, 12292.

de Boer, S.F., Buwalda, B., and Koolhaas, J.M. (2017). Untangling the neurobiology of coping styles in rodents: Towards neural mechanisms underlying individual differences in disease susceptibility. Neurosci. Biobehav. Rev. *74*, 401–422.

Bogdanova, O. V., Kanekar, S., D'Anci, K.E., and Renshaw, P.F. (2013). Factors influencing behavior in the forced swim test. Physiol. Behav. *118*, 227–239.

Bylsma, L.M., Morris, B.H., and Rottenberg, J. (2008). A meta-analysis of emotional reactivity in major depressive disorder. Clin. Psychol. Rev. *28*, 676–691.

Cabib, S., and Puglisi-Allegra, S. (1996). Stress, depression and the mesolimbic dopamine system. Psychopharmacology (Berl). *128*, 331–342.

Conrad, C.D., Magariños, A.M., LeDoux, J.E., and McEwen, B.S. (1999). Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. Behav. Neurosci. *113*, 902–913.

Dannlowski, U., Ohrmann, P., Bauer, J., Kugel, H., Arolt, V., Heindel, W., Kersting, A., Baune, B.T., and Suslow, T. (2007). Amygdala reactivity to masked negative faces is associated with automatic judgmental bias in major depression: A 3 T fMRI study. J. Psychiatry Neurosci. *32*, 423–429.

DeCola, J.P., and Rosellini, R.A. (1990). Unpredictable/uncontrollable stress proactively interferes with appetitive Pavlovian conditioning. Learn. Motiv. *21*, 137–152.

Denny, E.B., and Hunt, R.R. (1992). Affective valence and memory in depression: Dissociation of recall and fragment completion. J. Abnorm. Psychol. *101*, 575–580.

Der-Avakian, A., and Markou, A. (2012). The neurobiology of anhedonia and other reward-related deficits. Trends Neurosci. *35*, 68–77.

Dias-Ferreira, E., Sousa, J.C., Melo, I., Morgado, P., Mesquita, A.R., Cerqueira, J.J., Costa, R.M., and Sousa, N. (2009). Chronic Stress Causes Frontostriatal Reorganization and Affects Decision-Making. Science *325*, 621–625.

Dibbets, P., van den Broek, A., and Evers, E.A.T. (2015). Fear conditioning and extinction in anxiety- and depression-prone persons. Memory *23*, 350–364.

Duman, R.S., and Monteggia, L.M. (2006). A Neurotrophic Model for Stress-Related Mood Disorders. Biol. Psychiatry *59*, 1116–1127.

Ellis, B.J., Jackson, J.J., and Boyce, W.T. (2006). The stress response systems: Universality and adaptive individual differences. Dev. Rev. *26*, 175–212.

Ford, D.E., and Erlinger, T.P. (2004). Depression and C-Reactive Protein in US Adults. JAMA Intern. Med. *164*, 1010–1014.

Francis, T.C., and Lobo, M.K. (2017). Emerging Role for Nucleus Accumbens Medium Spiny Neuron Subtypes in Depression. Biol Psychiatry *81*, 645–653.

Francis, T.C., Chandra, R., Friend, D.M., Finkel, E., Dayrit, G., Miranda, J., Brooks, J.M., Iniguez, S.D., O'Donnell, P., Kravitz, A., et al. (2015). Nucleus accumbens

medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. Biol Psychiatry *77*, 212–222.

Francis, T.C., Chandra, R., Gaynor, A., Konkalmatt, P., Metzbower, S.R., Evans, B., Engeln, M., Blanpied, T.A., and Lobo, M.K. (2017). Molecular basis of dendritic atrophy and activity in stress susceptibility. Mol Psychiatry *22*, 1512–1519.

Von Frijtag, J.C., Reijmers, L.G.J.E., Van der Harst, J.E., Leus, I.E., Van den Bos, R., and Spruijt, B.M. (2000). Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. Behav. Brain Res. *117*, 137–146.

Von Frijtag, J.C., Van den Bos, R., and Spruijt, B.M. (2002). Imipramine restores the long-term impairment of appetitive behavior in socially stressed rats. Psychopharmacology (Berl). *162*, 232–238.

Gallistel, C.R., and Gibbon, J. (2000). Time, rate, and conditioning. Psychol. Rev. *107*, 289–344.

Golden, S.A., Covington, H.E., Berton, O., and Russo, S.J. (2011). A standardized protocol for repeated social defeat stress in mice. Nat. Protoc. *6*, 1183–1191.

Gotlib, I.H. (1983). Perception and recall of interpersonal feedback: Negative bias in depression. Cognit. Ther. Res. *7*, 399–412.

Goto, Y., and Grace, A.A. (2008). Limbic and cortical information processing in the nucleus accumbens. Trends Neurosci. *31*, 552–558.

Gourley, S.L., Swanson, A.M., Jacobs, A.M., Howell, J.L., Mo, M., DiLeone, R.J.,

Koleske, A.J., and Taylor, J.R. (2012). Action control is mediated by prefrontal BDNF and glucocorticoid receptor binding. Proc. Natl. Acad. Sci. U. S. A. *109*, 20714–20719.

Gruber, J., Oveis, C., Keltner, D., and Johnson, S.L. (2011). A discrete emotions approach to positive emotion disturbance in depression. Cogn. Emot. *25*, 40–52.

Gur, R.C., Erwin, R.J., Gur, R.E., Zwil, A.S., Heimberg, C., and Kraemer, H.C. (1992). Facial emotion discrimination: II. Behavioral findings in depression. Psychiatry Res. *42*, 241–251.

Haaga, D.A.F., Dyck, M.J., and Ernst, D. (1991). Empirical status of cognitive theory of depression. Psychol. Bull. *110*, 215–236.

Heim, C., Newport, D.J., Mletzko, T., Miller, A.H., and Nemeroff, C.B. (2008). The link between childhood trauma and depression: Insights from HPA axis studies in humans. Psychoneuroendocrinology *33*, 693–710.

Henriques, J.B., Glowacki, J.M., and Davidson, R.J. (1994). Reward Fails to Alter Response Bias in Depression. J. Abnorm. Psychol. *103*, 460–466.

Hodes, G.E., Pfau, M.L., Purushothaman, I., Francisca Ahn, H., Golden, S.A., Christoffel, D.J., Magida, J., Brancato, A., Takahashi, A., Flanigan, M.E., et al. (2015). Sex differences in nucleus accumbens transcriptome profiles associated with susceptibility versus resilience to subchronic variable stress. J. Neurosci. *35*, 16362–16376.

