2022

Double Trouble: The Development and Use of a Novel Spatial Memory Task to Study Depression in a Female Rodent Model

Ekaterina L. Koelliker
Colby College

Double Trouble: The Development and Use of a Novel Spatial Memory Task to Study Depression in a Female Rodent Model

Ekaterina L. Koelliker
Colby College

Abstract

Preclinical rodent models of depression are important for improving our understanding of the behavioral and neurobiological implications of the disorder. However, the current behavioral assays used to assess depressive symptoms in rodents have substantial shortcomings; they are basic, test animals individually, and do not evaluate animals for extended periods. The primary goals of the present study, which was divided into two experiments, were to develop a novel task that could be used to study spatial memory and to apply the task to rodent models of depression. Both experiments used a circular arena with 10 identical jars to analyze the spatial navigation abilities of rats. To target the intersection between social and cognitive changes that result from depression, the location of a food reward in the arena was signaled to the rats by a social cue. Unlike a traditional conditional discrimination task, the identity of a conspecific rat served as the context which indicated where an animal should navigate, and rats performed the task in pairs. In Experiment 1, we demonstrated that rats were able to use a social context to determine the location of the food reward. Moreover, rats performed better in the task when they were paired with their cagemate compared to when they were paired with a different rat. In Experiment 2, the task was applied to corticosterone models of depression. We found that performance on the task may have been impaired by exposure to high levels of corticosterone. Thus, the present thesis contributes to our understanding of the corticosterone model of depression and extends upon the current behavioral assessments used to study depression in rodents.

Introduction

Major Depressive Disorder (MDD) is the leading cause of disability worldwide (WHO, 2021) with a lifetime prevalence of 20% (Hasin et al., 2018). Distinct from typical changes in mood and temporary feelings of sadness in response to challenges in everyday life, depression is a debilitating, chronic mental health disorder characterized by decreased mood and anhedonia (i.e., loss of pleasure in activities that an individual once found enjoyable) that persists for most of the day over a period of at least two weeks (American Psychiatric Association, 2013). In addition to these symptoms, depression can result in concentration difficulties, excessive feelings of guilt, suicidal thoughts, or fatigue, which interfere with several aspects of an individual's life such as functioning at work (Kennedy, 2008). Depression presents in different populations at different rates. Notably, the disorder is nearly twice as prevalent in females compared to males (Cyranowski et al., 2000). Despite this, female rodent models of depression are severely understudied in neurobiological literature (Lopez & Bagot, 2021).

Individuals with depression display cognitive deficits such as memory and decision-making (Castaneda et al., 2008). In neurotypical individuals, the hippocampus and prefrontal cortex (PFC) are two brain regions that are important for cognition (Sigurdsson & Duvarci, 2016). Behavior-focused studies have revealed that the depressive state produced by chronic mild stress in rats impairs many reward-related learning tasks. While the acquisition of a Pavlovian stimulus-response association was not impacted by chronic stress, the formation of an operant conditioning association and goal-directed learning were both impaired (Xu et al., 2017). Spatial memory is also weakened in depression, as evidenced by stressed rats exploring the novel and alternate arms in a Y maze with similar frequencies in comparison to control rats who entered the novel arm more than the alternate arm (Kleen et al., 2006). While it is possible that

these deficits, as well as those identified concerning operant conditioning, are due to the hallmark depressive symptoms of anhedonia and decreased motivation, chronic stress did not disrupt a rat's incentive to explore nor its desire for a reward in operant tasks (Kleen et al., 2006). Altogether these findings suggest that depression and chronic stress result in an array of cognitive impairments.

There is a large body of research on the biological basis of depression that points to several key neurological findings (Krishnan & Nestler, 2008). One such finding is the disruption of neural plasticity, which is the brain's vital ability to adjust its activity in response to external and internal stimuli (Liu et al., 2017). Perhaps unsurprisingly, two neural regions that are significantly impacted by the disorder are the hippocampus and the prefrontal cortex (PFC) (Zhang et al., 2018). The hippocampus is especially vulnerable to changes induced by stress and depression, which points to how deficits in this neural structure maintain the disorder. Notably, depression is associated with reduced hippocampal volume (Chan et al., 2016) and neurogenesis (Jacobs et al., 2000).

The hippocampus plays a role in the regulation of the hypothalamic-pituitary-adrenocortical (HPA) axis, which is the system that is responsible for the stress response (Jankord & Herman, 2008). In reaction to acute stress, glucocorticoids are secreted to gather the energy resources required to meet an anticipated need (Lupien et al., 1998). The release of glucocorticoids is preceded by the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland, which is stimulated by corticotropin-releasing hormone (CRH) from the hypothalamus (Pariante & Lightman, 2008). Glucocorticoids are involved in feedback inhibition on CRF and ACTH as well as regulation of neuronal processes and anatomy including the survival of neurons, the size of the hippocampus, neurogenesis, and memory acquisition

(Pariante & Lightman, 2008). While the activity of the HPA axis is essential for organism survival, the elevation of glucocorticoid levels can have adverse health effects (McEwen, 2008). Moreover, increased levels of the primary glucocorticoids in humans (cortisol) and rats (corticosterone) as well as increased size and activity of the pituitary and adrenal glands are linked to depression (Vale, 2005).

The cognitive deficits observed in individuals with depression are consistent with chronic stress-induced changes in dendritic morphology in the hippocampus as observed in primate and rodent studies (Kleen et al., 2006). Specifically, elevated basal corticosteroid levels and, thus, stress result in hippocampal impairments of long-term potentiation (LTP), which is the process by which connections between neurons become stronger (Kim & Diamond, 2002). Moreover, chronic stress results in the enhancement of the process responsible for synaptic weakening, or long-term depression (LTD) (Xu et al., 1997). When hippocampal LTP is impaired as a result of stress, hippocampus-dependent explicit memory is also diminished, and several prominent theories of memory hypothesize that hippocampal LTP is a key neuronal substrate in memory processes (Hebb, 2002; Pittenger & Duman, 2008; Whitlock et al., 2006).

On a neurobiological level, finding one's way in an environment and accurately navigating involves an intricate network of neurons. Degradation in the hippocampus is significantly correlated with spatial learning deficits as well as elevated basal plasma corticosterone levels in rats (Arbel et al., 1994). Greater loss of neurons in the pyramidal cell fields of the hippocampus was observed in cognitively impaired rats compared to their cognitively unimpaired counterparts as assessed through a water maze task (Issa et al., 1990). Place cells in the CA1 region of the hippocampus are neurons that encode data about an animal's representation in an environment as displayed by action potential firing when that animal is in

the cell's "place field" and the absence of firing in response to being positioned elsewhere (O'Keefe, 1976). The existence of these neurons was initially discovered in the pyramidal cell layer of the hippocampus as rats foraged in a particular location of an environment (O'Keefe, 1976). Therefore, several prominent theories of spatial navigation have suggested that place cells could tell the animal about where they are located in space, forming the basis of a so-called "cognitive map," in reference to its possible connection to a cartographic map (e.g., allowing an animal to take novel shortcuts; O'Keefe and Nadel, 1978).

The prefrontal cortex allows a navigator to evaluate path options and make decisions about which they will select. In humans, increased activity is recorded in the prefrontal areas as navigators replanned a route (Javadi et al., 2017) or planned the shortest possible route to a goal location (Kaplan et al., 2017). Interestingly, the latter process of forming a shortcut was associated with increased coupling between the prefrontal region and the hippocampus (Kaplan et al., 2017). In rats, neuronal firing in the medial prefrontal cortex (mPFC) represented place fields that were consistent with the locations of fixed goal regions (Hok et al., 2005). As a result, it was concluded that this pattern of firing could be linked to the motivational salience of the locations and, thus, that the mPFC is important for encoding path planning (i.e., goals related to spatial navigation) (Hok et al., 2005).

Navigation is often framed as an individual process, especially in the scope of research with most wayfinding studies analyzing the cognitive processes employed by an individual as they navigate an environment alone (Dalton et al., 2019). This concentration on individual wayfinding is counterintuitive; much of the time animals (non-human and human alike) spend traveling through space is in the presence of others and, as a result, their movement is influenced by others (Dalton et al., 2019). Social wayfinding encompasses the behaviors, interactions, and

related cognitive processes as two or more animals navigate through an environment together (Montello & Sas, 2006).

The recent discovery of social place cells, which fire in response to the position of a conspecific, offers neurobiological evidence for social wayfinding (Bray, 2018). As a demonstrator rat turned either left or right in a T-maze with an observer rat watching, specific neurons in the dorsal CA1 (dCA1) of the observer rat fired, displaying place fields that corresponded with the demonstrator rat's position (Danjo et al., 2018). In bats, a spatial observational-learning task paired with hippocampal dCA1 recordings revealed the same subset of neuronal firing in the brain of an observer as it observed the location of its conspecific (Omer et al., 2018). Considering that the neurobiological structures that underpin depression and navigation overlap, and social processing is implicated in depression, it is reasonable to predict that social wayfinding may be impaired for individuals with depression.

Presently, the models by which researchers investigate depression impact and limit our understanding of the disorder as they do not encompass the multifaceted nature of depression. In rodent models, tasks to assess depression include fear conditioning (for evaluating cognition and emotion), forced swim test (for behavioral despair), shock avoidance (for hopelessness), elevated plus maze (for anxiety symptoms), and sucrose preference (for anhedonia) (Wang et al., 2017). These tasks are short in duration, utilize limited measures, and assess rats alone; however, rats are social animals like humans, pointing to the necessity for tasks which they perform together. A separate array of tests is utilized to understand memory and cognition such as the radial arm maze and the object recognition task, and these assessments are less often integrated with those for depressive symptoms (Price & Duman, 2020). Moreover, the study of spatial navigation and depression simultaneously is even less common (Keynejad et al., 2018). A major objective of the

present thesis is to expand upon the current assessments of depression in rodent models through the development of a novel task.

Previous neglect of the inclusion of female rodent models in neuroscience research resulted in male rodents being studied at disproportionately high rates. The sex bias in the literature is evidenced by a 5.5 to 1 ratio of male to female single-sex animal studies (Beery & Zucker, 2011). Given the higher prevalence of depression in females and the importance of preclinical research in treatment development, this discrepancy represents a prominent issue in health. Here, we use only female rats in an effort to compensate for this preference towards males that exists in the literature.

The goal of our first experiment was to develop a novel task that could be used to assess neurocognitive changes of depression in rat models and would target the intersection between social and cognitive deficits induced by the disorder. To achieve this, a spatial memory task that was guided by a social cue was developed. Instead of learning to associate visual or auditory cues with a particular response as in a traditional conditional discrimination task (e.g., Brown et al., 2005; Murray & Ridley, 1999), we intended for the identity of another rat to serve as the signal indicating where the animal should navigate to in a circular maze. The within-subjects social conditions that the rats learned were “Besties” and “Frenemies” in which the contexts indicated the location of a food reward in a spatial navigation maze. In the Besties condition, rats were exposed to and navigated the maze with their cagemates. Rats were paired with another rat who was not their cagemate in the Frenemies condition. For a given rat, a particular pot contained a food reward in the Besties condition that was distinct from the pot containing the reward in the Frenemies condition (Figure 1). The first experiment provided a baseline for the ability of rats to use a social cue to complete a maze task.

In Experiment 2, nearly all methodological components were kept consistent, except a postnatal drug-induced model of depression was employed to determine how a depressive phenotype would influence performance on the social context-based task. Corticosterone injections were administered to induce depressive symptoms as elevated levels of this hormone serve as a marker for depression (Johnson et al., 2006). To analyze the effects of corticosterone, the navigational abilities of a control group that received saline injections were compared against the experimental group in the maze task. Experiment 1 is novel in that we discovered that rats are sensitive to their social companion as a conditional discrimination cue in a navigation task. Moreover, in Experiment 2 we built on these findings to investigate whether depression leads to detrimental effects in using social cues to guide spatial memory. Together, we aimed to improve the current lens through which we study depression in rat models by integrating memory and social processes.

General Method

Animal and Housing Conditions

The subjects of the study were female Long-Evans rats (n = 24) from Charles River Laboratories (Stone Ridge, NY). Upon arriving at the vivarium, all rats were housed in pairs and reared to adulthood. Rats were housed in individually ventilated and clear polycarbonate cages (30.5 x 30.5 x 18.5 cm) (Thoren Caging Systems, Inc., Hazleton, PA) with access to food (Harlan Rat Chow) and water *ad libitum* until food restriction began (see below). The colony rooms were temperature controlled at a range of 22 ± 1º Celsius and 31-44% humidity, and a 12-hour-light cycle/12-hour-dark cycle (lights turned on at 0800 hr). All training and testing procedures were conducted during the light cycle. Research assistants performed enrichment handling five days per week (between 1000 and 1700 hr) in which groups of four rats at a time

were placed in a 101.5 cm x 46 cm bin lined with corncob bedding to explore and play with a variety of toys. The groupings of rats for enrichment were kept consistent throughout the experiment and were intentional; each group contained two pairs of cagemates whose cages were beside each other in the colony room. For each group, enrichment handling lasted 5-8 minutes. The Colby Institutional Animal Care and Use Committee approved all procedures.

