The Use of Repeated Forced Social Defeat as an Animal Stress Model

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Over 50% of patients are resistant to current treatments for depression. With a lifetime prevalence of 20.6% among US adults, it is evident that the present understanding of major depressive disorder is limited, and current experimental methods are inadequate to decode the complex pathology of depression. This study used a novel animal model, Repeated Forced Social Defeat, to induce depressive-like symptoms in mice in order to add to the current literature on depression. The study sample comprised forty wild type male C57BL/6 inbred mice. The Tube Test was utilized to determine the established social rankings within each group of mice. This was followed by administration of the Tail Suspension Test and a second administration of the Tube Test. Repeated Forced Social Defeat was administered a week after the second round of Tube Test administration, followed by one last round of the Tail Suspension Test. Results indicate that the dominant mice did not display significantly greater immobility times than the subordinate mice in the Tail Suspension test prior to- and after administration of Repeated Forced Social Defeat. However, there was a non-significant trend towards the dominant mice displaying greater immobility times in comparison to the subordinate mice post social defeat. Evidently, while there were no significant differences, dominant mice seem to display less resilience following social defeat in comparison to subordinate mice. A possible explanation for a lack of significant differences is a small sample size and short period of administration. These findings demonstrate the potential of utilizing Repeated Forced Social Defeat as a means to induce depressive-like symptoms in mice, as well as the relevance of dominance hierarchies in predicting susceptibility to specific stressors. Further investigation of Repeated Forced Social Defeat is warranted.

Abbreviations: ABT – Agonist Behavior Test; BA – Barber Assay; FST – Forced Swim Test; HPA – Hypothalamic-Pituitary-Adrenal; MDD – Major Depressive Disorder; RFSD – Repeated Forced Social Defeat; RIP – Resident-Intruder Paradigm; TST – Tail Suspension Test; TT – Tube Test; UMA – Urine Marking Assay; VBS – Visible Burrow System

Keywords: Depression; Animal Model; Social Stress; Social Hierarchy; Antidepressant

Introduction

The present study was designed to analyze the efficacy of a novel antidepressant screening method in mice, Repeated Forced Social Defeat (RFSD), to improve the current understanding of the psychopharmacological mechanisms of antidepressants and the current understanding of Major Depressive Disorder (MDD). According to results from a national survey taken by 36,309 US adults, the lifetime prevalence of major depressive disorder is 20.6% (Hasin et al., 2018). However, over half of the patients with MDD are unable to achieve remission from traditional treatments, making the introduction of a new treatment crucial (Trivedi et al., 2006). This present study utilized the Tube Test (TT) to determine dominance hierarchies in cohorts of mice. Once social rankings were determined, the new model of mild chronic stress, RFSD, was used to induce depressive-like symptoms in mice.

The limitations to current treatments for depression reflect the lack of understanding of...
MDD. This is in large part due to the complexity of the disorder, as demonstrated by the heterogeneity of symptoms found in patients (Goldberg, 2011). From a psychopharmacological standpoint, researchers have proposed numerous hypotheses for the causes of depression in addition to the various intracellular mechanisms downstream of antidepressant-receptor binding (Dale et al., 2015). Preclinical studies are used to investigate the antidepressant mechanisms of action as well as the safety of drugs, leading to more insight into the pathophysiology of the illness itself.

A process has been created to effectively filter out harmful and ineffective drugs before they are introduced into the market (Reddy et al., 2019). Out of 250 compounds tested in preclinical studies, approximately 5 are chosen for clinical trials. The small percentage of drugs that are approved for human consumption demonstrates the importance of preclinical studies in screening compounds. Animal antidepressant screening tests, used in preclinical studies, are categorized into despair-based tests and reward-based tests. Despair-based tests involve the induction of learned helplessness to study the antidepressant-like effects of drugs, most commonly in rodents (Kaur Multani et al., 2014). The term “learned helplessness” describes the changes in behavior an organism experiences from the inability to control outside stressors (Overnier and Seligman, 1967).