Hoffman, A.N., Lorson, N.G., Sanabria, F., Foster Olive, M., and Conrad, C.D. (2014). Chronic stress disrupts fear extinction and enhances amygdala and hippocampal Fos

expression in an animal model of post-traumatic stress disorder. Neurobiol. Learn. Mem. *112*, 139–147.

Holmes, N.M., Marchand, A.R., and Coutureau, E. (2010). Pavlovian to instrumental transfer: A neurobehavioural perspective. Neurosci. Biobehav. Rev. *34*, 1277–1295.

Iñiguez, S.D., Flores-Ramirez, F.J., Riggs, L.M., Alipio, J.B., Garcia-Carachure, I., Hernandez, M.A., Sanchez, D.O., Lobo, M.K., Serrano, P.A., Braren, S.H., et al. (2018). Vicarious Social Defeat Stress Induces Depression-Related Outcomes in Female Mice. Biol. Psychiatry *83*, 9–17.

Kendler, K.S., Karkowski, L.M., and Prescott, C.A. (1999). Causal relationship between stressful life events and the onset of major depression. Am J Psychiatry *156*, 837–841.

Kendler, K.S., Thornton M, L., and Gardner O, C. (2000). Stressful life events and previous episodes in the etiology of major depression in women: An evaluation of the “kindling” hypothesis. Am. J. Psychiatry *157*, 1243–1251.

Kessler, R.C. (1993). Sex and depression in the National Comorbidity Survey I: Lifetime prevalence, chronicity and recurrence. J. Affect. Disord. *29*, 85–96.

Kessler, R.C., Zhao, S., Blazer, D.G., and Swartz, M. (1997). Prevalence , correlates , and course of minor depression and major depression in the national comorbidity survey. J. Affect. Disord. *45*, 19–30.

Kleen, J.K., Sitomer, M.T., Killeen, P.R., and Conrad, C.D. (2006). Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore. Behav. Neurosci. *120*, 842–851.

Kraepelin, E. (1921). Manic-Depressive Insanity and Paranoia (Edinburgh: E. & S. Livingstone).

Krishnan, V., and Nestler, E.J. (2008). The molecular neurobiology of depression. Nature *455*, 894–902.

Krishnan, V., Han, M.H., Graham, D.L., Berton, O., Renthal, W., Russo, S.J., LaPlant, Q., Graham, A., Lutter, M., Lagace, D.C., et al. (2007). Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. Cell *131*, 391–404.

Kudielka, B.M., and Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. Biol. Psychol. *69*, 113–132.

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

LaPlant, Q., Chakravarty, S., Vialou, V., Mukherjee, S., Koo, J.W., Kalahasti, G., Bradbury, K.R., Taylor, S. V., Maze, I., Kumar, A., et al. (2009). Role of Nuclear Factor κB in Ovarian Hormone-Mediated Stress Hypersensitivity in Female Mice. Biol. Psychiatry *65*, 874–880.

Lemos, J.C., Wanat, M.J., Smith, J.S., Reyes, B.A., Hollon, N.G., Van Bockstaele, E.J., Chavkin, C., and Phillips, P.E. (2012). Severe stress switches CRF action in the nucleus accumbens from appetitive to aversive. Nature *490*, 402–406.

López, J.F., Akil, H., and Watson, S.J. (1999). Neural circuits mediating stress. Biol.

Psychiatry *46*, 1461–1471.

Mazure, C.M. (1998). Life stressors as risk factors in depression. Clin. Psychol. Sci. Pract. *5*, 291–313.

McGuire, J., Herman, J.P., Horn, P.S., Sallee, F.R., and Sah, R. (2010). Enhanced fear recall and emotional arousal in rats recovering from chronic variable stress. Physiol. Behav. *101*, 474–482.

Miczek, K.A., Nikulina, E.M., Shimamoto, A., and Covington, H.E. (2011). Escalated or suppressed cocaine reward, tegmental BDNF, and accumbal dopamine caused by episodic versus continuous social stress in rats. J. Neurosci. *31*, 9848–9857.

Miracle, A.D., Brace, M.F., Huyck, K.D., Singler, S.A., and Wellman, C.L. (2006). Chronic stress impairs recall of extinction of conditioned fear. Neurobiol. Learn. Mem. *85*, 213–218.

Mogil, J.S., and Chanda, M.L. (2005). The case for the inclusion of female subjects in basic science studies of pain. Pain *117*, 1–5.

Morgado, P., Silva, M., Sousa, N., and Cerqueira, J.J. (2012). Stress transiently affects pavlovian-to-instrumental transfer. Front. Neurosci. *6*, 1–6.

Morrissey, M.D., Mathews, I.Z., and McCormick, C.M. (2011). Enduring deficits in contextual and auditory fear conditioning after adolescent, not adult, social instability stress in male rats. Neurobiol. Learn. Mem. *95*, 46–56.

Muir, J., Lopez, J., and Bagot, R.C. (2019). Wiring the depressed brain: optogenetic and chemogenetic circuit interrogation in animal models of depression.

Neuropsychopharmacology *44*, 1013–1026.

Muir, J., Tse, Y.C., Iyer, E.S., Biris, J., Cvetkovska, V., Lopez, J., and Bagot, R.C. (2020). Ventral Hippocampal Afferents to Nucleus Accumbens Encode Both Latent Vulnerability and Stress-Induced Susceptibility. Biol. Psychiatry 1–12.

Olausson, P., Kiraly, D.D., Gourley, S.L., and Taylor, J.R. (2013). Persistent effects of prior chronic exposure to corticosterone on reward-related learning and motivation in rodents. Psychopharmacology (Berl). *225*, 569–577.

Panksepp, J., and Ikemoto, S. (1996). Dissociations Between Appetitive and Consummatory Responses by Pharmacological Manipulations of Reward-Relevant Brain Regions. Behav. Neurosci. *110*, 331–345.

Patten, S.B., Williams, J.V.A., Lavorato, D.H., Fiest, K.M., Bulloch, A.G.M., and Wang, J.L. (2015). The prevalence of major depression is not changing. Can. J. Psychiatry *60*, 31–34.

Pittenger, C., and Duman, R.S. (2008). Stress, depression, and neuroplasticity: A convergence of mechanisms. Neuropsychopharmacology *33*, 88–109.