Food Restriction. Because the task was appetitively motivated, rats were food restricted. A measured amount of Harlan Chow was placed inside each cage to maintain body weights that were 85-90% of pre-restriction weights. Body weights were measured daily. Four sucrose pellets were placed in each cage before habituation trials to familiarize the rats with the reward that was used for the task.

Social-Context-Guided Spatial Memory Task

The social-context-guided spatial memory task was developed for the present study to assess the ability of rats to use the identity of a conspecific rat as a conditional discrimination cue to locate a food reward.

Apparatus. A circular arena (150 cm in diameter, 37 cm high) lined with corncob bedding was used as the apparatus. 10 identical glass pots (6 cm in diameter, 5.5 cm high) were filled to the rim with corncob bedding and placed in distinct locations in the maze and the bottom of the pot was affixed to the floor of the arena using Velcro®. Each pot was designated a letter A through J and the locations were kept consistent throughout the experiment (Figure 1).

Materials. Sucrose pellets (Bio-Serv, Dustless Precision Pellets, 45 mg) were used as a food reward. The arena took up most of the room, with large, high-contrast images on the North and South walls. There were no visual cues within the interior of the maze, requiring that the rats learn the locations of the pots relative to extramaze cues. Between each trial, sucrose pellets that

were left over by the rats in the pots were removed. During probe testing, all the pots were removed from the maze, cleaned with OdoBan® spray, and filled with fresh corncob between each trial to eliminate the potential confound of scents of the pots and control for odor being used as a cue. A video camera (Logitech HD Pro Webcam C920, Fremont, CA), which was fixed above the maze on the ceiling and connected to a computer outside of the apparatus room, recorded each trial of training and probe testing. A Dell OptiPlex 5250 AIO and Logitech HD Pro Software version 2.51 were used to collect videos of each trial for later analysis.

Conditions. The within-subjects design consisted of two trial types for each rat. For one trial type, named "Besties", rats were tested with their cagemate. For the other trial type, "Frenemies", rats were paired with another rat who was not their cagemate, but instead a rat that they spent time with during enrichment. For the Besties trials, one pot contained the sugar food reward while a different pot contained the food reward in the Frenemies trials. As a result, the social condition (Besties or Frenemies) signaled the correct location of the food reward (Table 1).

Habituation. For four days before the start of training sessions, rats were put into the maze apparatus in pairs for 10 minutes. During these trials, all the pots contained four sucrose pellets. Each rat was exposed to the Besties condition for Days 1 and 2, and to the Frenemies condition for Days 3 and 4.

One-Baited Pot Training. On a given training day, all 24 rats were subject to either the Besties or Frenemies condition. Training for the one-baited pot task lasted for 10 days (five days of each Social condition) and the Social condition was counterbalanced across the 10 days. An experimenter moved each rat from their home cage in the colony room to a secondary cage with either their Bestie or their Frenemy depending on the assigned condition for the day. Before

placing the rats in the maze, an experimenter added sucrose pellets to one of the 10 pots. For a particular rat, a different pot was baited when they were paired with their Bestie compared to when they were with their Frenemy. For example, in the Besties condition, Rat 1 had pot I baited while in the Frenemies condition, the same rat had pot B baited. The sucrose was placed on top of the pot for the first two days of training (one day of each condition). Beginning on day three of training, the sucrose was buried just below the surface of the corncob bedding in the pot. After rats had spent five minutes in the secondary cage in their designated pairings, the experimenter brought them into the testing room and placed them in the maze at the same time, facing away from the pots. The rats were placed in the maze at a pseudorandom location chosen from eight coordinate directions (north, south, east, west, north-east, north-west, south-east, south-west). The experimenter took the rats out of the maze and returned them to the secondary cage when they had both successfully found the sucrose pellets in the baited pot. Three trials were conducted for each pair of rats per training day.

Probe Testing.

Paired Probe. Following 10 days of training on the one-baited pot task, all rats were assessed in their assigned pairings when none of the pots in the maze contained sucrose pellets to eliminate the possibility that they were relying on olfaction to find the correct pot (i.e. the Target Pot). To establish the social context cue, rats spent time in a cage with either their Bestie or Frenemy as they did in training before being put into the maze in the same pairing. The social condition (Besties or Frenemies) that rats were tested in for their knowledge of the pot's location was counterbalanced across days. Each probe trial lasted for 2 minutes. Videos were recorded of each paired probe trial and the number of incorrect pots that each rat visited before reaching the Target Pot was counted.

Single Probe. Rats were placed alone in the maze that contained no sugar pellets for 2-minute trials. As in training and the paired probe testing, to establish the social context cue, rats spent time in a cage with another rat (either their Bestie or Frenemy depending on the condition) before entering the maze.

Experiment 1

In the first experiment of this two-part study, we sought to verify whether rats could differentiate between two conspecifics and apply this distinction to navigate within a maze. Moreover, a secondary goal was to determine whether the condition (Besties or Frenemies) influenced their ability to use the social cue to navigate to the Target Pot. We predicted that as a result of the amount of time that they spent with their cagemate (i.e., the Besties condition) compared to their non-cagemate (i.e., the Frenemies condition), the rats would perform better on the navigation task when they were paired with their Bestie compared to their Frenemy. Because this was a novel task, we made methodological modifications to improve the experiment during the experiment. Specifically, as noted below, we initially began our training protocol with the Four Baited Pots condition. Then, we subsequently changed to the One Baited Pot condition because we realized this condition would allow us to better elucidate whether animals were choosing the correct pot, a contextual error pot, or a pot that was never rewarded.

Method

Experimental timeline. See Figure 2.

Training.

Four Baited Pots. Protocols for the Four Baited Pot task were identical to those of the One Baited Pot except four of the pots contained sucrose pellets (instead of one pot in the One

Baited Pot task). Rats underwent training on this task for 12 days, which were counterbalanced for Bestie and Frenemy days.

One Baited Pot. Pot assignments for the rats in their Bestie and Frenemy conditions were made such that one of the pots that had the food reward for a given rat in the Four Baited Pot task remained baited in the One Baited Pot task. Videos were recorded of each trial and viewed by research staff who recorded the number of incorrect pots that each rat visited before finding the Target Pot.

Probe Testing.

Paired Probe. Videos were recorded of each paired probe trial and the number of incorrect pots that each rat visited before reaching the Target Pot was counted.

Reminder trials. Following the paired probe trials, rats underwent four additional days of training (two days of Besties, two days of Frenemies). Protocols were kept consistent between the initial 10 training days and these additional training days.

Single Probe. Pots and the bedding within them were cleaned between each trial as described in Materials. Video recordings of the probe testing for Rats 1-12 were viewed by research staff who counted the number of errors that rats made before reaching the Target Pot and measured the latencies (i.e. the amount of time that elapsed) to the Target Pot and the Context Error Pot (i.e., the pot that would be baited for the opposite Social condition).

Statistical analyses. Statistical analyses were conducted using SPSS version 28.0.0.0. The variables that were analyzed were the Social condition (within-subjects) and the Pot Type (within-subjects). Graph Pad Prism (Version 9. 3. 1, 2021) was used to create graphs comparing the latencies to the Target Pot across Social conditions (Besties and Frenemies).

Single Probe. Latencies to the Target Pot were compared across the two Social conditions (Besties and Frenemies) by a paired samples t-test. To assess whether there was an interaction between the Pot Type and the Social Condition when latency was measured, a mixed ANOVA was conducted.

The time between reaching the Target Pot and the Context Error Pot was calculated by subtracting the Latency to the Target Pot from the Latency to the Context Error Pot. As a result, positive values of this measure indicate that the rats reached the Target Pot before the Context Error Pot while negative values indicate that they arrived at the pots in the opposite order. A paired t-test was run between the difference scores for the Social conditions.

Results

Body weights. The average body weights for the rats throughout the experiment are displayed in Figure 3.

Single Probe.

Latency to the Target Pot. To test whether the Social condition influenced how well the rats performed in the task, we conducted a paired samples t-test between Besties and Frenemies. We found a significant difference in latency to the Target Pot (t (11) = -1.976, p = 0.037) in which Besties (M = 4.0417, SD = 3.85686) were faster than Frenemies (M = 9.7833, SD = 8.86462) (Figure 4). Figure 4 shows that rats performed better in the Besties compared to the Frenemies condition.

Latency to the Context Error Pot. We ran a mixed ANOVA between the Pot Type (Target Pot, Context Error Pot) and the Social condition to test whether rats varied in how quickly they went to the Target pot or made a contextual error based on who they were paired with. We found a non-significant interaction (F (1,22) = 2.763, p = 0.111, partial η^2 = 0.112).

Time between reaching the Target Pot and Context Error Pot. To determine if rats were selecting the Target Pot or making a contextual error first, we conducted a paired t-test ($t(11) = 1.490$, $p = 0.082$) between the Social conditions that was non-significant but did display a statistical trend (Figure 5). Figure 5 shows that rats may be better able to make the distinction between the Target and Context Error Pots when assessed with their Bestie ($M = 2.5167$, $SD = 5.75766$) compared to when tested with their Frenemy ($M = -3.3000$, $SD = 10.66703$).

Discussion

This experiment aimed to establish protocols and measures for a novel spatial navigation task that, contrary to previous paradigms, assessed rats in pairs. This work provided us with a baseline of navigation performance in the task to reflect on when applying it to rodent models of depression.

First, our data provided evidence that rats were attuned to the social context (i.e., Besties or Frenemies) and were able to use this context to navigate to the correct pot. The development of this task contributes to the limited body of literature on social wayfinding in rat models. Experiment 1 allowed us to determine the amount of training (five days in each social condition) that would be necessary on the task before assessing performance on probe tests. The number of errors made before getting to the Target Pot and the latencies to the Target and Contextual Error Pots were measures that displayed how well rats navigated in the maze.

We predict that rats may navigate better with rat that they know well (i.e. in the Besties condition) compared to a rat that they don't know very well (i.e. in the Frenemies condition). The present experiment revealed that rats perform better in this spatial navigation task when paired with their cagemate compared to a different (non-cagemate) rat as evidenced by making fewer errors and navigating more quickly to the Target Pot in the Besties condition. The context

of the Besties condition may have been more salient compared to the Frenemies condition because the rats spent most of the time with their Bestie and were only exposed to their Frenemy for a limited amount of time before performing the maze task.

The present experiment is novel in that rats are not only assessed on a navigation task in pairs rather than individually but also reveals that rats can use the identity of a conspecific to make conditional discriminations in a spatial memory test.

Experiment 2

The goal of the second experiment was to determine whether depressive symptoms lead to impairment in performance on the social-context-guided navigation task. The impact of Social (Besties and Frenemies) and Treatment (Saline and Corticosterone) condition on the accuracy with which rats navigated the maze was analyzed.

Method

Experimental timeline. See Figure 6.

Corticosterone Model of Depression

Half of the rats (n = 12) were assigned to the control (Saline) condition and the other rats (n = 12) were given Corticosterone injections, serving as models of depression. Corticosterone (CORT, 4.0 mg/kg) was diluted in a solution of 10 ml saline and 40 μl tween. The injections were administered subcutaneously at an injection volume of 1 ml/kg. Saline (at 1 ml/kg) was delivered to the control group of rats. The dose was selected as it previously has been shown to induce depressive symptoms in rodents, indicating a disruption in HPA-axis functioning (Johnson, Fournier, & Kalynchuk, 2006). Injections were given between 0900 and 1000 hrs, which was consistent with previous studies involving CORT exposure (Kott et al., 2015).

Conditions. Pairings were made such that a given rat's Bestie and Frenemy were in the same Stress Condition as they were to avoid contact between the rats in the two stress conditions and to ensure that non-stressed (Saline) rats did not become stressed out (Table 2).

Training.

Pots were cleaned OdoBan® spray and filled with new corncob between each trial in training. The walls of the apparatus were extended to a height of 21.5 cm. Training was spaced out from injections on a given day, beginning between 1400 and 1600 hr and ending before 1900 hr.

Probe Testing

Single Probe. Each rat's behavior was tracked using ANY-maze® Video Tracking System version 6.05 (Stoelting Co., Wood Dale, IL), which generated the latencies to each pot. In addition, latencies to the Target Pot were compared to those of the Context Error Pot. For rats that never reached the Target Pot or Context Error Pot, the latency was scored as 120 seconds (which was the duration of an entire probe trial). The time between reaching the Target Pot and the Context Error Pot was calculated as in Experiment 1.

Paired Probe. Videos of each trial were recorded and viewed by the research staff. A stopwatch was used to measure the latencies to each pot. The frequencies of the following behaviors were documented: sniffing from behind, following each other, nose-to-nose sniffing, visiting the same pot, and visiting different pots. The proportion of pot visits for which the rats went to the same pot was calculated by dividing the number of times that the rats went to the same pot by the total pot visits.