Commonly used despair-based tests are the Forced Swim Test (FST) and the Tail Suspension Test (TST) (Steru et al., 1985). The FST was developed by Porsolt et al. (1977). They initially observed a portion of the rats escaping the Morris Water Maze - an assay to measure spatial learning of rodents (Vorhees and Williams, 2006) - slower than others. Following this observation, Porsolt et al. (1977) designed the FST to screen antidepressants. The immobile behavior or lack of swimming or climbing, is eventually adopted by mice after an initial burst of escape-oriented behavior and is described as behavioral despair as the animal is no longer attempting to escape (Porsolt et al., 1978).

Due to the complexity and heterogeneity of depression, a study that utilizes a combination of animal models would bring more insight into the pathophysiology of MDD. An animal that induces stress may also be very effective as the induction of stress plays an important role in the development of depression. Stress can induce physiological changes that involve increased hypothalamic-pituitary-adrenal (HPA) axis activation (Willner, 2005). According to Bao et al. (2008), the human body has a stress response system that maintains homeostasis when faced with adversity. However, excessive activity of the HPA axis may result in negative consequences. Hyperactivity of the HPA axis has been consistently found in patients with major depression, with patients exhibiting increased concentrations of cortisol, a stress hormone, in plasma, urine, and cerebrospinal fluid. With 60% of depressive episodes being preceded by exposure to stressors, a novel model that induces stress is warranted (de Carvalho Tofoli et al., 2011).

Humans experience stress from various forms of social stress such as bullying and workplace harassment. Rather than physical altercations, the most common forms of stressors encountered by humans are psychological or social (Aloe and Levi-Montalcini, 1977). Björkqvist (2001) found that victims of bullying were more likely to be depressed, experience low self-esteem, and have a negative view of themselves. Bullying in the workplace correlates with an increase in depressive-like symptoms after one year (Loerbroks et al, 2015). In addition to peer relations at work, socioeconomic status is another determinant of health in humans. In a meta-analysis study performed by Everson et al. (2002), a higher prevalence of depression was found in the lower class. Other social species have a similar correlation between social ranking and vulnerability to stress-related diseases (Sapolsky, 2005). There is considerable evidence of the formation of social hierarchies amongst male mice cage-mate, for example. According to Horii et al. (2017), group-housed mice tend to show aggressive behavior towards each other and form social hierarchies. These hierarchies tend to be linear and maintained through fighting and chasing (So et al., 2015), where the dominant mouse is more likely to display agonistic behavior such as chasing and aggressive grooming (i.e. barbering, pulling fur from the back of its subordinate). In one study, cohorts of four male mice were monitored over the course
of four days, and the average frequency of agonistic behaviors was recorded for each mouse. After determining the social rankings, the effects of social hierarchy on depressive-like behaviors were examined using the FST. Findings revealed that the subordinate mice displayed significantly longer immobility times in comparison to dominant mice (Horii et al., 2017).

Other studies have also demonstrated a connection between social ranking and depressive-like symptoms (Wood et al., 2012). Rygula et al. (2005) argued that a chronic stress model that uses social conflict to generate stress is preferable to a model that generates stress with physical stimuli such as foot shock and cold exposure. Currently, when studying social defeat in rodents, the Resident-Intruder Paradigm (RIP) is the most used model. To induce social stress, a male rodent (the intruder), is placed in the cage of another male rodent (the resident). The two rodents fight for a certain amount of time, and the defeated opponent is identified as the rodent being chased, the one that assumes a defensive posture such as rearing up on hind legs, or the rodent that is squeaking. The winner is referred to as the dominant male while the losing rodent is the subordinate and the one that experiences social stress. In the RIP, the resident male is from a highly aggressive strain to ensure that the resident reliably attacks the intruder mouse (Bartolomucci et al., 2009). In this way, the resident rodent will be territorial and force the intruder into submission.