Pizzagalli, D.A., Iosifescu, D., Hallett, L.A., Ratner, K.G., and Fava, M. (2008). Reduced hedonic capacity in major depressive disorder: Evidence from a probabilistic reward task. J. Psychiatr. Res. *43*, 76–87.

Pizzagalli, D.A., Holmes, A.J., Dillon, D.G., Goetz, E.L., Birk, J.L., Bogdan, R., Dougherty, D.D., Iosifescu, D. V., Rauch, S.L., and Fava, M. (2009). Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major

depressive disorder. Am. J. Psychiatry *166*, 702–710.

Post, R.M. (1992). Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. Am. J. Psychiatry *149*, 999–1010.

Prendergast, B.J., Onishi, K.G., and Zucker, I. (2014). Female mice liberated for inclusion in neuroscience and biomedical research. Neurosci. Biobehav. Rev. *40*, 1–5.

Qi, J., Zhang, S., Wang, H.L., Barker, D.J., Miranda-Barrientos, J., and Morales, M. (2016). VTA glutamatergic inputs to nucleus accumbens drive aversion by acting on GABAergic interneurons. Nat Neurosci *19*, 725–733.

Resendez, S.L., Jennings, J.H., Ung, R.L., Namboodiri, V.M., Zhou, Z.C., Otis, J.M., Nomura, H., McHenry, J.A., Kosyk, O., and Stuber, G.D. (2016). Visualization of cortical, subcortical and deep brain neural circuit dynamics during naturalistic mammalian behavior with head-mounted microscopes and chronically implanted lenses. Nat Protoc *11*, 566–597.

Reynolds, S.M., and Berridge, K.C. (2002). Positive and Negative Motivation in Nucleus Accumbens Shell: Bivalent Rostrocaudal Gradients for GABA-Elicited Eating, Taste “Liking”/“Disliking” Reactions, Place Preference/Avoidance, and Fear. J Neurosci *22*, 7308–7320.

Reynolds, S.M., and Berridge, K.C. (2008). Emotional environments retune the valence of appetitive versus fearful functions in nucleus accumbens. Nat Neurosci *11*, 423–425.

Riga, D., Theijs, J.T., De Vries, T.J., Smit, A.B., and Spijker, S. (2015). Social defeat-induced anhedonia: Effects on operant sucrose-seeking behavior. Front. Behav.

Neurosci. *9*, 1–12.

Roitman, M.F., Wheeler, R.A., and Carelli, R.M. (2005). Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. Neuron *45*, 587–597.

Rottenberg, J., Gross, J.J., and Gotlib, I.H. (2005). Emotion context insensitivity in major depressive disorder. J. Abnorm. Psychol. *114*, 627–639.

Russell, J.A. (1980). A circumplex model of affect. J. Pers. Soc. Psychol. *39*, 1161–1178.

Sandi, C., Merino, J.J., Cordero, M.I., Touyarot, K., and Venero, C. (2001). Effects of chronic stress on contextual fear conditioning and the hippocampal expression of the neural cell adhesion molecule, its polysialylation, and L1. Neuroscience *102*, 329–339.

Sangha, S., Robinson, P.D., Greba, Q., Davies, D.A., and Howland, J.G. (2014). Alterations in reward, fear and safety cue discrimination after inactivation of the rat prelimbic and infralimbic cortices. Neuropsychopharmacology *39*, 2405–2413.

Shansky, R.M. (2019). Animal studies in both sexes. Science *364*, 825–826.

Sial, O.K., Warren, B.L., Alcantara, L.F., Parise, E.M., and Bolaños-Guzmán, C.A. (2016). Vicarious social defeat stress: Bridging the gap between physical and emotional stress. J. Neurosci. Methods *258*, 94–103.

Soares-Cunha, C., Coimbra, B., Sousa, N., and Rodrigues, A.J. (2016). Reappraising striatal D1- and D2-neurons in reward and aversion. Neurosci Biobehav Rev *68*, 370–386.

Spijker, J., Bijl, R. V., De Graaf, R., and Nolen, W.A. (2001). Determinants of poor 1-year outcome of DSM-III-R major depression in the general population: Results of the Netherlands Mental Health Survey and Incidence Study (NEMESIS). Acta Psychiatr. Scand. *103*, 122–130.

Stuhrmann, A., Suslow, T., and Dannlowski, U. (2011). Facial emotion processing in major depression: A systematic review of neuroimaging findings. Biol. Mood Anxiety Disord. *1*, 1–10.

Stuhrmann, A., Dohm, K., Kugel, H., Zwanzger, P., Redlich, R., Grotegerd, D., Rauch, A.V., Arolt, V., Heindel, W., Suslow, T., et al. (2013). Mood-congruent amygdala responses to subliminally presented facial expressions in major depression: Associations with anhedonia. J. Psychiatry Neurosci. *38*, 249–258.

Takemoto, M., and Song, W.J. (2019). Cue-dependent safety and fear learning in a discriminative auditory fear conditioning paradigm in the mouse. Learn. Mem. *26*, 284–290.

Treadway, M.T., and Zald, D.H. (2011). Reconsidering anhedonia in depression: Lessons from translational neuroscience. Neurosci. Biobehav. Rev. *35*, 537–555.

Tsankova, N.M., Berton, O., Renthal, W., Kumar, A., Neve, R.L., and Nestler, E.J. (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat. Neurosci. *9*, 519–525.

Tye, K.M. (2018). Neural Circuit Motifs in Valence Processing. Neuron *100*, 436–452.

Walker, R.A., Andreansky, C., Ray, M.H., and McDannald, M.A. (2018). Early

adolescent adversity inflates threat estimation in females and promotes alcohol use initiation in both sexes. Behav. Neurosci. *132*, 171–182.

Wanat, M.J., Bonci, A., and Phillips, P.E. (2013). CRF acts in the midbrain to attenuate accumbens dopamine release to rewards but not their predictors. Nat Neurosci *16*, 383–385.

Warren, B.L., Vialou, V.F., Iñiguez, S.D., Alcantara, L.F., Wright, K.N., Feng, J., Kennedy, P.J., Laplant, Q., Shen, L., Nestler, E.J., et al. (2013). Neurobiological sequelae of witnessing stressful events in adult mice. Biol. Psychiatry *73*, 7–14.

Whiteford, H.A., Degenhardt, L., Rehm, J., Baxter, A.J., Ferrari, A.J., Erskine, H.E., Charlson, F.J., Norman, R.E., Flaxman, A.D., Johns, N., et al. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet *382*, 1575–1586.