Statistical analyses. Statistical analyses were conducted using the same version of SPSS as in Experiment 1. The independent variables that were analyzed were the Stress condition (between-

subjects) and Social condition (within-subjects). The same version of Graph Pad Prism as in Experiment 1 was used to produce graphs to compare rat performance across Social (Besties and Frenemies) and Stress conditions (Saline and Corticosterone).

Body weights. To determine whether the Cort exposure resulted in a change in body weight, we calculated the average body weights and ran independent samples t-tests between Saline and Cort groups for each week of the experiment.

Single probe tests. For each Latency to the Target Pot, a 2 x 2 mixed ANOVA was conducted. To further investigate our hypotheses related to the impact of Stress and Social condition on performance, we conducted planned comparison tests. Independent samples t-tests were run for the Latency to the Target Pot between Stress Conditions and paired samples t-tests were run to compare the latencies to the Target Pot and the Context Error pot between Stress and Social conditions. Two-tailed p-values were used as we hypothesized that the effects would tend towards a particular direction with Saline rats performing better than Cort rats.

Paired probe tests. In addition to the same set of 2 x 2 ANOVA's and planned comparison tests as in the Single Probe tests, social interaction behaviors were analyzed. Additional 2 x 2 mixed ANOVA's were conducted for the six interaction behaviors that were measured in the Paired Probe to determine whether the rats were interacting differently based on the Social or Stress condition that they were in. We further investigated the number of times that rats visited different pots through planned comparisons to see whether the Social or Stress conditions affected these interactions. Specifically, independent samples t-tests were run to determine if there was a difference in this behavior between the Besties and Frenemies rats as well as between the Cort and Saline rats within the Besties condition.

Results

Body weights. Figure 7 shows the change in the rats' body weights throughout the experiment in which week 1 represents the start of Cort injections. Cort rats had lower body weights than Saline rats on only weeks 2 (t (11) = -3.3192, p = 0.004) and 3 (t (11) = -2.922, p = 0.009) of Experiment 2 (Figure 7).

Single Probe I.

Latency to the Target Pot. To assess the effects of the Social and Stress conditions on how quickly a rat navigated to the Target Pot, a 2 x 2 mixed ANOVA was conducted. There was no significant main effect of Social condition (F (1,22) = 0.055, p = 0.816, partial η^2 = 0.003) or for Stress condition (F (1,22) = 2.634, p = 0.119, partial η^2 = 0.107), and there was no significant interaction (F (1,22) = 0.850, p = 0.366, partial η^2 = 0.037) (Figure 8). However, planned comparisons revealed that in the Besties condition, the latency to the Target Pot was higher for Cort rats compared to Saline rats (t (22) = 1.860, p = 0.038). A paired samples t-test was run to determine whether there was a significant difference between the two social conditions for Saline rats in the amount of time it took them to get to the Target Pot. There was no significant difference across social conditions on this measure in the Saline rats (t (11) = -1.433, p = 0.090); however, a statistical trend emerged. Figure 8 shows that the latency to the Target Pot was lower in the Besties condition (M = 7.6250, SD = 6.21247) compared to the Frenemies condition (M = 18.3167, SD = 25.31402).

Latency to the context error pot. Two 2 x 2 mixed ANOVA's were conducted to investigate whether there were differences in the speed with which rats went to the Target Pot and the Context Error Pot between the Stress Conditions. One ANOVA explored all rats assessed when they were assessed in the Besties condition and one for the Frenemies condition. For the Besties condition, the analyses yielded non-significant main effects of Pot Type (F (1,22) =

0.133, $p = 0.719$, partial $\eta^2 = 0.006$) and Stress condition (F (1,22) = 1.200, $p = 0.285$, partial $\eta^2 = 0.052$). For the Frenemies condition, the main effects of the Pot Type and Stress condition were also non-significant. The interactions between Pot Type and Stress condition were not significant for the Besties (F (1,22) = 1.992, $p = 0.172$, partial $\eta^2 = 0.083$) (Figure 9) and Frenemies (F (1,22) = 2.036, $p = 0.168$, partial $\eta^2 = 0.085$) (Figure 10).

A planned comparison comparing the latency to the Target Pot and the Context Error Pot for Saline rats in the Besties condition did not reveal statistical significance for a paired t-test (t (11) = -0.942, $p = 0.183$); however, a statistical trend emerged in which rats reached the Target Pot before the Context Error Pot. Although statistical significance was not achieved when the two pot types were compared for the Cort rats in the Besties condition using a planned comparison (t (11) = 1.571, $p = 0.072$), this does represent a statistical trend in which the rats went to the Context Error Pot before the Target Pot. Figure 9 shows that Saline rats tended to arrive at the Target Pot faster compared to the Context Error Pot in the Besties condition while rats exposed to Cort displayed the opposite tendency and may have gone to the Context Error Pot faster than the Target Pot.

In the Frenemies condition, planned comparisons between the latency to the Target Pot and Context Error Pot for both stress conditions failed to reach significance for both Saline rats (t (11) = -1.359, $p = 0.101$) and Cort rats (t (11) = 0.486, $p = 0.318$). There was a statistical trend in which Saline rats went to the Target Pot faster than they went to the Context Error pot, which is displayed in Figure 10.

Time Between Reaching the Target Pot and Context Error Pot. To test whether the Social and Stress conditions impacted a rat's decision to go to the Target Pot or the Context Error Pot first, we conducted a mixed ANOVA of the Time Between Reaching the Target Pot

and Context Error pot (i.e., Latency to the Context Error Pot – Latency to the Target Pot). We did not observe main effects of Social condition (F (1,22) = 0.567, p = 0.459, partial η^2 = 0.025) or Stress condition (F (1,22) = 2.698, p = 0.115, partial η^2 = 0.109), and the interaction was not significant (F (1,22) = 0.149, p = 0.704, partial η^2 = 0.007). Planned comparisons between the Stress conditions failed to meet significance for Besties condition (t (22) = -1.411, p = 0.086). Figure 11 shows the trend that emerged in the Besties condition in which Saline rats (M = 9.5917, SD = 35.29078) were more likely to go to the Target Pot before the Context Error Pot while the Cort rats (M = -5.6583, SD = 12.47342) tended to visit the pots in the opposite order. When being assessed in the Frenemies condition, rats demonstrated similar behavior, although it did not yield statistical significance (t (22) = -1.427, p = 0.084). Figure 11 displays the trend in which Saline rats (M = 17.0000, SD = 43.33862) tended to visit the Target Pot ahead of the Context Error Pot while the Cort rats (M = -3.2667, SD = 23.29535) went to the Context Error Pot first.

Retention Probe.

Following two weeks absent of training and testing on the task, rats were evaluated in a Retention Probe that was set up identically to the earlier Probe test to determine whether they were able to navigate to the Target Pot in either Social condition.

Latency to Target Pot. To determine whether Social and Stress conditions influenced how quickly a rat navigated to the Target Pot, we conducted a 2 x 2 mixed ANOVA. There was no significant main effect of Social condition (F (1,22) = 1.527, p = 0.223, partial η^2 = 0.067) or for Stress condition (F (1,22) =0.348, p = 0.561, partial η^2 = 0.016), and there was no significant interaction (F(1,22) = 1.096, p = 0.307, partial η^2 = 0.047).

Latency to the Context Error Pot. To explore whether latencies to the Target Pot and Context Error Pot varied based on the Stress condition, two 2 x 2 mixed ANOVA's were conducted: one in which Besties were assessed and one in which Frenemies were assessed. For the Besties, the ANOVA did not reveal a statistically significant interaction ($F(1,22) = 0.425$, $p = 0.521$, partial $\eta^2 = 0.019$) and the main effects of the Pot Type ($F(1,22) = 1.169$, $p = 0.291$, partial $\eta^2 = 0.050$) and the Stress condition ($F(1,22) = 0.000$, $p = 0.986$, partial $\eta^2 = 0.000$) were not significant.

When rats were assessed in the Frenemies condition, there was no main effect of Pot Type ($F(1,22) = 0.006$, $p = 0.939$, partial $\eta^2 = 0.000$) or Stress condition ($F(1,22) = 0.334$, $p = 0.569$, partial $\eta^2 = 0.015$). The interaction between Pot Type and Stress condition was statistically significant ($F(1,22) = 4.592$, $p = 0.043$, partial $\eta^2 = 0.173$) (Figure 12). Figure 12 shows that the Saline rats ($M = 12.7583$, $SD = 15.85247$) went to the Context Error Pot faster than the Cort rats ($M = 31.6417$, SD 35. 38970) did.

Paired Probe.

We aimed to develop a better understanding of how the rats were interacting with each other during the task by assessing them in pairs. Two re-training days (one day in each Social condition) preceded the Paired Probe. Unlike previous probe tests, the paired aspect of navigation on this test was consistent with how the rats were trained.

Latency to Target Pot. We ran a 2 x 2 mixed ANOVA to see whether Social and Stress conditions had an impact on the rats' latencies to the Target Pot. There was no significant main effect of Social condition ($F(1,22) = 0.007$, $p = 0.936$, partial $\eta^2 = 0.000$) or of Stress condition ($F(1,22) = 0.348$, $p = 0.561$, partial $\eta^2 = 0.016$), and there was no significant interaction ($F(1,22) = 1.203$, $p = 0.285$, partial $\eta^2 = 0.052$).

Social Interactions. To gauge whether the Social or Stress condition was influencing the frequency with which rats interacted with each other, five 2 x 2 mixed ANOVA's were conducted for the followings social interaction behaviors: sniffing from behind, following each other, nose-to-nose sniffing, visiting the same pot, and visiting different pots. The proportion of pot visits that the rats went to the same pot (i.e. the number of times at the same pot divided by total pot visits) was calculated and analyzed by a mixed ANOVA.

There was a significant interaction between the Social Condition and Treatment for the frequency with which rats visited different pots ($F(1,10) = 9.918$, $p = 0.010$, partial $\eta^2 = 0.498$). There was a main effect of Social Condition ($F(1,10) = 18.443$, $p = 0.002$, partial $\eta^2 = 0.648$), but no main effect was seen for Stress Condition ($F(1,10) = 0.839$, $p = 0.381$, partial $\eta^2 = 0.077$) (Figure 13). To further investigate our finding, we conducted a paired samples t-test across both Stress conditions which revealed that Frenemies ($M = 11.9167$, $SD = 0.66856$) were visiting different pots significantly more often ($t(11) = -3.191$, $p = 0.004$) than Besties ($M = 10.6667$, $SD = 1.30268$). These findings suggest that when rats were in the maze with their Frenemy, they were more frequently at different pots than when they were performing the task with their Bestie. Amongst rats assessed in the Besties condition only, Cort rats ($M = 11.3333$, $SD = 1.36626$) went to different pots significantly more times ($t(5) = 2.169$, $p = 0.041$) than Saline rats ($M = 10.0000$, $SD = 0.89443$). This indicates that, compared to the Saline rats, the rats exposed to Cort were more likely to be separated from the other rat that they were in the maze with. Figure 13 shows the relative frequencies with which rats in the Social and Stress conditions entered different pots.

The interactions between Social and Stress condition were not significant for the following behaviors: sniffing from behind ($F(1,10) = 0.085$, $p = 0.777$, partial $\eta^2 = 0.008$),

following each other (F (1,10) = 0.172, $p = 0.687$, partial $\eta^2 = 0.017$), nose-to-nose sniffing (F (1,10) = 0.678, $p = 0.429$, partial $\eta^2 = 0.063$), visiting the same pot (F (1,10) = 3.913, $p = 0.076$, partial $\eta^2 = 0.281$), and the proportion of pot visits at the same pot (F (1,10) = 0.881, $p = 0.370$, partial $\eta^2 = 0.081$). Across the social behavior measures, the interaction between the Stress and Social conditions influenced the number of times that the rats were at different pots in the maze, but did not have an impact on their other social interactions (e.g. sniffing, following, and visiting the same pot).

Single Probe II.

To further explore the behavioral patterns that we observed in the initial probe, we assessed rats individually following two days of re-training in each Social condition.

Latency to Target Pot. We conducted a 2 x 2 mixed ANOVA to see whether the Social and Stress conditions affected the rats' latencies to the Target Pot. We found a significant main effect of Stress condition (F (1,22) = 4.311, $p = 0.050$, partial $\eta^2 = 0.164$). There was no significant main effect of Social condition (F (1,22) = 1.945, $p = 0.177$, partial $\eta^2 = 0.081$) and there was no significant interaction (F(1,22) = 2.218, $p = 0.151$, partial $\eta^2 = 0.092$) (Figure 14). Figure 14 shows that Cort rats entered the Target Pot faster than Saline rats.