In a different study, the administration of a TCA, desipramine, attenuated the increased immobility time caused by chronic stress from exposure to the RIP in rats (Wood et al., 2012). While the RIP has been shown to produce depressive-like symptoms in rodents (Wood et al., 2012), this present study examined a less intrusive means to cause social stress as well as a model with more face validity.

The Tube Test (TT) was initially developed in 1961 to measure dominance between mouse strains (Lindzey et al., 1961), but the use of the TT has been expanded to measure social hierarchies among mouse cage mates of the same strain (Wang et al., 2011). In a study conducted by Wang et al. (2011), TT results were consistent with four other tests, including the Visible Burrow System (VBS) (Melhorn et al., 2017), the Agonist Behavior Test (ABT) (Wang et al., 2011), the Barber Assay (BA) (Long, 1972), and the Urine Marking Assay (UMA) (Drickamer, 2001). Subordinate mice experienced a greater weight loss in the VBS, where food and water are difficult to access; displayed fewer agonistic behaviors in a novel environment in the ABT; subordinate mice were more likely to be subjected to whisker barbering in the BA; and marked smaller territories with their urine in the UMA (Wang et al., 2011). Evidently, the TT provides a robust and valid means to determine the social ranking.

Unlike the RIP, the TT creates a mild level of stress and prevents rodents from inflicting injuries upon each other as there is not enough space for a violent confrontation (Zhou et al., 2018). TT results are also transitive and stable. Fan et al. (2019) demonstrated the transitivity of the TT results. After labeling every mouse in a cohort of four A through C, they found that if mouse A defeats mouse B and mouse B defeats mouse C, mouse A will defeat mouse C. These findings coincide well with claims that mice maintain linear hierarchies (So et al., 2015). Moreover, social ranking remains consistent once it has been established (Kunkel and Wang, 2018).

The present study investigated a new form of social stress, Repeated Forced Social Defeat (RFSD), that is created to disrupt pre-established dominance hierarchies determined by the TT in cage-mate mice. It was hypothesized that the disruption of social hierarchies will create significant differences in the immobility times of the affected rodents in the TST.

### Materials and Methods

Forty adult, wild-type male C57BL/6 inbred mice (Envigo, Indianapolis, IN) weighing 24.2-37.8 g were used in the study. Female mice were not used in the study as literature suggests that female mouse hierarchies are not as salient as those of male mice. For example, female hierarchies are less linear than male social hierarchies and have less directionality regarding the display of behaviors that maintain dominance, such as fighting, chasing, and mounting
All 40 of the mice were used in the TT procedure; 20 of the mice were used in RFSD; 20 (10 alpha mice and 10 omega mice) were used in the TST after the TT and before RFSD; 10 (5 alpha mice and 5 omega mice) of the mice were used in the TST after RFSD. Mice were maintained on a 12 to 12 h light-dark cycle (lights on 0600 h, lights off 1800 h). They were allowed 5 weeks to adapt to their new environment before experimentation. The mice were group-housed in a temperature-controlled vivarium at 22-24 °C. Nesting material and ad libitum access to food and water was provided in their home cage. The mice were group-housed on a 4 per cage basis.

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (protocol AM10284, J.H. Porter P.I.). The study was performed in agreement with the guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and all procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (IACUC Protocol AM10284).

Materials

A clear, Plexiglas tube (diameter, 3 cm; length, 30 cm) was used to perform the TT and RFSD test. The tube was placed on a 3D-printed stand attached to a white, plywood board. The diameter of the tube was sufficient for an adult mouse to move through it, but not to turn around. Paper towels soaked in a 10% ethanol solution were used to remove odors from the apparatus after each test. A plastic pipe, two tube stoppers, and a thin, circular piece of transparent plastic were used to prevent subject re-entry at each end of the tube. A timer was used to record the length (s) of the confrontation between two mice before one of the mice retreated. Two covered, empty transition cages containing bedding were used to house the selected mice after they were taken out of the cage for habituation to the TT apparatus.