Willner, P., Muscat, R., and Papp, M. (1992). Chronic mild stress-induced anhedonia: A realistic animal model of depression. Neurosci. Biobehav. Rev. *16*, 525–534.

Xu, P., Wang, K., Lu, C., Dong, L., Chen, Y., Wang, Q., Shi, Z., Yang, Y., Chen, S., and Liu, X. (2017). Effects of the chronic restraint stress induced depression on reward-related learning in rats. Behav. Brain Res. *321*, 185–192.

Zucker, I., and Beery, A.K. (2010). Males still dominate animal studies. Nature *465*, 690.

Figures

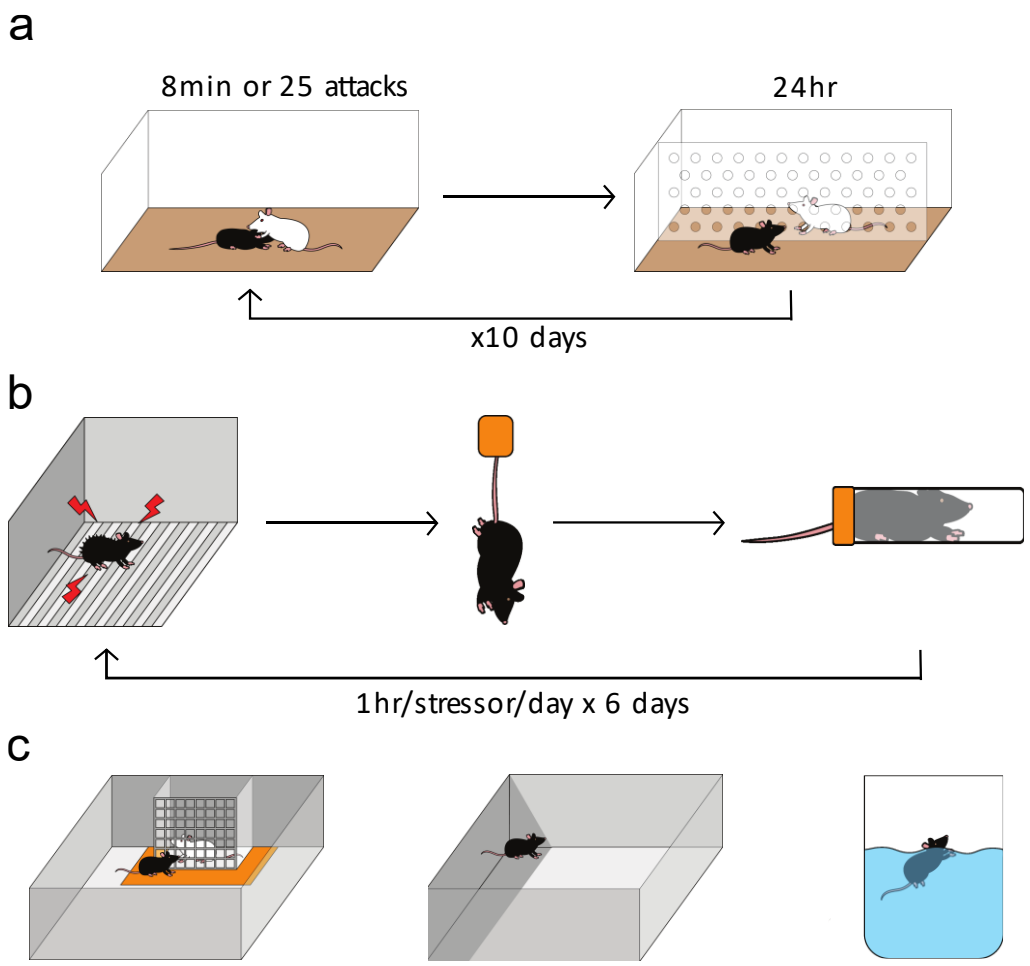

Figure 1. **Stress Protocols a)** Schematic of chronic social defeat protocol. **b)** Schematic of sub-chronic variable stress protocol. **c)** Stress behaviour battery. Left: social interaction test. Target mouse is a male CD1 in the CSDS experiment (as shown) or a female C57 in the SCVS experiment (not shown). Middle: open field test. Right: forced swim test.