Latency to the Context Error pot. Two 2 x 2 mixed ANOVA's (one for Besties and one for Frenemies) were run to determine whether the latency to enter the Target Pot and Context Error Pot were different depending on the Stress condition. In the Besties condition, the analyses yielded non-significant main effects of Pot Type (F (1,22) = 2.172, $p = 0.155$, partial $\eta^2 = 0.090$) and Stress condition (F (1,22) = 0.078, $p = 0.783$, partial $\eta^2 = 0.004$) and a non-significant interaction (F (1,22) = 0.150, $p = 0.702$, partial $\eta^2 = 0.007$).

For the Frenemies condition, there was a main effect of the Stress condition ($F(1,22) = 5.813$, $p = 0.025$, partial $\eta^2 = 0.209$). There was no main effect of Pot Type ($F(1,22) = 1.007$, $p = 0.326$, partial $\eta^2 = 0.044$) and the interaction between Pot Type and Stress condition was not significant ($F(1,22) = 2.036$, $p = 0.168$, partial $\eta^2 = 0.085$) (Figure 15). Figure 15 shows that Cort rats navigated to both the Target Pot and the Context Error Pot faster than Saline rats.

Discussion

The goal of Experiment 2 was to apply the social-context-guided spatial navigation task that we developed in Experiment 1 as a means of analyzing the behavior of rodent models of depression. The same trend involving Social Condition as Experiment 1 emerged in which Besties tended to navigate more accurately and quickly compared to Frenemies, supporting the consistency in methods across the two experiments.

Given the social and cognitive deficits associated with depressive symptoms, we predicted that exposure to high levels of Cort would impair performance on the task. While the rats were assessed in several probe tests throughout Experiment 2, the initial probe (Single Probe 1) yielded the most robust findings. Compared to those that received Saline injections, rats in the Cort condition were slower to get to the Target Pot when navigating with their cagemate. The rats exposed to high levels of Cort may be slower to reach the Target Pot in the Frenemies condition and may have been more likely to make contextual errors in both Social conditions. Overall, this experiment enriches the array of assessments that are currently used to study depression in rodents because it combines the cognitive and social aspects of depression, and assesses rats in pairs.

General Discussion

The overarching aim of the present thesis was to expand upon the current tasks that are used to assess rodent models of depression in the literature and develop a richer, more

comprehensive test in which animals perform in pairs rather than individually. Additionally, the present study investigated female rats to overcome the sex bias in neurobiology toward male rodent models.

As predicted, rats navigated more accurately when paired with a rat who they knew better compared to a rat who they were less familiar with. Perhaps the context of the Besties condition was more salient compared to the Frenemies condition because the rats spent most of the time with their Bestie and were only exposed to their Frenemy for a limited amount of time before performing the maze task. Through analyses of the rats' social interactions with each other as they navigated the maze in Paired Probe trials, we intended to determine whether there were differences in the frequencies of the following behaviors between the rats in the Besties and Frenemies conditions: following each other, sniffing (from behind and nose-to-nose), visiting different pots, and visiting the same pot. The proportion of pot visits for which rats in a pair went to the same pot was compared between the two Social conditions as well. There were no significant differences in the number of times that animals engaged in these behaviors besides the number of times that they visited different pots. Rats visited different pots than their co-navigator more often in the Frenemies condition compared to the Besties condition. While the implications of these findings taken together are complex, it is unlikely that the behaviors of following, sniffing, and visiting the same pot were responsible for the enhanced navigation in the Besties condition compared to the Frenemies condition. However, it seems possible that the higher frequency with which rats in the Frenemies condition visited different pots may play a role in the less accurate social wayfinding of the condition compared to the Besties condition.

Most spatial memory literature in humans and rodents up until this point focuses on animals as they navigate alone (Dalton et al., 2019). As a result, the difference in how well (human and

non-human) animals navigate with an animal with whom they have a close relationship compared to an animal who they don't know as well has not been previously explored. However, the presence and influence of others as we navigate is important due to its prevalence in daily life, and neglecting it leads to distortions in how we conceptualize the wayfinding process (Dalton et al., 2019).

The recent evidence of social place cells supports the idea that, when they navigate in the presence of a conspecific, rats are using the location of the other rat to make decisions about where to go in the environment (Danjo Teruko et al., 2018; Omer David B. et al., 2018). The co-presence of others has a significant influence on navigational decisions because individuals choose to either follow or avoid their co-navigator depending on the situation as evidenced by the wayfinding patterns of pairs of humans in a T-shaped virtual environment (Yassin et al., 2021). It was also noted that participants most often navigated together and reunited quickly in their dyad if separated during the task (Yassin et al., 2021). A future direction of the present study could involve looking for similar patterns in the rats as they navigated in the maze such as the establishment of hierarchical roles in which one rat acts as a follower and the other as a leader.

To address the primary goal of developing the navigation task, which was to study depression, we replicated the behavioral and neurobiological features of the disorder by exposing rats to high levels of Cort in Experiment 2. Because the same structures that exhibit deficits in depression are also implicated in spatial learning and memory, we hypothesized that rats subjected to Cort would have a more difficult time remembering the location of a particular pot in the maze. Since the location of the pot was signaled by the identity of another rat, the task also reflected the animals' abilities to make and apply social distinctions. The most prominent finding

relating to depressive symptoms in Experiment 2 was the significant difference between the Saline and Cort rats in the latency to the Target Pot which displayed that Cort exposure impaired navigational abilities on the task. While the investigation of social-context-informed spatial memory is novel, previous work concluding that chronic stress inhibits spatial memory supports this finding of the present study (Kleen et al., 2006). Additionally, earlier studies have displayed that CA3 hippocampal NMDA receptors are necessary for new learning on an object association task guided by context (Rajji et al., 2006) and hippocampal lesions lead to impaired performance on a spatial context task (Komorowski et al., 2013). Together, these studies indicate the importance of an intact hippocampus for context-guided tasks and provide reasoning behind the deficits exhibited by the Cort rats in the present thesis.

Making the distinction between the Target Pot and the Context Error Pot seemed to be more difficult for all subjects of the study as it required that they discern the social context that was signaled to them before entering the maze. Since the Saline rats did not display their ability to make this distinction, it was not surprising that there was no significant interaction between Stress Condition and Pot Type when analyzing the latencies to the Target and Context Error Pots. However, there were subtle patterns that emerged in which Cort rats tended to visit the Context Error Pot before the Target Pot and Saline rats tended to do the reverse. This hinted at the fact that the Cort rats may not have been picking up the social cue as well as the Saline rats were.

While the subtle outcomes of the experiment are likely a result of the more nuanced and richer task that was employed, there are several aspects of the methodology that could be expanded upon. To mention a few, the saliency of the context could be improved, and the necessity of accurate navigation could be made clearer to the subjects. Before entering the maze,

the rats spent approximately five minutes in their assigned pairings to alert them of the social context. However, this duration of time may not be sufficient for the rats to understand that the location of the food reward is determined by the rat they are with. The salience of the conspecific rat identity in the time before the rats enter the maze is especially important during the probe trial because they are assessed alone rather than in their pairings. Next, training utilized a discrete trial procedure in which rats were removed from the maze upon getting to the Target Pot. As a result, rats always got a reward (even if they made many errors or took a long time to get to the Target Pot) and, thus, receiving the food reward was not contingent on accurate navigation. Additionally, rats were fed following their completion of the task, which is important to keep in mind as they may have viewed the conclusion of the maze task as a signal that they would receive a larger amount of food rather than just the small sugar pellets that were in the arena. To indicate that direct navigation to the Target Pot was important, an experimenter could remove the rats during training as soon as they made an error in the maze. While this would require a great deal of hands-on work for the experimenter, a potential alternative could be a T-maze or radial arm maze in which the animals would only receive a reward if they go to the correct arm.

To further our understanding of the nuanced results of the initial Single Probe test, we conducted three additional probe tests throughout Experiment 2. The Retention Probe, which aimed to understand whether the rats had longer-term memory of the maze configuration and the social condition pairings, assessed the rats following two weeks without any training or exposure to other rats besides their cagemate. The outcome was not consistent with the initial Single Probe since Cort rats were not slow to reach the Target Pot compared to Saline rats in the Besties condition. Moreover, while rats performed better in the Besties condition in Experiment 1, we did not see this distinction between the Social conditions in the Retention Probe of Experiment 2.

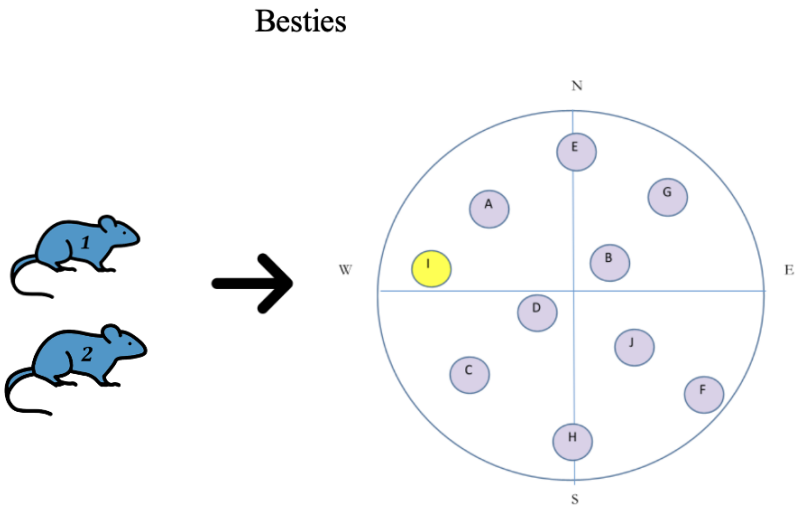
A probe test in which the rats entered the maze in their given pairings (as they would during training sessions) was conducted to see whether rats performed better when they were tested in pairs compared to individually. Additionally, we speculated that the rats may have formed an association between entering the maze alone and not receiving any food reward, which had led them to navigate less accurately in the Retention Probe. As with the results of the Retention Probe, our findings for the Paired Probe were inconsistent with the initial Single Probe and Experiment 1. Notably, the rats had only received two additionally days of training before this Paired Probe and, as a result, may not have re-learned what they had forgotten about the task and the social contexts throughout their two-week break.

A final probe that was identical in methodology to the initial Single Probe (i.e., rats entered the maze individually after being exposed to the social context) did not reveal significant statistical interactions between the Stress and Social Conditions. Graphically, the navigational abilities of the rats appear to be similar between the Saline and Cort conditions when they were paired with their Bestie. While this finding is not expected, it may point to the fact that the rats did not receive sufficient re-training following a two-week pause and that neither of the groups remembered the task well enough to navigate quickly to the Target Pot.

The objective of the two experiments of this thesis was to develop and apply a novel task to aid with the study of female rat models of depression. The task was created to meet the demand within neuroscience for behavioral assays that assess depression in richer and more extensive ways as well as the necessity to include female rodent models. As a first pass, the task that was developed is encouraging in that it targeted the social and cognitive deficits associated with depression and yielded findings that pointed to how spatial navigation is affected by the presence of another navigator.

While the present task adds to the current array of methods to study depression in rodent models, it is novel in its integrative assessment of the social and cognitive facets of the disorder. Thus, it builds upon the neurobiological basis for depression as the disorder impacts brain structures that are important for both memory and social interactions.

(a)



(b)

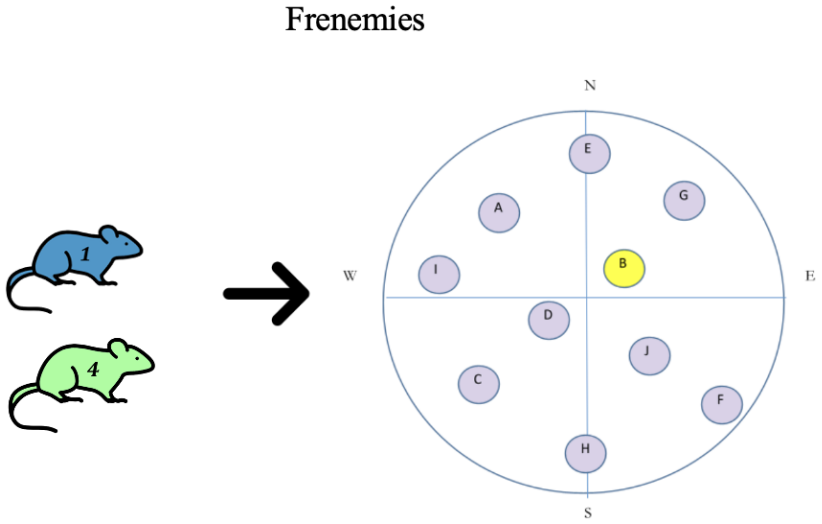


Figure 1. The maze configuration with Pots A through J that was used in Experiments 1 and 2 with sample pairings for the **(a)** Besties and **(b)** Frenemies conditions.

Table 1. Corresponding rat pairings, social conditions, and Target pots.