Four open, 3-walled, rectangular tail suspension boxes were used to measure the mice’s immobility while under inescapable tail suspension in the TST. The boxes were made of plywood and painted white with the dimensions of 55 cm by height, 18 cm by width, and 18 cm by depth. Each box was large enough to prevent any contact with the walls while the mouse was suspended, and the wall prevented them from seeing each other. A metal hook was attached at the top of each box's interior. To prevent the mice from climbing up their tails towards the hook as a means of escape, climb-stoppers were created from clear, hollow cylinders (5.5 cm, 0.9 g each) cut from plastic, 1 mL syringes. They were then placed onto the tails. Micropore surgical tape (1 in. wide) was cut into 17 cm pieces, marked with a Sharpie 2 cm from one end, and was used to attach the tail to the hook.

Procedures

The TT was performed on mice that had been cohabitating for 5 weeks. Each mouse was trained to move forward through the tube and to enter 5 times from each end for 2 consecutive days. If the mouse retreated or stopped moving for more than 1 min, the experimenter gently encouraged the mouse to move forward by touching the mouse’s tail with a smaller, plastic tube. A thin piece of plastic or rubber stopper was placed against the entryway to prevent reentry once the mouse exited the tube. The tube was cleaned and dried between each trial with the 10% ethanol solution to remove odor, urine, or feces. After the 2 day-habituation phase, social ranks per group were tested from 4 to 8 consecutive days, depending on the consistency of the social rankings in a given cohort of mice.

The TT was performed according to the protocol established by Fan et al. (2019). Using a round-robin design, pairs of mice from the same social group were tested, leading to 6 confrontations per cage of 4 mice during 1 testing day. Each mouse from the group was tested against the other, ensuring that all possible confrontations were observed and recorded. Prior to testing, each mouse was removed from the home cage and placed into an empty transition cage. Immediately after both mice were isolated from the home cage and each other, they were placed onto the TT apparatus at a time and encouraged to walk through each end of the tube 1 time. Each mouse was habituated to the testing apparatus for 5 min while the other stayed in the transition cage. After habituation to the testing apparatus, the mice were placed back into an empty cage, and the apparatus was cleaned with.
a 10% ethanol solution. The test was initiated after both mice walked through the tube during the habituation period.

Upon initiation of the TT, the 2 mice were held by the tail at opposite ends of the tube and released once their heads were inside the tube. The mice moved towards each other from both ends of the tube, and the researcher started a stopwatch when both mice met in the center. The timer was stopped once 1 of the mice retreated and all 4 of its paws exited the tube. The mouse that retreated from the tube was designated as the subordinate mouse while the mouse that remained in the tube or moved forward was designated as the dominant mouse. As each mouse was tested 3 times, the researcher placed the mouse facing the end of the tube opposite of the end it entered in the last trial. Mice were left in their home cage for 20 min prior to being tested again.

After all mice were tested, the number of times each mouse retreated, stayed in place, or moved forward were added together. The act of retreating out of the tube was designated as a loss. The act of remaining in the tube or moving forward was designated as a win. A retreat from the tube was scored as -1, and a win was scored as +1. The social ranking of each mouse was determined by comparing its score to the scores of its cage mates. Mice with the highest score received were given rank of “1” and regarded as the dominant mice. The mice with lower scores were ranked 2 through 4 and regarded as subordinates. The social ranking was considered stable if the ranks remained consistent for 4 consecutive days. If the social ranking changed prior to the 5th day, the entire process was repeated before the mice were used in RFSD (Fan et al., 2019). The TST was conducted on the day after social ranking was established.