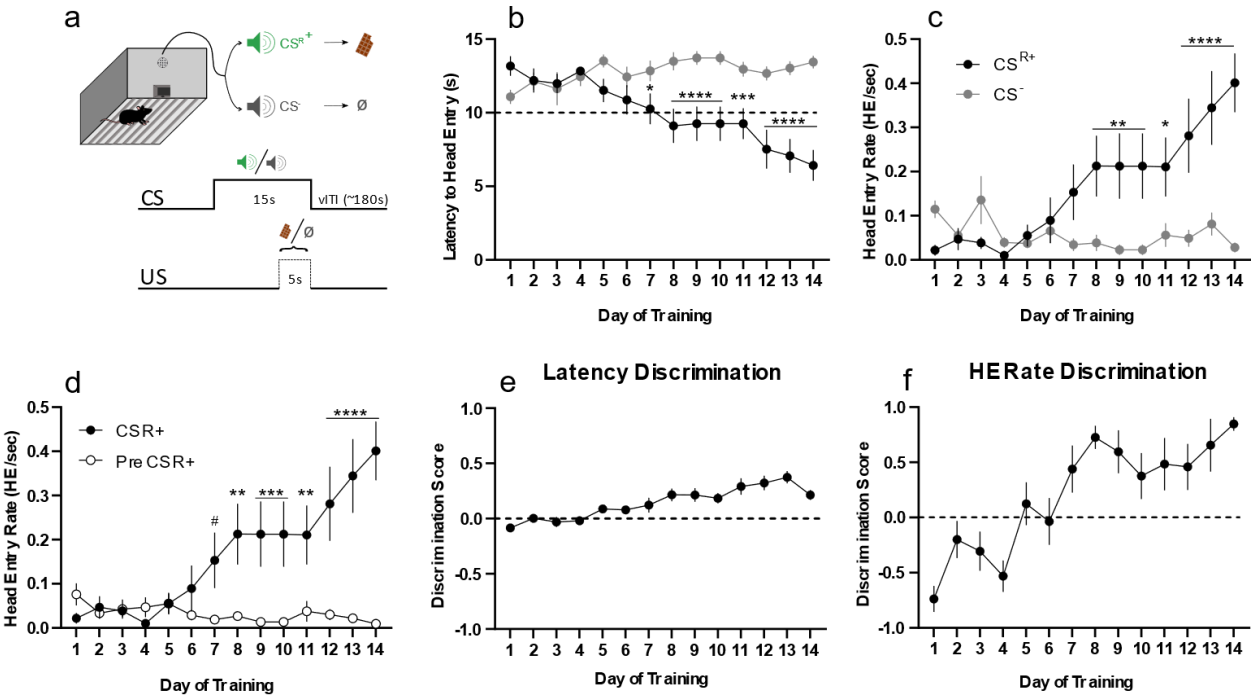

Figure 2. **Appetitive conditioning a)** Schematic of appetitive conditioning trial structure. **b)** Latency to enter the food port during CS^{R+} and CS^{-} trials across all days of training. The dotted line indicates the threshold where rewards become available. **c)** Head entry rate within the first 10 seconds of each CS^{R+} and CS^{-} trial across training. **d)** Head entry rate within the first ten seconds of CS^{R+} trials and the 10 seconds before the CS^{R+} (pre CS^{R+}). **e-f)** Discrimination scores comparing latency and head entry rates between CS^{R+} and CS^{-} trials. Data are expressed as mean ±s.e.m. All post-hoc comparisons are Sidak corrected. $p < 0.1$; #, $p < 0.05$; *, $p < 0.01$; **, $p < 0.001$; ***, $p < 0.0001$; ****.

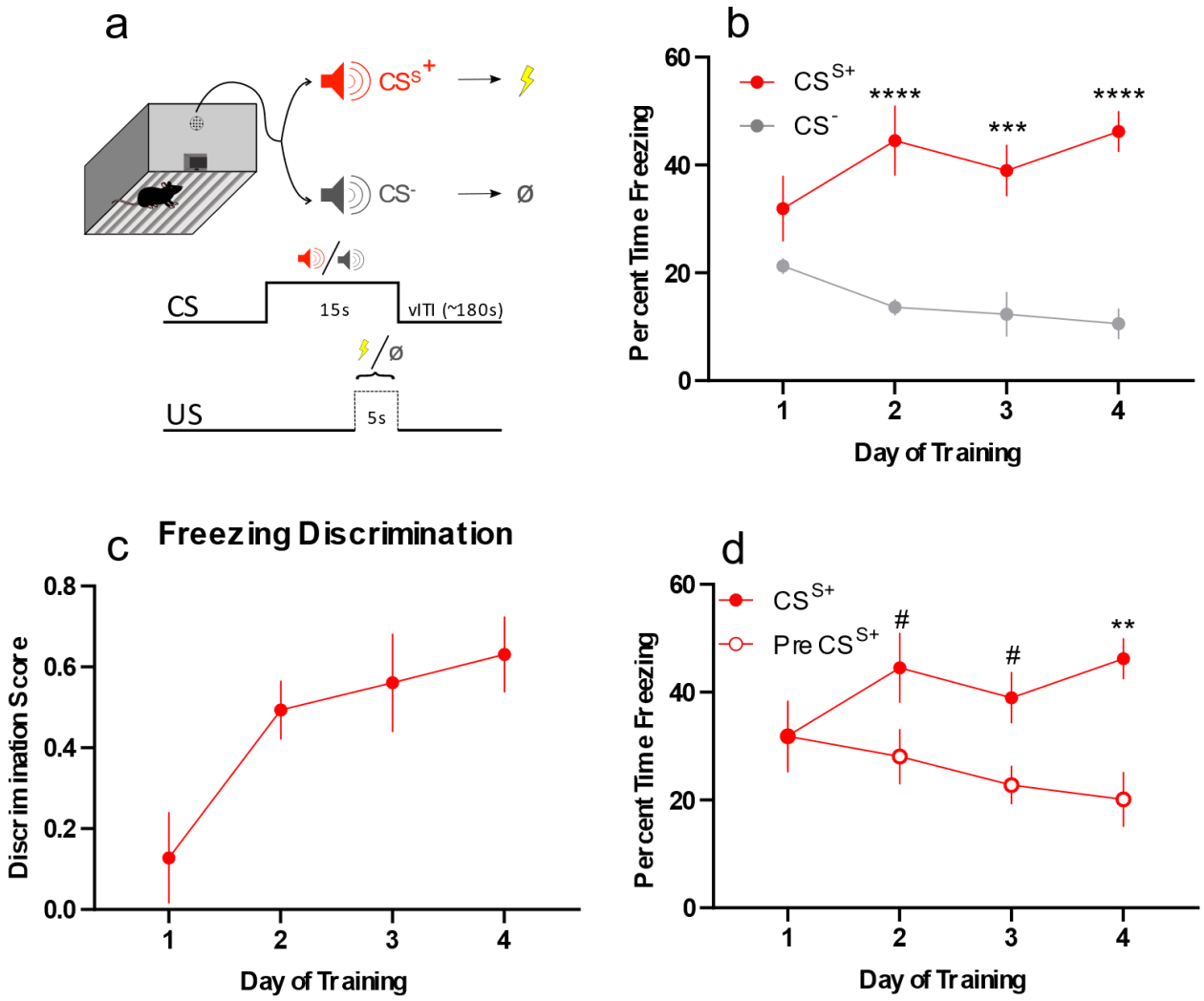

Figure 3. **Aversive conditioning a)** Schematic of aversive conditioning trial structure. **b)** Percent freezing during the first 10 seconds of CS^{S+} and CS^{-} trials across training. **c)** Discrimination scores comparing percent freezing between CS^{S+} and CS^{-} trials. **d)** Percent freezing during the first ten seconds of CS^{S+} trials and the 10 seconds before the CS^{S+} (pre CS^{S+}). Data are expressed as mean ±s.e.m. All post-hoc comparisons are Sidak corrected. $p < 0.1$; #, $p < 0.05$; *, $p < 0.01$; **, $p < 0.001$; ***, $p < 0.0001$; ****.