Rat Pairing	**Social Condition**	**Target Pot**
1 & 2	Besties	I

3 & 4	Besties	C
5 & 6	Besties	J
7 & 8	Besties	D
9 & 10	Besties	H
11 & 12	Besties	E
13 & 14	Besties	G
15 & 16	Besties	F
17 & 18	Besties	H
19 & 20	Besties	I
21 & 22	Besties	B
23 & 24	Besties	G
1 & 4	Frenemies	B
2 & 3	Frenemies	E
5 & 8	Frenemies	G
6 & 7	Frenemies	F
9 & 12	Frenemies	I
10 & 11	Frenemies	D
13 &16	Frenemies	A
14 & 15	Frenemies	D
17 & 20	Frenemies	A
18 & 19	Frenemies	C
21 & 24	Frenemies	J
22 & 23	Frenemies	F

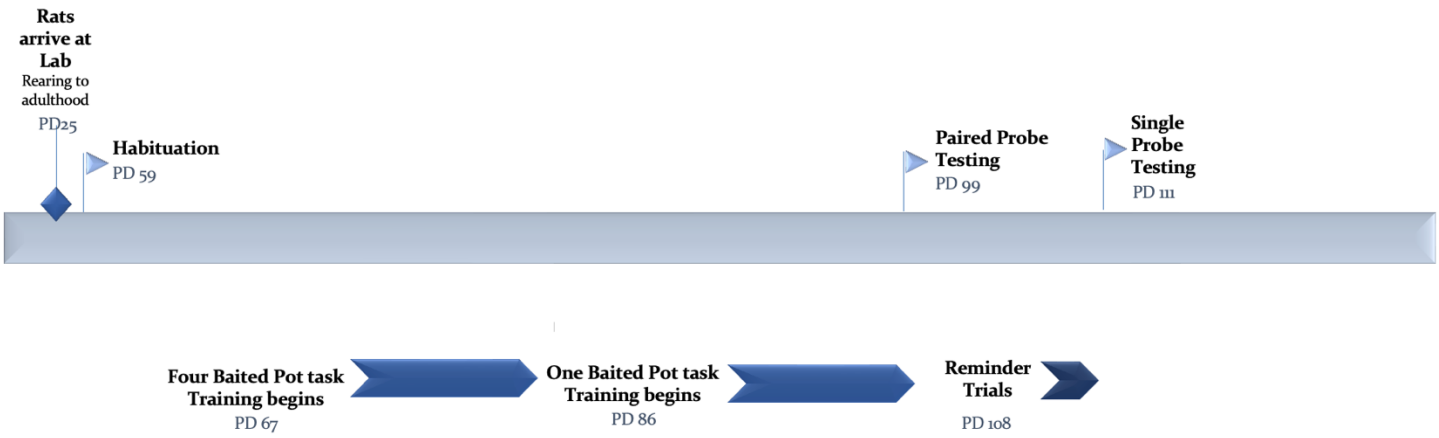


Figure 2. Experimental timeline for Experiment 1.

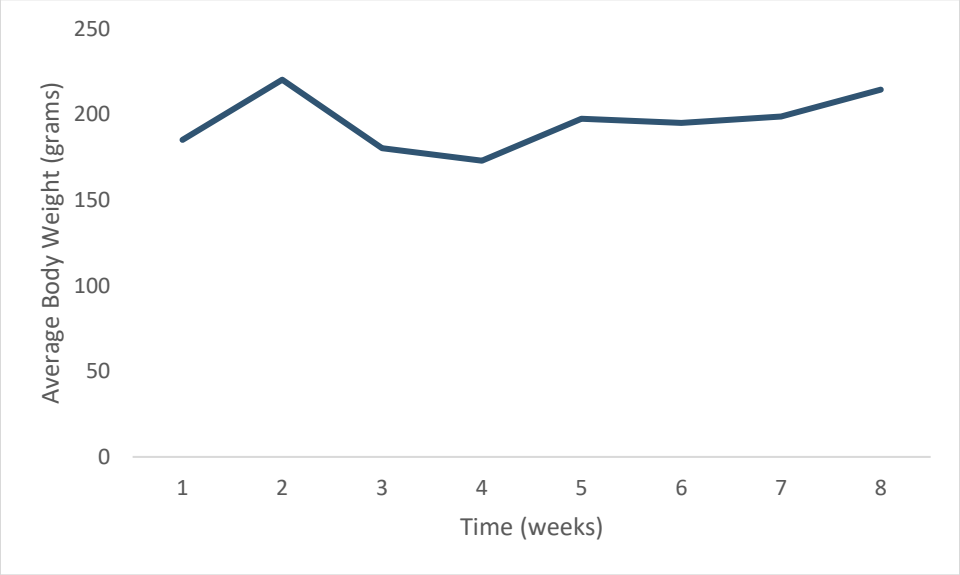


Figure 3. Average body weights (grams) as a function of time (weeks) during Experiment 1.

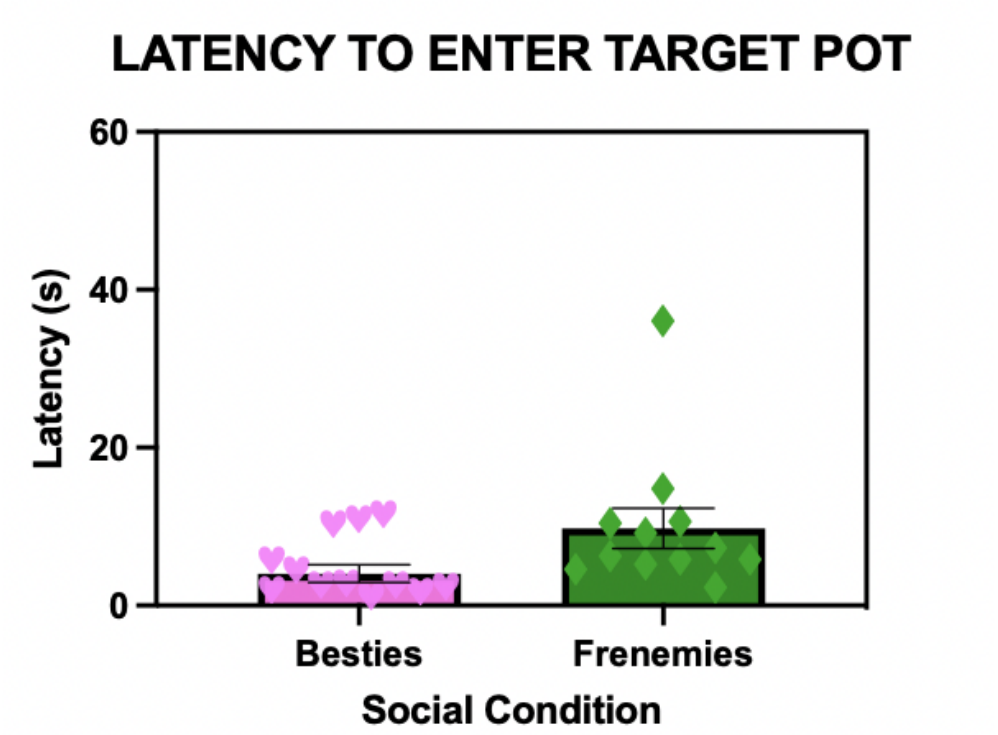


Figure 4. Latency to the Target Pot in the Single Probe of Experiment 1.

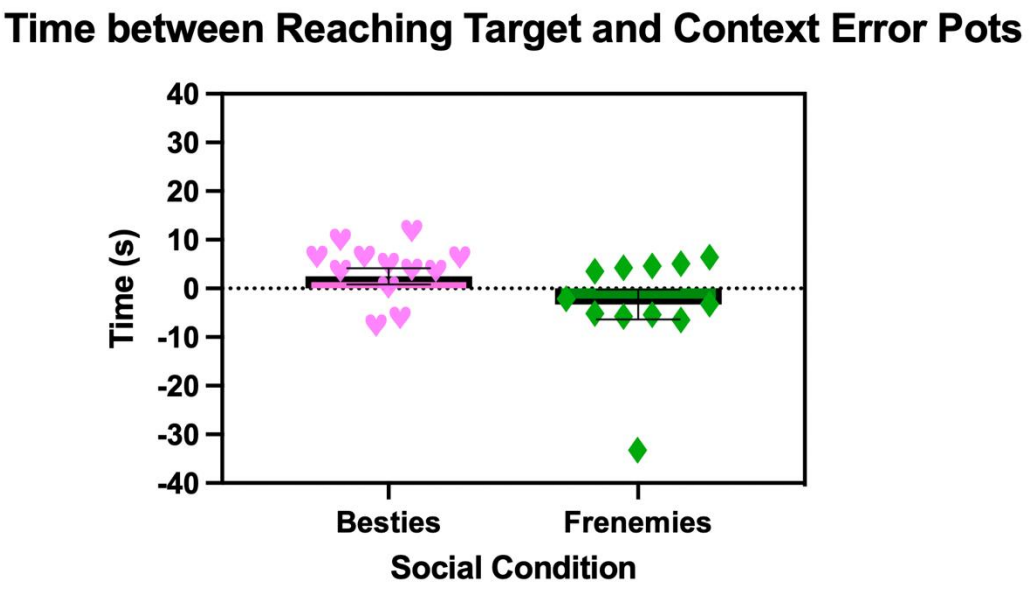


Figure 5. Time between reaching the Target and Context Error pots as a function of Social condition in the Single Probe of Experiment 1.

Table 2. Social and stress condition pairings for Experiment 2.

Rat Pairing	Social Condition	Stress Condition	Target Pot
25 & 26	Besties	Cort	I
27 & 28	Besties	Cort	C
29 & 30	Besties	Saline	J
31 & 32	Besties	Saline	D
33 & 34	Besties	Cort	H
35 & 36	Besties	Cort	E
37 & 38	Besties	Saline	G
39 & 40	Besties	Saline	F
41 & 42	Besties	Cort	H
43 & 44	Besties	Cort	I
45 & 46	Besties	Saline	B
47 & 48	Besties	Saline	G
25 & 28	Frenemies	Cort	B
26 & 27	Frenemies	Cort	E
29 & 32	Frenemies	Saline	G
31 & 32	Frenemies	Saline	F
33 & 36	Frenemies	Cort	I
34 & 35	Frenemies	Cort	D
37 & 40	Frenemies	Saline	A
38 & 39	Frenemies	Saline	D
41 & 44	Frenemies	Cort	A
42 & 43	Frenemies	Cort	C
45 & 48	Frenemies	Saline	J

46 & 47	Frenemies	Saline	F

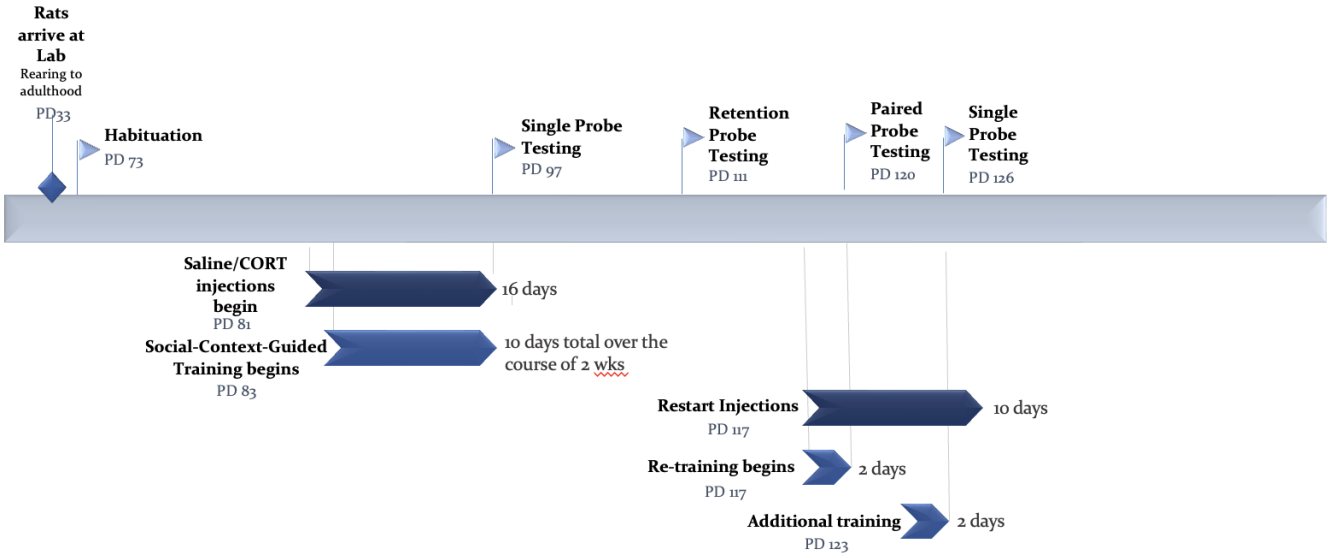


Figure 6. Experimental timeline for Experiment 2.