The TST was performed according to the protocol established by Can et al., (2012). A “Testing, No Interruption” sign was taped onto the door of the testing room. All testing boxes were placed side-by-side on a table, and a camera was placed across from the testing chambers. A household fan was turned on to create white noise. Metal collecting trays filled with cage bedding were placed at the bottom of each box. A 10% alcohol solution in a spray bottle, paper towels, and a bottle of bleach were used for cleaning the apparatus between sessions. The identification of each group of mice was written on a white board and video-recorded prior to initiating the TST.

Mice were brought into the testing room 30 min prior to testing. Only 1 group of 4 mice was brought in at a time, and each mouse was placed in a separate cage for the 30-min habituation period. Each cage contained a cotton, environment enrichment square and bedding. The fragments of tape used for suspension were prepared prior to the experiment. At the end of the 30-min habituation period, climb stoppers were placed onto each subject’s tail prior to tape application. In order to ensure the secure attachment of mice to the tail suspension apparatus, the ends of the tape were wrapped around the end of each tail so that the tape stuck to itself at the demarcation line with 2-3 mm of the tail remaining outside of the tape. The other end of the tape was adhered to the edge of each habituation cage to prevent the tape from becoming tangled. Once tape had been applied to each mouse’s tail, the camera was set to start recording. 1 by 1, the mice were suspended by wrapping the free end of the tape around each hook. The entire process, from applying the climb stopper to suspending the mice, took no longer than a min in order to avoid causing additional stress prior to the procedure. Once the last mouse was suspended, the researchers left the room and allowed the test to run for 6 min. At the end of each session, the recording was stopped, and the tape was carefully removed from each mouse. The mice were returned to their home cages, and the apparatus was wiped down with a 10% alcohol solution sprayed on paper towels. All climb-stoppers were placed in a 2% bleach solution for cleaning.

After all mice had been tested, the videos were uploaded to a shared Google Drive folder to be scored by raters that were blind to the experimental conditions. Prior to undergoing RFSD and the day after the TST, the mice were subjected to the TT protocol for 2 consecutive days to ensure that the social ranking remained the same after TST.

Mice were subjected to RFSD 1 week following the 2nd TT procedure. Prior to undergoing RFSD, each mouse was weighed, and its weight was recorded. The weight was
recorded 1 time per week for each cage, and the entire procedure lasted for 10 consecutive days. All procedures were repeated exactly 24 h apart. Thus, the alpha mice were subjected to a total of 10 repeated forced defeats before being tested again in the TST.

Each home cage of 4 mice was taken to the testing room and left to habituate for 20 min. The testing order was randomly determined and recorded on a chart. Post-habituation, the alpha mouse was identified and placed in a transition cage. The mouse it was paired with was placed in a separate transition cage. After a waiting period of approximately 5 min, the mice were placed at opposite ends of the tube. Each mouse was held by its tail and positioned facing into the tube, ensuring that each mouse entered the tube and confronted the other mouse midway. Once the head of each mouse was inside the tube, the mice were simultaneously released. Upon release, a rubber stopper was used to blockade the end behind the subordinate mouse. Gently, the stopper was pushed forward with either a pencil or a plastic pipe, preventing the subordinate mouse from backing out and forcing the alpha mouse to retreat. After all 4 paws of the alpha mouse exited the tube, both mice were taken back to their home cage. A 20-min timer was started, and the mice were left in the cage until the timer went off. The entire apparatus, including the probe, was cleaned with the 10% ethanol solution after each trial. Once the 20-min interval ended, the mice underwent a 2nd TST on the day after RFSD was concluded.

Data Analysis

Depressive-like behaviors were analyzed using the immobility time from the TST after determining the social rankings of each group of mice, prior to and after RFSD. The researcher that performed the TST did not score the videos. Raters (5-6 per video) were trained to score the TST videos prior to experimentation. When scoring the videos, the raters were unaware of the social rank of each mouse. The raters scored using a Google score sheet. The rows were used for tracking the time in s, and each column was designated to one mouse from each cage. The columns were organized into 12 groups of 4. The raters began scoring for mobility time once the last mouse had been suspended, for 6 min. Mobility was determined if both the front and hind paws of the animal were moving. For each s that the mice were mobile, the raters typed “1” into the score sheet. These were added together to calculate the total mobility time. The total immobility time was calculated by subtracting the total mobility time from 360 s. The total immobility time for each social ranking was then averaged between the raters’ scores.