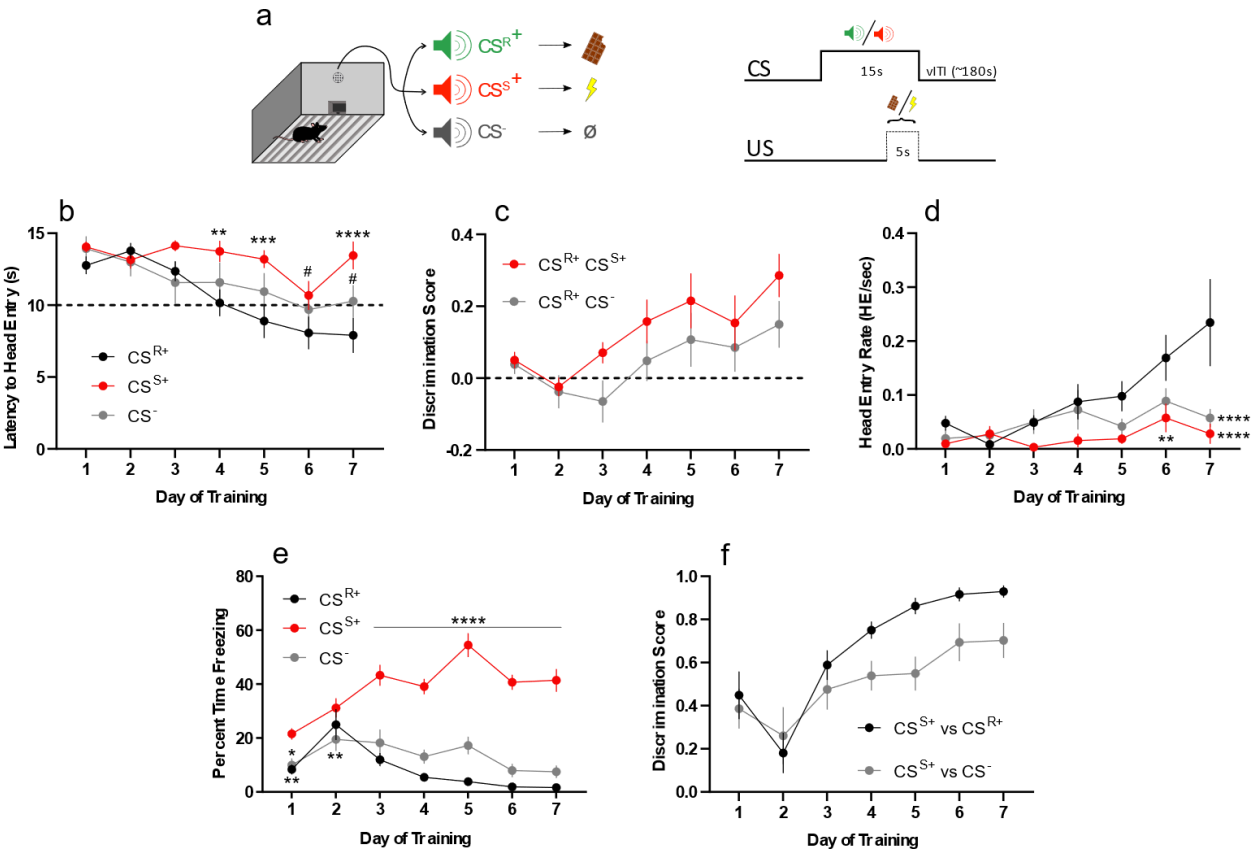

Figure 4. **Pavlovian Valence Discrimination Task a)** Schematic of the Pavlovian valence discrimination task trial structure. **b)** Latency to enter the food port during all trial types across training. Post-hoc comparisons with CS^{R+} values. **c)** Discrimination scores comparing latency to enter the food port between CS^{R+} and CS^{S+} trials and CS^{R+} and CS^{-} trials. **d)** Head entry rate within the first 10 seconds of each CS^{R+} and CS^{-} trial across training. Post-hoc comparisons with CS^{R+} values. **e)** Percent time freezing during the first 10 seconds of each trial type across training. Post-hoc comparisons with CS^{S+} values. **f)** Discrimination scores comparing percent of time freezing between CS^{S+} and CS^{R+} trials and CS^{S+} and CS^{-} trials. Data are expressed as mean ±s.e.m. All post-hoc comparisons are Sidak corrected. $p < 0.1$; #, $p < 0.05$; *, $p < 0.01$; **, $p < 0.001$; ***, $p < 0.0001$; ****.