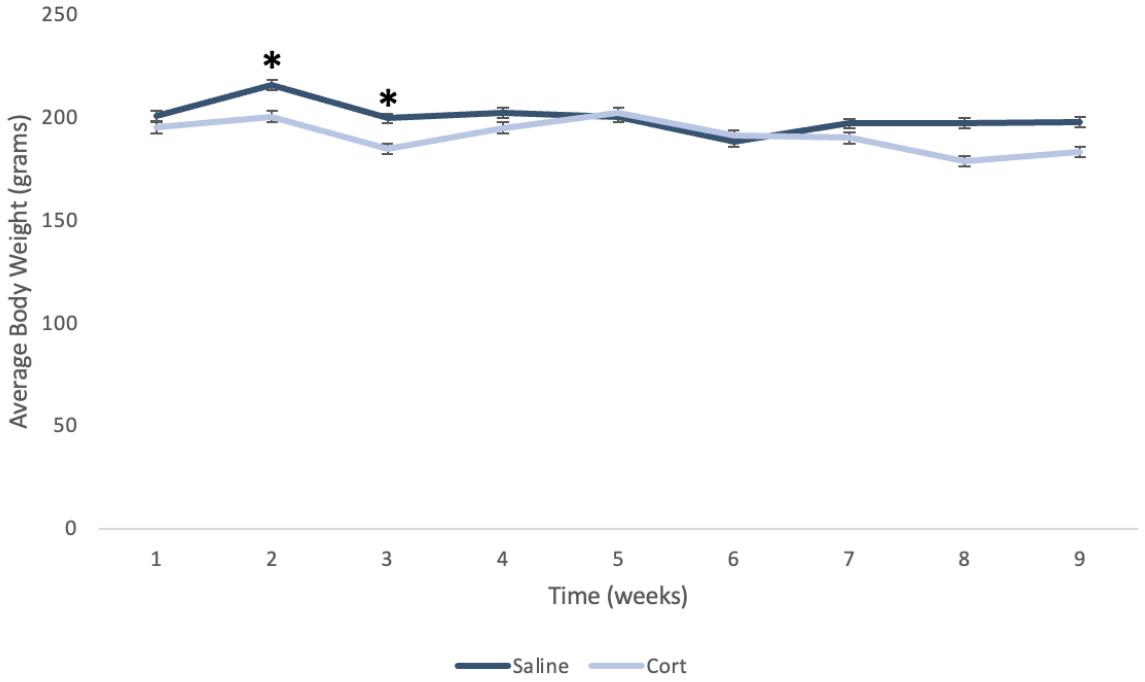


Figure 7. Average body weights (grams) as a function of time (weeks) in Experiment 2.

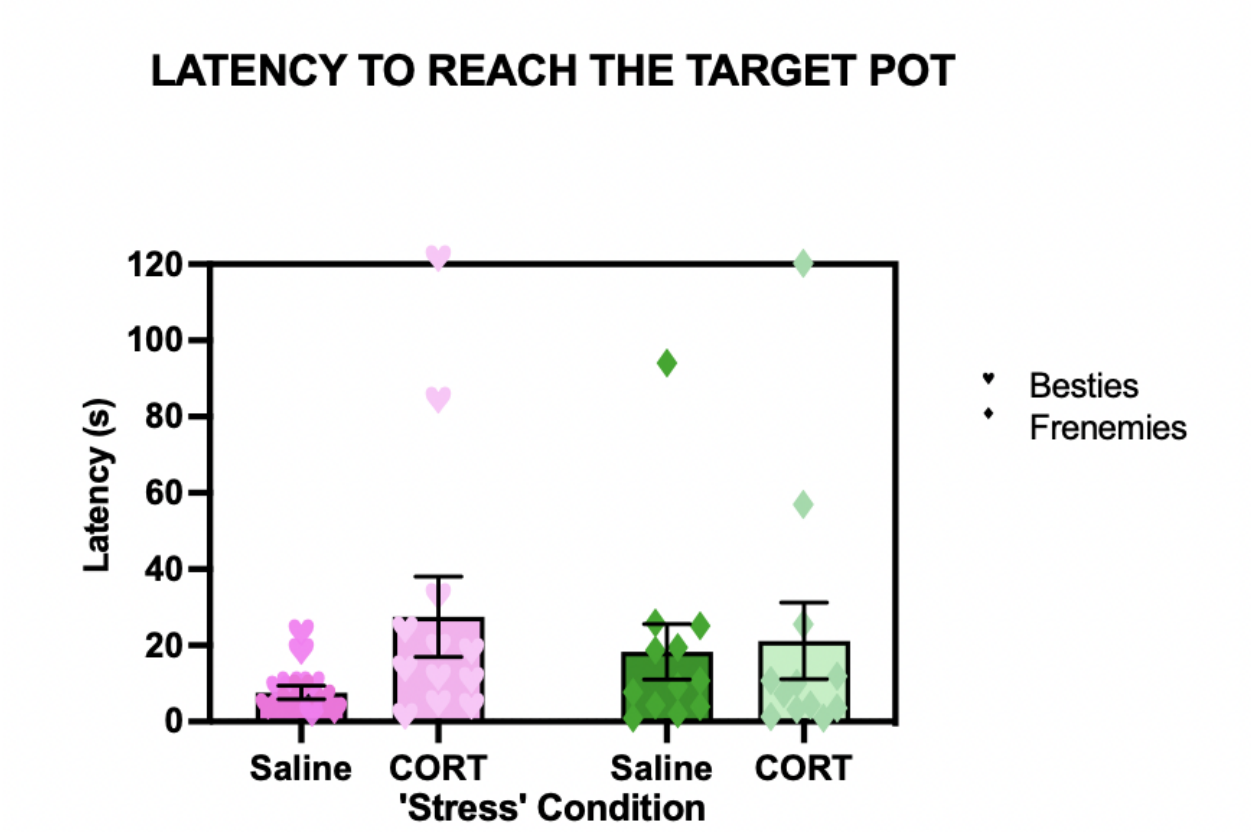


Figure 8. Latency to the Target Pot as a function of Social and Stress conditions in Single Probe 1 of Experiment 2.

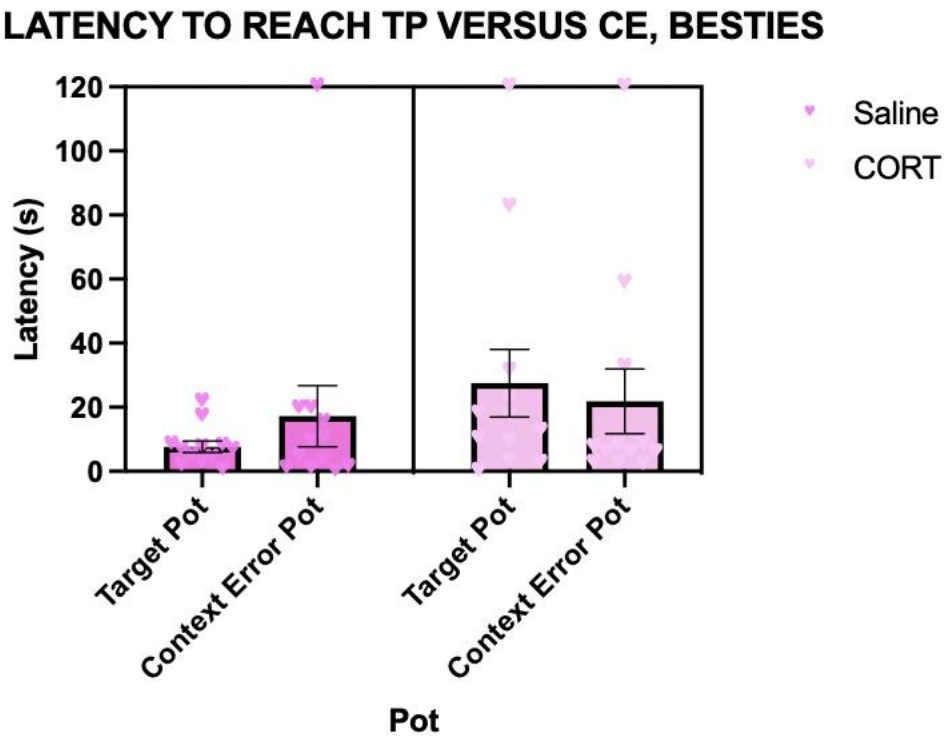


Figure 9. Latency to the Target and Context Error Pots as a function of Stress condition for rats being assessed with their Bestie in Single Probe 1 of Experiment 2.

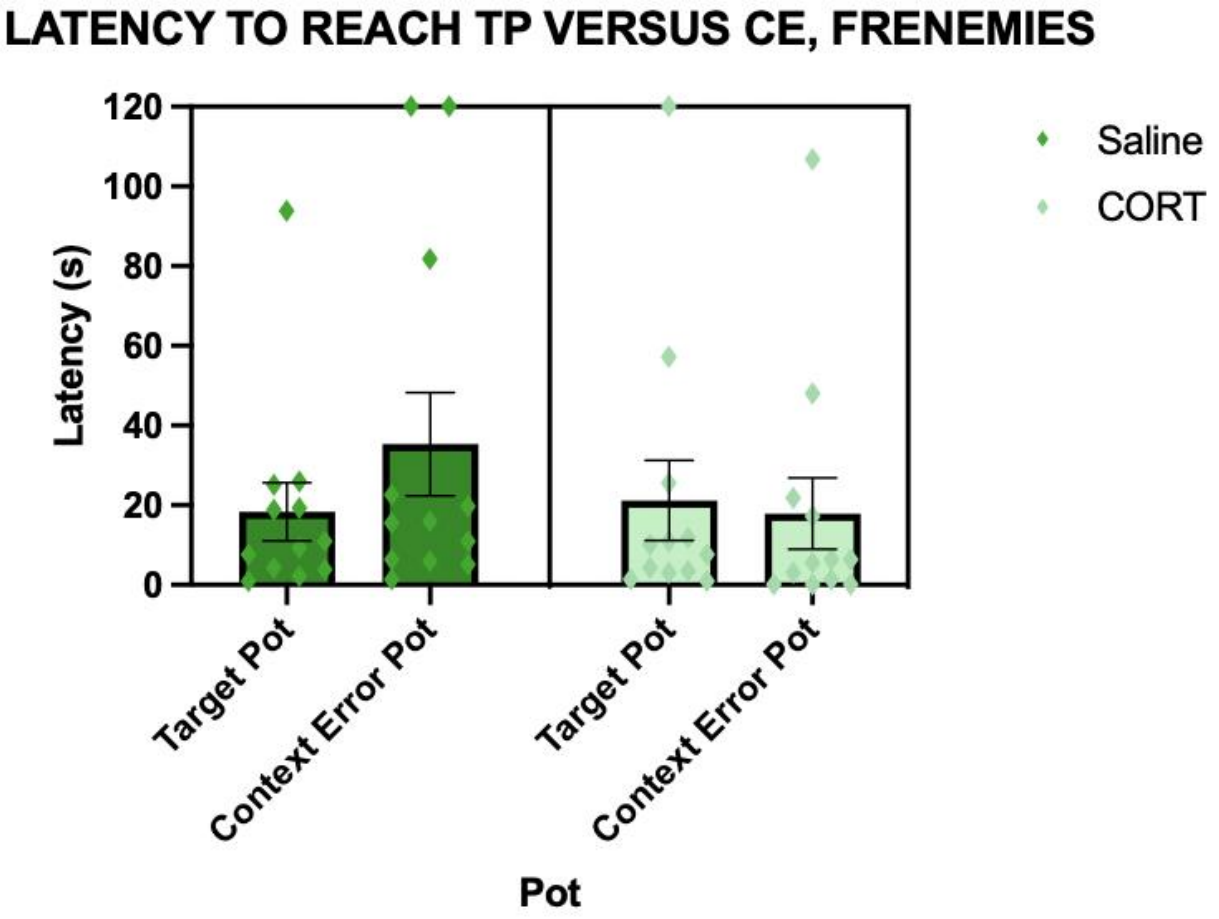
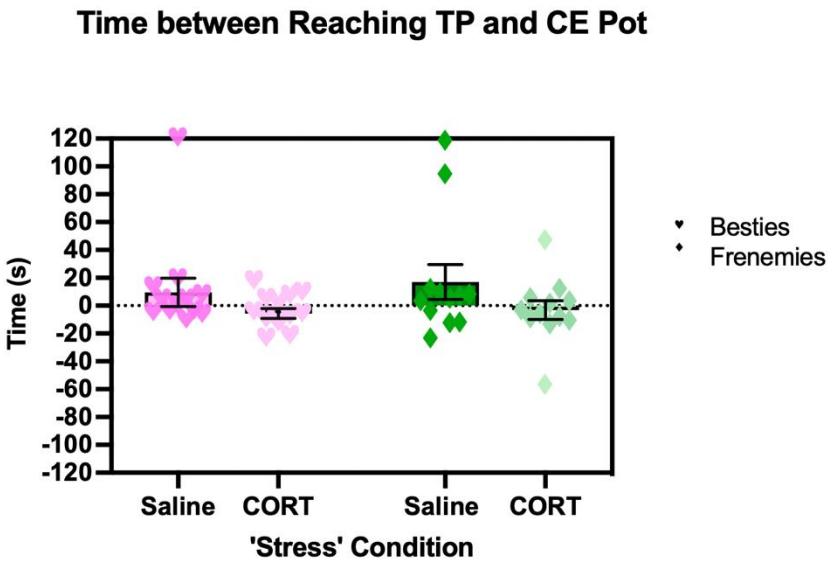


Figure 10. Latency to the Target and Context Error Pots as a function of Stress condition for rats being assessed with their Frenemy in Single Probe 1 of Experiment 2.