For the TST, the dependent variable was the immobility time. The independent variable was the social ranking of the mice: dominant (alpha) versus subordinate (omega). All data were expressed as means ± SEM. Immobility time data was analyzed using an unpaired, pooled variance, two-tailed t-Test. The criterion for significance was a \( p \)-value \( \leq 0.05 \). Data analysis for both studies were conducted with Prism version 8.0 for MacOS GraphPad Software (GraphPad Software Inc., La Jolla CA v. 5.0). All significant differences were at \( p \leq .05 \).

Results

An independent-samples t-test was conducted to compare the average immobility time of the alpha mice to the average immobility time of the omega mice prior to and after RFSD. There was no significant difference in the immobility times of the 10 mice determined to be dominant (alpha) \((M = 196.1, \text{SD} = 63.5)\) compared to the immobility times of the 10 mice determined to be fourth ranking subordinate or omega \((M = 202.8, \text{SD} = 49.26)\); \( t(18) = 0.26, p = 0.795 \). The results showed that the alpha and omega mice displayed similar immobility times prior to RFSD (see Figure 1).

An independent-samples t-test was conducted to compare the average immobility time of the alpha mice to the average immobility time of the omega mice after administration of RFSD. There was no significant difference in the
immobility times of the 5 alpha mice ($M = 212.3$, $SD = 59.21$) compared to the immobility times of the 5 omega (subordinate) mice ($M = 176.7$, $SD = 49.23$); $t(8) = 1.03$, $p = 0.332$. These results indicate that social stress did not cause differences in immobility times between the alpha and the omega mice in the TST (see Figure 2).

![Figure 1: Average immobility times of mice with rankings alpha and omega in the Tail Suspension Test prior to Repeated Forced Social Defeat. Data are expressed as means of immobility time in s (± SEM).](image)

![Figure 2: Average immobility times of mice with rankings of alpha and omega in the Tail Suspension Test post Repeated Forced Social Defeat. Data are expressed as means of immobility time in s (± SEM).](image)

**Discussion**

The aim of this study was to investigate a novel way to induce chronic social stress in rodents for screening novel antidepressant drugs. In their review, Hollis and Kabbaj (2014) established the validity of social defeat as a means of inducing depressive-like symptoms and the efficacy of tests that utilize behavioral despair (i.e. TST and FST) in measuring symptoms induced by social defeat. For example, RIP induces increased immobility time in the FST as well as depressive-like symptoms such as anhedonia and social avoidance (Hollis and Kabbaj, 2014). There is significant evidence that rodents are social animals and establish social hierarchies (Williamson et al., 2016). In their study, 10 cohorts of 12 mice formed a linear dominance hierarchy. Eight of the ten cohorts had mice that occupied a unique social rank, and all cohorts displayed social networks that had high transitivity. While the FST and TST have limitations as models of clinical depression due to their short-term induction of stress, they are useful in predicting the efficacy of most
antidepressants, and the immobility time determined by these tests is an indication of depressive-like behavior (see Lucki, 2010). The FST and TST are therefore more effective when used in conjunction with a model of depression that induces depressive-like symptoms over a longer period. The present study therefore focused on the utilization of a model that repeatedly induces social stress (RFSD for 10 days) followed by the administration of the TST. This study utilized the TT to measure the social dominance hierarchies of cohorts of mice (four per cage). Once social hierarchies within a cage were determined, the hierarchical status of the alpha (dominant) mouse was challenged as a means of inducing stress using the RFSD. The TST was used before the determination of social ranking and after RFSD as a means to demonstrate whether RFSD induced depressive-like symptoms in mice.