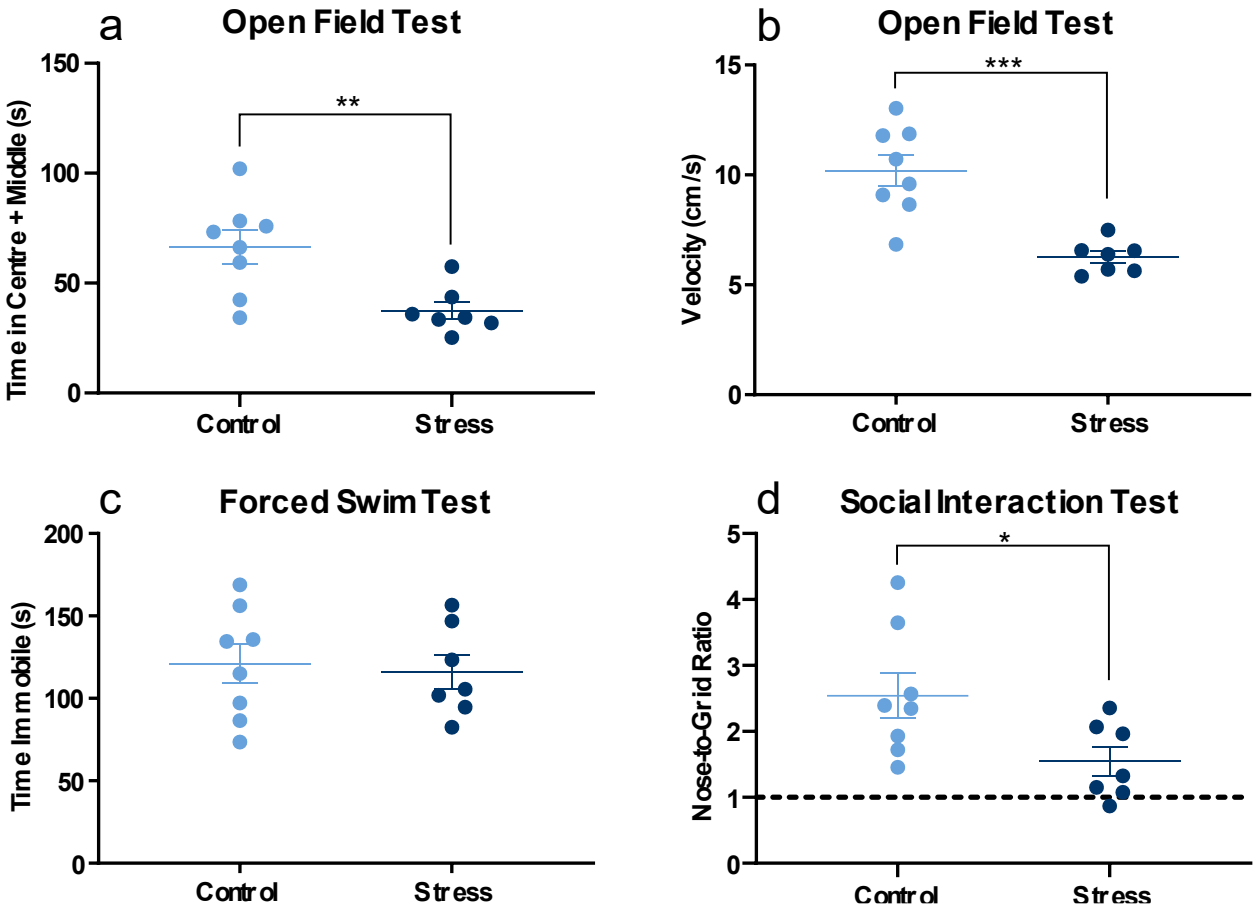

Figure 5. **Post-CSDS Behaviour a)** Time spent in the centre and middle portions of the open field. **b)** Mean velocity during the open field test. **c)** Time spent immobile during the forced swim test. **d)** Nose-to-grid ratio calculated after the social interaction test. Data are expressed as mean ±s.e.m. Data points represent individual animal means. $p < 0.05$; *, $p < 0.01$; **, $p < 0.001$; ***.

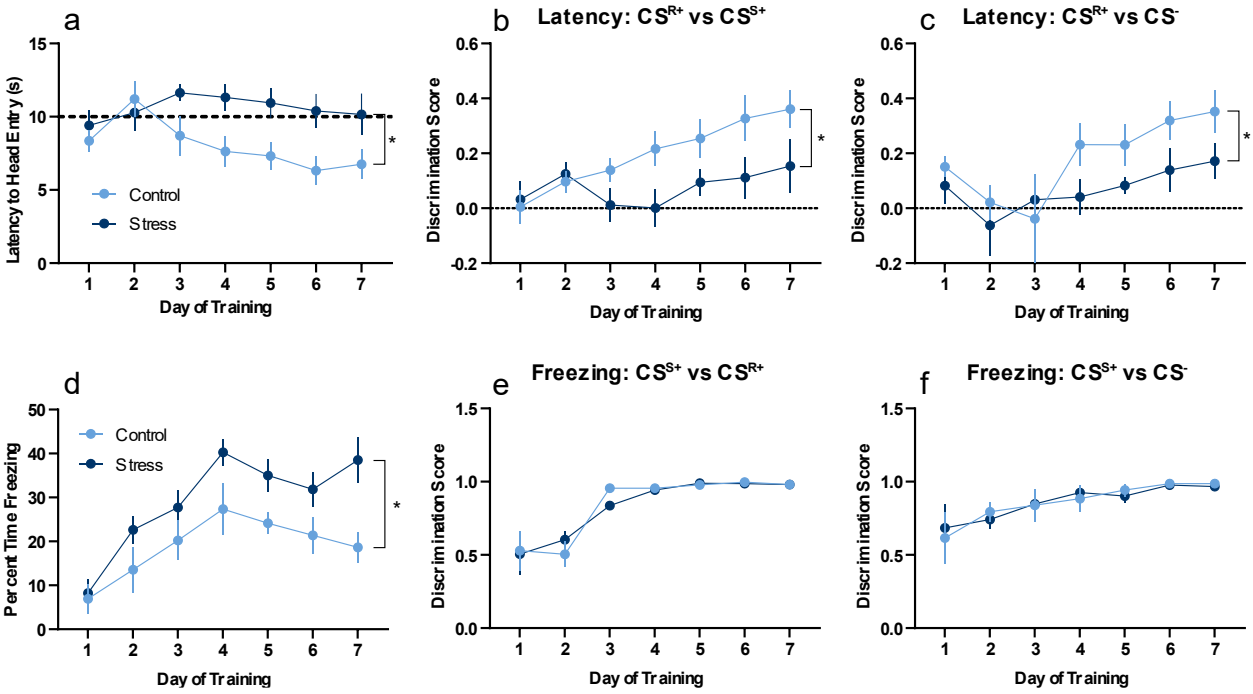

Figure 6. **Effect of CSDS on Appetitive and Aversive Conditioning a)** Latency to enter the food port during the CS^{R+} across training in control and stressed males. **b)** CS^{R+} vs CS^{S+} latency discrimination scores across training in control and stressed males. **c)** CS^{R+} vs CS^{-} latency discrimination scores across training in control and stressed males. **d)** Percent time spent freezing during the CS^{S+} across training in control and stressed males. **e)** CS^{S+} vs CS^{R+} freezing discrimination scores across training in control and stressed males. **f)** CS^{S+} vs CS^{-} freezing discrimination scores across training in control and stressed males. Data are expressed as mean ±s.e.m. $p < 0.05$; *.