(a)



(b)

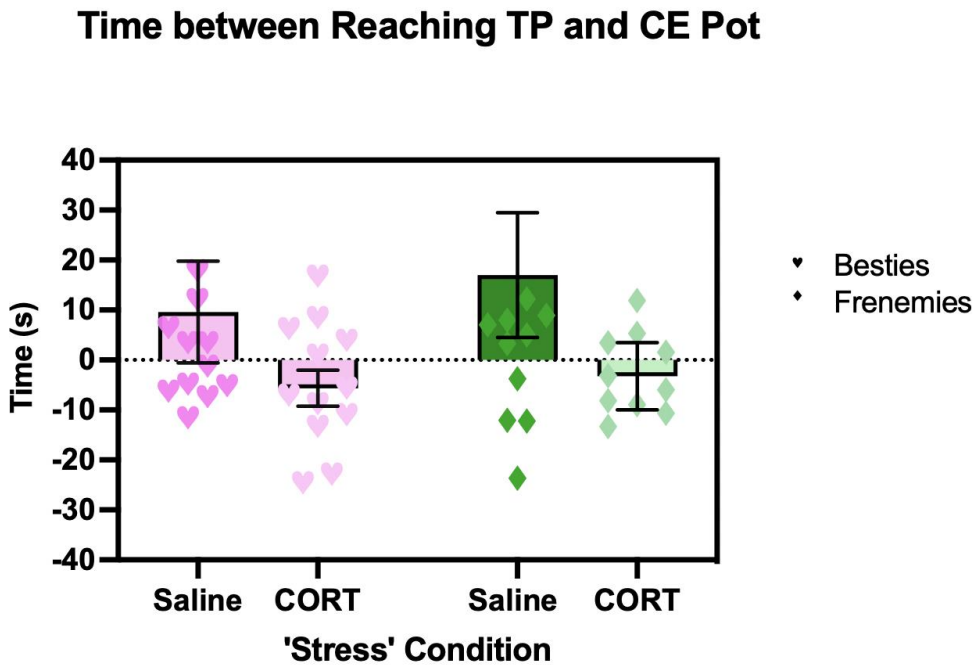


Figure 11. The time between reaching the Target Pot and the Context Error Pot as a function of Stress and Social conditions in Single Probe 1 of Experiment 2 in which **(a)** displays all data points and **(b)** zooms in for time (in seconds) between -40 and 40.

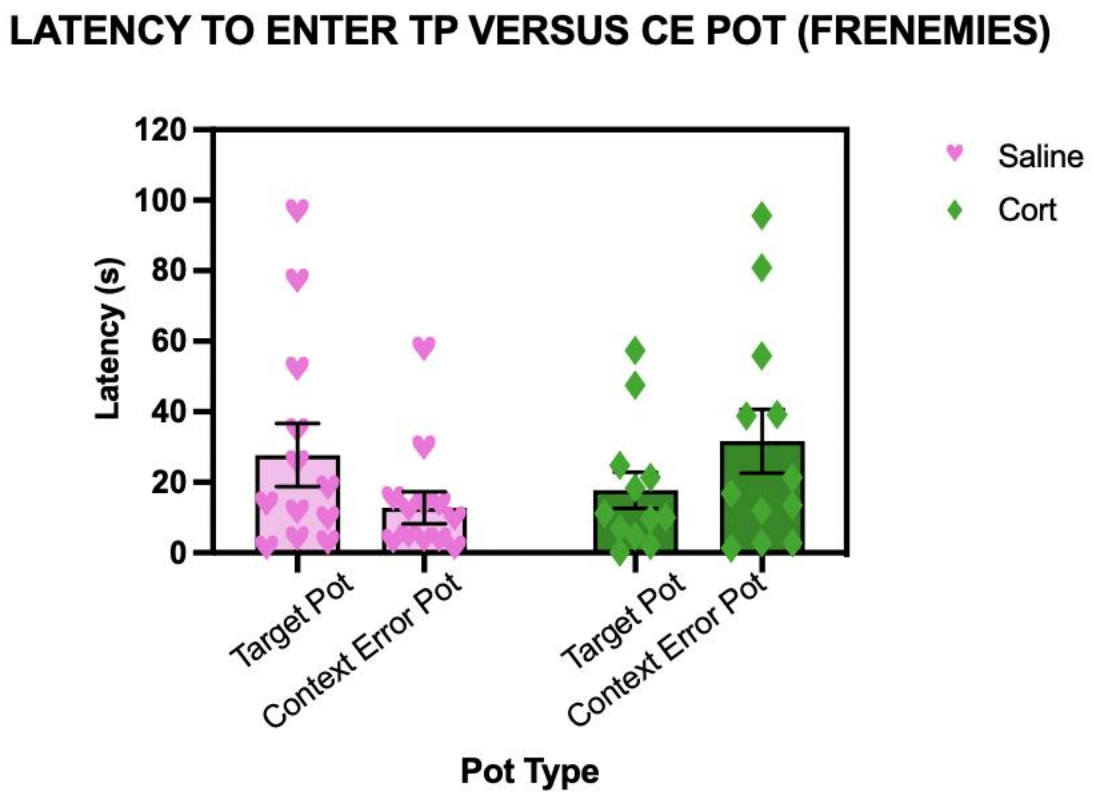


Figure 12. Latency to the Target and Context Error Pots as a function of Stress condition for rats being assessed with their Frenemy in the Retention Probe of Experiment 2.

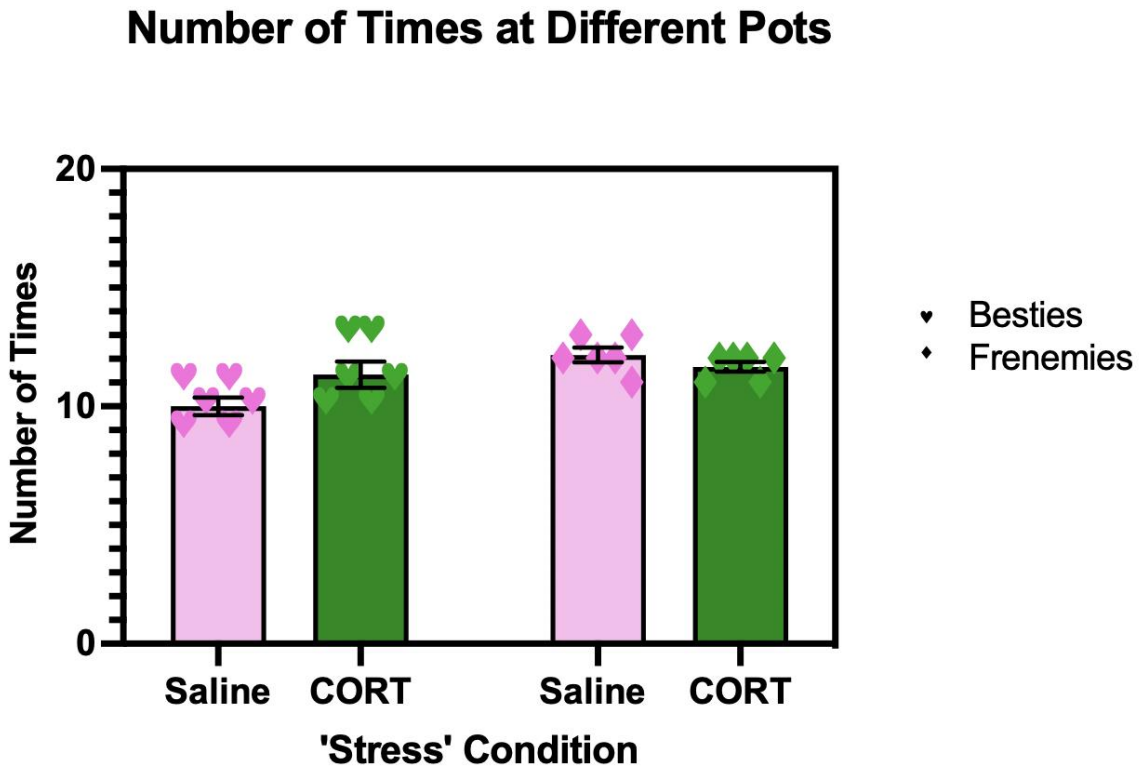


Figure 13. The number of times that rats entered different pots as a function of Stress and Social conditions in the Paired Probe of Experiment 2.

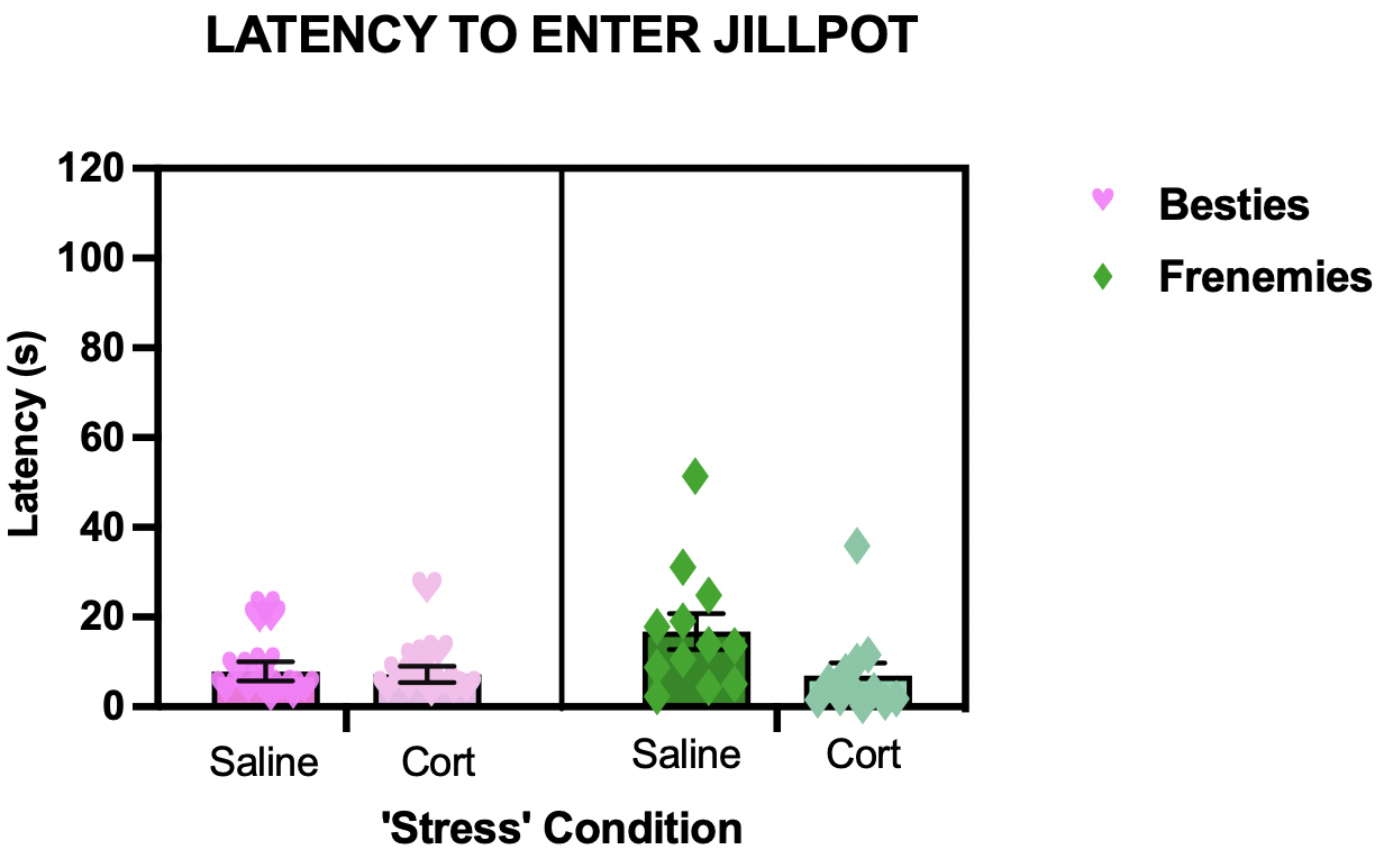


Figure 14. Latency to the Target Pot as a function of Social and Stress conditions in Single Probe 2 of Experiment 2.