It was hypothesized that the administration of RFSD would result in an increase in depressive-like behavior in the alpha mice (i.e. greater immobility time). Furthermore, subordinate mice should display significantly greater immobility times in comparison to the dominant mice prior to RFSD and similar or significantly smaller immobility times in comparison to the dominant mice after RFSD. In a different study, C57BL/6 mice of lower social ranking displayed greater immobility times in the FST and more depressive-like and anxiogenic-like symptoms than dominant mice (Horii et al., 2017). Moreover, Larrieu et al. (2017) found that mice determined to be dominant using the TT exhibited greater social avoidance post chronic exposure to an aggressive male in a social defeat procedure than subordinate mice. However, the results of the present study failed to these hypotheses.

Prior to undergoing RFSD, the alpha mice had an average immobility time of 196.1 seconds, and the omega mice had an average immobility time of 202.8 seconds (see Figure 1). The average immobility times did not significantly differ ($p > 0.05$), thereby demonstrating that the omega mice did not display significantly more depressive-like behaviors than the alpha mice. Higher levels of cortisol and corticotropin release factor mRNA have been reported in the medial and central amygdala of dominant mice (Williamson et al., 2016; So et al., 2015), indicating that dominant mice experience higher levels of HPA activity. Dominant animals experience higher levels of stress in groups where they must continuously establish their dominant status (Beery and Kaufer, 2015). While dominant mice may experience stress from establishing their social status, the subordinate mice experience stress from having a lower social ranking, leading to similar displays in immobility time.

The difference between the average immobility times of alpha and omega mice post RFSD was not significant ($p > 0.05$) (see Figure 2). The results were surprising as the alpha mice were the only mice subjected to social stress. However, there were no controls utilized in this present study. It therefore cannot be determined whether the lack of a significant difference between the alpha and omega mice demonstrates the lack of induction of depressive-like behaviors.

There is a possibility that the lack of significant differences in average immobility times of dominant and subordinate mice was due to the small sample size. The sample size consisted of 5 alpha mice and 5 omega mice in TST taken post RFSD, and the TST taken prior to RFSD consisted of 10 alpha and 10 omega mice. Of note, there was a non-significant trend towards decreased immobility time in the omega mice after RFSD, and the dominant mice displayed greater (but nonsignificant) immobility times in comparison to the subordinate mice post social defeat. Future studies with a larger sample size may reveal that omega mice significantly decrease in depressive-like symptoms after displaying behavior associated with social dominance.

The study is also limited by the time period for conducting RFSD. Procedures that involve chronic social stress generally last from 10 to 40 days, and models that involve chronic mild stressors, such as a wet cage, last from 5 to 9 weeks (Scheggi et al., 2018). The RFSD test was conducted for 10 days and may have produced significant results if conducted for a longer period. This limitation suggests that chronic social stressors may be more effective in causing depressive-like symptoms in mice than
acute stressors, and further investigation comparing both stressors is warranted.  

Other limitations involve the use of only male mice. Female mice subjected to chronic social instability displayed significantly more anxiety-like behaviors in comparison to controls, while stressed male mice did not differ significantly from their control counterparts (Saavedra-Rodriguez and Feig, 2013). Female mice may be more vulnerable to social stressors than male mice, and the effect of RFSD on female cohorts, as well as comparisons between the effects of RFSD on male and female mice, warrants further investigation.

While the results failed to support the hypothesis, they demonstrated the complex relationship between stressors and depressive-like symptoms. Social ranking may play a mediating role between the stressor and response to the stressor in animal models, and the effect of a stressor may vary in strength depending on the type of stressor (i.e acute or chronic, social stressor or physical stressors). Results from RFSD models should be compared to other animal models, including the TST as well as CMS. Lastly, clinical studies evaluating the effects of social stress on patients of lower status compared to those of higher social status, including ones for male and female cohorts, are potential avenues for future research.

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