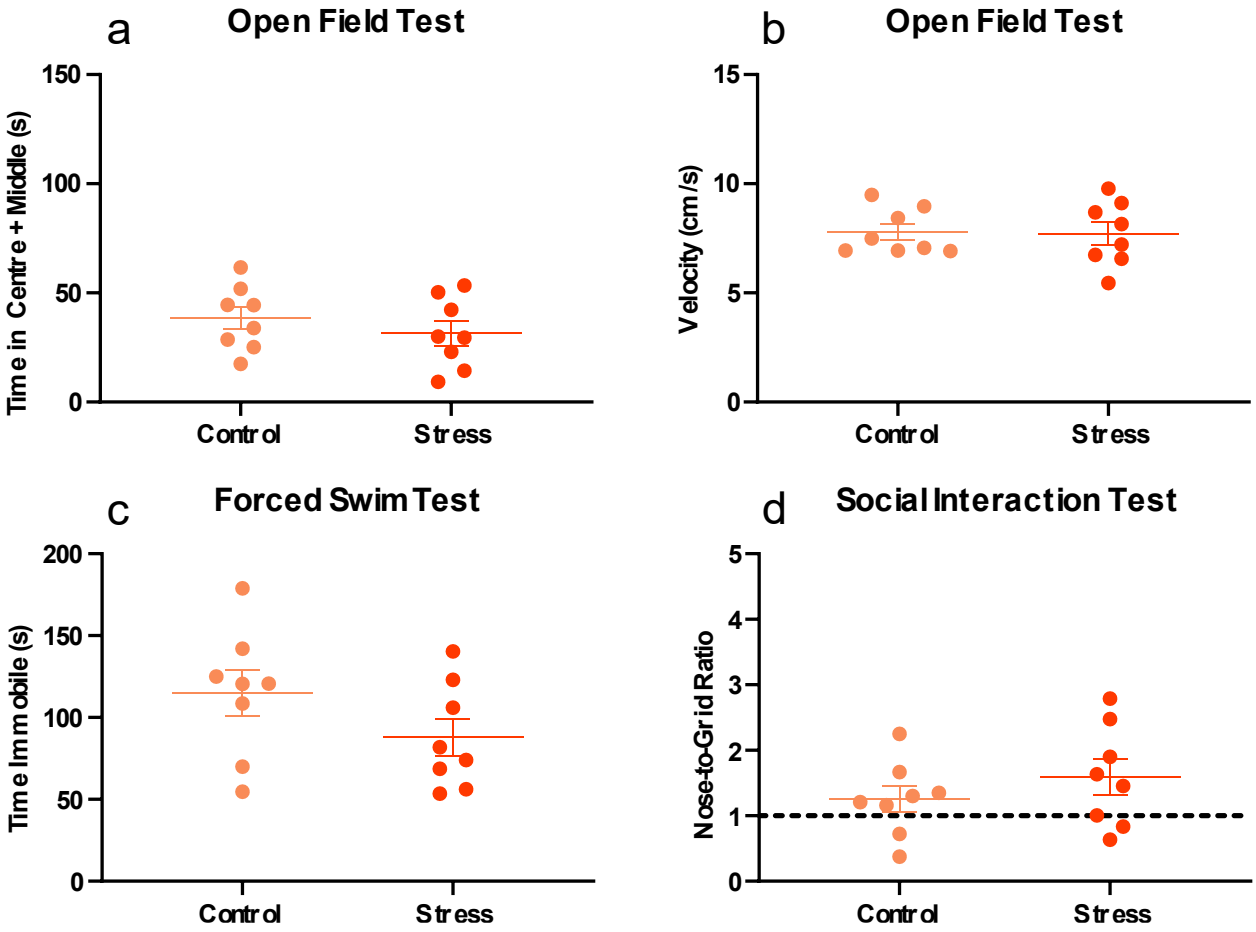

Figure 7. **Post-SCVS Behaviour a)** Time spent in the centre and middle portions of the open field. **b)** Mean velocity during the open field test. **c)** Time spent immobile during the forced swim test. **d)** Nose-to-grid ratio calculated after the social interaction test. Data are expressed as mean ±s.e.m. Data points represent individual animal means.

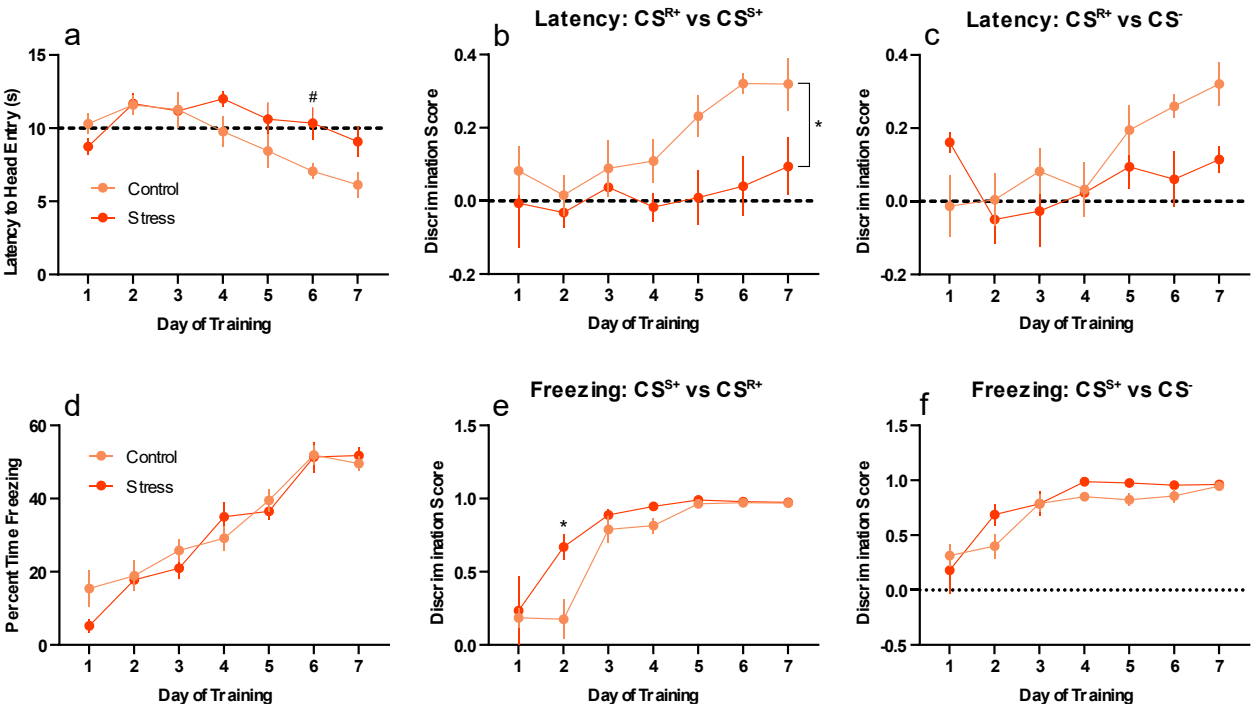

Figure 8. **Effect of SCVS on Appetitive and Aversive Conditioning a)** Latency to enter the food port during the CS^{R+} across training in control and stressed females. **b)** CS^{R+} vs CS^{S+} latency discrimination scores across training in control and stressed females. **c)** CS^{R+} vs CS^{-} latency discrimination scores across training in control and stressed females. **d)** Percent time spent freezing during the CS^{S+} across training in control and stressed females. **e)** CS^{S+} vs CS^{R+} freezing discrimination scores across training in control and stressed females. **f)** CS^{S+} vs CS^{-} freezing discrimination scores across training in control and stressed females. Data are expressed as mean ±s.e.m. All post-hoc comparisons are Sidak corrected. $p < 0.1$; #, $p < 0.05$; *.

Appendix A

Custom code for the PVD task, data translation and extraction can be found at the following repository:

https://github.com/tchclark/PVD_code_bagotlab