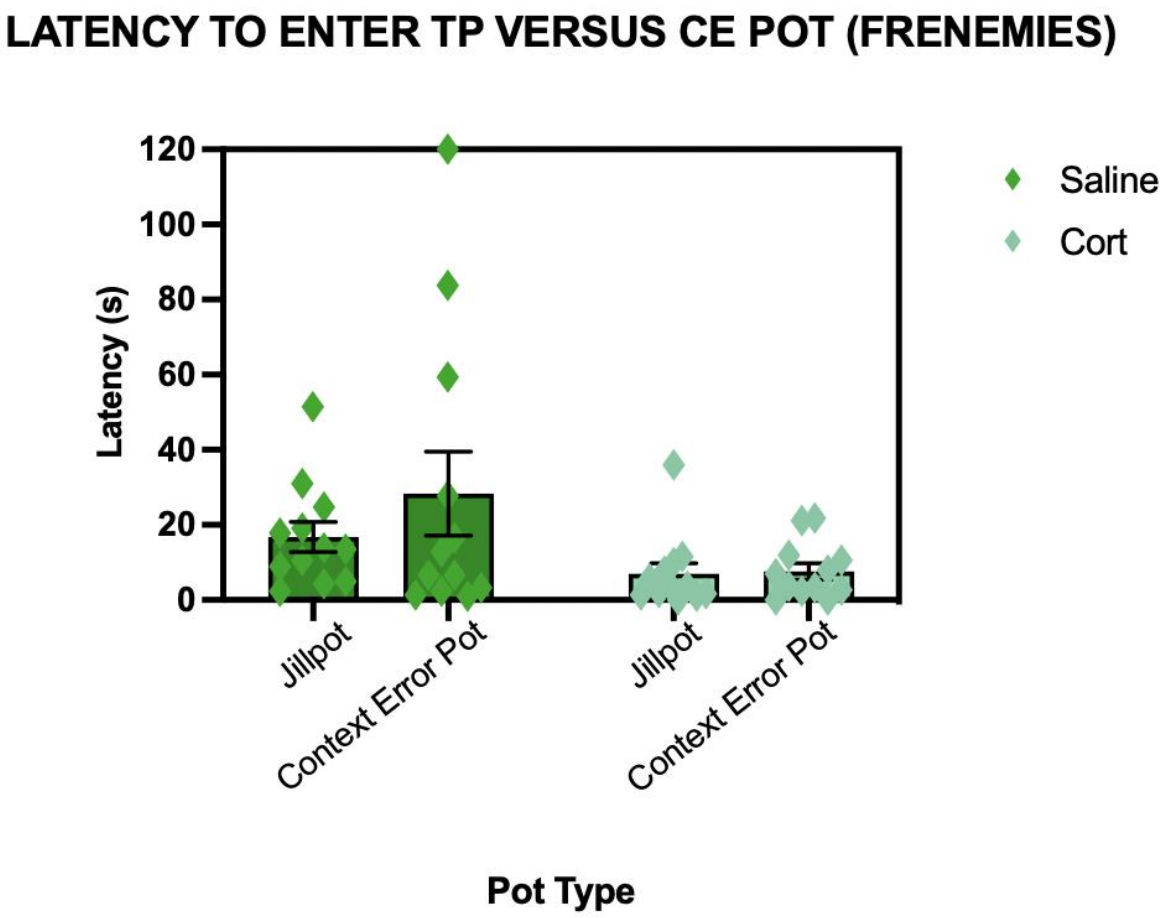


Figure 15. Latency to the Target and Context Error Pots as a function of Stress condition for rats being assessed with their Frenemy in the Single Probe 2 of Experiment 2.

References

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.).

Arbel, I., Kadar, T., Silbermann, M., & Levy, A. (1994). The effects of long-term corticosterone administration on hippocampal morphology and cognitive performance of middle-aged rats. *Brain Research, 657*(1–2), 227–235.

Beery, A. K., & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neuroscience & Biobehavioral Reviews*, *35*(3), 565–572. https://doi.org/10.1016/j.neubiorev.2010.07.002

Brown, K. L., Pagani, J. H., & Stanton, M. E. (2005). Spatial conditional discrimination learning in developing rats. *Developmental Psychobiology*, *46 2*, 97–110.

Castaneda, A. E., Tuulio-Henriksson, A., Marttunen, M., Suvisaari, J., & Lönnqvist, J. (2008). A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. *Journal of Affective Disorders*, *106*(1), 1–27. https://doi.org/10.1016/j.jad.2007.06.006

Chan, S. W. Y., Harmer, C. J., Norbury, R., O'Sullivan, U., Goodwin, G. M., & Portella, M. J. (2016). Hippocampal volume in vulnerability and resilience to depression. *Journal of Affective Disorders, 189,* 199–202. https://doi.org/10.1016/j.jad.2015.09.021

Cyranowski, J. M., Frank, E., Young, E., & Shear, M. K. (2000). Adolescent onset of the gender difference in lifetime rates of major depression: A theoretical model. *Archives of General Psychiatry, 57*(1), 21–27.

Hebb, D. O. (2002). *The Organization of Behavior: A Neuropsychological Theory* (1st ed.). Psychology Press. https://doi.org/10.4324/9781410612403

Teruko, D. Taro, T. & Shigeyoshi, F. (2018). Spatial representations of self and other in the hippocampus. *Science*, *359*(6372), 213–218. https://doi.org/10.1126/science.aao3898

Hasin, D. S., Sarvet, A. L., Meyers, J. L., Saha, T. D., Ruan, W. J., Stohl, M., & Grant, B. F. (2018). Epidemiology of Adult DSM-5 Major Depressive Disorder and Its Specifiers in the United States. *JAMA Psychiatry*, *75*(4), 336–346. PubMed. https://doi.org/10.1001/jamapsychiatry.2017.4602

Issa, A., Rowe, W., Gauthier, S., & Meaney, M. (1990). Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. *The Journal of Neuroscience*, *10*(10), 3247. https://doi.org/10.1523/JNEUROSCI.10-10-03247.1990

Jacobs, B. L., van Praag, H., & Gage, F. H. (2000). Adult brain neurogenesis and psychiatry: A novel theory of depression. *Molecular Psychiatry*, *5*(3), 262–269. https://doi.org/10.1038/sj.mp.4000712

Jankord, R., & Herman, J. P. (2008). Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. *Annals of the New York Academy of Sciences*, *1148*, 64–73. PubMed. https://doi.org/10.1196/annals.1410.012

Johnson, S. A., Fournier, N. M., & Kalynchuk, L. E. (2006). Effect of different doses of corticosterone on depression-like behavior and HPA axis responses to a novel stressor. *Behavioural Brain Research*, *168*(2), 280–288. https://doi.org/10.1016/j.bbr.2005.11.019

Kennedy, S. H. (2008). Core symptoms of major depressive disorder: Relevance to diagnosis and treatment. *Dialogues in Clinical Neuroscience*, *10*(3), 271–277. PubMed. https://doi.org/10.31887/DCNS.2008.10.3/shkennedy

Keynejad, R. C., Marková, H., Šiffelová, K., Kumar, N., Vlček, K., Laczó, J., & Kopelman, M. D. (2018). Spatial navigation deficits in amnestic mild cognitive impairment with neuropsychiatric comorbidity. *Aging, Neuropsychology, and Cognition*, *25*(2), 277–289.

Kim, J. J., & Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nature Reviews Neuroscience*, *3*(6), 453–462. https://doi.org/10.1038/nrn849

Kleen, J. K., Sitomer, M. T., Killeen, P. R., & Conrad, C. D. (2006). Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore. *Behavioral Neuroscience, 120*(4), 842–851. PubMed. https://doi.org/10.1037/0735-7044.120.4.842

Komorowski, R. W., Garcia, C. G., Wilson, A., Hattori, S., Howard, M. W., & Eichenbaum, H. (2013). Ventral hippocampal neurons are shaped by experience to represent behaviorally relevant contexts. *The Journal of Neuroscience*, *33*(18), 8079. https://doi.org/10.1523/JNEUROSCI.5458-12.2013

Krishnan, V., & Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature*, *455*(7215), 894–902. https://doi.org/10.1038/nature07455

Liu, W., Ge, T., Leng, Y., Pan, Z., Fan, J., Yang, W., & Cui, R. (2017). The Role of Neural Plasticity in Depression: From Hippocampus to Prefrontal Cortex. *Neural Plasticity*, *2017*, 6871089. https://doi.org/10.1155/2017/6871089

Lopez, J., & Bagot, R. C. (2021). Defining valid chronic stress models for depression with female rodents. *Biological Psychiatry*, *90*(4), 226–235. https://doi.org/10.1016/j.biopsych.2021.03.010

Lupien, S. J., de Leon, M., de Santi, S., Convit, A., Tarshish, C., Nair, N. P. V., Thakur, M., McEwen, B. S., Hauger, R. L., & Meaney, M. J. (1998). Cortisol levels during human

aging predict hippocampal atrophy and memory deficits. *Nature Neuroscience*, *1*(1), 69–73. https://doi.org/10.1038/271

McEwen, B. S. (2008). Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *European Journal of Pharmacology*, *583*(2–3), 174–185. PubMed. https://doi.org/10.1016/j.ejphar.2007.11.071

Montello, D., & Sas, C. (2006). *Human Factors of Wayfinding in Navigation*.

Murray, T. K., & Ridley, R. M. (1999). The effect of excitotoxic hippocampal lesions on simple and conditional discrimination learning in the rat. *Behavioural Brain Research*, *99*(1), 103–113. https://doi.org/10.1016/S0166-4328(98)00077-1

O'Keefe, J. (1976). Place units in the hippocampus of the freely moving rat. *Experimental Neurology*, *51*(1), 78–109. https://doi.org/10.1016/0014-4886(76)90055-8

Omer, D. B., Maimon, S. R., Liora, L., & Ulanovsky, N. (2018). Social place-cells in the bat hippocampus. *Science*, *359*(6372), 218–224. https://doi.org/10.1126/science.aao3474

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: Classical theories and new developments. *Trends in Neurosciences*, *31*(9), 464–468. https://doi.org/10.1016/j.tins.2008.06.006

Pittenger, C., & Duman, R. S. (2008). Stress, depression, and neuroplasticity: A convergence of mechanisms. *Neuropsychopharmacology*, *33*(1), 88–109. https://doi.org/10.1038/sj.npp.1301574

Price, R., & Duman, R. (2020). Neuroplasticity in cognitive and psychological mechanisms of depression: An integrative model. *Mol Psychiatry*, *25*(3), 530–543. https://doi.org/doi:10.1038/s41380-019-0615-x

Rajji, T., Chapman, D., Eichenbaum, H., & Greene, R. (2006). The role of CA3 hippocampal NMDA receptors in paired associate learning. *The Journal of Neuroscience, 26*(3), 908. https://doi.org/10.1523/JNEUROSCI.4194-05.2006

Dalton, R., Hölscher, C., & Cath, D. (2019). Wayfinding as a Social Activity. *Frontiers in Psychology, 10,* 142. https://doi.org/10.3389/fpsyg.2019.00142

Sigurdsson, T., & Duvarci, S. (2016). Hippocampal-prefrontal interactions in cognition, behavior and psychiatric disease. *Frontiers in Systems Neuroscience, 9*. https://www.frontiersin.org/article/10.3389/fnsys.2015.00190

Vale, W. (2005). The neurobiology of depression: Inroads to treatment and new drug discovery. *The Journal of Clinical Psychiatry, 66 Suppl 7,* 5–13.

Wang, Q., Timberlake II, M., Prall, K., & Dwivedi, Y. (2017). The recent progress in animal models of depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *77*, 99–109. https://doi.org/10.1016/j.pnpbp.2017.04.008

Whitlock, J. R., Heynen, A. J., Shuler, M. G., & Bear, M. F. (2006). Learning induces long-term potentiation in the hippocampus. *Science*, *313*(5790), 1093–1097. https://doi.org/10.1126/science.1128134

WHO. (n.d.). *Depression*.

Xu, L., Anwyl, R., & Rowan, M. J. (1997). Behavioural stress facilitates the induction of long-term depression in the hippocampus. *Nature*, *387*(6632), 497–500. https://doi.org/10.1038/387497a0

Xu, P., Wang, K., Lu, C., Dong, L., Chen, Y., Wang, Q., Shi, Z., Yang, Y., Chen, S., & Liu, X. (2017). Effects of the chronic restraint stress induced depression on reward-related

learning in rats. *Behavioural Brain Research*, *321*, 185–192. https://doi.org/10.1016/j.bbr.2016.12.045

Yassin, M., El Antably, A., & Abou El-Ela, M. A. S. (2021). The others know the way: A study of the impact of co-presence on wayfinding decisions in an interior virtual environment. *Automation in Construction*, *128*, 103782. https://doi.org/10.1016/j.autcon.2021.103782

Zhang, F.-F., Peng, W., Sweeney, J. A., Jia, Z.-Y., & Gong, Q.-Y. (2018). Brain structure alterations in depression: Psychoradiological evidence. *CNS Neuroscience & Therapeutics*, *24*(11), 994–1003. PubMed. https://doi.org/10.1111/cns.12835